A STUDY OF GROWTH, DEVELOPMENT AND N-FIXATION OF SEVERAL WHITE CLOVER (*Trifolium repens* L.) CULTIVARS UNDER DIFFERENT WATER DEFICIT AND PHOSPHORUS LEVELS

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ABSTRACT

White clover (*Trifolium repens* L.) is one of the most important pasture plants in New Zealand. It contributes nitrogen, high quality forage and seasonal spread of production. However, it has high requirements for phosphate and does not persist well under moisture stress conditions. In this study the effects of water deficit level and phosphorus (P) level on the growth, development and N-fixation of different white clover cultivars have been studied. Several New Zealand and overseas white clover cultivars of contrasting morphological characteristics were selected for this study. These cultivars were Kopu, Pitau, Dusi, Haifa, Huia, Tahora and Whatawhata.

The main objectives of this study were to determine the effects of water stress and phosphorus levels on growth, development, and N-fixation of these cultivars and to identify the plant characteristics most effective in distinguishing the cultivars used in the study. The research approach was to measure the responses of these cultivars to water deficit and P fertility treatments; and the responses were then related to selected plant characteristics, particularly morphological characteristics. Attempt was also made to distinguish the cultivars using multivariate analysis techniques.

The results of this study showed that the cultivars had responded differently to water deficit treatment and P treatments. The retardation they suffered in their growth and development, expressed by both dry weight (DW) and growth rate of stolon components (leaf, petiole and branch), was significantly different and, in many cases, could be related back to their morphology and development characteristics. Cultivars of small stature, such as Whatawhata and Tahora, were generally less affected by the stress treatments. Cultivars grown well under control conditions, such as Kopu and Haifa, suffered more severely under stress conditions. For all cultivars, parameters associated with leaf area were more sensitive to water deficit stress than those associated with growing points.

The study of plant water status of these cultivars under progressively decreasing water availability failed to identify any significant difference between the cultivars in their ability to avoid dehydration at medium to high water deficit stress levels. So it could be suggested that the difference between the cultivars represented their ability to tolerate water stress and was unlikely to be associated with their dehydration avoidance ability but

more likely to be associated with morphological characteristics such as deep root and reducing leaf area when water deficit stress occurred. The cultivars were different in their N-fixation ability, as measured by the acetylene reduction analysis (ARA), and this difference was related strongly to leaf size. But when expressed as ARA per unit DW, the difference between the cultivars was non-significant.

Overall, under control conditions, mainly the morphological characteristics, such as leaf size, leaf weight and petiole length, caused the difference between cultivars, other characteristics, such as the plant DW components, DW partitioning, and P and N partitioning in plant components, were less important. Among the morphological characteristics, leaf size and leaf weight per stolon were the most important characteristics differentiating between cultivars. These two characteristics were also the most important for determining plant yield. Under water deficit stress and P deficiency treatment, root DW and branch number were the two most important characteristics differentiating between the cultivars.

CHAPTER ONE
INTRODUCTION

While white clover is grown across a broad spectrum of climatic conditions (Gibson and Cope, 1985), it succeeds best in cool moist climates. It is not productive or even persistent under certain stress conditions; for instance, during summer drought or with severe soil phosphorus(P) and potassium(K) deficiency (Johns, 1978; Caradus, 1980; Thomas, 1984). Although such problems are relatively minor under normal farming conditions in New Zealand, summer drought can cause poor growth (Radcliffe, 1982) and so prevent the fulfilment of the advantage of a better seasonal spread of white clover (Brougham, 1966). Poor growth can also lead to poor persistence, especially of white clover cultivars selected under adequate environmental conditions for high yielding ability (Charlton, 1984). The white clover's productivity and persistency under poor conditions has been indicated as an important breeding objective by some workers (eg. Rhodes, 1984, 1985), however, the information of