GENOTYPIC VARIATION IN GROWTH AND
CARBON ISOTOPE DISCRIMINATION

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by

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## Chapter One

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Statement

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

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Summary

A series of studies was undertaken in order to examine the physiological basis for the relationship between growth and carbon isotope discrimination ($\Delta$). The recent finding that the carbon isotope composition of $C_3$ plants is negatively related to transpiration efficiency ($W$, the ratio of dry matter accumulated to water transpired) has led to a number of studies which have shown that there is wide variation in $\Delta$ (and $W$) within species and the suggestion that $\Delta$ can be used as a selection criterion for $W$. In addition positive, negative or no relationships between $\Delta$ (or $W$) and growth have been demonstrated, depending on the species investigated. Carbon isotope discrimination is a complex characteristic which depends on stomatal and photosynthetic attributes. Hence, it is possible for a number of different relationships between growth and $\Delta$ to occur. This thesis examines such relationships, the understanding of which are crucial for correct agronomic or ecological interpretation of genotypic variation in $\Delta$. Fundamentally the question is: what sort of plant is obtained when $W$ is selected for?

To answer this question a pilot study on sunflower was undertaken. The results of this study showed that amongst six genotypes, during vegetative growth there was a positive relationship between biomass and $W$ (negative with $\Delta$). In a subsequent experiment this relationship was further analysed using growth analysis techniques and leaf gas exchange on seven sunflower genotypes. The results of the second experiment were consistent with those of the first. Furthermore, there was a negative relationship between relative growth rate ($RGR$) and $\Delta$. The variation in $\Delta$ was related negatively to photosynthetic rate ($A$), measured under high light and ambient CO$_2$ conditions, but not related to stomatal conductance ($g$). The variation in $RGR$ between these genotypes was due to variation in net assimilation rate ($NAR$) rather than leaf area ratio ($LAR$). In turn, there was a close positive relationship between $A$ and $NAR$. These results led to
the conclusion that variation in photosynthetic capacity ($A_{\text{max}}$) within sunflower accounts for that in $\Delta$ and $RGR$ during vegetative growth.

Further experiments were conducted to test whether the above results were typical of sunflower. A larger range of germplasm including a wild accession and an *Helianthus argophyllus* accession was examined for variation in $\Delta$ and $RGR$. In order to compare with the results of other published growth analyses under (low light) growth cabinet conditions a shade treatment was also imposed on eight of the genotypes. There was significant genotypic variation in both $\Delta$ and $RGR$ and the two were negatively related as above. Under either natural or low light (30% of natural) conditions, there was a positive relationship between $RGR$ and $NAR$. These results, taken together, were further evidence of the importance of $A_{\text{max}}$ in determining differences in $RGR$ and $\Delta$ between genotypes of sunflower. Yet a further experiment showed that $\Delta$ was negatively related to $A_{\text{max}}$ (obtained under saturating $CO_{2}$ and light conditions using an oxygen electrode) amongst a composite set of genotypes selected from the above three experiments. These results are different from those of other studies (either between or within species) which have shown that differences in partitioning normally determine differences in growth. A simulation based on the results of the sunflower experiments suggested that variation in the apparent nitrogen use efficiency ($NUE$, ratio of $A_{\text{max}}$ to nitrogen per unit leaf area) may explain the positive relationship between photosynthetic capacity and growth, and would be a fruitful area of further research.

Rather than continue with this line of research it was decided to determine, at the species level, whether the same relationships held as for sunflower. A preliminary experiment showed that $\Delta$ was negatively related to $W$ amongst a range of pasture cultivars (26 cultivars belonging to 11 species). The intra-species variation in $\Delta$, while significant, was not large due to the narrow range of genotypes available. However, it is likely that
some of this variation was due to genotype by environment interaction as indicated by comparison with subsequent results. A further experiment was aimed at analysing a subset of the above cultivars (23 cultivars from 10 species) for variation in $RGR$ and $\Delta$ during early growth. There was significant variation in $\Delta$ and $RGR$. Differences in $RGR$ arose because of $LAR$ which, in turn, was related to variation in both specific leaf area ($SLA$ leaf area per leaf biomass) and leaf weight ratio ($LWR$, leaf biomass as a proportional of total plant biomass). When legumes and grasses were analysed separately, no clear relationship between $RGR$ and its components emerged. Neither could $\Delta$ be related to any of the growth analysis parameters (excepting $SLA$). However, there were relationships between biomass accumulation and $\Delta$: positive for the grass cultivars; negative for the legume cultivars. Hence, the results obtained depended very much on the type of comparison being made. As a generalisation, it seemed that if NAR (and $A_{max}$) caused differences in $RGR$ then it was likely that there would be a negative relationship between $RGR$ and $\Delta$. In contrast if $LAR$ was responsible for variation in $RGR$ no relationship could be found between $\Delta$ and $RGR$.

A further experiment was aimed at contrasting ecotypes of two very different Mediterranean pasture species selected from the above experiment. *Trifolium subterraneum* and *Phalaris aquatica* were selected because a range of collected ecotypes was readily available. Within a considerable range of ecotypes (50 for *T. subterraneum* and 26 for *P. aquatica*), there was wide variation in $\Delta$. Subsets of these ecotypes (15 for *T. subterraneum* and 11 for *P. aquatica*) were found to vary significantly in $RGR$. For *T. subterraneum* the variation in $RGR$ was accounted for by variation in NAR whereas for *P. aquatica* the variation in $RGR$ could be accounted for that in $LAR$. In addition $RGR$ and $\Delta$ were negatively related amongst the *T. subterraneum* ecotypes whereas there was no relationship between the two amongst *P. aquatica* ecotypes. The results for *T. subterraneum*, just as with sunflower, indicated that $A_{max}$ may be the cause
The most important point to be made from this work is that variation in $A_{\text{max}}$ should be reconsidered as the cause of variation in both $\Delta$ and $RGR$ especially within some species. This is an unexpected finding and is discussed with respect to the large number of studies which have shown that variation in $LAR$ causes variation in $RGR$. Hence, the type of plant we obtain when selecting for high $W$ (low $\Delta$) will very much depend on the species that is being examined. It is suggested that the basis for variation in $\Delta$ and $RGR$ should be known across a wide range of material before selection for $W$, via $\Delta$, takes place. These results also pose an important question for future research, that is, why do species differ in the amount of variation in those characters which determine differences in $RGR$ and $\Delta$?
CHAPTER ONE

INTRODUCTION

The aim of the work presented in this thesis is to gain a greater understanding of how growth and carbon isotope discrimination are related both between and within species. It suffices to say here that carbon isotope discrimination ($\Delta$) is closely related to whole plant transpiration efficiency ($W$, the ratio of plant biomass to water transpired). A detailed explanation will be given below. I shall firstly concentrate on the historical context viz. why this particular problem has arisen at this time. To do this, the notion of selection for physiological traits in plants and the impact on the pattern of science is examined. I then focus on the development of $W$ as a trait particularly with regard to its relationship with $\Delta$. Because it is crucial to understanding possible links between $W$ and growth the analysis of plant growth is then briefly reviewed, especially with respect to the characteristics that provide variation in growth rate among and between species. Finally, a statement of experimental objectives is presented.

1.1 Physiological Traits

In the past 20-30 years there has been a significant emphasis in plant physiology and agronomy on selection and breeding of plants with superior physiological attributes and hopefully enhanced agronomic performance. There are probably many reasons for this, including economic imperative, availability of appropriate technology, and even curiosity. Whatever the cause, large amounts of time and money have been expended in the search for such traits. Predictably, the progression of work done has conformed to the specific requirements of both plant breeding and agronomy.
The necessary criteria that need to be satisfied for the successful use of a physiological trait in a breeding program have been outlined by Garrity et al. (1982) and Mahon (1983). For such a physiological trait there needs to be:

1) Development of a hypothesis concerning the potential contribution of the trait
2) Genetic variability available
3) Characterisation of genetic control including both narrow and broad sense heritabilities and selection of an appropriate breeding method
4) An appropriate screening technique which allows selection to take place in large scale field trials
5) Proof of agronomic benefit. This includes studies on pleiotropic effects (or unintended consequences) of selecting for the trait.

This list of criteria determines not only the ultimate fate of the trait but is also a guide to understanding the interconnection between various studies on the usefulness of traits. By way of example, the development of two traits will be examined according to these criteria. Firstly, osmoregulation, which has been proposed as a physiological trait that may provide higher yield under water limited conditions (Morgan 1984) is examined. Basic theory and genotypic variation were demonstrated (Morgan 1977). Genetic control and broad sense heritabilities have been assessed (Morgan et al. 1986; Grummet et al. 1987; Morgan 1991) as have both agronomic performance including some work on pleiotropic effects (Quisenberry et al. 1984; Morgan and Condon 1986; Grumet et al. 1987) and assessments of the screening technique (Morgan 1983; Morgan et al. 1986). Secondly, the pattern can also be shown in the attempts to use photosynthetic rate as a selection criterion for increased yield. Mahon and Hobbs have published a number of papers that addressed these criteria for pea (e.g. Mahon and Hobbs 1981, 1987; Hobbs and Mahon 1982, 1985). Much of the work in selecting for photosynthetic rate
in other crops may be categorised according to the same criteria: basic work on genetic variability and physiology (e.g. El Sharkawy et al. 1965; Dornhoff and Shibles 1970; 1976; Cook and Evans 1983); genetic studies (e.g. Byrne et al. 1981; Yamauchi and Yoshida 1985); appropriateness of screening techniques (e.g. Pearce et al. 1968; Secor et al. 1982); and the difficult area of relationship to agronomic performance (e.g. Crosbie et al. 1982; Ford et al. 1983). In this case the lack of any demonstrated benefit of selection for higher photosynthetic rate (e.g. Elmore 1980; Lambers 1987; Nelson 1988; Austin 1989) also led to both a re-examination of the theoretical aspects (e.g. Day and Chalabi 1988) and of pleiotropic effects, such as the relationship with leaf nitrogen content (e.g. Evans 1983). In short, the lack of any demonstrated agronomic benefit has curtailed the use of photosynthetic rate (or capacity) as a physiological trait.

The above examples of trait development are presented as a pattern for the treatment of yet another trait central to this thesis - Transpiration efficiency

1.2 Transpiration efficiency

1.2.1 Background

Transpiration efficiency ($W$) can be defined as the ratio of plant biomass to total water transpired. Alternatively it has been called water-use efficiency ($WUE$). It is sometimes defined as the ratio of above ground biomass to water transpired (e.g. Passioura 1977). To both physiologists and agronomists $W$ has been a source of interest for some time. The reason for this interest is obvious enough especially where water is in short supply. Comprehensive studies by Briggs and Shantz (1914), Shantz and Piemeisel (1927) and Richardson and Trumble (1928) using large pots under glasshouse conditions showed that $W$ (or its inverse, transpiration ratio) varied both between and within species and under varying levels of nutrition.
After this early work these methods were abandoned as workers concentrated more on micro-meteorological aspects of water-use efficiency (Stanhill 1986). In later times $W$ was shown to vary within and between species in both glasshouse and field environments (Anderson and Read 1966; Downes 1967; Dobrenz 1971; Bleak and Keller 1973; Johns and Lazenby 1973) but could not be directly selected for because of difficulties associated with field measurement (Hubick et al. 1986). Barker, Frank and Berdahl (1989) have also recently recognised the need for a rapid screening technique, especially since genotypic ranking based on single leaf gas exchange measurements is unreliable (Frank et al. 1987). An early study on heritability had found that (the inverse of) $W$ was under genetic control (Hunt 1962). However, overall the major difficulty with $W$ as a physiological trait was the lack of an appropriate screening technique.

Alternative strategies to achieve greater $W$ via indirect selection have been suggested. For example reduced stomatal conductance should often favour CO$_2$ assimilation over evaporation at the leaf level (Cowan and Troughton 1971). As a result, many attempts were made to select for stomatal characteristics which would lead to lower conductance. Generally these were unsuccessful (Jones 1987) and probably due to the problems of advection (Jarvis and McNaughton 1986) and difficulties with both screening and genotype by environment interaction (Jones 1987). Some success has been gained using selection for glaucousness to improve plant $W$ (Ferguson 1974; Richards et al. 1986). But overall the lack of an appropriate screening technique has been the largest hurdle to overcome.

Tanner and Sinclair (1983) identified that modification of $p_i/p_a$ (the ratio of the partial pressure of CO$_2$ in the intercellular air spaces ($p_i$) of the leaf to ambient ($p_a$), see explanation below) was a possible, but unlikely, means of improving crop water use efficiency. At about the same time Farquhar et al. (1982) suggested that carbon isotope discrimination ($\Delta$) could be used as a
technique by which plants could be screened for variation in $W$ amongst $C_3$ species. In the section that follows the development of $\Delta$ as a screening method is presented.

1.2.2 The relationship between Carbon isotope discrimination and Transpiration efficiency

1.2.2.1 Theoretical aspects

Transpiration efficiency ($W$) and $\Delta$ are negatively related because both in turn are directly related to $p_i/p_a$ (Farquhar et al. 1982b; O'Leary 1988) and expressed more simply by Farquhar and Richards (1984) and Hubick et al. (1986). The basis of the relationship can be more readily understood when the independent relationships between $W$ and $\Delta$ versus $p_i/p_a$ are considered separately.

The relationship between $\Delta$ and $p_i/p_a$ in $C_3$ plants in its simplest form is:

$$\Delta = a + (b - a) \frac{p_i}{p_a}$$  \hspace{1cm} (1)

where $a$ is the fractionation due to diffusion in air (4.4%o) and $b$ is the net fractionation caused by carboxylation (values of approximately 27%o have been experimentally obtained). Carbon isotope discrimination is calculated from the isotope composition ($\delta$) with respect to PeeDee Belemnite (PDB) of both air and the plant material such that

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}$$  \hspace{1cm} (2)

where subscripts $a$ and $p$ refer to plant and air respectively. More detailed analyses are available (e.g. Evans et al. 1986; Farquhar et al. 1989a; von Caemmerer and Evans 1991) but for our purposes this will suffice.
An instantaneous measure of \( W \) at the leaf level, obtained by gas exchange techniques can be defined as the ratio of the CO\(_2\) assimilation rate \((A)\) to the evaporation rate \((E)\) and is related to \( p/p_{a} \) thus:

\[
\frac{A}{E} = \frac{p_{a}(1 - p_{i}/p_{a})}{1.6v} \tag{3}
\]

where \( v \) is the difference between intercellular and ambient vapour pressures (Farquhar et al. 1989a). To express \( A/E \) in terms of whole plant \( W \) a correction has to be made for respiratory losses and evaporation not related to CO\(_2\) uptake:

\[
W = \frac{A (1 - \phi)}{E (1 + \phi_{w})} \tag{4}
\]

where \( \phi \) is the proportion of carbon that is fixed during the day but then respired by photosynthetic organs at night and non-photosynthetic organs over the whole period and \( \phi_{w} \) is the proportion of water lost from the plant that is not related directly to stomatal uptake of CO\(_2\), e.g., due to cuticular evaporation, or night time losses due to incompletely closed stomata. When combining equations 2 and 3 we obtain an expression for \( W \) in terms of \( p/p_{a} \),

\[
W = \frac{p_{a}(1 - p_{i}/p_{a})(1 - \phi)}{1.6v (1 + \phi_{w})} \tag{5}
\]

Based on equations 1 and 4 we would expect \( \Delta \) to be positively related to \( p/p_{a} \) and \( W \) to be negatively related to \( p/p_{a} \) and, hence, \( W \) and \( \Delta \) to be negatively related to one another.

Correlations between \( \Delta \), \( W \) and \( p/p_{a} \) have been demonstrated using a variety of methods ranging from gas exchange techniques at the single leaf level to crop measurements. In many of these studies a second aim has been to demonstrate genotypic variation in \( W \) and or \( \Delta \).
1.2.2.2 At the gas exchange level

Positive correlations have been found between \( p/p_a \) measured under optimum conditions and \( \Delta \) from plant dry-matter (Farquhar et al. 1982a; Condon et al. 1990). It is possible also to estimate \( \Delta \) from the isotopic composition of air before and after it leaves the gas exchange cuvette. These so-called 'on-line' studies have also yielded positive \( p/p_a \) relationships largely in accordance with theory (Evans et al. 1986; Hubick et al. 1988; von Caemmerer and Evans 1991; Ehleringer et al. 1991). Meinzer et al. (1990) have found a strong negative relationship between \( \Delta \) and the ratio of \( A \) to stomatal conductance which is, in turn, negatively related to \( p/p_a \). In field studies Dingkuhn et al. (1991) found that \( \Delta \) was negatively related to \( A/E \), measured on leaves of vegetative plants, amongst rice cultivars. Over a longer period, Brugnoli et al. (1988) found that \( \Delta \) measured on sugars and starch accumulated over one day was positively correlated with the CO\(_2\)-assimilation weighted value of \( p/p_a \) calculated from gas exchange data.

1.2.2.3 Longer term studies.

The above studies were important in confirming the general predictions of Farquhar et al. (1982b). However, it is over the longer time periods (life cycle) that the \( W \) versus \( \Delta \) relationship needed to be confirmed in order to assess the adequacy of \( \Delta \) as a screening technique for \( W \). Negative \( W \) versus \( \Delta \) relationships, at the pot level, have been demonstrated for genotypes of wheat (Farquhar and Richards 1984; Condon et al. 1990), peanut (Hubick et al. 1986; Hubick et al. 1988; Wright et al. 1988; Hubick 1990), tomato (Martin and Thorstenson 1989), cotton (Hubick and Farquhar 1987), barley (Hubick and Farquhar 1989), common bean (Ehleringer et al. 1991) potato (Vos and Groenwold 1989) and a range of pasture grasses (Johnson et al. 1990; Johnson and Basset 1991; Read et al. 1991a). When focusing on genotypic variation in \( W \) and \( \Delta \) it is important to note that in some studies the relationship between \( \Delta \) and \( W \) has largely been achieved by the
imposition of drought stress (Farquhar and Richards 1984; Johnson et al. 1990). However, whether within well-watered or drought-stressed treatments, genotypic variation in $W$ was negatively correlated with $\Delta$ at the single plant level within a range of species. And, like the initial studies (e.g. Richardson and Trumble 1928), there was considerable genotypic variation in $W$.

1.2.2.4 At the crop level

Of the many studies at the single plant level, few have been complemented by field studies. Wright et al. (1988) grew peanut genotypes in mini-lysimeters embedded in the ground and surrounded by normal canopy. Soil evaporation was minimised by placing a thin plastic sheet on the soil between the plants. Under these conditions there was a negative relationship between $W$ and $\Delta$. Likewise, using cotton, Yakir et al. (1990) found that $\Delta$ and $W$ were negatively correlated when $W$ was calculated as the ratio of total "seed-cotton yield" to total water applied during the season. Soil evaporation was not taken into account. This contrasts with Condon (1988) who, using different methods, found no relationship between $\Delta$ and $W$ amongst field grown wheat genotypes. Crop transpiration in this study was calculated from estimates of total evaporation, estimated using neutron moisture meter measurements, and soil evaporation, obtained using a model of evaporation from bare soil under canopies (Cooper et al. 1983). This result may have been confounded by a number of factors including: 1) the fact that $\Delta$ was positively correlated with biomass accumulation which provided problems in the partitioning of evaporation between soil and crop, 2) no account was taken of root biomass, 3) the extent to which boundary layer conductance influenced canopy transpiration was unknown. Later studies, taking evaporation from soil into account, have shown a negative relationship between $W$ and $\Delta$ between two wheat genotypes (G.D. Farquhar, pers. comm.).
For the most part, despite potential problems at the leaf level (von Caemmerer and Evans 1991) and at the crop level (Condon 1988), the bulk of evidence shows a strong negative correlation between $\Delta$ and $W$, whether based on genotypic variation alone or in combination with drought or nutrient treatments. It seemed that an adequate screening method for $W$ had been demonstrated especially as measurements using $\Delta$ were relatively inexpensive and repeatable (Farquhar et al. 1989b). However, to satisfy the criteria for a physiological trait, two further areas had to be addressed: the characterisation of genetic control and the relationship with agronomic benefit.

1.3 Genetic control of transpiration efficiency and carbon isotope discrimination

With the aid of an adequate screening tool studies on the genetic control of $W$ became much less cumbersome. Genotypic rankings of $\Delta$ have been conserved across different environments whether they be levels of drought stress (Hubick et al. 1988; Condon et al. 1990; Hubick and Farquhar 1989; Johnson et al. 1990), mineral nutrition (Hubick 1990) or multi-site field trials (Hubick et al. 1988; Condon et al. 1987; Johnson and Basset 1991). These studies have complemented earlier work by Hunt (1962) and Bleak and Keller (1973) which also concluded that $W$ (in one form or another) was under strong genetic control. In two studies where high and low $\Delta$ parents have been crossed the $F_1$ generation appears to be intermediate but closer to the low $\Delta$ parent indicating possible dominance of characteristics that contribute to low $\Delta$ (Hubick et al. 1988; Martin and Thorstenson 1989).

Hubick et al. (1988) used an $F_2$ of such a cross in peanut and demonstrated that $\Delta$ was a quantitative trait. Amongst this $F_2$ population there was a strong negative correlation between $W$ and $\Delta$ indicating the potential for selecting for greater $W$ amongst segregating populations.
In contrast to the above findings, a number of workers have found significant genotype by environment interactions. Neither Δ nor yield were consistent amongst genotypes of bean grown under irrigated and non-irrigated conditions (White et al. 1990). Likewise, amongst cowpea and barley genotypes, rankings have significantly differed between irrigated and non-irrigated treatments or sites (Hall et al. 1990; Craufurd et al. 1991). These results should not be seen as "exceptions to the rule" but rather as clear evidence that very little is presently understood about the fundamental basis of variation in Δ (or W) and how this affects growth characteristics. One way to overcome this problem is to study the physiological basis for relationships between Δ and growth.

1.4 Pleiotropic effects

Pleiotropic effects can broadly be defined as unintended consequences of selection for a character. While selection for greater W using Δ appears possible, the desirability of plants with greater W is another question. For example, it may be that the plants with greater W are less productive, or more productive under water-limited conditions but not under high rainfall conditions. Likewise, there may be some relationship between flowering time and W or Δ (e.g. Craufurd et al. 1991). The question arises as to what sort of plant we obtain under selection pressure for increased W? To answer, the plant characteristics which influence ρ/ρa need to be understood.

1.4.1 Theoretical considerations

At the leaf level, two major factors influence ρ/ρa, CO₂ assimilation rate (A) and the stomatal conductance (g) to diffusion of water vapour (the inverse of the often-used stomatal resistance). The relationship between A, g and ρ/ρa can be expressed as

\[ A / (1.6g) = \rho_a (1 - \rho/\rho_a) \]  

(6)
An increase in $A$ relative to $g$ will produce a lower $p/p_a$ whereas an increase in $g$ relative to $A$ will have the opposite effect. It should be noted that $A$ and $g$ often change together (Wong et al. 1979). There may be considerable ramifications for relationships between $W$ and growth depending on whether $A$ or $g$ is the source of variation in $W$. However, $A$ is not independent of $g$ and a better measurement is photosynthetic capacity ($A_{\text{max}}$) which is made under saturating light and CO$_2$ conditions. Two simplified scenarios relating to single plants can be considered. Firstly, one where the source of variation in $p/p_a$ is $g$ with $A_{\text{max}}$ and all other parameters involved in plant growth held constant. In this case a reduction in $g$ produces a decrease in $p/p_a$, but, as a consequence, a reduction in $A$ and hence in growth rate. Alternatively if $A_{\text{max}}$ were the sole source of variation in $p/p_a$ (assuming $g$ and all other parameters involved in plant growth to be constant), an increase in $A_{\text{max}}$ would produce a decrease in $p/p_a$ and also an increase in growth rate. In the first scenario fast growth is associated with greater $p/p_a$ (smaller $W$), but in the second scenario faster growth is associated with smaller $p/p_a$. These two scenarios are obviously oversimplifications (e.g. one general problem being the absence of correlations between $A_{\text{max}}$ and growth rate), but they do provide some insight into relationships between $W$ and growth. This is further discussed below.

1.4.2 Experimental observations

There is an array of data showing relationships between $W$ (or $\Delta$) and either gas exchange characteristics or growth in controlled and field environments. These can be conveniently divided into those that have explored relationships between $A$ or $g$ and $\Delta$ and those that have shown correlations between $W$ or $\Delta$ and biomass and/or yield.
1.4.2.1 Gas Exchange

Relationships between $A$ and/or $g$ and $\Delta$ depend on species and in some cases the environmental conditions used in the relatively few studies completed. Amongst genotypes of lima beans (*Phaseolus lunatus*) and dry beans (*Phaseolus vulgaris*) $g$ and $\Delta$ were positively correlated under well-watered conditions in the field (Ehleringer 1990; Ehleringer *et al.* 1990). Likewise amongst coffee genotypes grown under frequent (weekly) irrigation $g$ was found to be the major source of variation in $\Delta$ (Meinzer *et al.* 1990). Similarly, $g$ was positively correlated with $\Delta$ under either well-watered or droughted conditions amongst clones of *Agropyron desertorum* (Read *et al.* 1991a). Under field conditions where water supply was either limited or uncontrolled, $g$ and $\Delta$ were also positively correlated amongst *Agropyron desertorum* clones and a range of shrub and conifer species (DeLucia *et al.* 1988). Where water is not in plentiful supply, it is difficult to separate the genotypic and environmental components that may give rise to such relationships. Initially Condon *et al.* (1987) also found a close relationship between $\Delta$ ($p/p_a$) and abaxial $g$ amongst wheat genotypes. Further studies (Condon *et al.* 1990; Morgan and LeCain 1991) showed that genotypic variation in $\Delta$ or $p/p_a$ could be due to either $A_{\text{max}}$ or $g$. Other studies have shown no correlation between $\Delta$ (or $W$) and $g$, e.g., between *Festuca* species (Silcock and Wilson 1981), peanut genotypes (Hubick 1990), rice (Dingkuhn *et al.* 1991) and snap beans (*Phaseolus vulgaris*) (Ehleringer *et al.* 1990). Amongst potato cultivars (for which there was a negative $\Delta$ - $W$ relationship), there was no relationship between $g$ and $\Delta$ but there was a negative relationship between $g$ and $W$ (Vos and Groenwold 1989).

1.4.2.2 Biomass or Yield

Many studies have reported correlations between biomass accumulation or yield and $W$ or $\Delta$. As Johnson *et al.* (1990) pointed out "comparative studies between $WUE$ and forage yield.....will be unique to a
particular species. Some early studies on $W$ provide insight into the relationship between it and biomass accumulation amongst parents and their open-pollinated progeny within the species, *Elymus junceus* and *Agropyron intermedium* (Fig. 1.1a derived from data in Hunt 1962) and amongst *Dactylis glomerata* genotypes (Fig. 1.1b derived from data in Keller 1953). In both cases the strong positive relationships between biomass and $W$ were obtained from plants in pots under well-watered conditions (Fig. 1.1).

![Figure 1.1 Plots of transpiration efficiency versus biomass accumulation for a) parents and their open pollinated progeny within the species *Elymus junceus* and *Agropyron intermedium* (from Hunt 1962) and b) genotypes of *Dactylis glomerata* (from Keller 1953). Lines in each case represent significant linear regressions.](image)

The experimental protocol was sound and has been outlined by Keller (1954). Similar results have been found amongst peanut genotypes (Hubick *et al.* 1990; Wright *et al.* 1988; Hubick 1990), a range of forage grass species (Johnson and Basset 1991) and, using $A/E$ as a measure of $W$, amongst a population of *Xanthium strumarium* (Farris and Lechowicz 1990).

A number of studies have found positive relationships between $\Delta$ and yield or biomass. Amongst wheat genotypes under well-watered and drought conditions, biomass or grain yield and $\Delta$ have been positively correlated (Condon *et al.* 1987; Condon 1988). Likewise, Read *et al.* (1991b) using lines differing in abscisic acid (ABA) accumulation under stress also obtained a
positive relationship between grain yield and $\Delta$ under both irrigated and drought conditions. Positive relationships between $\Delta$ and yield or biomass accumulation have also been presented amongst common bean (*Phaseolus vulgaris*) genotypes (White *et al.* 1990) and amongst clones of *Agropyron desertorum* (Johnson *et al.* 1990). However, amongst the common bean genotypes this relationship was only found under rain-fed conditions at one site but not under irrigated conditions at the same site (White *et al.* 1990). Likewise, the apparent positive relationship between forage yield and $\Delta$ amongst *Agropyron desertorum* clones (Johnson *et al.* 1990) was largely a result of the three watering regimes used rather than a correlation due to genotypes alone, i.e., within any watering regime there appeared to be no correlation at all. However, a further study found that shoot biomass and $\Delta$ were positively related amongst nine of these clones under well watered conditions (Read *et al.* 1991a). In a recent paper, Craufurd *et al.* (1991) examined the relationship between $\Delta$ (measured on the grain) and yield from crops grown in England and Syria. They found that $\Delta$ was positively correlated with yield under droughted conditions and negatively correlated with yield under less droughted conditions. Unfortunately, by measuring $\Delta$ of the grain, as they did, there was no opportunity to directly compare trials independent of drought effects. Variation in time to maturity may have confounded these results.

1.4.2.3 Other relevant characteristics

Variation in yield among crop plants can be due to a number of factors independent of biomass accumulation (Austin *et al.* 1980). One of these is harvest index (HI, the ratio of harvestable products to total biomass (Donald 1968)). In peanut $\Delta$ was negatively related to biomass but not to pod yield because of a positive association between $\Delta$ and HI (Wright *et al.* 1988). However, this was not the case with wheat (Condon 1988). The time to ear emergence has been negatively correlated with $\Delta$ amongst barley genotypes
grown at different sites (Craufurd et al. 1991). Interestingly, when closely analysed there was no significant relationship between $\Delta$ and days to ear emergence under irrigated conditions at Cambridge. Hence the relationship may have been caused by drought simultaneously accelerating plant development and reducing $\Delta$.

Other plant characteristics important to growth have been related to $\Delta$. Specific leaf area ($SLA$, the ratio of leaf area to leaf dry weight) has been positively related to $\Delta$ amongst peanut cultivars (Wright et al. 1988; Wright and Rao, unpublished data) and wheat cultivars (Lopez-Castaneda, unpublished results). Similar results have been found by Gutschick (1988) who found a positive relationship between $SLW$ (specific leaf weight, inverse of $SLA$) and $p_i$ in *Medicago sativa*. These results can theoretically be explained by assuming a significant proportion of the variation in $\Delta$ ($p_i/p_a$) is due to $A_{max}$ (e.g. Pearce et al. 1969) and, hence, nitrogen content per unit leaf area (Evans 1989). In contrast there are some experiments which have shown no relationship between $A$ and $SLA$ (Baghsari et al. 1977). Other studies have shown no relationship between $SLA$ and $\Delta$ (White et al. 1990). The relationship between $SLA$ and growth will be addressed below.

Carbon-isotope discrimination has been positively correlated with root length density amongst bean genotypes (White et al. 1990). This relationship only held under rain-fed conditions and may be indicative of the effects of drought stress on both $p_i/p_a$ and root length density. On a physiological level, no relationship was found between abscisic acid (ABA) levels and $\Delta$ in field-grown wheat (Read et al. 1991b). Although the relationship between $\Delta$ and yield was altered by ABA, those accumulating the most ABA in the field displayed a much steeper regression between $\Delta$ and grain yield.

It is difficult to draw conclusions from a comparatively small number of studies on a limited set of species. This is made more difficult by the range of experimental conditions described. I have been careful to note the conditions
under which results have been obtained (and have omitted some) in an effort to differentiate between real physiological causes of pleiotropy, on the one hand, and apparent relationships caused by environmental conditions, on the other. The theoretical analysis presented above suggests that if there is genotypic variation at the leaf level in either $A$ or $g$ then a range of relationships between $\Delta$ and growth can be obtained. This seems to be the case and the nature of these relationships varies with species. The majority of the studies showing relationships between $\Delta$ (or $W$) and growth have been done at the crop level (e.g. Condon et al. 1987; Wright et al. 1988; White et al. 1990; Craufurd et al. 1991). Hence, measurements of growth have usually been restricted to either yield or biomass at maturity (but see Wright et al. 1988). One recent study has shown that amongst genotypes of barley there was a positive relationship between absolute growth rate and $\Delta$ (Favero et al. unpublished data). The components of variation in $\Delta$ ($A$ and $g$) are rate processes and, hence, are easier to relate to rate processes, like growth rate rather than the integrated result of growth rate and partitioning such as yield. Therefore if we wish to understand the fundamental links between growth and $W$ then this has to be done at a level where other processes, e.g. phenological and reproductive partitioning, will not interfere. This means examining the relationships between $\Delta$ and growth characteristics during early vegetative growth. In the following section aspects of the measurement and variation in growth rate are explored.

1.5 Analysing Growth

To be able to relate $\Delta$ to growth at a physiological level, the measurement and analysis of growth must first be understood. To do this growth must first be defined and then split into components which are both measurable and can be related to the factors which determine variation in $\Delta$. 
1.5.1 Measuring growth

As a term 'growth' can refer to any number of processes but for the purposes of this thesis is defined as the irreversible increase in plant size as measured by dry weight (Hunt 1978) which will be referred to as biomass. The field of growth analysis owes its origin to the ideas of Blackman (1919) who, drawing from the analogy of compound interest, derived a formula to represent the efficiency of biomass production, i.e. the efficiency index:

\[ B_t = B_0 e^{rt} \]  

where \( B_t \) is the final weight after time, \( t \), \( B_0 \) is initial weight and \( r \) was originally termed the efficiency index. Blackman (1919) made two other pertinent observations. He noted that for high vegetative production two factors were necessary - large seeds and a high efficiency index. In addition he came to the "obvious" conclusion that "some plants can work with far greater economy than others".

Following Blackman (1919), a range of developments in growth analysis were made by deriving methods for calculating \( RGR \) and splitting it into its components leaf area ratio (\( LAR \), leaf area per unit whole plant biomass) and net assimilation rate (\( NAR \), the rate of biomass increase per unit leaf area, sometimes referred to as unit leaf rate) (Briggs et al. 1920; Fisher 1921). The development of growth analysis techniques has been reviewed by Evans (1972). The formulae for the instantaneous values of the quantities normally calculated using growth analysis are:

\[ RGR = \frac{1}{B}\frac{dB}{dt} \]  

\[ LAR = \frac{LA}{B} \]  

\[ NAR = \frac{1}{LA}\frac{dB}{dt} \]

where \( LA \) is leaf area and \( B \) is total plant biomass. Net assimilation rate, \( NAR \), at a physiological level, is the difference between photosynthetic uptake and respiratory loss on a leaf area basis (Evans 1972). Leaf area ratio, \( LAR \), can be split into specific leaf area (\( SLA \), leaf area per unit leaf
weight) and leaf weight ratio ($LWR$, the fraction of total plant biomass in the leaves):

$$SLA = \frac{L}{B_\ell}$$  
$$LWR = \frac{B_\ell}{B}$$

where $B_\ell$ is the dry weight of the leaves and hence,

$$RGR = LAR \cdot NAR$$

1.5.1.1 Classical analysis

There are two main methods used to calculate these quantities. The first, referred to as 'classical growth analysis' (Hunt 1978), uses data from weekly harvests of a relatively large number of plants. Relative growth rate is calculated by dividing the difference between the average logarithmically (Ln) transformed biomass at each harvest by the length of the time interval. This is carried out for each harvest. In order to calculate $NAR$, $LAR$ is assumed to be either constant over the time interval or to have changed linearly (Williams 1946; Radford 1967; Evans 1972). The calculation of errors from these analyses is difficult and leads to approximations, especially in the case of $NAR$ (Causton and Venus 1981).

1.5.1.2 Functional analysis

The second method (developed by Vernon and Allison 1963; Hughes and Freeman 1967; and Hunt and Parsons 1974) has been termed 'functional analysis' (Hunt 1978). Polynomial functions are fitted between both $L$ and $B$ (Ln transformed) versus time (see Fig 1.2a,b). By differentiating the equation used to describe Ln $B$ versus time, $RGR$ can be derived at any time during the growth period. Also $LAR$ can be calculated at any time during the course of growth as can $LWR$ and $SLA$. As a result, a continuous time-course for $NAR$ can also be derived (see Hunt 1982). In addition, this method provides the means for calculating errors for each of
the characters (see Causton and Venus 1981). Although some pit-falls of this type of methodology have been pointed out, especially with reference to the choice of polynomial (Poorter 1989), it is the most popular method and, as long as it is carefully applied, provides a powerful tool for the analysis of growth data.

![Graphical presentation of the standard functional analysis](image)

**Figure 1.2** Graphical presentation of the standard functional analysis, see text for explanation.

### 1.5.1.3 Statistical considerations

Many attempts have been made to develop appropriate statistical techniques applicable to growth analysis (e.g. Garretsen and Keuls 1986; Spitters and Kramer 1986). However, these are complex and have generally not been adopted either in ecological or physiological studies (e.g. Rice and Bazzaz 1988; Dijkstra and Lambers 1989; Poorter and Remkes 1990).

Recently, Poorter and Lewis (1986) have suggested a simple technique to test for differences in RGR. This method uses time and treatments (e.g. genotypes) as factors and $B$ (Ln transformed) as a variable in a simple factorial analysis of variance. Hence, a significant time x genotype interaction would indicate significant variation in RGR. Analysis of variance of $LAR$, $LWR$ or $SLA$ is more straightforward but can also be analysed over time (some of the difficulties with ontogenetic drift will be discussed below). There is still no widely accepted method for testing differences in $NAR$ and even the most complex calculations of the error term involve some level of approximation (Causton and Venus 1981). It is not certain how much this matters. For
example, once significant variation in $RGR$ is established, the major concern is to locate (by means of regression) whether this is due to $LAR$ (and its components) or $NAR$ (and its components) (e.g. Bush and Evans 1988; Poorter and Remkes 1990).

### 1.5.1.4 Ontogenetic Drift

Many of the complexities of growth analysis can be avoided if plants are analysed during the early vegetative stage when growth is (near) exponential. However, one further complication needs to be accounted for. Each of the growth characteristics has been shown to drift with either time, size or development (Briggs et al. 1920; Williams 1946; Poorter and Pothman 1992). Such changes have been termed 'ontogenetic drift'. Many factors are involved in producing such drift; e.g. in longer term studies, reproductive growth affects the changes in $LAR$ (Evans 1972). For the most part such effects will be avoided by focusing on vegetative growth but here again ontogenetic drift has been shown. In this case variation in plant size (measured as biomass) can give rise to apparent differences which, when size is corrected for, disappear (Dijkstra and Lambers 1989). In addition relationships between either seed or seedling size and seedling $RGR$ have also been shown between and within species (Cook and Evans 1983, 1988; Shipley and Peters 1990). As Evans (1972) pointed out, 'drift' does not convey any sense of direction. Hence 'drift' in characteristics such as $LAR$ or $NAR$ may be either upward or downward (Evans 1972; Poorter and Pothman 1992). A number of studies have taken this into account and involved analysis of plants either at a fixed biomass (Rice and Bazzaz 1988) or over a biomass range (Bush and Evans 1983; Evans and Bush 1985; Dijkstra and Lambers 1989; Poorter and Remkes 1990).

Some alterations to the functional technique can be made in order to analyse plants at a fixed biomass. Firstly, what biomass will the plants be analysed at? Rice and Bazzaz (1988) analysed at the average biomass of all
plants used in the experiment, termed grand mean biomass, $B^*$. This is an acceptable method as long as $B^*$ falls within the range of the first and final harvests of all treatments. The calculation of $RGR$ can be made after solving the simultaneous equation for $t$ once the value of $B^*$ is determined. Furthermore, instead of the technique outlined in Fig. 1.2, $LA$ is determined at $B^*$ using a Ln transformed relationship between the two (Fig. 1.3). The advantage of this technique over that of Hunt and Parsons (1974) is that the variance that arises between individuals at each harvest is only counted once (i.e. Ln $B$ versus $t$) rather than twice (i.e. both Ln $LA$ and Ln $B$ versus $t$). In practice this method does not greatly affect the value of either $LAR$, $NAR$ etc but it does minimise the variance. The same techniques apply for the calculation of $LWR$ and $SLA$.

![Graphical presentation of an alternate method of functional analysis](image)

Figure 1.3 Graphical presentation of an alternate method of functional analysis, where leaf area ratio is calculated from the relationship between biomass and leaf area (both Ln transformed) directly (see text for details).
1.5.1.5 Some Objections

Some general reservations about these techniques have been expressed (e.g. Hardwick 1984; Korner 1991). However, these tend to stem from either a mistaken view of growth analysis itself (i.e. either that it is "too empirical" or lacks statistical rigour) or a misunderstanding of the techniques per se. The answers gained from growth analysis are more like first approximations which can lead to mechanistic insights rather than final conclusions, especially in genotypic comparisons. For instance the question of why one genotype grows faster than another requires growth analysis which "can be interpreted in terms of the more explicit treatments developed within more complex models" (Warren Wilson 1981). An example of this is a study of herbaceous species which used growth analysis as well as gas exchange and chemical composition in order to explain differences in RGR (Poorter and Remkes 1990; Poorter et al. 1990).

One problem that occurs at a fundamental level in growth analysis is that NAR and LAR are not independent. The theoretical basis for this has been outlined by Causton and Venus (1981). Moreover, in practice, NAR and LAR tend to be negatively related (Lambers and Poorter 1992) although not always (Poorter and Remkes 1990). Again this further emphasises the notion that the dividing of RGR into its components should be seen as the 'first step on a journey' rather than 'arrival at the destination'. Ultimately it is the components of NAR and LAR that are of interest in explaining differences in growth rate. It is worth noting that on an agronomic scale it is impractical to select for NAR but possible to select for its components - either photosynthetic rate or respiration (Volencec et al. 1984; Mahon and Hobbs 1987; Nelson 1988). Much then will depend on the aim of the experimenter. In this case the aim is to give an account of how growth and Δ may be related.
1.5.2 Causes of variation in relative growth rate within and between C₃ species

The main focus in this section will be herbaceous C₃ plants as these are most relevant to the relationship between Δ and growth in crop or pasture plants. The factors associated with greater RGR depend very much on the comparison being made. In a review of a wide range of growth analysis experiments, Poorter (1989b) found that in most cases variation in LAR was more closely related to RGR while variation in NAR was the cause in fewer cases.

1.5.2.1 Leaf area ratio and its components

Many comparisons have shown the importance of LAR in determining differences in RGR either within species (e.g. Shibles and MacDonald 1962, Brewster and Barnes 1981; Dijkstra and Lambers 1986,1989) or between species (Blackman and Wilson 1951; Cooper 1967; Potter and Jones 1977 [although C₄ species were included]; Poorter and Remkes 1990). Earlier field work showed that in crops, leaf area development was the main source of variation in crop growth rates (Watson 1947,1952) and it is possible that these differences when traced back to individual plants were due to differences in LAR.

As shown above LAR can be divided into LWR and SLA. In the cases quoted above RGR has been positively related to either SLA (Potter and Jones 1977; Dijkstra and Lambers 1986,1989) or both SLA and LWR (Brewster and Barnes 1981; Poorter and Remkes 1990) but not LWR itself. To generalise, leaves with greater SLA ('thin' leaves) return more to the plant in terms of growth, than smaller SLA ('thick leaves'). Generally between genotypes high SLA leaves will have lower photosynthetic rates than low SLA leaves (e.g. Pearce 1969) but not always (Bhagsari et al. 1977). This is the basis for the conclusion reached in many studies that photosynthesis is either
unrelated or negatively related to growth or yield amongst plants (e.g. Nelson 1988; Austin 1989). It occurs because of what has been termed the 'dilution effect', i.e., the gain to the plant in terms of total photosynthesis is greater when the photosynthetic machinery is spread over a larger area (see Sinclair and Horie 1989; Evans 1990).

One important factor is the conditions under which comparisons are made. Some of the studies showing LAR to be the cause of variation in RGR have been made under relatively low irradiance (e.g. Dijkstra and Lambers 1989; Poorter and Remkes 1990) and this may affect the expression of both leaf growth rate and photosynthetic rate. Patterson et al. (1978) found that the influence of LAR on RGR declined as irradiance increased. Where possible, in comparing genotypes or species it is important to match experimental conditions, such as irradiance to those normally experienced. In summary LAR, SLA, and LWR can be important determinants of variation in RGR. However, in some situations NAR has also been shown to be important.

### 1.5.2.2 Net assimilation rate and its components

There are cases in which variation in RGR has been caused by variation in NAR either within or between species. (Blackman and Black 1959; Thorne 1960; Eagles 1967; Pons 1977; Masle 1992). The physiological basis of this relationship can be further analysed by splitting NAR into its photosynthetic and respiratory components. Net assimilation rate can be expressed in terms of carbon as:

$$NAR = A.L.(1-\phi)$$

where $A$ is the net rate of carbon assimilation during the day, $L$ is the light period as a proportion of the day, and $\phi$ is the proportion of carbon assimilated by the leaves during the day that is subsequently respired (Masle and Farquhar 1988).
In the context of crop performance, many attempts have been made to relate \( A \), whether measured on single well illuminated leaves or on canopies, to growth, biomass or yield and yet few positive relationships have emerged (for reviews see: Evans 1976, 1981, Elmore 1980; Gifford and Evans 1981; Eagles and Wilson 1982, Gifford 1987; Lambers 1987; Nelson 1988; Austin 1989; Snaydon 1991) although one exception has been amongst pea genotypes (Hobbs and Mahon 1987). In addition, amongst genotypes of pima cotton (*Gossypium barbadense* L.), genetic advances in yield have been associated with increases in both photosynthetic capacity and stomatal conductance (Cornish *et al.* 1991). In cases where apparent relationships have emerged (for example) between canopy photosynthesis during reproductive growth and final yield (e.g. Wells *et al.* 1982, 1986), this has been due more to developmental differences rather than as a result of variation in photosynthesis *per se* (Evans 1990).

Variation in NAR can also be due to variation in respiration as up to 50\% of photosynthate produced daily may be subsequently respired (Lambers and Dijkstra 1987). The variation in respiration between lines of *Lolium perenne* (Wilson 1975) has been directly related to differences in growth (Wilson 1982; Robson 1982a, b). Few other species have been examined in such detail and hence little is known about the genetic variation in respiration within other species (Kraus *et al.* 1989).

In conclusion, the causes of variation in RGR are variable and depend on the species or set of species being compared and the conditions under which such comparisons have been made. With this in mind it is perhaps possible to speculate on the physiological bases for the relationship between growth and \( \Delta \).
1.6 Carbon isotope discrimination and growth

Genotypic variation in both growth and $W$ can be caused by a number of factors. So how is it that growth (usually measured as cumulative biomass) can be either negatively or positively correlated with $W$ (or $\Delta$)? This question can be addressed with respect to the three factors that have been shown to be correlated with growth rate - photosynthetic rate, respiratory rate and leaf area ratio. While it is unlikely that $\Delta$ could be directly related to respiration rate (except through interrelationships with photosynthetic rate or partitioning), both photosynthetic rate and $LAR$ require further consideration.

In the case of photosynthetic rate, a scenario was presented above that related $A_{\text{max}}$ to $\Delta$ independently of $g$ and concluded that faster growth could be related to low $\Delta$ (section 1.4.1). As pointed out above, higher $A$ is usually achieved at a cost in terms of $SLA$ (Dornhoff and Shibles 1976) or leaf size (Evans and Dunstone 1970) because of nitrogen partitioning. This has not been a common experience with most crop plants. Some recent work has suggested that selection for high $A$ could be of benefit in terms of growth under high light conditions where expression of photosynthetic variation is maximised (Day and Chalabi 1988).

The other scenario in section 1.4.1, where $g$ varied while $A_{\text{max}}$ was held constant, predicted that $\Delta$ and growth could be positively related. Unlike the situation above, the increase in leaf $A$ is not dependent on greater investment in leaf nitrogen content and is, therefore, unlikely to have a cost in terms of growth. This may well explain the relationship between growth and $\Delta$ found by Condon et al. (1987). Likewise, Read et al. (1991a) have found positive relationships between $\Delta$ and $g$ and between $\Delta$ and shoot biomass amongst clones of *Agropyron desertorum*.

In many cases variation in $RGR$ is due to $LAR$. How might this affect the $RGR$ - $\Delta$ relationship? This can be more easily discussed if it is assumed
that the variation in \( LAR \) is largely attributable to \( SLA \) (e.g. Poorter 1989b) and \( LWR \) is relatively constant. A high \( SLA \) plant will have faster \( RGR \) (e.g. Poorter and Remkes 1990) and may have a greater \( \Delta \) as a result of the positive relationship between \( \Delta \) and \( SLA \) (Wright \textit{et al.} 1988). In contrast, where \( SLA \) is negatively related to \( RGR \) (Pons 1977) then it is possible that \( \Delta \) will be negatively related to \( RGR \). However, this is just another restatement of the photosynthetic capacity scenario presented above. It has been assumed that there is no intrinsic relationship between \( g \) and \( SLA \).

I have presented a few possible suggestions in order to account for the array of relationships between growth and \( \Delta \) encountered above. What has become evident is that data have to be collated from very different studies in order to formulate hypotheses. In effect, no one study has given a clear account of the causes of variation in both \( \Delta \) (or \( W \)) and growth either within or between species.

1.7 Thesis overview

This thesis is an attempt to explain empirically observed relationships of growth (or yield) with \( W \) on a physiological basis. This review has shown that one of the main problems with in using \( W \) (or \( \Delta \)) as a selection criterion is the consequences in terms of growth. In the chapters (each presented as a separate paper) that follow, an account is given of the relationship at the empirical level between growth and \( \Delta \) amongst sunflower genotypes (Chapter 2). This relationship is then analysed in terms of the underlying physiological causes using both growth analysis and gas exchange techniques (Chapters 3 and 4). A similar analysis is then carried out on a range of species (Chapter 5) to explore if the relationships found within sunflower hold over a range of
species. Two of these species were then chosen for further analysis (Chapter 6) of both the causes of variation in $RGR$ and how these relate to $\Delta$, if at all.

2.1 ABSTRACT

Transpiration efficiency of any matter production (ME), carbon assimilation, and water use efficiency during plant development are measured on six sunflower (Helianthus annuus L.), genotypes grown for 50 days in a greenhouse. Two watering regimes were used: one well watered (WW) and the other delivering half the water used by the WW plants (HW), while imposed.

Four major results emerged from this study: 1) there was significant genotypic variation in WW sunflower and this was closely reflected in $\Delta$ for both watering treatments; 2) the low-watering regime rendered a decrease in $\Delta$ but no change in $W$ and assimilation; the genotypic ranks for either $\Delta$ or $W$ was not significantly altered by either stress; 3) a positive correlation between $W$ and biomass accumulation occurred among genotypes of HW plants; and, the ratio of total plant carbon assimilation to leaf area, was positively correlated with $W$ and negatively correlated with $\Delta$.

These results are discussed in reference to the connection between transpiration efficiency and plant growth. In short, $\Delta$ can be used to select for $W$ among young vegetative sunflower plants. However, selection for $W$ may be accompanied by changes in other important plant growth characteristics such as...
CHAPTER TWO


2.1 ABSTRACT

Transpiration efficiency of dry matter production ($W$), carbon-isotope discrimination ($\Delta$) and dry matter partitioning were measured on six sunflower (Helianthus annuus L.) genotypes grown for 32 days in a glasshouse. Two watering regimes, one well watered ($HW$) and the other delivering half the water used by the $HW$ plants ($LW$), were imposed.

Four major results emerged from this study: 1) there was significant genotypic variation in $W$ in sunflower and this was closely reflected in $\Delta$ for both watering treatments; 2) the low watering regime caused a decrease in $\Delta$ but no change in $W$; nonetheless the genotypic ranking for either $\Delta$ or $W$ was not significantly altered by water stress; 3) a positive correlation between $W$ and biomass accumulation occurred among genotypes of $HW$ plants; 4) $\varphi$, the ratio of total plant carbon content to leaf area, was positively correlated with $W$ and negatively correlated with $\Delta$.

These results are discussed with reference to the connection between transpiration efficiency and plant growth. In short, $\Delta$ can be used to select for $W$ among young vegetative sunflower plants. However, selection for $W$ may be accompanied by changes in other important plant growth characteristics such as $\varphi$. 
2.2 INTRODUCTION

The recent finding that transpiration efficiency ($W$, the ratio of carbon accumulation to transpiration) is negatively related to carbon-isotope discrimination ($\Delta$) in C$_3$ plants has led to a renewed interest in $W$ as a possibly exploitable crop character. Genetic variation in $W$ has been known for some time (Briggs and Shantz 1913, 1914) and its association with $\Delta$ has been highlighted in several studies (Farquhar and Richards, 1984; Hubick et al., 1986, 1988; Hubick and Farquhar, 1987; Wright et al., 1988; Martin and Thorstenson, 1988 and Hubick and Farquhar, 1989). However, because variation in $W$ (and $\Delta$), at the leaf level, may occur via variation in photosynthetic capacity or stomatal conductance or both, there is no a priori reason for assuming high or low $W$ should be linked with either fast or slow relative growth rate when water supply is not limiting. It is also possible that characters associated, but not mechanistically linked, with $\Delta$ or $W$ may be important in determining plant productivity. One such character is $q$, the ratio of plant carbon to leaf area, which affects relative growth rate, $RGR$, via

$$RGR = \frac{AL(1-\phi)}{q}$$  \hspace{1cm} (1)

where $A$ is the net rate of carbon assimilation during the day, $L$ is the light period as a proportion of the day and $\phi$ is the proportion of net assimilation by leaves in the daytime which is then lost by respiration (Masle and Farquhar 1988).

Masle and Farquhar (1988) found a negative correlation between $q$ and $\Delta$ in wheat plants grown in soils of differing strength. For vegetative plants, $q$ may be divided into three components, $q_r$, $q_s$ and $q_l$ which are the ratios of root, stem and leaf carbon, respectively, to leaf area. Wright et al. (1988) found that specific leaf weight, which corresponds to $q_l$, was negatively correlated with $\Delta$ among peanut genotypes. As mentioned
above, many crop species have displayed genetic variation in $W$, but most experiments showing this have taken the plant through a full life cycle with harvests for yield and biomass at the end of this period. As a consequence of senescence and abscission, parameters like $Q$ have been difficult to obtain. To avoid these problems, relationships between $Q$ (and its components) and $W(\Delta)$ were examined during the vegetative stage only. We used sunflower genotypes in order to explore any genetic variation in $W$ within this species and also because $Q$ in sunflower was comparatively easy to divide into its components. Two watering regimes were added to the design to increase the range of $\Delta$ and $W$ and to explore the relationship between the two under water stress.

2.3 MATERIALS AND METHODS

Seeds of six sunflower genotypes (Guayacan, Morden, Impira Inta, Contiflor 3, 880B and 206R) were sown on 21 Nov. 1986 in pots holding 5.5 kg dry soil containing little organic matter which were placed in a naturally-lit glasshouse in Canberra, Australia. Day temperature was nominally 25°C and night temperature was 20°C. Pots were covered with grey plastic over the tops and seedlings were allowed to grow through small holes in the plastic to minimise evaporation from the soil. Two watering regimes were imposed: 1) watering daily to field capacity ($HW$) and 2) half the water required for the $HW$ treatment ($LW$). Six replicates of each genotype, for both treatments, were arranged in three randomised blocks. Seedlings were thinned to one per pot on 30 Nov. Loss of water from each pot was monitored from ten days after sowing, when a measurable weight difference had occurred, until the conclusion of the experiment. Plants were initially watered to field capacity every second day and later in the experiment, daily. Plants were harvested on 22 Dec. and separated into roots, leaves and stems. Leaf areas were measured immediately with a
LiCor (Li3000) meter and then all parts were dried at 80 °C for 48 hours. Dry weights were recorded for the plant parts and then converted to a carbon basis after obtaining an average carbon content of 39.7% (range 38.7%-40.8%) using a Carlo Erba (model 1108) elemental analyser. Total carbon content for each plant was used to calculate the transpiration efficiency as the molar ratio of total carbon to water used. Water used was the sum of the total water added plus the difference between the initial and final pot weights, expressed on a molar basis.

Carbon-isotope ratios were measured on 5-10 mg samples of finely ground leaf material bulked from all the leaves of single plants. Each sample was combusted in the elemental analyser to produce CO₂ which was purified and analysed for carbon-isotope ratio using a VG SIRA 24 ratio mass spectrometer with dual inlets and triple collectors. The isotope composition of the sample gas was compared, by the mass spectrometer, with a working standard of CO₂ gas with a composition, relative to Pee Dee Belemnite (δ¹³C of -35.08‰). Carbon-isotope discrimination, Δ, was calculated from the isotopic composition of the leaf material and that of air, which was taken to be -8‰ relative to PDB (Hubick et al. 1986).

2.4 RESULTS

Relationship between W and Δ

Transpiration efficiency (W) and carbon isotope discrimination (Δ) were negatively correlated amongst genotypes for LW and HW plants (Fig. 2.4).
2.1). The relationship between $W$ and $\Delta$ appeared to alter depending on watering regime but neither slopes nor intercepts of these lines differed significantly (Fig. 2.1). However, analysis of variance showed that the effect of decreasing the water supply was to significantly decrease $\Delta$ without any corresponding change in $W$ in all but one genotype (*Guayacan*). Importantly, analysis of variance also showed that genotype ranking for $\Delta$ or $W$ was not significantly altered by the *LW* watering regime.

![Figure 2.1](image.png)

Figure 2.1 Plot of mean transpiration efficiency of whole plants versus mean carbon-isotope discrimination measured in the dried leaf material of different sunflower genotypes under *HW* and *LW* watering conditions. Regression equations: 1) for *HW* plants, $W = 9.51 - 0.38\Delta$, $r^2 = 0.95$; 2) for *LW* plants $W = 10.84 - 0.48\Delta$, $r^2 = 0.72$. Neither the slopes nor the intercepts of these equations differed significantly from each other. Bars indicate ± one standard error.

Relationships between biomass accumulation, $W$ and $q$ amongst *HW* genotypes.

The genotypes which accumulated the most biomass, expressed as total plant carbon, also had the greatest $W$ (Fig. 2.2). The correlation coefficient, on an individual plant basis, between total biomass and $W$ was $r$
= 0.90. There was also a strong correlation between total biomass and $q$ ($r = 0.87$).

![Graph showing the relationship between plant biomass and transpiration efficiency](image)

Figure 2.2 Plot of total plant biomass versus the transpiration efficiency of whole plants of different sunflower genotypes under the HW conditions. Symbols as in Fig. 2.1.

The ratio of total plant carbon to leaf area, $q$, was positively correlated with $W$ among these genotypes (Fig. 2.3). As mentioned earlier, $q$ can be subdivided into three components, $q_r$, $q_s$ and $q_l$ which are the ratios of the root, stem and leaf carbon, respectively, to leaf area. Each of these components was positively related to $W$ (Fig. 2.4). Therefore, the relationship in Fig. 2.3 between $q$ and $W$ is contributed to by all three components and does not arise because of an overriding relationship of any one of these components with $W$. Each of these components was strongly correlated with $q$: $q_l$ versus $q$, $r^2 = 0.71$; $q_s$ versus $q$, $r^2 = 0.89$; $q_r$ versus $q$, $r^2 = 0.80$. Each of these correlations were significant at $P < 0.01$. 
Figure 2.3 Plot of $q$ versus transpiration efficiency of whole plants of different sunflower genotypes under HW conditions. Symbols as in Fig. 2.1.

Figure 2.4 Plots of $q_l$, $q_s$ and $q_r$ versus transpiration efficiency of whole plants of different sunflower genotypes under HW conditions. Symbols as in Fig. 2.1.
2.5 DISCUSSION

Sunflower can be added to the list of C₃ crop species that display genetic variation in $W$ and $\Delta$, e.g. wheat, tomato, peanuts, cotton and barley (Farquhar and Richards 1984; Hubick et al. 1986; Hubick and Farquhar 1987; Martin and Thorstenson 1988; Hubick and Farquhar 1989). As with the work cited above, there was a close negative correlation between $W$ and $\Delta$ among these sunflower genotypes. The basis for this relationship has been explained elsewhere (Farquhar et al. 1988).

However, while the relationship has been confirmed for young glasshouse-grown plants, the ultimate application of $\Delta$ for use as a selection criterion for high $W$ will be dependent both on the stability of the relationship under different environmental conditions and on how other important plant attributes correlate with $W$ and/or $\Delta$ (e.g. Condon et al. 1987).

The results show that $\Delta$ can be varied independently of $W$, i.e. reducing the supply of water to the plants decreased $\Delta$ without a corresponding increase in $W$. This may be due to many factors, of which we consider the major two to be: 1) water-stress induced increase in leaf-to-air vapour-pressure difference and/or 2) an increase in respiratory losses due to water stress. The closure of stomata in water-stressed plants leads to a rise in leaf temperature which, in turn, causes an increase in leaf-to-air vapour-pressure difference. Thus some of the increase in $W$ that may accrue from a decline in stomatal conductance is forfeited in comparison with an extremely well-ventilated plant. In this way $\Delta$ may decline in water-stressed plants with $W$ being relatively less affected. An increase in the proportion of plant carbon lost by respiratory processes in stressed plants could also explain why $W$ did not increase under water stress. Depending on the species and the conditions under which measurements are made, water stress may either increase or decrease the proportion of carbon gained which is subsequently lost in respiration (McCree 1986). If $\phi$ (see
equation (1)) for HW plants were, say, 0.3 it would have to increase to 0.43 (i.e. an increase of 42%) to fully explain the absence of a response of W to water stress among LW plants. While this is certainly out of the bounds of most experience (McCree 1986), it is possible that a lesser increase in \( \phi \) combined with a stress induced increase in leaf-to-air vapour-pressure difference could explain the absence of a stress effect on \( W \). Irrespective of the reasons for the change in \( \Delta \) but not \( W \), it is of more importance that the LW watering regime did not significantly alter the genotype ranking for \( W \) or \( \Delta \). Therefore, under both watering regimes, sampling for \( \Delta \) amongst these genotypes should enable a reliable ranking for \( W \) to be obtained.

The influence of dry-matter allocation on relative growth rate \( (R) \) is represented by the parameter \( Q \) in equation (1). If \( A \) and \( \phi \) were to remain constant, fast growth (large \( R \)) would be associated with low \( Q \) (i.e. low plant carbon to leaf area ratio) and vice versa. Therefore, any association between \( Q \) and \( W \) or \( \Delta \) may be of importance for an understanding of the relationship between plant water use and productivity. In this case the plants which were more conservative in terms of the ratio of water use to carbon fixed (high \( W \), low \( \Delta \)) displayed high \( Q \). This relationship is similar to the observed negative correlation between \( Q \) and \( \Delta \) obtained for young wheat plants grown on soils of differing "strength" (Masle and Farquhar 1988), but differs importantly in that the sole source of variation in \( Q \) and \( \Delta \) was genetic. This relationship has since been confirmed among six wheat genotypes during early growth (Virgona and Farquhar, unpublished data). Condon et al. (1987) found a positive relationship between final above ground biomass and \( \Delta \) for a wide range of wheat genotypes grown under well-watered conditions in the field. This may have been caused by a negative relationship between \( \Delta \) and \( Q \), with high \( \Delta \) genotypes growing faster due to their having small \( Q \) (see equation 1). A theoretical basis for this relationship has been provided by Cowan (1986). He suggested that the compromise between water conservation and assimilation of carbon at
the stomatal level should be matched by the compromise between carbon allocation for the exploration of soil water, and the carbon allocation to leaves to increase relative growth rate. The compromise at the stomatal level should be reflected in $W$ and $\Delta$, water conservation being associated with large $W$ and small $\Delta$. The compromise in carbon partitioning should be seen in $\varnothing$ (Masle and Farquhar 1988), with large $\varnothing$ and $W$ occurring together.

Cowan's theoretical prediction is based on optimisation principles and contains no information on the mechanistic relationship between $\varnothing$ and $W$. It could be hypothesised that a positive relationship between $\varnothing$ and $W$ may arise from genetic variation in photosynthetic capacity, with greater capacity being associated with greater $\varnothing_i$ (see Gutschick 1988). However, there may be some other fundamental link in the present case, because $\varnothing_s$ and $\varnothing_r$ are also positively related to $W$. Each of the three components of $\varnothing$ were closely correlated with $\varnothing$ itself. This may explain why a relationship between one component and $W$ is reflected in the overall $\varnothing$ versus $W$ relationship (Fig. 2.3). There is a need for a study which will elucidate the nature of these relationships. This is especially necessary because it is known that neither $\varnothing$ nor its components remain constant with either plant age or size (Evans 1972). Therefore, it may be necessary to analyse for genotypic variation in $\varnothing$ amongst plants of similar size or developmental stage (see also Rice and Bazzaz 1989).

The degree to which $\varnothing$ affects $RGR$ will depend on the other parameters in equation (1); if $A$ and $\phi$ are constant, plants with large $\varnothing$ would grow at a slower rate than those with small $\varnothing$. However, in this study, the sunflower plants which accumulated the most biomass also displayed large values of $\varnothing$. Although $\phi$ may have varied among genotypes this cannot have been the main, systematic, source of variation because $\Delta$ changed with $W$ in a rather predictable manner, e.g. in the $HW$ plants $W$
approached zero as $\Delta$ approached $24.8 \times 10^{-3}$ (Fig. 2.1), c.f. $27-29 \times 10^{-3}$ in the simplest theory (Farquhar and Richards 1984). Put simply, imagining lines isogenic except for $\phi$, we would find that $W$ and $RGR$ would vary but not $\Delta$. However, this did not happen in this experiment where the $W$ versus $\Delta$ relationship could largely be predicted ignoring variation in $\phi$. While we cannot exclude the possibility of genotypic variation in $\phi$, we may state that genotypic variation in $\phi$ was not the main source of variation in $W$ (nor, probably in $RGR$). Therefore, genotypic variation in $A$, great enough to negate the effects of $\phi$ on $R$, may explain our results. On the other hand, final biomass at 32 days may not reflect $R$ at all. This can happen if seed size or time to emergence varies with $W$ among genotypes in a systematic manner. This issue will be addressed in a further study designed to measure classical growth parameters as well as photosynthetic gas-exchange.

The proposed use of $\Delta$ in breeding programs must be approached carefully. For sunflower, as with many other C$_3$ crop species, genetic ranking in $W$ of isolated plants can be achieved using $\Delta$. The usefulness of such rankings will heavily depend on what characters are associated with $W$ and the relative importance of variation in the stomatal and photosynthetic characteristics that determine variation in $W$. Therefore, while increased $W$ may be a desirable trait in some systems (Passioura 1977), direct selection among existing lines of sunflowers for increased $W$, in isolation from other traits, may be difficult since at least one important growth characteristic ($\phi$) appears to be mechanistically related and/or genetically associated with $W$. 
CHAPTER THREE

The relationship between growth rate, carbon isotope discrimination and photosynthetic gas exchange amongst seven sunflower genotypes

3.1 ABSTRACT

Growth analysis was carried out on seven sunflower (*Helianthus annuus* L.) genotypes to examine the relationships between growth, transpiration efficiency (*W*, the ratio of dry matter accumulation to transpiration) and leaf area ratio (*LAR*, ratio of leaf area to whole plant biomass). The growth analysis consisted of five sequential harvests every 6-7 days until 31 days after emergence. Carbon-isotope discrimination, *Δ*, *W*, and photosynthetic rate per unit leaf area (*A*) at ambient concentration of CO₂ were measured on plants that made up the final harvest of the growth analysis. Six of the seven genotypes were also grown under a low nitrogen regime and harvested at 30 days after emergence. When plants from the final harvest were analysed, both *W* and whole-plant biomass (*B*) were negatively correlated with *Δ*, and *LAR* was positively correlated with *Δ*. Throughout the growth period or when analysed at a common biomass (*B'*) which occurred approximately mid-way through the experiment, relative growth rate (*RGR*) and net assimilation rate (*NAR*) were positively correlated. Likewise, RGR and *Δ* were negatively correlated but not when analysed at 30 days after emergence. When *LAR* was calculated at a common biomass there was no relationship between it and *Δ*. Gas exchange data showed that genotypic variation in *A* (51%) was greater than that in leaf conductance (*g*, 32%). The CO₂ assimilation rate was positively correlated with both *NAR* and *RGR* and negatively correlated with *Δ*. Under low *N* conditions, *A* and *g* declined by 33% and 12%, respectively, across all genotypes producing a significant rise in *Δ* of 1.1%.

These results indicate that variation in photosynthetic capacity can account for genotypic variation in both *Δ* and *RGR* during vegetative growth in sunflower.
3.2 INTRODUCTION

Sunflower is one among a number of species within which genotypic variation in transpiration efficiency ($W$, ratio of dry matter accumulated to water transpired) exists (Chapter 2). In that study, under either well-watered or droughted conditions, there was a negative relationship between carbon isotope discrimination ($\Delta$) and $W$ amongst the genotypes used. The relationship arises because both $\Delta$ and $W$ are related to $p/p_a$, the ratio of the partial pressure of CO$_2$ inside ($p$) to that outside ($p_a$) the leaf.

Theory and experimental evidence have shown that $p/p_a$ is positively related to $\Delta$ and negatively related to $W$ (see Farquhar et al. 1989a). In the earlier study (Chapter 2), the genotypes that accumulated the most biomass over a fixed period during vegetative growth had the smallest $\Delta$, largest $W$ and the least leaf area per unit whole-plant dry weight (leaf area ratio, LAR) (These results are summarised in Fig. 3.1).

The basis for genotypic differences in $\Delta$ lies in photosynthetic capacity and/or stomatal conductance to the diffusion of water vapour ($g$). For instance, Ehleringer (1990) found a positive relationship between $\Delta$ and $g$ amongst common bean cultivars. Amongst wheat cultivars grown under glasshouse conditions, variation in $p/p_a$ could be approximately equally attributed to photosynthetic capacity and stomatal conductance (Condon et al. 1990; Morgan and LeCain 1991). A range of relationships have been demonstrated between biomass accumulation (and/or yield) and $\Delta$ (or $W$): positively amongst field grown wheat genotypes (Condon et al. 1987) and Agropyron desertorum clones (Read et al. 1991a); negatively amongst sunflower genotypes (Chapter 2), peanut genotypes (Wright et al. 1988), and amongst and across pasture species (Keller 1954; Hunt 1962; Johnson and Bassett 1991); or no relationship, amongst cultivars of common bean (White et al. 1990).

In Chapter one, two simple scenarios were proposed in an attempt to explain how $\Delta$ and growth may correlate. The first is one in which variation in
photosynthetic capacity, \( A_{\text{max}} \), (with \( g \) constant) produces differences in \( \Delta \) amongst genotypes. If other factors contributing to growth are held constant (e.g. phenology, partitioning and respiration) then plants with large \( A_{\text{max}} \), and hence large \( A \), would have small \( \Delta \) and would also grow faster. In contrast, if \( A_{\text{max}} \) were constant and \( g \) allowed to vary and again all other growth parameters were constant, then \( \Delta \) would be positively correlated with \( g \). As a corollary, these low \( g \)-low \( \Delta \) plants would have a lower \( A \) and hence grow more slowly. It is likely that both of these scenarios oversimplify the complex linkage between \( \Delta \) and growth. However, they can provide a first step in understanding how \( \Delta \) may correlate with growth.

The sunflower genotypes which had large biomass and low \( \Delta \) also displayed small \( LAR \) (leaf area per unit total biomass, the inverse of \( g \) as presented in Chapter 2 is used here because of its linear relationship to relative growth rate, \( RGR \), the rate of dry weight increase per unit dry weight). It is uncertain whether this reflected a possible mechanistic link between \( LAR \) and \( \Delta \) or whether it arose because of changes in \( LAR \) with ontogeny. Leaf area ratio and \( RGR \) are known to change with environment, phenology, size and age (See Evans 1972 especially chapters 13 & 17). One way of partially avoiding the confounding effects of the variation due to size is to analyse at a fixed biomass (Evans 1972; Rice and Bazzaz 1989) or over a fixed biomass range (e.g. Evans and Bush 1985; Bush and Evans 1988; Chapin et al. 1989; Poorter and Remkes 1990).

In this chapter, seven sunflower cultivars, three of which with wide genotypic variation in \( \Delta \) (Chapter 2), are examined for relationships between \( RGR \), \( \Delta \), and \( LAR \). Using data from sequential harvests it is possible to partially correct for ontogenetic drift in \( LAR \) and \( RGR \) and hence verify whether relationships between \( LAR \), \( RGR \) and \( \Delta \) are independent of size effects. In order to explore the interrelationships between rate of photosynthesis, stomatal conductance, \( \Delta \) and growth, gas exchange was measured in all seven
genotypes. Furthermore, a low nitrogen ($N$) treatment was added to the experimental design to test the coupling between $A$ and $g$ under low $N$ among genotypes.

Figure 3.1 Plot of transpiration efficiency ($W$), Biomass ($B$) and leaf area ratio ($LAR$) versus carbon isotope discrimination ($\Delta$). Symbols around the broken line represent data originally presented in Chapter 2 and the solid line represents results from the final harvest of the plants used in this experiment. For all correlations, $p < 0.05$. Genotype symbols as in Table 3.1 as well as Contiflor 3 (h), 206R (i) and 880 (j). Mole-based units are based on an assumed dry-matter carbon content of 40%.
3.3 MATERIALS AND METHODS

Experimental design and plant culture. Seven genotypes of sunflower (*Helianthus annuus* L.) were grown in a glasshouse (temperature range 15 - 35 °C, maximum irradiance 1150 µmol quanta.m⁻².s⁻¹) during autumn in Canberra (see Table 3.1 for a list of the genotypes and corresponding symbols used in text and figures). The daily total irradiance levels outside the glasshouse during the experiment are shown in Fig. 3.2.

Plants were harvested sequentially during early growth. Seeds were sown into 4.5 L pots, containing a 1:1 mixture of river sand and sandy-loam, for all but the first two harvests, for which 0.5 L and 0.75 L pots (respectively) were used. All pots were randomised except for the smaller pots which were grouped together. The emergence date (taken as the full appearance of both cotyledons) was noted for each plant. The experiment commenced on 14th of March, the date at which, on average, all genotypes had emerged except for *Morden* and *Impira Inta* which emerged one day later. Plants were fertilised with full strength nutrient solution (Hewitt 1966) at least once every two days commencing at day 5 after emergence. At each fertilisation, pots were flushed with excess solution. Six of the genotypes (all but genotype *Impira Inta*) were also grown under low nitrogen (N) conditions, i.e. as above with 1 mM instead of 12 mM N, until the final harvest of the plants used for growth analysis. The eight replicates of the low N treatment were randomised with the plants used for growth analysis.
Harvests. Plants were randomly allocated to harvests at the beginning of the experiment. Harvests took place on days 5, 11, 18, 25, 31 after the commencement of the experiment and day 30 for the low N plants. Replication varied between harvests and genotypes but was greater than or equal to 8 (except for genotype Suncross 150 at harvest one, n=5). At each harvest plants were separated into leaves, stems and roots (which were obtained after separation from the soil by gentle washing). Leaf area was obtained for each plant using a Li-Cor (Li-3000) leaf area meter. Samples were dried at 80 °C for at least 2 days before weighing for dry weight.

Water use, gas exchange and isotope analysis. Water use was measured in plants allocated to the final harvest (but not low N plants). Soil evaporation was minimised by placing a 6 cm layer of fine gravel on the soil surface. Pots were fertilised as above and were weighed before flushing (in late afternoon) and early the next morning from time of first fertilisation to the conclusion of the experiment. Transpiration efficiency ($W$) was calculated as total biomass over cumulative water lost from the pot. Gas exchange was measured during the final week of the experiment on the plants used for the final harvest of the growth analysis (on 6 replicates) and on the low N plants (5 replicates). The 7th
or 8th leaves of all genotypes were measured except on Morden where the 5th or 6th leaves were used. Gas exchange was carried out under a photon flux density of 1800 µmol quanta.m⁻².s⁻¹, at 25 °C and 10-12 mbar leaf-to-air vapour pressure difference. A detailed description of the gas exchange system has been made by Brugnoli et al. (1988). Two clamp-on chambers were used to measure two replicates at a time. Generally leaves took 20 minutes to equilibrate (flow rates were kept constant to avoid wide variation in boundary layer conductance). The calculation of parameters from the gas exchange is as per von Caemmerer and Farquhar (1981).

Carbon isotope discrimination (Δ) was measured on the leaves of the plants from final growth analysis harvest and on the low N plants. For two of the genotypes, Suncross 40R and Impira inta, Δ was measured on leaf and root dry matter of plants from all but the first harvest. The method was exactly as described in Chapter 2. The δ¹³C of carbon dioxide in air was assumed to be -8.0 ‰.

Growth and statistical analysis. For this analysis relative growth rate (RGR) was split into net assimilation rate (NAR, the rate of dry weight production per unit leaf area) and leaf area ratio (LAR). Leaf area ratio, in turn, was split into specific leaf area (SLA, the ratio of leaf area to leaf dry weight) and leaf weight ratio (LWR, the ratio of leaf dry weight to plant dry weight), hence,

\[ RGR = NAR \times LAR = NAR \times SLA \times LWR \]

The use of fitted functions allows a simple method whereby each of these parameters can be estimated at particular times or at a particular biomass. In this case calculations of these parameters were made at a common total dry weight which we will denote \( B^* \). We chose to analyse plants at a biomass (\( B \)) near to the size which occurred mid-way throughout the growth period. In essence the \( B \) (Ln transformed) averaged across all genotypes over the second and third harvests (days 12 and 17) was used because there was on average a larger number of replicates in the first harvest. Quadratic functions
were fitted for Ln (B) versus time from emergence and Ln (LA, leaf area) versus Ln (B). The calculations of RGR, LAR, NAR, SLA and LWR and their respective variances were made as per Causton and Venus (1981) (namely pages 52-56 & 84; see also Hunt and Parsons 1974), except that the estimates of LA and its variance were made using the Ln (LA) versus Ln (B) relationship. Likewise LWR was estimated from the relationship between B and leaf dry weight (both Ln transformed). Genstat V5.02 was used to fit relationships, determine the level of significance of polynomial terms and for analysis of variance.

3.4 RESULTS

In order to explain the results from Chapter 2, it was important that a similar set of relationships be obtained. Figure 3.1 shows a comparison between the earlier data and results from the final harvest of plants used for the present analysis. The results are consistent in that the nature of the relationships of transpiration efficiency (W), biomass (B) and leaf area ratio (LAR) versus Δ is conserved (Fig. 3.1). The absolute value of Δ differed between experiments and was much greater in the present one (grown during April) than in the former (grown during December). Differences in vapour pressure deficit could probably account for this result. Similarly the higher values of LAR during the present experiment may have been a response to the lower light levels received during April in Canberra. In both experiments there was a negative correlation between LAR and B (Fig. 3.3).
The relationship between $LAR$ and $B$ at the final harvest (Fig. 3.3) may have been indicative of a close correlation between $LAR$ and $B$ throughout the growth period. However, a time course of changes in $LAR$ for each cultivar (Fig. 3.4a) shows that genotypic ranking changed markedly with time. Furthermore, there was less spread in the data when $LAR$ was analysed according to size (Fig. 3.4b).
To correct for this ontogenetic drift in LAR and its components (and hence RGR and NAR) cultivars were analysed at a common B (in this case 0.49 g, B*). The genotypic variation in RGR and NAR was much larger than for LAR, SLA and LWR (Table 3.1). A comparison of the changes in relationships between NAR, LAR and \( \Delta \) versus RGR over time (at 10, 20 and 30 days after emergence) and at \( B^* \) is made in Figure 3.5. Throughout the growth period there was a significant positive relationship between NAR and RGR and, at days 10 and 20 after emergence, a negative relationship between \( \Delta \) and RGR (Fig. 3.5). This also occurred for the analysis at \( B^* \) (which occurred between 12-17 days after emergence depending on genotype). At no time, nor at \( B^* \), was there a relationship between LAR and RGR. Figure 3.5 also shows the decline in RGR and NAR from day 10 to day 30. Most of the variation in LAR was due to SLA (\( r=0.83, p < 0.05 \)) rather than LWR (ns).

Table 3.1 Genotype means (± one standard deviation) for relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR) and specific leaf area (SLA) all calculated at a biomass of 0.49 g (B*).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RGR (g.kg(^{-1}).d(^{-1}))</th>
<th>NAR (g.m(^{-2}).d(^{-1}))</th>
<th>LAR (m(^{2}).kg(^{-1}))</th>
<th>LWR</th>
<th>SLA (m(^{2}).kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suncross 40R  (a)</td>
<td>246±4</td>
<td>14.7±0.3</td>
<td>16.7±0.2</td>
<td>0.49±0.01</td>
<td>33.9±0.3</td>
</tr>
<tr>
<td>Hysun 31 (b)</td>
<td>226±4</td>
<td>14.3±0.3</td>
<td>15.8±0.2</td>
<td>0.46±0.01</td>
<td>34.1±0.1</td>
</tr>
<tr>
<td>Polestar (c)</td>
<td>222±6</td>
<td>12.9±0.6</td>
<td>17.2±0.7</td>
<td>0.48±0.02</td>
<td>35.6±0.3</td>
</tr>
<tr>
<td>Guayacan (d)</td>
<td>219±4</td>
<td>15.2±0.4</td>
<td>14.5±0.2</td>
<td>0.45±0.01</td>
<td>31.9±0.3</td>
</tr>
<tr>
<td>Suncross 150 (e)</td>
<td>214±6</td>
<td>13.1±0.4</td>
<td>16.4±0.2</td>
<td>0.47±0.01</td>
<td>34.6±0.3</td>
</tr>
<tr>
<td>Morden (f)</td>
<td>191±3</td>
<td>12.1±0.3</td>
<td>15.8±0.3</td>
<td>0.51±0.02</td>
<td>31.3±0.8</td>
</tr>
<tr>
<td>Impira Inta (g)</td>
<td>148±4</td>
<td>8.5±0.2</td>
<td>17.5±0.1</td>
<td>0.48±0.01</td>
<td>36.8±0.7</td>
</tr>
</tbody>
</table>
Figure 3.5 Net assimilation rate (NAR) versus relative growth rate (RGR), leaf area ratio (LAR) versus RGR and RGR versus carbon isotope discrimination (Δ) at 10, 20, and 30 days after emergence and at the grand mean weight of all plants (B* i.e. 0.49 g). Units are as in Fig. 3.6 and errors of growth parameters can be found in Table 3.1.
Using the growth analysis parameters calculated at $B^*$, $RGR$ and $NAR$ were negatively correlated with $\Delta$ amongst the genotypes (Fig. 3.6). There was no relationship between $LAR$ and $\Delta$ when $LAR$ was calculated at $B^*$ as opposed to the highly significant relationship at the final harvest (contrast Figs 3.1 and 3.6).

![Graph showing correlations between growth rate and assimilation rate.](image)

Figure 3.6 Relative growth rate ($RGR$), net assimilation rate ($NAR$) (molar units are also on a 24 hour basis) and leaf area ratio ($LAR$) corrected for ontogenetic drift (analysed at $B^*$) versus carbon isotope discrimination ($\Delta$). Symbols as in Table 3.1.

When $CO_2$ assimilation rate ($A$) was measured at an ambient $CO_2$ concentration of 350 µbar.bar$^{-1}$ there were positive relationships found between $A$ and both $NAR$ and $RGR$ (Fig. 3.7). The rate of $CO_2$ assimilation and $\Delta$ were negatively correlated (Fig. 3.7). The range in $A$ (51%) between genotypes was greater than that of stomatal conductance $g$ (32%) (Table 3.2). Six of the genotypes (all but Impira Inta, genotype g) were grown to the same age as the
last harvest of the growth analysis plants under low nitrogen (N) conditions. The low N produced significantly lower A and g for each genotype (Table 3.2); averaged across all genotypes A was 33% less and g was 12% less. As a result Α increased under low N conditions by an average of 1.1%/o. There was no genotype by N interaction for A, g, or Δ.

Table 3.2. Genotype means for CO₂ assimilation rate (A), leaf conductance (g) and carbon isotope discrimination (Δ) of plants grown under low and high nitrogen conditions.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CO₂ Assim. rate (A)</th>
<th>Conductance (g)</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µmol.m⁻².s⁻¹)</td>
<td>(mol.m⁻².s⁻¹)</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>Low N</td>
<td>High N</td>
<td>Low N</td>
</tr>
<tr>
<td>Hysun 31 (b)</td>
<td>20.7</td>
<td>35.5</td>
<td>0.59</td>
</tr>
<tr>
<td>Guayacan (d)</td>
<td>23.6</td>
<td>34.2</td>
<td>0.73</td>
</tr>
<tr>
<td>Suncross 150 (e)</td>
<td>18.8</td>
<td>33.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Suncross 40 (a)</td>
<td>17.5</td>
<td>32.8</td>
<td>0.66</td>
</tr>
<tr>
<td>Polestar (c)</td>
<td>20.4</td>
<td>29.5</td>
<td>0.57</td>
</tr>
<tr>
<td>Morden (f)</td>
<td>15.2</td>
<td>25.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Impira Inta (g)¹</td>
<td>-</td>
<td>(23.5)</td>
<td>-</td>
</tr>
<tr>
<td>Means¹</td>
<td>19.3</td>
<td>31.8</td>
<td>0.63</td>
</tr>
<tr>
<td>LSD(0.05)²</td>
<td>7.23</td>
<td>6.59</td>
<td>0.17</td>
</tr>
<tr>
<td>LSD(0.05)³</td>
<td>6.92</td>
<td>0.17</td>
<td>0.34</td>
</tr>
</tbody>
</table>

¹Impira Inta was not included in the ANOVA or calculation of treatment means, the means shown are for completion only.
²For comparison within nitrogen treatments.
³For comparison between nitrogen treatments.
Carbon isotope discrimination of leaf dry matter was measured on a low \( \Delta \) genotype, \textit{Suncross 40R} and a high \( \Delta \) genotype, \textit{Impira Inta} from earlier harvests (Fig. 3.8). There was no time trend for \textit{Suncross 40R} but \( \Delta \) did change with time for \textit{Impira inta}. Importantly these genotypes were consistently ranked through time. There was a positive correlation between \( \Delta \) measured on roots and that of leaves (\( r = 0.92, p < 0.01 \)).
3.5 DISCUSSION

Among sunflower genotypes, photosynthetic rate is positively correlated with relative growth rate (RGR) and negatively correlated with carbon isotope discrimination ($\Delta$). Before exploring the implications of this finding it is appropriate to assess both the methods used and some limitations to this result.

In calculating the growth analysis parameters it was decided that some adjustment should be made for the size of the plant. This is by no means an original idea (Evans 1972) and has been used in a variety of studies (Evans and Bush 1985; Chapin et al. 1989; Rice and Bazzaz, 1989). The relative growth rate and allocation pattern of plants are known to be functions of size or age and phenology (Evans 1972). Accordingly, in their recent work with Plantago, Dijkstra and Lambers (1989) were able to account for some of the inter-ecotype variation in RGR simply by comparing across a similar biomass range. In this experiment we compared plants at a particular biomass ($B^*$) which was calculated to occur approximately mid-way through the growth period. This meant that the genotypes were between 12-17 days after emergence and were planted in a greenhouse. Hence, while representing the ideal, it is not a controlled experiment.
emergence and were probably still vegetative. A similar approach was taken by Rice and Bazzaz (1989), who used the mean of all plants from all harvests.

An example of the effect of size can be seen in the change in the relationship between \( RGR \) and \( \Delta \) with time. Although there is a strong correlation at days 10 and 20 and at \( B^* \), there is no relationship at day 30 (Fig. 3.5). It is likely that at this stage the large-sized, small \( \Delta \), genotypes began to grow more slowly and hence the relationship no longer held. The issue of whether the ranking for \( \Delta \) amongst genotypes changes with time is dealt with below.

There are possibly some problems with this method of analysis. Firstly, as with most growth studies, it would have been ideal if this type of experiment were carried out under constant conditions, but our experiment was performed in a glass house. Hence, while temperature was reasonably controlled, light levels were not (Fig. 3.2). As a result both \( NAR \) and \( RGR \) decreased quite dramatically with time (Fig. 3.5). Net assimilation rate, \( NAR \), is in dry matter terms the difference between photosynthesis and respiration on a leaf area basis (see Poorter 1989b) and has been shown in sunflower to increase linearly with irradiance (Warren Wilson 1967, 1971). In this case it is likely that the drop in \( NAR \) and \( RGR \) was caused by falling irradiance throughout the experiment. Net assimilation rate decreases with size/phenology in plants (Watson 1947; Evans, 1972). Notwithstanding this, the fall in both \( NAR \) and \( RGR \) was much larger than that usually reported (e.g. Causton and Venus 1981) for the size/phenology aspect alone and can be largely ascribed to the fall in light levels (Fig. 3.2), caused both by decreasing day length and increasing cloudiness.

Secondly, we correct for ontogenetic drift because we are interested in mechanistic links between photosynthesis, \( \Delta \), allocation and growth rate. If this were not the case and, for instance, we were more concerned with time-trends of \( A \) or of phenology then the performance of the plant at a particular
The aims of the study were to further analyse the positive relationship between LAR and $\Delta$ and the negative relationship between $B$ and $\Delta$ among sunflower genotypes (Chapter 2). In the case of the former, when analysed at $B^*$, no relationship was apparent. The correlation between LAR, measured at the final harvest, and $\Delta$ thus appears to be an artefact of a further more basic linkage between factors which affect size (and/or phenology) and $\Delta$. Simply put, the bigger low-$\Delta$ genotypes achieved low LAR sooner (compare Figs 3.4a and 3.4b). In fact, there was no relationship between $\Delta$ at final harvest and LAR at the penultimate harvest, indicating that the relationship in Fig. 3.1 only came about because of changes in LAR during the last week of growth (Fig. 3.4). The negative relationship between $B$ and $\Delta$ is more straightforward. It can be explained by the close correlation of RGR with $\Delta$ between these genotypes. The rest of this section will deal with this result.

There was a strong positive relationship between RGR and NAR (Fig. 3.5). Such relationships have been found in other species but generally the view is expressed that variation in LAR is the main determinant of different growth rates among or within species (e.g. Poorter 1989b). Furthermore, there was a positive correlation between both NAR and RGR versus photosynthetic rate ($A$). Fast growth and low $\Delta$ were related to higher $A$ (Fig. 3.7). Higher peak rates of photosynthesis or NAR are generally regarded as liabilities to crop and plant growth (e.g. Watson 1952; Elmore 1980; Evans 1976; Gifford 1987; Nelson 1988; Austin 1989) because in many cases they are associated with lower area per leaf or per plant (e.g. Bhagsari and Brown 1986). In this case no such effects were apparent. Indeed the high $A$ plants had gained the most biomass by 31 days after emergence (Fig. 3.3) and had achieved the highest leaf areas (data not shown, though it can be inferred from Fig. 3.3). There are other instances where high $A$ is associated with high RGR (e.g. Pisum sativum,
Hobbs and Mahon 1982; also see review by Poorter 1989b). We may expect a negative relationship between $A$ and SLA as has generally been found (e.g. Pearce et al. 1969, Morgan and LeCain 1991). This was the case when SLA was calculated at $B^*$ and at the final harvest (data not shown) but only when genotype Morden was excluded. However SLA calculated this way is an average among all leaves on the plant and not just the leaf on which photosynthetic rate was measured. Further analysis of the relationship between allocation, growth rate and rate of photosynthesis is needed.

Generally selection for high $A$ has not increased yield or biomass production in either C3 or C4 crop species (e.g. Nelson et al. 1975; Crosbie and Pearce 1982; Ford et al. 1983). After analysing the potential flow-on effects of increased leaf photosynthesis, Day and Chalabi (1989) suggested that selection for high $A$ would be most promising in terms of crop performance in high radiation environments. Sunflower is one such crop in which there is also demonstrated variation in $A$ (Lloyd and Canvin 1977) and a tendency to maximise $A$ "almost regardless of conditions" (Rawson and Constable 1980). The relationship between $A$ and leaf area per leaf or plant (e.g. in wheat, Evans and Dunstone (1970) and rice, Cook and Evans (1983)) which has been used to explain the absence of a positive correlation or the presence of a negative correlation between $A$ and growth does not apply in this situation. Blum (1990) has recently reported a wheat cultivar with both high $A$ and high yield and cites as a possible explanation selection in a high irradiance environment (Day and Chalabi 1988).

Variation in $A$ can be due to changes in photosynthetic capacity ($A_{\text{max}}$) or in conductance ($g$). In the present case the negative correlation between $A$ and $\Delta$ (Fig. 3.6) and the absence of any correlation between $g$ and $\Delta$ indicates (see Fig. 3.9) that variation in $A$ was mainly due to changes in $A_{\text{max}}$. This was also the case with the differential effects of N supply on $A$ and $g$ and the resultant shift in $\Delta$. Lowering the nitrogen supply caused a decline in
photosynthetic rate and conductance (Table 3.2; Fig. 3.9). However, \( \Delta \) increased under low \( N \) (i.e. \( \rho/\rho_a \) increased) due to the much lower \( A \), rather than declining because of lower \( g \). Thus by varying \( N \) supply the correlation between \( A \) and \( g \) could be broken. Although the relationship between \( A \) and \( g \) was thought to be constant (at a given temperature and humidity) producing near-constant \( \rho_i \) (Wong et al. 1979), later studies have found this not to be the case (e.g. Sage and Pearcy 1987; for brief review see Farquhar et al. 1989a). Species may well differ in this regard. In short, both genotype and nutritional effects on \( A \) were much more prevalent than those on \( g \).

![Figure 3.9](image)

Figure 3.9 Plots of carbon isotope discrimination (\( \Delta \)) versus A) CO\(_2\) assimilation rate (\( A \)) and B) leaf conductance (\( g \)), under low nitrogen (closed symbols) and high nitrogen (open symbols) conditions. All genotypes except Impira Inta (a) are included.

All relationships with \( \Delta \) have been made based on two assumptions: that \( \Delta \) of leaf dry matter is representative of the whole plant and that genotypic differences in \( \Delta \) at the final harvest mirrored those at other time during the growth period. Carbon isotope discrimination of the leaves and roots were compared amongst Suncross 40R and Impira Inta. A close correlation between \( \Delta \) of leaves and that of roots was obtained. Hubick et al. (1986) obtained similar results with peanut. While the magnitude of the differences in \( \Delta \) between these
two genotypes increased with time (Fig. 3.8), the differences were at all times significant and consistent. The initial increase in \( \Delta \) with time by cultivar *Impira inta* can most likely be attributed to a low \( \Delta \) of the seed rather than a real trend in \( \Delta \) per se. It is unlikely then that the genotypic ranking for \( \Delta \) significantly changed with time. This is also the case with wheat (Condon 1988).

In the *Introduction* I proposed two simple scenarios which could be used to explain correlations between \( \Delta \) and growth. One of these in which \( A_{\text{max}} \) varied and \( g \) was held constant provides outcomes very similar to the results from this experiment. Although \( A_{\text{max}} \) was not measured, the relationship between \( A \) and \( \Delta \) and the lack of any correlation between \( g \) and \( \Delta \) (Fig. 3.9) provides evidence that genotypic variation in \( \Delta \) was due mostly to \( A_{\text{max}} \). Likewise \( RGR \) was correlated with \( A \) (and most likely \( A_{\text{max}} \)). Although earlier these scenarios were referred to as over simplistic, at least in the case of sunflower during early growth and, for that matter, under low \( N \) conditions, the concept of a varying \( A_{\text{max}} \) with constant \( g \) can adequately account for genotypic variation in both growth and \( \Delta \).
CHAPTER FOUR

Genotypic variation in growth and carbon isotope discrimination, and its relationship to photosynthetic capacity amongst sunflower germplasm

4.1 ABSTRACT

To verify the results obtained in the previous chapter a wider range of sunflower germplasm was screened for variation in relative growth rate (RGR) and carbon isotope discrimination (\(\Delta\)). A growth analysis was carried out on thirteen sunflower (Helianthus annuus) genotypes, a wild sunflower accession and an Helianthus argophyllus accession grown in a glasshouse with peak irradiance of 1200 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\). Relative growth rate (RGR) was positively correlated with net assimilation rate (NAR) but no relationship was found with leaf area ratio (LAR). This was also the result for eight of the sunflower lines grown at a lower light level (i.e. 30\%, peak irradiance of 400 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\)) adjacent to the above experiment. Under both low and high light intensities, NAR was negatively related to LAR. For the 'high light' plants, both RGR and NAR were negatively correlated with \(\Delta\).

In a second experiment, 13 sunflower lines and a wild accession were grown under high irradiance conditions (peak irradiance of 1400 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\)) in a glasshouse during summer. There was a close negative correlation between \(\Delta\) and photosynthetic capacity (\(A_{\text{max}}\)), measured with an oxygen electrode. In turn, \(A_{\text{max}}\) was negatively related to the specific leaf area, and positively related to both nitrogen content and total chlorophyll content on a leaf area basis. A simulation is presented which examines the nature of the relationship between LAR and NAR.

These results provide further evidence that genotypic variation in transpiration efficiency, as reflected by \(\Delta\), and RGR, during the vegetative stage can be directly related to photosynthetic rate in sunflower.
4.2 INTRODUCTION

In the preceding chapter relative growth rate (\textit{RGR}) was negatively correlated with carbon isotope discrimination (\(\Delta\)) amongst seven sunflower genotypes. Because \(\Delta\) has been shown to be negatively related to transpiration efficiency (\(W\), the ratio of biomass accumulated per unit water transpired) in sunflower (Chapter 2) and other species (see Farquhar \textit{et al.} 1989a; Ehleringer \textit{et al.} 1990), this means that the faster growing genotypes used water more efficiently. The photosynthetic rate, measured at ambient CO\(_2\) and the net assimilation rate, \textit{NAR}, were also negatively correlated with \(\Delta\) and positively related to \textit{RGR}. It should also be noted that leaf conductance to water vapour (\(g\)) was not related to \(\Delta\) amongst the genotypes tested (Chapter 3). Because this is a somewhat novel result, one aim of this work is to simply confirm this set of results using a substantially different set of genotypes.

In most cases genotypic variation in photosynthetic rate or net assimilation rate is either unrelated or negatively related to biomass accumulation or relative growth rate (for reviews see Nelson 1988; Poorter 1989b). There are exceptions such as pea (Mahon and Hobbs 1987) but generally higher \textit{RGR} is related more closely to variation in leaf area ratio (\textit{LAR}, the ratio of leaf area to whole plant biomass) e.g. (Bhagsari and Brown 1986). However, some of the work showing \textit{LAR} to be the major cause of differences in \textit{RGR} within or between species (e.g. Dijkstra and Lambers 1989; Poorter and Remkes, 1990) has been done under low light conditions (i.e. 270-400 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\)). In a comparison of four species (three C\(_3\) and one C\(_4\)) the extent to which \textit{NAR} and \textit{LAR} influenced \textit{RGR} depended on irradiance (Patterson \textit{et al.} 1978). At low light \textit{LAR} was the major factor whereas at high light \textit{NAR} dominated. Accordingly, this study examines to what extent the relationships between \textit{RGR}, \textit{NAR} and \textit{LAR} change under reduced irradiance amongst sunflower genotypes.
A commonly held view of the role of photosynthetic rate in determining differences in growth is that the 'cost' outweighs the 'benefit'. The 'cost' may be seen in terms of the extra nitrogen needed to sustain the higher photosynthetic rate being diverted from leaf area or tiller production (e.g., Heide et al. 1985). The extent to which the 'benefits' of extra investment in photosynthetic machinery are realised may be dependent on the growth conditions. Day and Chalabi (1988) recently pointed out that the potential for realising the gain from a higher photosynthetic rate in canopies is much greater in an environment of high irradiance. Photosynthetic rate has been closely related to $RGR$ amongst seven sunflower genotypes (Chapter 3). Given that photosynthetic rate is closely related to leaf nitrogen content (Field and Mooney 1986; Evans 1989; Sinclair and Horie, 1989), the question arises as to why the 'costs' in terms of leaf nitrogen and whole plant productivity were not greater than the 'benefits' in terms of increased $RGR$ within sunflower. A further aim of this work is to examine the nature of the relationship between photosynthetic capacity ($A_{\text{max}}$) and nitrogen content and whether this can explain why there is not an overriding trade-off between growth and photosynthesis.

Two experiments are presented. Firstly, a growth analysis during the early vegetative stage on 15 sunflower lines, grown under high light conditions, and a subset of 8 grown under low light conditions in a glasshouse. Growth analysis parameters are estimated at a common biomass (Chapter 3) in order to avoid some of the effects of ontogenetic drift (Evans 1972; Poorter and Pothman 1992). The second experiment uses a composite set of genotypes drawn from those used in the previous paper and from the first experiment. The plants were raised in a glasshouse under comparatively high light conditions and analysed for variation in photosynthetic capacity and $\Delta$. 
4.3 MATERIALS AND METHODS

Experiment One. Seeds of thirteen cultivated sunflower (*Helianthus annuus* L.) lines, a wild accession and an accession of *H. argophyllus* (see Table 4.1) were germinated in petri-dishes. The wild and *H. argophyllus* accessions were imbibed for two days (22 °C) and were then treated with cold temperature (4 °C) for one day, followed by a further three days at 22 °C before being planted on 18/2/90. All other genotypes were placed in petri-dishes on 18/2/90 and were planted on 22/2/90. When both cotyledons had emerged from the seed coat the seedlings were transferred to pots. The experiment consisted of four harvests (dates: 28/2, 6/3, 12/3, 17/3) of between 4-8 replicates depending on harvest and genotype. Pot size varied according to harvest: 0.75 l for the first, 1 l for the second and 4.5 l for the third and fourth harvests. Pots were flushed daily with nutrient solution (Hewitt 1966), commencing one week after planting. Plants were grown in a glasshouse with temperature range of 17-35 °C and maximum irradiance of 1200 µmol quanta m\(^{-2}\) s\(^{-1}\). Eight of the genotypes (see table 4.1) were also grown under shade conditions (maximum irradiance of 400 µmol quanta m\(^{-2}\) s\(^{-1}\)) after 28/2. Until this time they were treated exactly as above. Only three harvests were made over a period which allowed these 'shade' plants to become a similar size to those growing under high light conditions. These harvests took place on 8/3, 15/3 and 23/3. At each harvest the soil was washed from the roots and the plants were separated into roots, stems and leaves. Leaf area was measured using a Li-Cor leaf area meter (Li 3000) and all plant parts were dried in an oven at 80 °C for at least two days before determining dry weight.

Experiment Two. Seeds of twelve sunflower lines and a wild accession were germinated in petri-dishes (as above, seeds of the wild accession were exposed to cold temperature, 1 °C for one day) and planted in 10 l pots in a glasshouse (temperature range 17-35 °C, maximum irradiance 1400 µmol quanta.m\(^{-2}\).s\(^{-1}\)). Seedlings were sown on 7/1/91 except for the wild accession
and cv. *Impira Inta* which were sown two days later. Plants were fertilised at least every two days after 14/1/91 by flushing the pots with nutrient solution (Hewitt 1966). Pots were arranged in six blocks each containing one replicate of each genotype. Between 30/1 and 1/2 the rate of oxygen evolution was measured on leaf discs from leaves 7 or 8 (5 and 6 for cv. *Morden*) on each plant using an oxygen electrode (Delieu and Walker 1981). Measurements were made at 25 °C, 2050 µmol quanta m⁻² s⁻¹ and 5% CO₂. After measurement, the leaf discs were dried at 80 °C for at least two days, weighed, ground and then analysed for nitrogen content using a Carlo Erba model 1108 elemental analyser. Two leaf discs were also sampled in parts of the leaf adjacent to that used for photosynthetic measurement and were analysed for chlorophyll content (Porra et al. 1989).

**Analysis** Differences in RGR were tested using the method of Poorter and Lewis (1986). Growth analysis parameters were calculated at a particular biomass (*B*; the average biomass of all plants used in the experiment irrespective of treatment or harvest). The method of calculation for the growth analysis parameters was that used in Chapter 3. Analysis of variance was carried out using Genstat V5.02.

**Carbon isotope discrimination** The dried leaf material (from the final harvest - experiment one) was finely ground and sub-sampled (0.6 ±0.2 mg) for carbon isotope analysis. Each sample was combusted in a Carlo Erba (model 1108) elemental analyser, used to chromatographically separate CO₂ which was then analysed by continuous flow isotope mass spectrometry by a VG isomass mass spectrometer. Carbon-isotope discrimination, Δ, was calculated from the isotopic composition of the leaf material and that of air, which was taken to be -8.0 % relative to PeeDee Belemnite (Hubick et al. 1986).
4.4 RESULTS

There was significant inter-genotype variation in RGR (p<0.001). When calculated at a common biomass, RGR ranged from 174 (cv. Ha 336) to 255 g.kg⁻¹.d⁻¹ (cv. Advance). Relative growth rate was positively correlated with NAR but unrelated to LAR (Fig. 4.1). The range of NAR was much higher in percentage terms than for LAR, specific leaf area (SLA) and leaf weight ratio (LWR) (Table 4.1).

Table 4.1. Genotype means (± one standard deviation) for relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) when analysed at a common biomass.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RGR (g.kg⁻¹.day⁻¹)</th>
<th>NAR (g.m⁻².day⁻¹)</th>
<th>LAR (m².kg⁻¹)</th>
<th>SLA (m².kg⁻¹)</th>
<th>LWR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advance</td>
<td>255±7</td>
<td>10.2±0.3</td>
<td>25.0±0.2</td>
<td>43.3±3.8</td>
<td>0.58±0.05</td>
</tr>
<tr>
<td>Contiflor</td>
<td>253±9</td>
<td>10.2±0.4</td>
<td>24.7±0.2</td>
<td>46.8±1.7</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>A/I HRS A</td>
<td>251±5</td>
<td>9.2±0.3</td>
<td>27.4±0.8</td>
<td>45.4±0.4</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>Wild</td>
<td>244±8</td>
<td>11.0±0.4</td>
<td>22.1±0.2</td>
<td>42.3±1.7</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>Ha 89</td>
<td>243±8</td>
<td>8.9±0.3</td>
<td>27.3±0.4</td>
<td>46.5±0.5</td>
<td>0.59±0.01</td>
</tr>
<tr>
<td>H. argophyllus</td>
<td>240±10</td>
<td>10.1±0.6</td>
<td>23.8±0.9</td>
<td>34.4±2.0</td>
<td>0.69±0.01</td>
</tr>
<tr>
<td>Guayacan</td>
<td>230±8</td>
<td>10.7±0.4</td>
<td>21.6±0.2</td>
<td>43.7±1.3</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>Hysun 44</td>
<td>227±9</td>
<td>8.5±0.3</td>
<td>26.9±0.3</td>
<td>49.1±1.4</td>
<td>0.55±0.01</td>
</tr>
<tr>
<td>A/I 26-4</td>
<td>227±7</td>
<td>8.2±0.3</td>
<td>27.5±0.2</td>
<td>51.6±1.5</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>Rha 346</td>
<td>215±7</td>
<td>8.6±0.4</td>
<td>25.0±0.7</td>
<td>46.3±2.3</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td>CM 400</td>
<td>212±8</td>
<td>8.6±0.4</td>
<td>24.8±0.6</td>
<td>45.6±0.1</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td>Rha 266</td>
<td>212±8</td>
<td>8.3±0.3</td>
<td>25.4±0.4</td>
<td>46.8±1.1</td>
<td>0.54±0.02</td>
</tr>
<tr>
<td>Ha 232</td>
<td>206±9</td>
<td>8.0±0.4</td>
<td>25.9±0.6</td>
<td>42.2±1.9</td>
<td>0.61±0.04</td>
</tr>
<tr>
<td>CM 586</td>
<td>199±13</td>
<td>8.7±1.5</td>
<td>23.6±4.0</td>
<td>43.4±1.0</td>
<td>0.55±0.10</td>
</tr>
<tr>
<td>Ha 336</td>
<td>174±8</td>
<td>6.5±0.8</td>
<td>27.0±2.8</td>
<td>49.7±6.3</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td><strong>Under Low Light (30% of above)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>H annuus Wild</strong></td>
<td><strong>198±9</strong></td>
<td><strong>6.3±0.3</strong></td>
<td><strong>31.4±0.4</strong></td>
<td><strong>55.5±1.1</strong></td>
<td><strong>0.57±0.01</strong></td>
</tr>
<tr>
<td><strong>A/I 26-4</strong></td>
<td><strong>174±9</strong></td>
<td><strong>5.6±0.3</strong></td>
<td><strong>30.9±1.0</strong></td>
<td><strong>62.5±5.87</strong></td>
<td><strong>0.50±0.03</strong></td>
</tr>
<tr>
<td><strong>Contiflor</strong></td>
<td><strong>173±15</strong></td>
<td><strong>5.8±0.6</strong></td>
<td><strong>30.0±1.3</strong></td>
<td><strong>56.5±2.3</strong></td>
<td><strong>0.53±0.01</strong></td>
</tr>
<tr>
<td><strong>CM 400</strong></td>
<td><strong>170±11</strong></td>
<td><strong>6.3±0.4</strong></td>
<td><strong>27.1±0.3</strong></td>
<td><strong>56.1±3.7</strong></td>
<td><strong>0.49±0.03</strong></td>
</tr>
<tr>
<td><strong>Rha 266</strong></td>
<td><strong>168±15</strong></td>
<td><strong>5.9±0.7</strong></td>
<td><strong>28.5±2.2</strong></td>
<td><strong>58.5±2.2</strong></td>
<td><strong>0.49±0.02</strong></td>
</tr>
<tr>
<td><strong>Ha 89</strong></td>
<td><strong>165±8</strong></td>
<td><strong>5.1±0.3</strong></td>
<td><strong>32.5±0.5</strong></td>
<td><strong>64.1±10.8</strong></td>
<td><strong>0.52±0.09</strong></td>
</tr>
<tr>
<td><strong>A/I HRS A</strong></td>
<td><strong>159±6</strong></td>
<td><strong>4.8±0.2</strong></td>
<td><strong>33.3±0.2</strong></td>
<td><strong>59.9±4.73</strong></td>
<td><strong>0.56±0.04</strong></td>
</tr>
<tr>
<td><strong>Hysun 44</strong></td>
<td><strong>158±10</strong></td>
<td><strong>5.2±0.3</strong></td>
<td><strong>30.1±0.3</strong></td>
<td><strong>61.8±4.1</strong></td>
<td><strong>0.49±0.03</strong></td>
</tr>
</tbody>
</table>
Eight of the genotypes were also grown under low light conditions in the same glasshouse. The imposition of shade made little difference to the dependence of RGR on NAR (Fig. 4.2). Under these low light conditions, there was no genotypic relationship between LAR and RGR but there was a broad relationship between treatments (Fig. 4.2). These growth parameters were estimated at a common biomass (384 mg) for both high light and low light treatments.

Carbon isotope discrimination (Δ) was measured on leaves of the 'high light' plants from the final harvest. The range in Δ was comparatively small in contrast with a previous study (Chapter 2) from 20.4 (CM 400) to 21.2 (HA 336) but there was significant inter-genotypic variation (p < 0.01). There were negative relationships between both RGR and NAR versus Δ (Fig. 4.3) but no relationship between LAR and Δ.
Figure 4.2 Plots of (a) Net assimilation rate (NAR) and (b) leaf area ratio (LAR) versus relative growth rate (RGR) under high (solid line, small letters) and low (dashed line, capital letters) conditions. Symbols as in Table 4.1. The fitted lines represents a linear regression. The alternate molar units are based on an assumed carbon content of 40% and for NAR are on a 24 hour basis.

Figure 4.3 Plots of carbon isotope discrimination (Δ) versus (a) relative growth rate (RGR) and (b) net assimilation rate (NAR). The fitted lines represent linear regressions. The alternate molar units are based on an assumed carbon content of 40% and are on a 24 hour basis.
In the second experiment, 14 sunflower lines, including a wild accession, were analysed for differences in photosynthetic capacity ($A_{\text{max}}$, rate of $O_2$ evolution at 5% $CO_2$ and 2050 $\mu$mol quanta m$^{-2}$ s$^{-1}$). Significant genotypic variation was found in each of the attributes measured: $\Delta$, $A_{\text{max}}$, nitrogen content per unit leaf area ($N_a$), total chlorophyll per unit leaf area ($Chl$) and specific leaf area ($SLA$) (Table 4.2). A correlation matrix is presented in Table 4.3 to show the interrelationships between these attributes. Carbon isotope discrimination was negatively related to $A_{\text{max}}$, $N_a$, $Chl$ and positively related to $SLA$. Photosynthetic capacity was positively correlated with $Chl$ and $N_a$ and, accordingly, negatively correlated with $SLA$ (Table 4.3).

Table 4.2 Genotype means for photosynthetic capacity ($A_{\text{max}}$), carbon isotope discrimination ($\Delta$), Specific leaf area ($SLA$), nitrogen content per unit leaf area ($N_a$) and total chlorophyll content ($Chl$)

<table>
<thead>
<tr>
<th></th>
<th>$A_{\text{max}}$</th>
<th>$\Delta$</th>
<th>$SLA$</th>
<th>$N_a$</th>
<th>$Chl$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$mol.m$^{-2}$.s$^{-1}$</td>
<td>$^\circ$/o</td>
<td>m$^2$.kg$^{-1}$</td>
<td>mol.m$^{-2}$</td>
<td>mmol.m$^{-2}$</td>
</tr>
<tr>
<td>Advance</td>
<td>75.3</td>
<td>20.6</td>
<td>30.8</td>
<td>0.156</td>
<td>0.482</td>
</tr>
<tr>
<td>Hysun 54</td>
<td>70.1</td>
<td>20.5</td>
<td>25.9</td>
<td>0.159</td>
<td>0.477</td>
</tr>
<tr>
<td>Ha 232</td>
<td>68.5</td>
<td>20.7</td>
<td>28.2</td>
<td>0.152</td>
<td>0.421</td>
</tr>
<tr>
<td>H. Annuus Wild</td>
<td>67.4</td>
<td>20.6</td>
<td>28.0</td>
<td>0.148</td>
<td>0.466</td>
</tr>
<tr>
<td>Hysun 33</td>
<td>65.3</td>
<td>21.0</td>
<td>28.2</td>
<td>0.149</td>
<td>0.455</td>
</tr>
<tr>
<td>Contiflor</td>
<td>65.2</td>
<td>20.9</td>
<td>27.3</td>
<td>0.152</td>
<td>0.471</td>
</tr>
<tr>
<td>Charata</td>
<td>63.8</td>
<td>20.8</td>
<td>28.9</td>
<td>0.150</td>
<td>0.389</td>
</tr>
<tr>
<td>CM 400</td>
<td>62.6</td>
<td>21.4</td>
<td>31.3</td>
<td>0.138</td>
<td>0.406</td>
</tr>
<tr>
<td>Ha 336</td>
<td>62.1</td>
<td>20.7</td>
<td>31.2</td>
<td>0.142</td>
<td>0.410</td>
</tr>
<tr>
<td>Argo/Impira Hrs A</td>
<td>61.9</td>
<td>21.2</td>
<td>29.3</td>
<td>0.158</td>
<td>0.371</td>
</tr>
<tr>
<td>HA 89</td>
<td>61.0</td>
<td>21.0</td>
<td>24.3</td>
<td>0.167</td>
<td>0.499</td>
</tr>
<tr>
<td>Impira Inta</td>
<td>55.8</td>
<td>22.1</td>
<td>34.6</td>
<td>0.131</td>
<td>0.238</td>
</tr>
<tr>
<td>Rha 266</td>
<td>50.9</td>
<td>21.4</td>
<td>39.9</td>
<td>0.136</td>
<td>0.375</td>
</tr>
<tr>
<td>Morden</td>
<td>45.6</td>
<td>23.4</td>
<td>34.8</td>
<td>0.125</td>
<td>0.320</td>
</tr>
</tbody>
</table>

LSD (0.05%) 5.8  0.5  3.0  0.016  0.046
Table 4.3 Correlation matrix for carbon isotope discrimination (Δ), photosynthetic capacity ($A_{\text{max}}$), nitrogen content per unit leaf area ($N_a$), total chlorophyll content per unit leaf area ($Chl$) and specific leaf area ($SLA$).

<table>
<thead>
<tr>
<th></th>
<th>Δ</th>
<th>SLA</th>
<th>$N_a$</th>
<th>$Chl$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ</td>
<td>-0.85***</td>
<td>-0.63*</td>
<td>0.71**</td>
<td>0.70**</td>
</tr>
<tr>
<td>$A_{\text{max}}$</td>
<td>0.71**</td>
<td>-0.75**</td>
<td>-0.73**</td>
<td></td>
</tr>
<tr>
<td>SLA</td>
<td>-0.95***</td>
<td>-0.87***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$N$</td>
<td>0.78***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, **, *** significant at p < 0.05, p < 0.01 and p < 0.001 respectively.

4.5 DISCUSSION

Fast growth amongst sunflower genotypes is associated with high rates of dry matter production per unit leaf area (NAR) rather than a high leaf area to biomass ratio (LAR) (Fig. 4.1). While not a unique result, this is unusual. In most cases NAR is either unrelated or negatively related to RGR (see review by Poorter 1989b). These results were obtained partly to confirm a similar finding (Chapter 3) on a smaller set of genotypes. Although the absolute range of RGR and NAR was greater in the previous paper, the nature of the result was conserved.

This association may have arisen because the plants were grown at relatively high irradiances enabling greater expression of differences in photosynthetic capacity. Some of the studies showing the dominance of LAR in determining differences in RGR have been done under low light conditions (270-400 µmol quanta m$^{-2}$ s$^{-1}$; Dijkstra and Lambers 1989; Poorter and Remkes 1990). A lower irradiance did not induce greater genotypic variation in LAR (Fig. 4.2, Table 4.1) amongst eight of the sunflower genotypes. In fact,
there was still a positive correlation between \( NAR \) and \( RGR \). This indicates that by simply reducing the irradiance we were unable to greatly influence the factors which determine genotypic differences in \( RGR \). This contrasts with the findings of Patterson \textit{et al.} (1978) who found in a between-species comparison that the dependence of \( RGR \) on leaf area partitioning, (which is equal to \( LAR \) if plants are grown approximately in steady state; see Poorter and Remkes 1990), increases as irradiance decreased.

The basic growth analysis equation, i.e.,

\[
RGR = NAR \times LAR
\]

has recently been re-written in terms of carbon instead of dry matter, to link \( NAR \) with gas exchange parameters, as:

\[
RGR = \frac{A \cdot L \cdot (1 - \phi)}{Q}
\]

where \( A \) is the net rate of carbon assimilation during the day, \( L \) is the light period as a proportion of the day, \( \phi \) is the proportion of net assimilation by the leaves in the daytime which is then lost by respiration and \( Q \) is the ratio of plant carbon to leaf area (Masle and Farquhar 1988). The numerator is \( NAR \) in terms of carbon and the denominator is the inverse of \( LAR \), again in terms of carbon. Genotypic variation in \( NAR \) can be due to \( A \) and/or \( \phi \). Unfortunately neither \( A \) nor \( \phi \) were measured in this experiment. However, the relationship between \( NAR \) and \( \Delta \) (Fig. 4.3) can help in further tracing the likely dependence of \( NAR \) on \( A \) and/or \( \phi \).

Carbon-isotope discrimination (\( \Delta \)) can be directly related (positively) to the ratio of intercellular to ambient CO\(_2\), \( p/p_a \). (Farquhar \textit{et al.} 1982b). Because \( p/p_a \) is negatively related to whole-plant transpiration efficiency (\( W \), the ratio of plant biomass to water transpired), \( \Delta \) and \( W \) are negatively related (Farquhar \textit{et al.} 1989a). Both \( RGR \) and \( NAR \) were negatively related to \( \Delta \). (Fig. 4.2). Hence, the genotypes which grew faster had lower \( p/p_a \) and higher \( NAR \).
Over a range of genotypes in which \( \Delta \) varied more widely than in the first experiment, there was little variation in stomatal conductance (Chapter 3). Assuming this is the case for this set of genotypes (with a smaller range of \( \Delta \)) then low \( p/p_a (\Delta) \) appears to indicate higher photosynthetic capacity. Hence, it is fair to assume that the systematic variation in \( NAR \) was largely due to more to \( A \) rather than \( \phi \). It cannot be assumed that \( \phi \) was constant but rather that it was probably not responsible for the relationships between \( NAR, RGR \) and \( \Delta \). In short, the results of experiment two confirmed that variation in \( A_{max} \) caused variation in \( \Delta \) amongst sunflowers (Table 4.3).

There are so few published results that it is hard to speculate on why in one species variation in \( g \) accounts for that in \( \Delta \) (e.g. common bean, Ehleringer et al. 1990) and, in another, that variation in \( A_{max} \) accounts for that in \( \Delta \) or \( p/p_a \) (Chapter 3 and Table 4.3). For wheat there seems to be little correlation of either \( A \) or \( g \) with \( \Delta \) (Condon et al. 1990; Morgan and LeCain 1991). An assumption underpinning any conclusion from these observations is that the germplasm screened is representative of the variation present in the species. The cultivars and lines used in this work represent wild, parental inbred, hybrid and open pollinated types from various backgrounds bred in different countries (D. George, pers. comm.). It may be assumed that no bias in the results has arisen as a function of the material screened. Hence, it appears that the extent to which either \( A_{max} \) or \( g \) influence \( \Delta \) is species dependent. Any explanation of this phenomenon will need to consider the ecology of wild progenitors, inheritance of \( A_{max} \) and/or \( g \) and current breeding/agronomic objectives.

On an agronomic level, it seems that if a breeding program for more water-use efficient sunflower cultivars were established, the result would be a faster growing plant. All the results presented thus far have dealt with characteristics that are important during vegetative growth. As a result, it is not yet possible to establish if a faster growing plant would also be a high yielding
plant. Moreover, there are situations in which a faster growing plant may not be desirable. If water is limited, a low Δ fast growing genotype may use water more efficiently but at a faster rate and hence hasten the onset of drought. Likewise, for a crop growing while VPD is declining, faster growing/low Δ plants will use more water earlier when it is more 'expensive' in terms of carbon. At the same time a slower growing/high Δ plant, while being less efficient, would conserve water which could be 'spent' under lower VPD conditions later in the season. Admittedly these are only possible scenarios which have not taken into account possible connections between growth rate and phenology on one hand, nor the complicating factor of interaction between the rate of canopy development and direct loss of water from the soil, on the other. It is interesting to note that many of the currently recommended varieties in Australia were large RGR/small Δ cultivars (e.g. Advance, Suncross 40R and Hysun 44).

Many studies and reviews have shown that there is either no relationship or a negative relationship between A or \( A_{\text{max}} \) and growth (e.g. Eagles and Wilson 1982). Examples to the contrary are rare (e.g. Mahon and Hobbs 1987). The most common explanation has been that there is a trade-off between leaf area and photosynthetic rate (sometimes referred to as the 'dilution effect' - Evans 1990). In experiment one there was a trade off between LAR and NAR (Fig. 4.4, for reasons set out below 1/LAR or ϕ, has been presented). In terms of plant growth, this means that the benefit from increased leaf area generally outweighs that of increased photosynthetic capacity as suggested by evolutionary studies in wheat (Evans and Dunstone 1970) and physiological studies on Poa pratensis (Heide et al. 1985). However, in sunflower there was a positive relationship between NAR and RGR (and between A and RGR in Chapter 3).
To explain the nature of the trade-off between \( LAR \) and \( NAR \), a simulation (see Appendix for details) is presented based on the results of experiments one and two. The basic assumption is that N content per unit leaf dry weight (\( N_w \)) is constant. In fact, there was no significant genotypic variation in \( N_w \) in the second experiment. The daily \( NAR \) is calculated for a sinusoidally varying irradiance with photosynthetic capacity of the leaf proportional to the nitrogen content per unit leaf area (\( N_a \)) and with \( LWR \) held constant. In order to fit theoretical lines to observed data, the relationship between \( A_{\text{max}} \) and \( N_a \) was allowed to change with light treatment (equations 3 and 4, appendix). As \( SLA \) (and hence \( LAR \)) increases, \( N_a \) and \( A_{\text{max}} \) decrease (e.g. Table 4.3) resulting in lower \( NAR \). In Figure 4.4, the results of this simulation and the raw data are presented. The inverse of \( LAR \) is used so that a the slopes of straight lines through the origin represent \( RGR \). Because of the curvilinear relationship between \( A_{\text{max}} \) and irradiance, the increases in \( SLA \) and \( LAR \) (reduction in
Fig. 4.4) are greater than the reduction in NAR, and are associated with higher RGR. This result is more apparent for the low light treatment (Fig. 4.4, dashed line).

While the data in Fig. 4.1 show that RGR increases with increasing NAR, RGR in the simulation declines with increasing NAR (compare slopes of dashed and solid lines in Fig. 4.4). In an attempt to account for this, a further condition was imposed on the simulation. While regression analysis showed that the intercept of the relationship between $A_{\text{max}}$ and $N_a$ was not significantly different from zero, analysis of variance showed that there was significant variation in $A_{\text{max}}/N_a$ (which will be referred to as apparent nitrogen use efficiency, NUE). Further analysis showed that one genotype (HA 89) had a large influence over the relationship between $A_{\text{max}}$ and $N_a$. When this genotype was ignored, the $A_{\text{max}}$ versus $N_a$ relationship had a significant positive intercept (equation 5, appendix, see also Sincair and Horie 1989)). Hence, the conditions for the simulation were changed from one of constant NUE (above) to variable NUE. When this was taken into account there was a much closer fit between the model and the experimentally determined data (solid line Fig. 4.4). It is important to note that this does not necessarily mean that there are fundamental differences in the use of nitrogen at the biochemical level between genotypes, although this may be possible. Rather, it may be indicative of differences between genotypes in nitrogen partitioning within leaves. Further work on the nature of the $A_{\text{max}}$ - $N_a$ relationship in sunflower is needed in order to address this problem.

**Conclusion** On the basis of the genotypes that have been screened, genotypic variation in RGR and $\Delta$ is due to photosynthetic capacity. With regard to RGR it is hypothesised that this comes about partially because of the differences in apparent NUE between sunflower genotypes. Hence in sunflower it appears that greater $A_{\text{max}}$ is not a burden to whole plant growth. However, further work is required on genotypic variation in nitrogen partitioning to further
understand the positive correlation between RGR and NAR. With regard to Δ, 
the close relationship between A_{max} and Δ indicates that genotypic variation in g 
is limited.

4.6 APPENDIX

The aim of the simulation was to derive NAR as a function of LAR. To 
do this a constant mass of nitrogen per unit leaf dry weight, N_{w}, was assumed 
(4286 mmol kg^{-1}, there was no significant variation in N_{w} between genotypes in experiment 2) as SLA varied. Photosynthetic capacity, in terms of electron 
transport (J_{max}) was derived initially (Fig. 4.4, dashed line) as directly 
proportional to N_{a}, i.e. for high light treatment (experiment 1):

\[ J_{\text{max}} (\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) = 1.23 \cdot N_{a} (\text{mmol} \cdot \text{m}^{-2}) \]  

(3)

and the low light treatment (experiment 1):

\[ J_{\text{max}} = 0.95 \cdot N_{a} (\text{mmol.m}^{-2}) \]  

(4)

The simulation was also run using a linear relationship between J_{max} and N_{a} 
with a positive intercept derived from the results of experiment two excluding 
HA 89 (Fig. 4.4, solid line), i.e.,

\[ J_{\text{max}} = 2.6 \cdot (N_{a} - 53) \]  

(5)

Irradiance was assumed to vary sinusoidally through a 12 h day with noon 
irradiance 1200 (high light) or 400 (low light) μmol quanta.m^{-2}.s^{-1} The light 
response curve used for photosynthetic electron transport was (Farquhar and 
Wong 1984),

\[ J = \frac{I_{2} + J_{\text{max}} - \sqrt{(I_{2} + J_{\text{max}})^{2} - 4 \cdot 0.7 \cdot I_{2} \cdot J_{\text{max}}}}{1.4} \]  

(6)

with irradiance, I_{2}, absorbed by photosystem II is calculated as:

\[ I_{2} = \frac{I_{0} \cdot (1-r) \cdot (1-f)}{2} \]  

(7)

where I_{0} is incident irradiance, r and f are both 0.15 and represent reflectance 
and spectral quality corrections and the 2 in the denominator results from
there being two photosystems. This was converted to the rate of CO₂ assimilation by (von Caemmerer and Farquhar 1981),

\[
A = \frac{J \cdot (p_i - 40)}{4.5 \cdot p_i + 420}
\]

(8)

where \( p_i \) is assumed to be 262 µbar (i.e. \( p_i/p_a = 0.75 \)).

The integrated value of \( A \) was converted to \( NAR \) assuming that dry matter was 40% carbon and that \( \phi \) (see equation 2) was 0.35 or 0.2 for the high and low light treatments respectively. These values, of \( \phi \), would also take into account other factors that are important when converting the theoretically calculated \( A \) into \( NAR \) (as obtained in experiment one) such as of cloudy days and self-shading.

Using this method it was possible to obtain \( NAR \) as a function of \( SLA \). Net assimilation rate was further related to \( LAR \) by imposing constant \( LWR \) at 0.56 and 0.53, for high and low light conditions respectively.
CHAPTER FIVE

Variation in relative growth rate and carbon isotope discrimination amongst a range of pasture cultivars

5.1 ABSTRACT

Two experiments, examine the relationships between growth, transpiration efficiency and carbon isotope discrimination between and within species, are presented. In the first, transpiration efficiency ($W$) and carbon isotope discrimination ($\Delta$) are measured on 26 cultivars representing 11 species of pasture plants. Transpiration efficiency, calculated as either total biomass per unit water used or shoot biomass per unit water used, was negatively related to $\Delta$. Biomass accumulation was also negatively related to $\Delta$ in this experiment.

In a second experiment, the causes of differences in relative growth rate in relation to $\Delta$ were examined for 23 cultivars representing 10 species (a subset of the above) during early growth. The differences in $RGR$ were accounted for by differences in the leaf area ratio ($LAR$). In turn, variation in $LAR$ was due to variation in both specific leaf area ($SLA$) and leaf weight ratio ($LWR$). These relationships did not hold if grass and legume cultivars were analysed separately. None of the growth parameters could be related to $\Delta$ even though biomass accumulation in this experiment was negatively related to $\Delta$ amongst legume cultivars and positively related amongst grass cultivars. The ranking for $\Delta$ was not consistent between experiments. Reasons for this are analysed and discussed.
5.2 INTRODUCTION

The previous chapters have shown that increased transpiration efficiency \((W)\) is accompanied by increased growth amongst sunflower genotypes. In other species, such as common bean (Ehleringer et al. 1990) or wheat (Condon et al. 1987), the opposite is the case. The development of a rapid screening technique for genotypic differences in \(W\) using carbon isotope discrimination, \(\Delta\) (Farquhar et al. 1989a), has made studies on the relationship between \(W\) and growth much less cumbersome. This means that instead of large experiments where constant monitoring of water use is required, plants can be harvested at the end of the growth period and the dry matter analysed for the carbon isotope ratio. On this basis cultivars within a species have been accurately ranked for \(W\) (e.g. Hubick et al. 1986). Most studies to date have concentrated on within-species variation in \(\Delta\) because of the potential of selecting higher \(W\) plants on the basis of \(\Delta\) in segregating populations (Hubick et al. 1988). As a result, very little is known about the relationship between \(\Delta\), \(W\) and growth between species.

In this chapter a range of pasture species, (and cultivars within species), commonly grown in temperate Australia, are examined for variation in \(\Delta\), \(W\) and relative growth rate \((RGR)\). This choice meant that a diversity of species and cultivars within species could be used. The set of species also allows a comparison of legumes versus grasses as these are the major two groups within this set.

Differences in growth are examined during the seedling stage at the maximum (or near) relative growth rate. This both allows comparison with the earlier sunflower work and avoids some of the effects of variation in phenology. Multi-species comparisons also enable an examination of what plant characteristics influence \(RGR\). A recent multi-species comparison (Poorter and Remkes 1990) found a strong positive relationship between \(RGR\) and leaf area ratio \((LAR,\) the ratio of leaf area to plant biomass). However,
these results differed from those presented in chapters 3 and 4 where, amongst a range of sunflower genotypes, net assimilation rate (NAR, the rate of dry matter accumulation per unit leaf area) was the cause of differences in RGR. As noted above, depending on species, there may be a positive, negative, or no relationship between W and growth. Amongst the sunflower genotypes a negative relationship between RGR and ∆ meant that biomass was positively correlated with W (chapters 2 and 3). Hence, selection for plants of increased transpiration efficiency may also mean selection for increased size during early growth. It may be that amongst a range of cultivars from various species, no particular relationship between growth and ∆ will arise. This would be the case if the extent to which factors which produce variation in RGR and ∆ varied across species. Therefore, when considering W or ∆ as a useful trait, it is crucial to understand the relationship between W or ∆ and RGR.

Two experiments are presented. In the first a range of pasture species is screened for intra- and inter-specific variation in W and ∆. This permits an analysis of the nature of the relationship between ∆ and W across species. The second experiment, using a similar set of genotypes to the first, is concerned with variation in RGR and its components within and amongst the species.

5.3 MATERIALS AND METHODS

Experiment one Seeds of 26 cultivars belonging to 11 species (see Table 5.1) were germinated in petri-dishes at 25 °C. Seedlings were selected for uniformity and planted into 0.5 L plastic planter bags (150 mm high X 70 mm radius) containing 1:1 sandy-loam:sand mixture. Plants were grown in a naturally-lit glasshouse with temperature range 15-32 °C and a peak irradiance of 1200 µmol quanta.m⁻².s⁻¹ during October and November in Canberra. Plants were initially fertilised daily (commencing one week after
planting) by flushing pots with full-strength nutrient solution (Hewitt 1966). Three weeks after planting, each bag, after its base was removed using a razor-blade, was placed in a 4.5 L pot. Care was taken to ensure minimal disturbance of the roots. The 4.5 L pots, four replications per cultivar, containing the same soil mixture as the planter bag, were randomly allocated to benches in the same glasshouse. A 100 mm layer of coarse gravel was placed on the surface of each of these pots to minimise direct evaporation.

Water-use was measured (commencing 25 days after planting) by flushing pots with nutrient solution in the late evening and allowing them to freely drain overnight. The following morning (7 am - 9 am) pots were weighed and plastic bags placed over the drain-holes in case of extra drainage. Pots were weighed again just prior to the next nutrient flush, which initially occurred at 3-5 day intervals reducing later to 1-2 day intervals. Water-use was calculated by subtracting the pot weight at the evening weighing from that in the morning after the last flushing. One check pot (containing no plants but otherwise identical to the pots with plants) was placed on each bench. Water-use was also measured for each of these and was used as an estimate of direct evaporation. Cumulative transpiration was calculated as the difference between total water loss and direct evaporation of a check pot on the same bench.

Harvesting took place, over a four day period, starting 47 days after planting. Plants were cut at the soil level and separated into leaves and stems. Leaf area was measured, using a Li-Cor (Li-3000) leaf area meter, on a subsample of leaves (at least 30%). Roots were washed free of the soil and together with leaves and stems dried at 80 °C for at least 48 h. Carbon isotope discrimination was measured on the leaves following the method set out in chapter 4.

Experiment Two  Seedlings of 23 cultivars from 10 species (see table 5.2) were germinated, selected for uniformity and planted into 1 L planter bags
(300 mm X 70 mm radius). Initially four seedlings were planted per pot and were thinned one week later to one per pot. Pots were flushed with nutrient solution (Hewitt 1966) once every two days, commencing one week after planting, up until three weeks after planting and from then on daily. Pots were placed randomly on benches in the glasshouse described above. Four harvests took place, commencing one week after planting, 5-6 days apart. At each harvest plants were separated into roots (obtained by washing), stems and leaves. Leaf area was measured on all leaves using a Li-Cor leaf area meter. Plant parts were dried in an oven at 80 °C for at least 48 h before weighing.

Carbon isotope discrimination was measured on the leaves of plants from the final harvest following the method presented in chapter 4. Estimates of the growth analysis parameters (RGR etc.) were made at a common biomass following the method presented in chapters 3 and 4. In this case the biomass at which these parameters was estimated was the average of all plants used in the experiment (e.g. Rice and Bazzaz 1988). Differences in RGR were tested using the method of Poorter and Lewis (1986). Analysis of variance was carried out using Genstat 5.02.

5.4 RESULTS

Experiment One There was significant variation in Δ and W at both the cultivar and species levels. At the cultivar level the analysis could be carried out in two ways: either by considering the variation across cultivars of all species, or testing for inter-cultivar variation within each species separately; even so, both gave the same result and only the former is presented in Table 5.1. *Trifolium subterraneum* was the only species in which cultivars varied in both Δ and W. The two *Medicago truncatula* cultivars were significantly different from each other for Δ but not for W. There was significant variation within grasses and legumes at the species level. For example, within the
legumes only *M. truncatula* and *T. subterraneum* differed significantly in either *W* or *Δ*. Amongst the grasses, *Dactylis glomerata* was the most water-use efficient species and *Phalaris aquatica* the most inefficient. Generally the grasses were more water-use efficient than the legumes and the large variation in *Δ* and *W* at this family level (*Poaceae* versus *Fabaceae*) accounted for much of the variation at the species and cultivar levels.

Table 5.1 Transpiration efficiency (*W*, mmol C. mol H₂O⁻¹) and carbon isotope discrimination (Δ, ‰) at the cultivar, species and family levels.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>CULTIVAR</th>
<th>W</th>
<th>Δ</th>
<th>SPECIES</th>
<th>CULTIVAR</th>
<th>W</th>
<th>Δ</th>
<th>FAMILY</th>
<th>W</th>
<th>Δ</th>
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There was a negative relationship between $W$ and $\Delta$, whether expressed at the cultivar (Fig. 5.1a), species or genus level (figure not shown but see caption Fig. 5.1). Qualitatively the relationship did not change when $W$ was based on above-ground dry matter ("tops") only ($W_t$, Fig. 5.1b). Biomass accumulation was negatively related to $\Delta$ (Fig. 5.2) but only when grass and legume cultivars were considered together. Within either of these groups there was no association between $\Delta$ and biomass.

**Figure 5.1** Plots of transpiration efficiency calculated from a) total biomass including roots ($W$) or b) biomass of the tops only ($W_t$) versus carbon isotope discrimination ($\Delta$). The molar ratio for $W$ is calculated assuming dry matter contains 40% carbon. The lines represent linear regressions: a) $W = 9.50 - 0.33.\Delta$, b) $W = 7.42 - 0.26.\Delta$

**Figure 5.2** Plot of carbon isotope discrimination versus total biomass. The line represents a significant linear regression.
**Experiment Two** Significant variation in RGR was found both within and between species. Again, these tests at the cultivar level were made both across all species and within each species separately. The results of the latter analysis can be found in Table 5.2. Grass cultivars grew faster than legume cultivars and this was due to the grasses displaying a greater LAR even though they tended to have lower NAR (Fig. 5.3a,b). The two components of LAR, SLA and LWR were also significantly positively related to RGR (Fig 5.3c,d). The same relationships were evident when analysed at the species level.

Interestingly, if we consider the legumes and the grasses separately, the source of variation in RGR could not be attributed to any one of its components (Fig. 5.3). Although most species were represented by only two cultivars, it appeared that the dependence of RGR on either NAR or LAR varied between species (Table 5.2). For instance, within the two *M. littoralis* cultivars, greater RGR was achieved by greater NAR, whereas the difference in RGR (although insignificant) between the two *P. aquatica* cultivars was due to LAR.

As with the first experiment there was significant within- and between-species variation in $\Delta$ (Table 5.2). Biomass was positively related to $\Delta$ amongst the grass cultivars and negatively related to $\Delta$ amongst the legume cultivars (Fig. 5.4). However, there was no relationship between $\Delta$ and RGR, LAR, NAR or LWR although there was a negative relationship between $\Delta$ and SLA. ($r = -0.46; p<0.05$)
Table 5.2 Growth analysis and carbon isotope discrimination ($\Delta$) results from experiment 2. Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA, g.m$^{-2}$) and leaf weight ratio (LWR) were calculated at a common biomass (65 mg). In all cases the coefficient of variation was less than 10% so standard deviations are not shown. Carbon isotope discrimination was measured on leaves of plants from the final harvest. The discrepancy ($\Delta_{2-1}$) between $\Delta$ of cultivars used in the second and first experiments is also presented.

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LSD (0.05) 0.4
Figure 5.3 Plots of relative growth rate (RGR) versus a) leaf area ratio (LAR), b) net assimilation rate (NAR), c) leaf weight ratio (LWR) and d) specific leaf area (SLA) at the cultivar level. All parameters were measured at a common biomass (65 mg). Symbols represent different grass (open) or legume (closed) cultivars. Lines represent linear regressions for each of the significant relationships.
5.5 DISCUSSION

Many studies have demonstrated the negative relationship between $\Delta$ and $W$ using a range of cultivars within species (Farquhar et al. 1989a) but few of these have included multi-species comparisons. A negative relationship between $\Delta$ and $W$ has been shown across two closely related *Lycopersicon* species and their F$_1$ progeny under well-watered conditions (Martin and Thorstenson 1988). Johnson and Bassett (1991) compared high and low $\Delta$ lines of three different species (*Lolium perenne*, *Dactylis glomerata* and *Festuca arundinacea*) under well-watered conditions. When further analysed, there was a significant negative relationship between $\Delta$ and $W$ ($r = -0.95$; $p < .01$). Our results show that $\Delta$ can be used as a selection (or ranking) criterion between species as well as within species (Fig. 5.1). One test, apart from the significance of the correlation coefficient, of the nature of the relationship between $W$ and $\Delta$ is the value of the x-axis intercept (i.e. when $W$ is zero). The value of 29.0 is close to the in vitro value for discrimination by
Rubisco (ribulose bisphosphate carboxylases) (Farquhar et al. 1989a) and certainly within the range of values recorded from previous experiments (Farquhar and Richards 1984; Hubick et al. 1986). There is also a possibility that the theoretical value may be dependent on the species being examined i.e. that the discrimination due to carboxylation varies between and even perhaps within species as large variation in discrimination between taxonomically widely separated Rubiscos have been reported (Farquhar et al. 1989a).

Within species, where a broad range of cultivars (or genotypes) has been screened, variation in $\Delta$ has been found (e.g. Hubick et al. 1986; Hall et al. 1990; White et al. 1990). For the most part there was little within-species variation in either $\Delta$ or $W$ even though $W$ or $\Delta$ have previously been shown to significantly vary in some of the species examined, e.g., Dactylis glomerata (Keller 1953) and Lolium perenne (Downes 1967). The explanation for this may lay in close genetic relationships between cultivars and/or in the small range of cultivars used. Trifolium subterraneum, which was the species represented by most cultivars, was the only species within which both $W$ and $\Delta$ varied significantly. The two cultivars which differed most in $\Delta$, Mount Barker (high $\Delta$) and Nungarin (low $\Delta$) also differ widely in time to flowering, the former being a long season cultivar and the latter a short season cultivar. The cultivars that were intermediate for $\Delta$ and $W$ are also intermediate in length of season (Oram 1990). In order to determine the extent to which there is a relationship between $\Delta$ and time to flowering a larger number of cultivars would need to be examined.

On an agronomic scale, $\Delta$ may be useful in determining the relative productivity of different species growing on limited water supply. With present-day techniques, accurate assessment of $W$, as defined (i.e. including roots), in pastures, in the field, is very difficult. When $W$ was estimated using only tops of the plant, $W_t$, there was still a strong negative correlation with $\Delta$ (Fig. 1b).
Variation in root:shoot ratio was present at the species and cultivar level (data not presented), but was insufficient to cause any loss in correlation between $W$ and $\Delta$.

An important characteristic of any physiological trait, or for that matter any trait proposed as a selection criterion, is that heritability be high, i.e., low genotype-by-environment interaction (Garrity et al. 1982). In most cases rankings between genotypes for $\Delta$ have been highly consistent across different environments including different sites (e.g. Condon and Richards 1992) or under various levels of drought stress (e.g. Ehleringer et al. 1990). Because two experiments were carried out for different lengths of time (and hence plants experienced different environments) there is an opportunity to compare $\Delta$ from the cultivars that were used for the $W$ study (ca. 50 days) and those used in the growth study (ca. 25 days). In this case $\Delta$ was not consistent between cultivars used in both experiments. As well as changes in ranking, $\Delta$ from the $W$ study was, on average, much lower than that of the growth study (compare tables 5.1 and 5.2). The discrepancy ($\Delta_{2-1}$) in $\Delta$ for cultivars between the two experiments has been calculated by subtracting the average $\Delta$ of each cultivar in the first experiment from that of the second (Table 5.2). Many factors could contribute to such a result. Perhaps the plants in the longer term experiment may have experienced some degree of water stress or root restriction. Either have been shown to induce lower $\Delta$ (e.g. Hubick et al. 1986; Masle and Farquhar 1988). Furthermore, it is likely that the magnitude of $\Delta_{2-1}$ was greatest in plants which reached the largest size, whether measured by total biomass or leaf area, and, if root restriction were a factor (e.g. Carmi et al. 1983; Robbins and Pharr 1988), root mass (Fig. 5.5a,b). In fact all three were positively related to $\Delta_{2-1}$ (see caption Fig. 5.5). Hence, it appears that some of the variation in $\Delta$ found in the second experiment was environmentally induced. However, if the legume and grass cultivars are considered separately, there was no relationship between either root mass or leaf area and $\Delta_{2-1}$ (Fig. 5.5).
average $\Delta_{2-1}$ was much greater for the grass species than the legume species, 1.7 $\%_{\text{oo}}$ and 0.3 $\%_{\text{oo}}$ respectively. It is possible that the grasses were more susceptible to any periodic experience of water deficit as they generally displayed higher leaf area ratios than the legumes (Fig. 5.3, Table 5.2) and like root biomass, leaf area and total biomass, $LAR$ was also positively correlated with $\Delta_{2-1}$ (Fig 5.5c). In this case $LAR$ calculated at a common biomass (from experiment two) has been used to characterise differences in allocation between the cultivars.

It is also possible that the growth conditions, i.e. glasshouse during October-November in Canberra, may have played some role in the discrepancy between the $\Delta$ results from both experiments. The growth analysis plants were harvested by the 21st of October whereas the $W$ plants were grown under conditions of increasing temperature and decreasing humidity for a further month. It may have been that some of the cultivars...
responded differently to these conditions thereby causing a discrepancy between the two sets of results. Another possibility is that the discrepancy arises as a function of the differing effects of age and/or phenology on W. However, in the few studies where $\Delta$ has been followed through time (e.g. Condon 1988; Chapter 3) genotypic rankings have been conserved.

As there is usually considerable variation in $\Delta$ within species, comparisons of species differences between studies may be unsound. Nonetheless if the species ranking for $\Delta$ from experiment two is compared with that from the study by Johnson and Bassett (1991), there is no agreement. In fact, the results are opposed: in experiment two the ranking between the species (small $\Delta$ to large) was $F. arundinacea$ $D. glomerata$ and $L. perenne$, whereas Johnson and Bassett (1991) found that $L. perenne$ had the smallest $\Delta$ and $F. arundinacea$ had the largest.

In summary, $W$ varied both within and between species and carbon isotope discrimination can be used to screen both species and cultivars for differences in $W$. However, it is important to take into account the growing conditions under which the experiment is conducted and, in order to avoid erroneous conclusions, to check for environmentally induced changes in $\Delta$ by including a number of harvests, especially during early growth.

Whatever the cause(s) of the discrepancy in the $\Delta$ values between the two experiments it is likely that the relationship between biomass accumulation and $\Delta$ was also affected. In the first experiment there was a negative relationship between biomass accumulation and $\Delta$ but no relationship when grasses and legumes were analysed separately. In experiment two, where plants were younger and there was much less chance of genotype-by-environment interaction, $\Delta$ was positively related to biomass amongst the grasses and negatively related amongst the legumes (Fig. 5.4). Given the probable environmental effects on both $\Delta$ and biomass accumulation it is unlikely that results from experiment one represent a true
genetic relationship between \( \Delta \) and growth. The two opposing relationships in Fig. 5.4 mirror the results of other published work. Some studies have shown that the most water-use efficient cultivars or ecotypes either grew the most, e.g. amongst and within various pasture grasses (Keller 1954; Hunt 1962; Johnson and Basset 1990), peanut cultivars (Wright et al. 1988) and sunflower cultivars (Chapters 2-4), or grew the least, e.g., in wheat (Condon et al. 1987) and *Agropyron desertorum* (Read et al. 1991a).

Another measure of plant productivity is relative growth rate \((RGR)\), which has been used as an indicator of growth potential and competitive ability between species (Grime 1979). Physiologically, \( \Delta \) can be more readily related to \( RGR \) (Masle and Farquhar 1988) than biomass production. However, in the case of experiment two, there was no correlation between \( \Delta \) and \( RGR \) either across all cultivars or within grasses and legumes separately. This is in contrast to the close negative relationship between \( \Delta \) and \( RGR \) amongst sunflower cultivars (Chapters 3 and 4). In fact, the only growth analysis parameter that did correlate (negatively) with \( \Delta \) was \( SLA \). This has also been shown with sunflower (Chapter 2) and peanut (Wright et al. 1988). Before examining why there was no apparent relationship between \( RGR \) and \( \Delta \), results of the growth analysis itself will be examined.

Across either cultivars or species the variation in \( RGR \) could be accounted for by \( LAR \) (Lambers and Dijkstra 1989; Poorter and Remkes 1990). However, other studies have shown \( RGR \) to be dependant on \( NAR \), e.g. between sun and shade species (Pons 1977) or within sunflower (Chapters 3 and 4). On closer examination the positive relationship between \( LAR \) and \( RGR \) is largely the result of the grass species displaying higher \( LAR \) than the legume species. This result may be regarded as relevant to this set of species only. A wider range of monocots and dicots showed that, if at all, dicots grew faster than monocots (Poorter and Remkes 1990). However, a contrasting result was obtained by Elias and Chadwick (1979) who compared
grasses and legumes for differences in $RGR$ and allocation. Further analysis of their data shows that the grass cultivars grew faster ($171 \pm 5$ g.kg$^{-1}$.d$^{-1}$, mean ± standard error of 28 cultivars from various species) than the legume cultivars ($156 \pm 6$ g.kg$^{-1}$.d$^{-1}$). In other studies where legume and grass species have been directly compared, no clear differences have emerged (Grime and Hunt 1975; Poorter 1989b). Most legume-grass comparisons have focused on the community level (see Harper 1977; Haynes 1980) and, hence, comparisons for $RGR$ are scarce. The absence of any correlation amongst the grass and legume cultivars separately may have been due to the small range of $RGR$ within these groups (Table 5.2). Perhaps species are naturally different in the way that they achieve variation in $RGR$; for example $LAR$ appears to dominate within rice (Cook and Evans 1983) while $NAR$ is more important within sunflower (Chapters 3 and 4). The result, when a number of different cultivars representing different species are analysed together, is that neither $NAR$ or $LAR$ dominate. It should also be noted that in some other cases neither $NAR$ nor $LAR$ clearly dominate in influencing variation in $RGR$ (e.g. Higgs and James 1969; Woodward 1983; Paul et al. 1984).

The characteristically high $LAR$ of the grasses may be important in situations (i.e. pastures) where grasses and legumes compete. In competition, displaying greater $LAR$ would tend to increase success (Poorter 1989b). More importantly, it appears that the reason for making growth comparisons, and hence the choice of species, may affect the result. Poorter and Remkes' (1990) comparison of species was made in order to understand the causes of variation in $RGR$ across a range of habitats. As a result, a broad range of species was used from resource-poor, resource-rich and disturbed habitats. In contrast, one aim of the present work was to test whether high $\Delta$ species and cultivars within species grew faster or slower. Although there was no relationship between $\Delta$ and $RGR$, $\Delta$ and biomass were positively related amongst grasses and negatively related amongst legumes (Fig. 5.4). This disparity occurred even though $RGR$ and biomass were positively correlated.
(r=0.60, p<0.01). It is also interesting to note that the relationships between RGR and Δ, although insignificant, were positive for the grass cultivars (r=0.1 ns) and negative for the legume cultivars (r=-0.33 ns).

There may be a number of reasons why the biomass versus Δ relationships were not reflected in the RGR versus Δ relationships. Depending on the cultivar, either linear or quadratic relationships between biomass (Ln transformed) and time were used to derive RGR. Accordingly, the degree to which RGR changed with ontogeny (if at all, i.e. not in linear relationships) varied between cultivars. Hence, RGR calculated at a common biomass may avoid effects of ontogenetic drift (e.g. Dijkstra and Lambers 1989) but may not always be an appropriate indicator of integrated performance. Variation in seed size is another factor that could contribute to any disparity between these relationships.

**Conclusion** A range of species and cultivars within species were screened for variation in Δ and growth. There was a large genotype-by-environment interaction on Δ, when cultivars were sampled at two different times, 25 and 50 days after sowing. At the earlier sampling time, for which it is assumed that plants did not experience any water deficit or root restriction, there was significant within and between species variation in Δ. Carbon isotope discrimination was negatively related to W but these data came from the later harvest and there is most likely to have been some mixture of environmental and genetic effects in this result. Differences in RGR were caused by LAR which was generally greater for the grass cultivars than the legumes. Biomass accumulation (at 25 days) was positively related to Δ amongst the grasses and negatively related to Δ amongst the legumes. This result could not be explained by the growth analysis results as there was no relationship between Δ and RGR when calculated at a common biomass. Care must be taken in studies on W and Δ not to confound environmental with genetic effects.
Variation in relative growth rate and carbon isotope discrimination within natural populations of *Trifolium subterraneum* and *Phalaris aquatica*

### 6.1 ABSTRACT

Natural populations of *Trifolium subterraneum* and *Phalaris aquatica* were examined for variation in carbon isotope discrimination (Δ) and relative growth rate (RGR). Amongst a wide range of accessions (50 - *T. subterraneum* and 26-*P. aquatica*) there was significant variation in Δ when grown under glasshouse conditions in a common environment. Separate studies, using subsets of these accessions (15-*T. subterraneum* and 11-*P. aquatica*), also found significant variation in RGR. There was a negative relationship between Δ and RGR amongst the *T. subterraneum* accessions but no relationship at all amongst the *P. aquatica* accessions. Amongst the *T. subterraneum* accessions, net assimilation rate (NAR, the rate of biomass production per unit leaf area) was positively correlated with RGR. In contrast, amongst the *P. aquatica* accessions, leaf area ratio (LAR, the ratio of leaf area to total biomass) was positively correlated with RGR. It is likely that photosynthetic capacity was the underlying systematic cause of variation in both Δ and RGR amongst *T. subterraneum* accessions as there was a close negative relationship between NAR and Δ. The variation in Δ amongst the *P. aquatica* accessions may have been caused by variation in stomatal conductance as there was no relationship between specific leaf area and Δ.

Amongst the *P. aquatica* accessions, there was some indication of adaptation, in terms of Δ, to the climate of the site of origin; large Δ being associated with more arid environments. However, there are reservations about this result due to the restricted range of ecotypes and the limited climatic information available.
6.2 INTRODUCTION

In the previous chapter a number of pasture species were screened for variation in carbon isotope discrimination ($\Delta$) and transpiration efficiency ($W$). Within only two of these species, *Trifolium subterraneum* and *Lolium perenne*, was there significant variation in $\Delta$ (during early growth, experiment two). Each of the species examined was represented by only a few genotypes and this may explain why very little intra-species variation in $\Delta$ was found. To detect substantial variation in $\Delta$ and hence $W$, within such species, a closer look at native populations is necessary.

Many of the pasture species naturalised in Australia originate from the Mediterranean area. From 1951 up to the present a number of plant collecting trips have been made (Donald 1970; Read and Dear 1991). The aim of these expeditions has been to bring back a large range of material from various locations to be used as breeding material or for direct selection. Two species have been chosen from these collections to be screened for variation in $\Delta$. *Trifolium subterraneum* has been chosen because a wide range of material is available and because of its adaptation across a wide range of environments of southern Australia (Morley 1961). *Phalaris aquatica* has been chosen firstly, because it provides a contrast with *T. subterraneum*, in that it is a monocot and is perennial and secondly, because previous work (Cooper and McWilliam 1966) characterised some of the accessions available and the environments in which they were found.

Studying the accessions of these species may also help in further understanding the characteristics which influence growth rate and how these are related to $\Delta$. Carbon isotope discrimination and relative growth rate ($RGR$) and/or biomass were negatively related amongst sunflower lines (Chapters 2-4). In contrast, when a number of different species were examined (Chapter 5), there was no relationship between $RGR$ and $\Delta$, but there was a positive relationship between $\Delta$ and biomass amongst the grass cultivars and, like
sunflower, a negative relationship between Δ and biomass amongst the legume cultivars. There is also variation in the characteristics that most influence RGR. Amongst the sunflower lines, net assimilation rate (NAR) and photosynthetic rate were shown to be closely correlated with RGR (Chapters 3 and 4). In contrast, amongst a range of pasture cultivars from various species, leaf area ratio (LAR) was positively associated with RGR (Chapter 5; see also Poorter and Remkes 1990). The choice of species may have much to do with the results obtained. Hence, the ecotypes of these two species were examined for variation in RGR and its causes.

Four experiments are presented. In experiments one and two, 50 accessions of *T. subterraneum* and 26 accessions of *P. aquatica*, respectively, were screened for variation in biomass production and Δ. In the third and fourth experiments, subsets of the above collections, 15 of *T. subterraneum* and 11 of *P. aquatica* respectively, were grown and analysed for variation in RGR and its components.

### 6.3 MATERIALS AND METHODS

**Experiment one** Fifty accessions of *Trifolium subterraneum* were obtained from the Australian Trifolium Genetic Resources Centre (Western Australian Department of Agriculture, Perth). The accessions originated in the Mediterranean region and were collected from sites of less than 500 m altitude (Table 6.1). Seeds were scarified and placed on moist paper in petri-dishes at 22 °C. Five days later, 8 seedlings were planted into 10 L pots in a glasshouse (temperature range over the entire experiment, 15-32 °C) on 20/10/90. Plants were thinned to 4 per pot 9 days later. Pots were organised in a nearest-neighbour design (Gleeson and Cullis 1987; Lill *et al.* 1988) with two replicate pots per accession (each containing four plants). Pots were flushed daily with nutrient solution (Hewitt 1966) commencing 6 days after planting. Harvest took
place over two days, 46-47 days after planting. For this harvest the tops of the plants were removed and dried in an oven at 80 °C for at least 48 h and then weighed. Carbon isotope discrimination was measured as in chapter 4 on sub samples (4-6 mg) of the tops after grinding.

**Experiment two** Twenty-four accessions of *Phalaris aquatica* originally collected from the Mediterranean region were obtained from CSIRO Division of Plant Industry, Canberra. Seeds of these, together with two local cultivars, *Australian* and *Sirolan*, were placed on damp filter paper in petri-dishes and exposed to a cold temperature treatment of 1 °C for three days and then germinated at 22 °C. Eight seedlings of each accession were planted into 10 L pots on 13/10/90. Two replicate pots were sown per accession. Pots were thinned to four plants at 13 days after sowing. Experimental design and fertilisation were as for experiment one, but fertilising commenced 9 days after planting. Plants were harvested over two days, 48-49 days after planting. At this harvest tiller numbers per plant were counted and the tops of the planted removed and dried at 80 °C for at least 48 h. Carbon isotope discrimination was measured as in experiment one.

**Experiment Three** Fifteen of the *T. subterraneum* accessions (Table 6.1) used in experiment one were germinated as above and planted into 0.75 L plastic, free-draining "planter" bags containing a 1:1 mixture of sandy-loam and sand. Pots were arranged in a randomised complete block design with five blocks. Initially two plants per pot were sown (on 20/10) and these were later thinned to one per pot (29/10). Plants were fertilised as above. Four harvests took place weekly commencing on 1/11. At each harvest plants were separated into leaves (including cotyledons) stems and roots. Leaf area was measured using a Delta-T (Mk2) leaf area meter, (which proved very accurate at low leaf areas) for the first harvest and for each subsequent harvest using a Li-Cor (Li 3000) leaf area meter. Plant matter was then dried at 80 °C for at least 48 h.
and then weighed. The leaves of plants from the final harvest were ground and sub sampled for carbon isotope analysis as above.

Experiment Four Eleven of the *P. aquatica* accessions used in experiment two (Table 6.2) which was identical in most aspects to that of experiment three. However, this experiment occurred eight days earlier and seeds were cold-treated as in experiment two.

Statistical analysis Growth analysis parameters were estimated at a common biomass, calculated as the average of all plants used in each experiment, i.e. 75 mg for *T. subterraneum* and 107 mg for *P. aquatica*. The calculation of these parameters and their error terms were as described in Chapter 1 and outlined by Causton and Venus (1981). Analysis of variance was carried out using Genstat version 5. Nearest neighbour analysis was carried out using SAFE (a program designed by NSW Department of Agriculture).

6.4 RESULTS

Experiments one and two For both *T. subterraneum* and *P. aquatica* there was significant ecotypic (from here on accessions will be referred to as ecotypes) variation in $\Delta$ and shoot biomass (Tables 6.1 and 6.2). The range of $\Delta$ was greater amongst the *T. subterraneum* ecotypes (20.3-22.9 $^\circ/_{oo}$) than for *P. aquatica*. In both experiments the choice of nearest-neighbour design and analysis was justified in that there were significant position effects. There was no relationship between $\Delta$ and biomass in either experiment. However, if the ecotypes of *T. subterraneum* var *subterraneum* are excluded then $\Delta$ was negatively associated with shoot biomass production ($r = -0.40$, $p < 0.05$). For the *P. aquatica* ecotypes, $\Delta$ was positively related to tiller number ($r = 0.74$, $p < 0.01$) but not to biomass production.
Table 6.1 Carbon isotope discrimination (Δ) and shoot biomass at ca. 45 days of *T. subterraneum* accessions in experiment one. Accessions marked with * are those used in experiment three. The place names of locations are part of a database, whereas the longitudes and latitudes were read from maps. As a result of both duplication and changes in place names of original collection sites, two locations (marked with #) could not be specifically identified.

<table>
<thead>
<tr>
<th>CPI Number</th>
<th>Taxonomic variety</th>
<th>Country or region</th>
<th>Long. and Lat.</th>
<th>Δ (‰)</th>
<th>Shoot Biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP 9 Brachycl.</td>
<td>subterraneum</td>
<td>Sardinia, 39°34' N, 8°57' E</td>
<td>20.3</td>
<td>3.48</td>
<td></td>
</tr>
<tr>
<td>70001a</td>
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<td>EP 53 White C</td>
<td>yanninicum</td>
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<td>4.74</td>
<td></td>
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<tr>
<td>GF168-1</td>
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<td>1.6</td>
<td></td>
</tr>
<tr>
<td>83966a</td>
<td>subterraneum</td>
<td>Greece 39°40' N, 20°40' E</td>
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<td>3.85</td>
<td></td>
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<tr>
<td>GF 105-11*</td>
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<td>3.21</td>
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<tr>
<td>84003c*</td>
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<td>2.84</td>
<td></td>
</tr>
<tr>
<td>103960a*</td>
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<tr>
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</tr>
<tr>
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<td>3.38</td>
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</tr>
<tr>
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<td>2.5</td>
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</tr>
<tr>
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<tr>
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<td>3.55</td>
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<tr>
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</tr>
<tr>
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<td>3.13</td>
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<td>21.6</td>
<td>3.32</td>
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<td>3.37</td>
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<tr>
<td>70035</td>
<td>brachycl.</td>
<td>Turkey 41°15' N, 29°2' E</td>
<td>21.7</td>
<td>1.78</td>
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<tr>
<td>GF148-2</td>
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<td>France 44°39' N, 0°36' W</td>
<td>21.7</td>
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</tr>
<tr>
<td>70003b*</td>
<td>subterraneum</td>
<td>Turkey 41°0' N, 39°43' E</td>
<td>21.7</td>
<td>3.08</td>
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</tr>
<tr>
<td>103942a</td>
<td>subterraneum</td>
<td>Spain 37°37' N, 7°15' W</td>
<td>21.7</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>103890a</td>
<td>subterraneum</td>
<td>Portugal 41°5' N, 7°12' W</td>
<td>21.7</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>103922a*</td>
<td>subterraneum</td>
<td>Portugal 39°11' N, 7°26' W</td>
<td>21.8</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>69993a</td>
<td>subterraneum</td>
<td>&quot;Dotyrol&quot; 21.8</td>
<td>2.8</td>
<td></td>
<td></td>
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<tr>
<td>84449b</td>
<td>subterraneum</td>
<td>Spain 42°41' N, 8°58' W</td>
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<td>2.04</td>
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</tr>
<tr>
<td>GF150-1</td>
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<td>France &quot;St Medard&quot; 21.8</td>
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<td></td>
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<tr>
<td>65195c</td>
<td>subterraneum</td>
<td>Tunisia 36°57' N, 8°45' W</td>
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<td>3.19</td>
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</tr>
<tr>
<td>GF017-1</td>
<td>brachycl.</td>
<td>Corsica 41°55' N, 8°44' E</td>
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<td>3.05</td>
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<tr>
<td>70001c</td>
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<td>Turkey 41°0' N, 39°43' E</td>
<td>22.0</td>
<td>2.91</td>
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</tr>
<tr>
<td>83965a*</td>
<td>subterraneum</td>
<td>Greece 39°40' N, 20°40' E</td>
<td>22.0</td>
<td>3.96</td>
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<tr>
<td>70004</td>
<td>subterraneum</td>
<td>Turkey 41°0' N, 39°43' E</td>
<td>22.0</td>
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<tr>
<td>GF108-3</td>
<td>subterraneum</td>
<td>France 43°50' N, 0°48' W</td>
<td>22.1</td>
<td>3.60</td>
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</table>
Table 6.1 (continued)

<table>
<thead>
<tr>
<th>CPI1 Number</th>
<th>Taxonomic variety</th>
<th>Country or region</th>
<th>Long. and Lat.</th>
<th>Δ</th>
<th>Shoot Biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>84000</td>
<td>subterraneum</td>
<td>Canary Is.</td>
<td>40°48' N, 3°56' W</td>
<td>22.1</td>
<td>2.99</td>
</tr>
<tr>
<td>EP18 Brachy a</td>
<td>brachycalycinum</td>
<td>Sardinia</td>
<td>39°27' N, 8°44' E</td>
<td>22.2</td>
<td>2.49</td>
</tr>
<tr>
<td>EP01 Brachy b</td>
<td>brachycalycinum</td>
<td>Sardinia</td>
<td>39°22' N, 9°10' E</td>
<td>22.3</td>
<td>2.04</td>
</tr>
<tr>
<td>84450d*</td>
<td>subterraneum</td>
<td>Spain</td>
<td>42°41' N, 8°58' W</td>
<td>22.9</td>
<td>1.25</td>
</tr>
</tbody>
</table>

LSD (5%) 0.7 1.04

Table 6.2 Place of origin, carbon isotope discrimination (Δ), shoot biomass, tiller number and length of summer drought (see text) in place of origin for the *Phalaris aquatica* ecotypes screened in experiment two. Ecotypes marked with # are those used for growth analysis in experiment four. Weather data were not available (n/a) or incomplete (*) for some of the sites. For one site "Yesilkoy", weather data was available but not a definite latitude and longitude.

<table>
<thead>
<tr>
<th>CPI1 Number</th>
<th>Country</th>
<th>Longitude, and latitude</th>
<th>Δ (‰)</th>
<th>Shoot Biomass (g)</th>
<th>Tiller number</th>
<th>Length of drought (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19344#</td>
<td>Portugal</td>
<td>38°53' N, 7°10' W</td>
<td>20.6</td>
<td>2.78</td>
<td>14.8</td>
<td>5</td>
</tr>
<tr>
<td>14495#</td>
<td>Algeria</td>
<td>35°37' N, 0°31' W</td>
<td>20.7</td>
<td>2.38</td>
<td>13.1</td>
<td>4</td>
</tr>
<tr>
<td>14279#</td>
<td>Greece</td>
<td>38°54' N, 22°26' E</td>
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<td>3.20</td>
<td>16.9</td>
<td>*</td>
</tr>
<tr>
<td>19350</td>
<td>Greece</td>
<td>39°38' N, 22°25' E</td>
<td>20.8</td>
<td>3.38</td>
<td>15.7</td>
<td>*</td>
</tr>
<tr>
<td>19357</td>
<td>Italy</td>
<td>41°54' N, 12°29' E</td>
<td>20.8</td>
<td>3.21</td>
<td>19.9</td>
<td>3</td>
</tr>
<tr>
<td>14693</td>
<td>Morocco</td>
<td>30°05' N, 9°09' W</td>
<td>20.9</td>
<td>3.45</td>
<td>12.9</td>
<td>4</td>
</tr>
<tr>
<td>19351</td>
<td>Greece</td>
<td>39°42' N, 21°38' E</td>
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<td>3.15</td>
<td>14.1</td>
<td>1</td>
</tr>
<tr>
<td>14072</td>
<td>Italy</td>
<td>41°15' N, 15°20' E</td>
<td>21.0</td>
<td>2.24</td>
<td>13.1</td>
<td>3</td>
</tr>
<tr>
<td>19289</td>
<td>Algeria</td>
<td>36°08' N, 2°55' E</td>
<td>21.2</td>
<td>3.14</td>
<td>14.9</td>
<td>*</td>
</tr>
<tr>
<td>14419#</td>
<td>Portugal</td>
<td>39°42' N, 8°35' W</td>
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<td>2.83</td>
<td>16.6</td>
<td>3</td>
</tr>
<tr>
<td>19275</td>
<td>Algeria</td>
<td>36°15' N, 2°10' E</td>
<td>21.3</td>
<td>3.03</td>
<td>11.5</td>
<td>4</td>
</tr>
<tr>
<td>Australian</td>
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<td>--</td>
<td>21.4</td>
<td>2.60</td>
<td>17.5</td>
<td>n/a</td>
</tr>
<tr>
<td>15220</td>
<td>Spain</td>
<td>38°53' N, 6°58' W</td>
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<td>2.70</td>
<td>15.9</td>
<td>4</td>
</tr>
<tr>
<td>14696</td>
<td>Morocco</td>
<td>33°26' N, 6°01' W</td>
<td>21.4</td>
<td>2.65</td>
<td>13.9</td>
<td>4</td>
</tr>
<tr>
<td>19268</td>
<td>Libya</td>
<td>32°30' N, 20°50' E</td>
<td>21.4</td>
<td>3.07</td>
<td>22.3</td>
<td>5</td>
</tr>
<tr>
<td>19299#</td>
<td>Algeria</td>
<td>36°44' N, 7°06' E</td>
<td>21.4</td>
<td>2.34</td>
<td>15.1</td>
<td>4</td>
</tr>
<tr>
<td>19264#</td>
<td>Israel</td>
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<td>3.50</td>
<td>17.1</td>
<td>7</td>
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<tr>
<td>Sirolan</td>
<td>--</td>
<td>--</td>
<td>21.5</td>
<td>4.59</td>
<td>15.9</td>
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<tr>
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<td>2.30</td>
<td>18.8</td>
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</tr>
<tr>
<td>14496*</td>
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<td>36°16' N, 2°45' E</td>
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<td>3.00</td>
<td>18.1</td>
<td>*</td>
</tr>
<tr>
<td>19280#</td>
<td>Algeria</td>
<td>35°04' N, 1°02' E</td>
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<td>2.45</td>
<td>21.1</td>
<td>5</td>
</tr>
<tr>
<td>14498</td>
<td>Algeria</td>
<td>34°17' N, 2°59' E</td>
<td>21.6</td>
<td>2.72</td>
<td>17.8</td>
<td>6</td>
</tr>
<tr>
<td>19305#</td>
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<td>&quot;Yesilkoy&quot;</td>
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<tr>
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<td>2.49</td>
<td>21.5</td>
<td>5</td>
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<tr>
<td>19315#</td>
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<td>30°50' N, 8°20' W</td>
<td>21.9</td>
<td>3.27</td>
<td>14.9</td>
<td>5</td>
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</tbody>
</table>

LSD (5%) 0.7 0.54 4.5
Experiments three and four  Growth analysis was carried out on 15 of the *T. subterraneum* ecotypes and 11 of the *P. aquatica* ecotypes (Tables 6.3 and 6.4). There was no significant block effect in either experiment. For both species there was significant variation in \( RGR \), ranging from 109-159 g.kg\(^{-1}\).d\(^{-1}\) amongst the *T. subterraneum* ecotypes and from 178-239 g.kg\(^{-1}\).d\(^{-1}\) amongst the *P. aquatica* ecotypes. Hence, all of the *P. aquatica* ecotypes grew faster than the *T. subterraneum* ecotypes (not only as presented in Tables 3 and 4 but also when analysed at a common biomass across species, data not presented). For *T. subterraneum* the variation in \( RGR \) was dependent on variation in \( NAR \) and not \( LAR \) (Fig. 6.1). For *P. aquatica* the opposite was the case; \( RGR \) being positively associated with \( LAR \) (Fig. 6.2b) and \( SLA \) (Fig. 6.2d) and not at all with \( NAR \) or \( LWR \) (Figs 6.2a and 6.2c).

![Figure 6.1](image)

Figure 6.1 Plots of \( RGR \) as a function of a) \( NAR \) and b) \( LAR \) amongst 15 ecotypes of *Trifolium subterraneum*. Growth parameters were analysed at a common biomass (75 mg). The line in (a) represents a significant linear regression.
Figure 6.2 Plots of $RGR$ as a function of a) $LAR$, b) $NAR$, c) $LWR$ and d) $SLA$ amongst 11 ecotypes of *Phalaris aquatica*. Growth parameters were analysed at a common biomass (107 mg). The lines represent significant linear regressions.
Table 6.3 Growth analysis on 15 *T. subterraneum* ecotypes. Means (± one standard deviation) for relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) when analysed at a common biomass. Carbon isotope discrimination was measured on leaves of plants from the final harvest.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RGR (g.kg⁻¹.d⁻¹)</th>
<th>NAR (g.m⁻².d⁻¹)</th>
<th>LAR (m².kg⁻¹)</th>
<th>SLA (m².kg⁻¹)</th>
<th>LWR</th>
<th>Δ (%/oo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>84450d</td>
<td>109±5.0</td>
<td>6.91±0.33</td>
<td>15.8±0.2</td>
<td>35.7±0.1</td>
<td>0.44±0.01</td>
<td>23.7</td>
</tr>
<tr>
<td>103880</td>
<td>110±4.6</td>
<td>7.49±0.33</td>
<td>14.7±0.2</td>
<td>34.8±0.2</td>
<td>0.42±0.01</td>
<td>23.7</td>
</tr>
<tr>
<td>103922a</td>
<td>119±5.7</td>
<td>8.47±0.44</td>
<td>14.1±0.3</td>
<td>31.8±0.3</td>
<td>0.44±0.01</td>
<td>23.4</td>
</tr>
<tr>
<td>103940a</td>
<td>125±7.5</td>
<td>8.80±0.63</td>
<td>14.2±0.5</td>
<td>30.3±2.8</td>
<td>0.47±0.03</td>
<td>23.2</td>
</tr>
<tr>
<td>GF39-2</td>
<td>133±5.1</td>
<td>10.71±0.46</td>
<td>12.4±0.2</td>
<td>29.2±0.4</td>
<td>0.43±0.01</td>
<td>23.4</td>
</tr>
<tr>
<td>103942b</td>
<td>134±7.6</td>
<td>8.15±0.58</td>
<td>16.4±0.7</td>
<td>38.1±1.1</td>
<td>0.43±0.01</td>
<td>23.4</td>
</tr>
<tr>
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<td>8.34±0.50</td>
<td>16.1±0.5</td>
<td>37.6±1.8</td>
<td>0.43±0.01</td>
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<td>9.41±0.39</td>
<td>14.8±0.3</td>
<td>34.1±0.2</td>
<td>0.44±0.01</td>
<td>22.8</td>
</tr>
<tr>
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<td>10.09±0.56</td>
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<td>0.47±0.07</td>
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<tr>
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<td>14.9±0.3</td>
<td>35.1±0.9</td>
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</tr>
<tr>
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<td>15.6±0.9</td>
<td>34.4±0.6</td>
<td>0.45±0.02</td>
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</tr>
<tr>
<td>84003 c</td>
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<td>12.4±0.3</td>
<td>31.2±0.2</td>
<td>0.40±0.01</td>
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<tr>
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<td>30.9±0.4</td>
<td>0.45±0.01</td>
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</tr>
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<td>31.1±4.4</td>
<td>0.43±0.05</td>
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</tbody>
</table>

Table 6.4 Growth analysis on 15 *Phalaris aquatica* ecotypes. Means (± one standard deviation) for relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) when analysed at a common biomass. Carbon isotope discrimination was measured on leaves of plants from the final harvest.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RGR (g.kg⁻¹.d⁻¹)</th>
<th>NAR (g.m⁻².d⁻¹)</th>
<th>LAR (m².kg⁻¹)</th>
<th>SLA (m².kg⁻¹)</th>
<th>LWR</th>
<th>Δ (%/oo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14419</td>
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<td>37.1±0.8</td>
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<td>22.2</td>
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<tr>
<td>14495</td>
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<td>39.1±0.6</td>
<td>0.56±0.02</td>
<td>22.0</td>
</tr>
<tr>
<td>19315</td>
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<td>10.66±0.71</td>
<td>19.3±0.8</td>
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<td>0.50±0.02</td>
<td>22.3</td>
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<tr>
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<td>19.0±0.6</td>
<td>37.1±0.5</td>
<td>0.51±0.02</td>
<td>21.9</td>
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<tr>
<td>14279</td>
<td>213±5</td>
<td>8.83±0.24</td>
<td>24.1±0.1</td>
<td>44.6±0.7</td>
<td>0.54±0.01</td>
<td>22.0</td>
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<tr>
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<td>43.4±0.1</td>
<td>0.52±0.04</td>
<td>22.1</td>
</tr>
<tr>
<td>15022</td>
<td>218±6</td>
<td>10.55±0.43</td>
<td>20.7±0.5</td>
<td>40.5±0.4</td>
<td>0.51±0.02</td>
<td>21.8</td>
</tr>
<tr>
<td>19299</td>
<td>220±6</td>
<td>10.68±0.41</td>
<td>20.6±0.3</td>
<td>39.9±0.2</td>
<td>0.52±0.01</td>
<td>22.8</td>
</tr>
<tr>
<td>14496</td>
<td>222±8</td>
<td>10.17±0.53</td>
<td>21.9±0.6</td>
<td>41.0±0.6</td>
<td>0.53±0.02</td>
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<td>19305</td>
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<td>9.29±0.43</td>
<td>25.7±0.6</td>
<td>48.2±0.3</td>
<td>0.53±0.02</td>
<td>22.0</td>
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</tbody>
</table>
Carbon isotope discrimination was also measured on the leaves of plants from the final harvest of the growth analysis. Amongst *T. subterraneum* ecotypes, RGR and $\Delta$ (measured on leaves of plants from the final harvest) were negatively correlated (Fig. 6.3) as were total biomass and $\Delta$ ($r = -0.67, p < 0.01$) and NAR ($r = -0.70, p < 0.01$). For *P. aquatica*, $\Delta$ was not related to RGR or any of its components or to total biomass.

The stability of ecotype ranking for $\Delta$ and productivity (in terms of either shoot biomass or total biomass) was tested by correlating between experiments. For *T. subterraneum* ecotypes common to experiments one and three, both $\Delta$ and biomass were positively related between experiments ($r = 0.54, p < 0.05$ and $r = 0.84, p < 0.001$, respectively). In contrast, ecotypes of *P. aquatica* common to experiments two and four showed no consistency in either $\Delta$ or biomass accumulation.

Figure 6.3 Plot of the relationship between RGR versus carbon isotope discrimination ($\Delta$) amongst 15 *Trifolium subterraneum* ecotypes. The line represents a linear regression.
6.4 DISCUSSION

In all the C₃ species that have been screened so far, variation in Δ (and hence, W) has been found (e.g. Hubick et al. 1986; Hall et al. 1990; Ehleringer et al. 1990; Craufurd et al. 1991). The experiments presented here have also shown that there is significant ecotypic variation in Δ within both T. subterraneum and P. aquatica. In contrast, when a limited set of Australian cultivars of these species was screened (Chapter 5) there was little variation compared to sunflower (e.g. Chapters 2-4).

There were ecotypes of P. aquatica found with Δ higher and lower than two of the currently recommended cultivars with which they were compared (Table 6.2). This indicates that it may be possible to select or breed cultivars that use water more efficiently than those presently available. The absence of any correlation (positive or negative) between Δ and either shoot biomass (experiment 2), RGR or total biomass (experiment 4) indicates that increased W can be achieved without any penalty in productivity, at least during the vegetative stage. There was a positive relationship between final tiller number and Δ in experiment two which may have implications for selection of more water-use efficient cultivars. However, as there was no relationship between biomass production and Δ this may be of less importance.

In general the T. subterraneum results lead to similar conclusions. Unfortunately no Australian cultivars were included in the comparison and although there was a large range in Δ (20.3 - 22.9%/w), the relevance to current recommended cultivars is limited. If we assume that the results from both experiments reported in chapter 5 can be directly compared with the present set of results then the four cultivars that were screened in chapter 5 (average Δ of 21.1), would rank in the upper quarter of the collected ecotypes. The conditions and time of year of both sets of experiments (chapter 5 and here) are not so different as to rule out such a comparison. Therefore, scope exists for breeding and selecting more water-use efficient T. subterraneum cultivars.
Across the broad spectrum of germplasm screened (experiment one) there was no relationship between $\Delta$ and biomass production, which means that there would be no penalty in terms of biomass by selecting for greater $\Delta$. There are, however, some reservations about this conclusion.

*Trifolium subterraneum* has been divided into three taxonomically distinct varieties (var., previously sub-species). Most of the ecotypes screened were of the var. *subterraneum* with fewer from var. *brachycalyclinum* and only two from var. *yanninicum*. Across all varieties there was no relationship between shoot biomass and $\Delta$. Only when vars *brachycalyclinum* and *yanninicum* were considered did a negative relationship appear. This is relevant because breeding and selection of cultivars occurs distinctly within these three groups. In fact, var. *brachycalyclinum* does not interbreed with the two other varieties.

The stability of $\Delta$ and productivity is also extremely important. In experiments one and two plants were grown in small communities (4 to a pot) and were harvested after ca. 46-49 days. In contrast, in experiments three and four plants were grown as individuals until only 30 days. The 15 *T. subterraneum* ecotypes common to both experiments (one and three) showed reasonable agreement for $\Delta$ and close agreement for biomass (shoot versus whole plant, comparing experiments one and three, respectively) between the two experiments. Many other studies have shown the robust nature of $\Delta$ across both experimental conditions and sites (e.g. Ehleringer 1990; Condon and Richards 1991). Amongst the 15 *T. subterraneum* ecotypes, the relationship between $\Delta$ and biomass production was not consistent between experiments, i.e., in the first there was no relationship (amongst the same 15 ecotypes) whereas in the third $\Delta$ and biomass production were negatively related. It must be remembered that in experiment one plants were grown in small communities whereas in experiment three only single plants were grown. Comparisons of
results from these two experiments may be complicated by the effects of competition especially amongst the faster growing ecotypes.

For the *P. aquatica* ecotypes, neither biomass nor $\Delta$ were related across experiments two and four. It should be noted that pots were well watered throughout the experiment and therefore it is unlikely that any difference in $\Delta$ or biomass arose because of drought-stress. Other factors such as pot size, competition and ontogenetic drift, may explain the lack of consistency between the results. While it is not possible to locate the reason(s) for the lack of consistency in ranking for $\Delta$ between experiments, it is interesting to note that under field conditions there is a high broad-sense heritability for $\Delta$ amongst a range of species (e.g. wheat, Ehdaie *et al.* 1991; *Agropyron desertorum*, Read *et al.* 1991a). Perhaps the absence of stability across environments is a function of factors peculiar to pot experiments. Furthermore, the range in $\Delta$ amongst the 11 *P. aquatica* ecotypes common to both experiments was comparatively low, i.e. 1.3% in experiment two and 1.0% in experiment four and this too may also account for a lack of consistency between the experiments. Another possible explanation is that variation in $\Delta$ was mainly due to $g$ as there was no relationship between SLA (as a crude inverse measure of photosynthetic capacity) and $\Delta$. It may be that genotypic variation in $g$ may not be as stable as that in photosynthetic capacity. Notwithstanding this, the objective of finding genetic variation in $\Delta$ within both *P. aquatica* and *T. subterraneum* has been demonstrated, and it is likely that this variation could be exploited to increase the transpiration efficiency ($W$) of the species. However the extent to which $W$ is a desirable trait will depend on its relationship with other important agronomic characters.

A further aim of this work has been to identify factors which influence the relative growth rate of the species. Previously, working with sunflowers, this aim arose in order to explain the correlation of plant biomass with $\Delta$ (Chapters 2 and 3). For the present experiments, the aim was to test the hypothesis that
the characteristics which lead to greater \textit{RGR} depend on the species or set of species being examined. The results of the growth analysis show that in one species, \textit{T. subterraneum}, variation in \textit{NAR} caused variation in \textit{RGR} (Fig. 6.1) whereas in the other species, \textit{P. aquatica}, variation in \textit{RGR} was caused by variation in \textit{LAR} (Fig. 6.2b). For both species these relationships can be further analysed to reveal more explicitly those characteristics which were important in conferring greater \textit{RGR}.

For the \textit{T. subterraneum} ecotypes, the rate at which biomass was produced per unit leaf area (\textit{NAR}) was the underlying cause of variation in \textit{RGR}. In physiological terms, \textit{NAR} is the difference between plant photosynthesis and respiration (Evans 1972). Amongst sunflower ecotypes, \textit{NAR} was closely related to the leaf CO\textsubscript{2} assimilation rate, whereas within \textit{Lolium perenne} it (or at least growth rate) has been more closely related to the respiration rate (Robson 1982a). No measurements of either respiration or photosynthesis were made in this study. However, there was a negative relationship between \textit{NAR} and \textit{Δ} indicating that the ecotypes with the lowest \textit{Δ} and hence \textit{p/p\textsubscript{a}} had the greatest \textit{NAR}. It is likely that this result would only arise when variation in photosynthetic rate caused variation in both \textit{p/p\textsubscript{a}} and \textit{NAR}. In short, these results are very similar to those of sunflower (Chapters 2-4) although no direct measurements of photosynthetic rate were made.

The ability to produce leaf area per unit biomass (\textit{LAR}) was the factor that caused differences in \textit{RGR} amongst the \textit{P. aquatica} ecotypes. When analysed further, the specific leaf area (\textit{SLA}) was more closely related to both \textit{RGR} and \textit{LAR} rather than to \textit{LWR}. This is in agreement with the findings of other intra-specific studies (e.g. Dijkstra and Lambers 1989). The spread in \textit{SLA} may be indicative of the "dilution effect" where plants which spread nitrogen over a larger leaf area grow faster than those which do not (see Sinclair and Horie 1989; Evans 1990). As further evidence of the fundamental differences between species, there was no relationship between \textit{Δ} and \textit{SLA}. 
This contrasts with other species such as peanut (Wright et al. 1988) and sunflower (Chapter 2) where positive relationships between SLA and $\Delta$ have been demonstrated and may indicate that the variation in $\Delta$ could partially be accounted for by variation in stomatal conductance to water vapour ($g$). This would be in contrast to sunflower but similar to common bean (Ehleringer 1990).

Why then, in one species is $NAR$ more important and in another $LAR$? In both experiments plants were grown under almost identical conditions, with the $P. aquatica$ experiment (four) commencing eight days before the $T. subterraneum$ experiment (three) in the same glasshouse. Hence, these two very different results are unlikely to be artefacts of any of the environmental conditions placed upon the plants, e.g., pot size, watering, fertilising etc. There are many differences between the species, one being a monocot the other a dicot; one being an annual ($T. subterraneum$) the other a perennial; one being a legume ($T. subterraneum$) the other a grass. However, the dependence of RGR on either $LAR$ or $NAR$ has never been separated among any of these classifications (Poorter 1989b) and was not in evidence amongst the range of grasses and legumes examined in Chapter 5. A recent analysis by Garnier (1991), using data from several sources, showed that for the same maximum RGR, monocots tended to have a higher root to leaf mass ratio and higher $NAR$ (on a mass basis) than dicots. However, his analysis drew upon a range of studies conducted under different conditions and did not address the important issue of partitioning to leaf area. As Garnier (1991) rightly pointed out there are not enough data available to enable any conclusion to be reached regarding broad taxonomic differences in the relationship of RGR to its components.

Poorter (1989b) suggested that the components of RGR and not RGR itself have been the targets of natural selection (see also Grime 1979; Lambers and Dijkstra 1987). He went on to describe the situations in which high and low $LAR$ species may have evolved in an effort to explain his and other similar
results (e.g. Dijkstra and Lambers 1989). The ecotypes of both species examined in the present experiments were from a range of environments from high (1700 mm) to low rainfall (200 mm) from a range of latitudes and longitudes (Tables 6.1 and 6.2), (little information is available regarding soil type or condition). Because of this, it would probably be incorrect to assume that *P. aquatica* ecotypes have evolved in more competitive habitats (like those of the high *LAR* species of Poorter and Remkes 1990) than the low *LAR/high NAR* ecotypes of *T. subterraneum*. In further considering this problem it may be more useful to question why amongst the *T. subterraneum* ecotypes variation in *LAR* was so limited and, likewise, why amongst the *P. aquatica* ecotypes variation in *NAR* was so limited? Hence, while the components of *RGR* may be the targets for selection (Poorter 1989b) it may also be important to focus on why there is limited genetic variation in these components in some species.

It is also possible that the variation available in one characteristic is greater because of the interaction between the plant type and environment. For example, it is likely that natural selection amongst these two pasture species has taken place under conditions of various levels of herbivory. Hence the ability to recover from grazing events through maintaining higher *NAR* may have been important in *T. subterraneum*, an annual with limited potential for carbohydrate storage. In contrast, the selection pressure on a perennial plant, presumably able to remobilise reserves, like *P. aquatica*, under various levels of herbivory might have favoured variation in leaf area production and hence *LAR*. There is a general tendency for perennials to store carbohydrate whereas annuals in many cases do not (Bloom *et al.* 1985).

The characteristics which influence *RGR* and hence some degree of ecological fitness (Grime and Hunt 1975; Poorter and Remkes 1990) differ between species. I am aware of no other studies which find that the influence of these characteristics differ so markedly between species under near-identical conditions. From an agronomic perspective these results may also be important
in that for *T. subterraneum* during early growth there is a negative relationship between $RGR$ and $\Delta$. Hence selection for greater water-use efficiency may *de facto* be selection for increased growth. These results are novel and further challenge the prevailing view that fast growth is achieved through increased leaf area or $LAR$. On the other hand, it should be noted that when the two species were directly compared (i.e., at the same biomass and averaged across ecotypes), the greater $RGR$ of *P. aquatica* was caused by greater $LAR$ with $NAR$ being approximately equal.

There is one other area of potential importance for some of these results. As mentioned in the *Introduction*, one reason for choosing *P. aquatica* for this study is that some of the lines available and the environments from which they were collected had previously been characterised by Cooper and McWilliam (1966). They found that the cold requirement for floral induction was related to the temperature during winter of the site of origin for a range of *P. aquatica* ecotypes from the Mediterranean region. Likewise the time taken to flowering in a common environment was closely related to the average length of summer drought in the place of origin. Therefore some of these ecotypes had shown a degree of adaptation in terms of reproductive characteristics to their environments.

As previously explained (chapter one) $\Delta$ can be used as an integrated measure of plant transpiration efficiency (e.g. Farquhar *et al.* 1989). Read and Farquhar (1991) compared $\Delta$ of a range of *Nothophagus* spp. collected from differing climates and then grown in a common environment in order to examine the adaptation of $W$ to climate. They found that $\Delta$ was negatively related to a range of climatic parameters from their sites of origin including mean annual rainfall, mean annual temperature, and rainfall from December to March. In short the species from the hotter and drier environments tended to have the highest $\Delta$ (lowest $W$) when grown in the common environment. In somewhat of a contrast, using ecotypes of the desert shrub *Hymenoclea*
salsola, Comstock and Ehleringer (1992) found that $\Delta$ was lowest in those environments where leaf-to-air vapour-pressure-deficit was highest regardless of rainfall. There is no real conflict here as the species are from widely different habitats and, in terms of $\Delta$ ($W$) may have been subjected to very different selection pressures.

For the *P. aquatica* ecotypes, weather data were available for most of the sites and included mean monthly rainfall, maximum and minimum temperature and saturation deficit. Sites were classified according to the length of the summer drought (in months) that characterises Mediterranean environments (Table 6.2). A month of drought defined as:

$$P / Sd^{0.75} < 9$$

where $P$ is precipitation (in mm) and $Sd$ is saturation deficit (in mm Hg) (Prescott 1958; Cooper and McWilliam 1966). Attempts were made to correlate either $\Delta$ and above-ground biomass (from experiment two) with a range of climatic parameters including, length of drought (in months), seasonal rainfall, total rainfall and average temperature. There was no relationship between above-ground biomass production and any of the climatic parameters. Carbon isotope discrimination was negatively related to autumn rainfall ($r = -0.42$ $p < 0.05$) but not rainfall in any other season or total rainfall. The length of drought as defined by Prescott (1958) was positively related to $\Delta$ but this was insignificant (Fig 6.4a). Only when $\Delta$ was averaged within each drought class group i.e. $\Delta$ averaged across ecotypes from sites with summer droughts of 3, 4 or 5 months duration was there a significant (positive) relationship between $\Delta$ and drought duration (Fig. 6.4b). This result may be an artefact of the absence of any replication at drought duration's of 1, 6 and 7 months. Certainly future studies should include more ecotypes from climatic extremes.
If we assume that the analysis is valid and represents an adaptation in terms of $W$ to climate, then the result is similar to that found by Read and Farquhar (1991), i.e., plants with genetically higher $\Delta$ were from the most arid environments. In other words, those plants from environments with the shortest growth period are less water-use efficient. Because there was no correlation between growth and $\Delta$ (in either experiments 2 or 4) it is not possible to link the need to grow fast (i.e. to avoid drought) with greater $\Delta$ in the more drought-prone environments. Likewise, Cooper and McWilliam (1966) found that relative growth rate was not linked to the aridity of the environments. It is also worthwhile noting that these type of studies have been done in one environment only (Read and Farquhar 1991; Comstock and Ehleringer 1992) and this too may affect the results. Although $\Delta$ may be a powerful tool in furthering the understanding of plant adaptation (Osmond 1987; Mooney 1991).
much more information of the ecophysiological significance of water-use efficiency will be needed before the nature of such adaptations can be appreciated and perhaps, exploited. However, if mechanistic links between $W$ and growth occur either within or between species then growth itself (e.g. leaf area production) may override $W$ as an adaptation to length of season or drought.

In conclusion, significant variation in $\Delta$ has been found in natural populations of $P.\ aquatica$ and $T.\ subterraneum$. The source of this variation and its effects on plants growth amongst $T.\ subterraneum$ is similar to that of sunflower in that $\Delta$ was negatively related to $RGR$ and $NAR$. In contrast there were no relationships between $\Delta$ and any of the growth characteristics of the $P.\ aquatica$ ecotypes. Although grown under similar conditions, the source of variation in $RGR$ was $NAR$ amongst the $T.\ subterraneum$ ecotypes and $LAR$ amongst the $P.\ aquatica$ ecotypes. There was a tendency for the $P.\ aquatica$ ecotypes from the more arid environments to have the highest $\Delta$. 

The initial studies focused on sunflower. In common with many studies which preceded it, a relationship for the source of variation of $RGR$ between sunflower ecotypes was found as $\Delta$ was negatively related to $RGR$ and $NAR$. An examination of the nature of the relationship showed that the variation in $\Delta$ could occur between ecotypes and within ecotypes. The nature of the relationship between $\Delta$ and $RGR$ was positive and consistent. This was in contrast to the examination under different light conditions between individuals of sunflower. The nature of the relationship between $\Delta$ and $RGR$ was also consistent with that found in other studies.
CHAPTER SEVEN

GENERAL DISCUSSION

The aim of the work presented in the preceding chapters was to provide an understanding of physiological links between theoretical and experimental leaf-level physiology and empirical relationships between $\Delta$ and growth at the crop level. The underlying question, in one sense, was whether or not $W$ is a useful physiological trait in plants. Many studies have found that, using $\Delta$ as a screening technique, $W$ has some potential for use as a selection criterion. However, the major problem has been that $\Delta$ (and more so $W$) are complex characteristics that are often associated either positively or negatively with growth. This study was initiated to examine relationships between growth, its determinants and $\Delta$. In doing so, a simplified system of examining such relationships during the vegetative stage of growth was used. This avoided some of the complications which may arise due to variation in phenology in longer term field studies.

The initial studies focused on sunflower. In common with the many studies which preceded it, a relationship (in this case negative) between growth and $W$ was found (e.g. Keller 1953; Hunt 1962). An investigation of the causes of this relationship showed that the variation in $\Delta$ (and hence, $W$) between these species was largely due to photosynthetic capacity ($A_{max}$). Hence, sunflower genotypes with the greater $A_{max}$ had lower $\Delta$. In turn the variation in $A_{max}$ was positively related to $RGR$. This was also reflected in the close relationship (even under different light conditions) between $RGR$ and $NAR$. The early finding that $\phi$ was positively related to $W$ was shown to be an artefact of ontogeny. The outstanding characteristic of these results is that they do not conform to what is considered 'normal' experience. 'Normally' when the relationship between growth and photosynthetic rate is examined, no (or a negative) relationship is apparent (e.g. Cook and Evans 1983; exhaustive lists can be found in Elmore 1980; Eagles and Wilson 1982).
For the sunflower genotypes examined, variation in the apparent nitrogen use efficiency (NUE, ratio of $A_{\text{max}}$ to $N_a$) was suggested as the main reason for the absence of an overriding trade-off between $LAR$ and $NAR$. This occurred because there was a positive intercept in the $A_{\text{max}}$ versus $N_a$ relationship. By incorporating this feature into a simulation, the nature of the negative relationship between $NAR$ and $LAR$ (a general feature, see Lambers and Poorter 1992), could be explained. This was a simple mathematical consequence of the conditions used for the simulation (in Chapter 4), i.e., if NUE increased with $A_{\text{max}}$, then the trade-off between $NAR$ and $LAR$ does not become so great and can result in a positive $RGR-NAR$ relationship. Just what the basis of variation in NUE is remains unknown and would be a fruitful area for further research.

The positive correlation between growth and $A$ suggests that sunflower differs from most crops (but see Mahon and Hobbs 1987). However, some qualifications are necessary as the sunflower studies have not been extended either to field performance or seed production. It is also unknown whether there is any link between drought resistance and $W$. Hence it is not possible to assume that high $W$ sunflower cultivars will be superior under field and/or drought conditions.

One important conclusion to be reached from this work is that no generalisations can be reached concerning the relationship between growth and $W$ (or $\Delta$). The $T. \text{subterraneum-P. aquatica}$ comparison in Chapter 6 is a good illustration of this point. The causes of differences in $RGR$ within the two species were very different. For $T. \text{subterraneum}$ a pattern of relationships similar to sunflower emerged. This meant that low $\Delta$ (high $W$) ecotypes grew faster and had greater $RGR$ due, in turn, to greater $NAR$. The simplest explanation for both $T. \text{subterraneum}$ and sunflower is that the variation in photosynthetic capacity was the basis for variation in $RGR$ and $p_r/p_a$. In contrast, there were no such relationships amongst 11 $P. \text{aquatica}$
ecotypes in which differences in RGR were due to LAR (and SLA). The real basis for variation in \( \Delta \) amongst the *P. aquatica* ecotypes was not examined. However, the absence of any relationship between \( \Delta \) and either NAR or SLA indicates that variation in \( g \) may be a major cause of that in \( \Delta \). Agronomically, selection for low \( \Delta \) amongst the *T. subterraneum* ecotypes would produce faster growing cultivars and, in contrast, amongst the *P. aquatica* ecotypes would appear to produce no differences in growth.

It appears, simply, that species are different. A comparison of the sunflower studies (Chapters 2-4) with those on common bean (Ehleringer 1990; Ehleringer *et al.* 1990; White *et al.* 1990; Ehleringer *et al.* 1991) also clearly shows this. In sunflower \( \Delta \) was closely related to \( A_{\text{max}} \) and positively related to either biomass production or RGR. In contrast, amongst common bean genotypes, \( \Delta \) was closely related to \( g \) (Ehleringer 1990) and not at all to biomass under irrigated conditions (White *et al.* 1990). The statement by Ehleringer *et al.* (1990) which proposed that "because of differences in the length of life cycle or differences in phenological patterns among plants there may be no unique relationship between dry matter production and transpiration efficiency" is undoubtedly true. Moreover, based on the results presented in the previous chapters and of other studies (e.g. Condon *et al.* 1987), the differences in the fundamental causes of variation in \( \Delta \), either through variation in photosynthetic or stomatal characteristics, can also explain the absence of a unique relationship between \( \Delta \) and biomass production.

These results, showing fundamental links between \( \Delta \) and growth, have far-reaching consequences. Studies on \( \Delta \) have not been confined to agronomic usefulness of \( \Delta \) as a physiological trait (Chapter 1) but have also included ecological studies (e.g. Ehleringer *et al.* 1986; 1988; Korner *et al.* 1988; 1991; Toft *et al.* 1989; Read and Farquhar 1991; Comstock and Ehleringer 1992). Hence, the existence of very different \( \Delta \)-growth
relationships within species and, perhaps, even between certain sets of species, may limit the ability to interpret the importance of differences in $\Delta$ amongst or between species under field conditions. This should be kept in mind when the scope for $\Delta$ in eco-physiological studies is being promoted (e.g. Osmond 1987; Mooney 1991).

Based on the variation in relationships between $\Delta$ and growth, a protocol for examining the use of $\Delta$ as a selection criterion can be proposed. As in Chapter one all the criteria for the successful adoption of a physiological trait apply. In addition, when dealing with a new species, future studies should concentrate on establishing whether or not there is a relationship between growth and $\Delta$ under well-watered conditions. This will avoid the almost inevitable positive relationship between $\Delta$ and biomass accumulation which results from genotypes being screened over a range of irrigation treatments (e.g. Johnson et al. 1990). Such studies should establish, across a broad range of genotypes, the source(s) of variation in both $\Delta$ and growth (probably $RGR$) and how they interrelate and should precede field testing across variable conditions. This would allow a more educated interpretation of apparent relationships between growth and $\Delta$ at the field screening stage.

As an attempt to determine what caused the relationships between $\Delta$ and growth, a number of growth analyses were carried out on plants during early vegetative growth (Chapters 2-6). The results of these studies have often been compared to those of Poorter (Poorter and Remkes 1990; Poorter et al. 1990) who produced a thorough analysis of the causes of variation in $RGR$ between a wide range of herbaceous species. The result depended on the comparison being made. At the (herbaceous) inter-species level variation in $LAR$ was most important in determining differences in $RGR$ (Chapter 5; Poorter and Remkes 1990) whereas within species either $NAR$ (Sunflower and T. subterraneum, Chapters 3,4,6) or $LAR$ (P. aquatica,
Chapter 6) was most important. Similarly, other studies have found that either between or within species, $A$ could not be related to growth (e.g. Fischer et al. 1981; Poorter et al. 1990) whereas, in contrast, between either diverse groups of plants (Shulze and Chapin 1987) or within species (Chapter 3), positive relationships have been found. Therefore, just as with $\Delta$, the result very much depends on the comparison being made.

Furthermore, given that there is, within species, variation in the causes of differences in growth rate, an area for future productive research would be to examine why this is so. Such studies would need to span fields of plant physiology, genetics and ecology to properly grasp the basic differences in genetic variation in these characteristics within species. Only such a study could mould some order out of what admittedly appear to be scattered results (including this thesis and other studies). One possible avenue may be to analyse the variation in growth and $\Delta$ found within species across different habitats (e.g. Chapter 6; Read and Farquhar 1991; Comstock and Ehleringer 1992). Such studies are only in their infancy but if combined with analyses of the physiological basis for variation in $\Delta$ and $RGR$, could provide a powerful tool for explaining differences in growth-$\Delta$ relationships between species.

**Conclusion** The initial aim of this work was to understand the relationship between growth and $\Delta$. At least for sunflower, the finding that variation in $A_{\text{max}}$ could explain variation in both $RGR$ and $\Delta$ provided a satisfactory explanation for the negative relationship between biomass and $\Delta$. However, this is only one species and it was quite apparent when considering other studies (e.g. Read et al. 1991a) that variation in $g$ can provide a very different scenario. Within the gene pool of each species there must be limited variation in some of the components of $\Delta$ and/or $RGR$ (or other factors which determine differences in biomass). This leads to the dominance of other component characteristics in determining differences in $\Delta$ and biomass, and hence, various relationships between the two. The final word is that there is no final word. Each species has to be considered.
separately and not as a basis for sweeping generalisations. When this is accepted then the important work of examining why the gene pools so differ can commence.
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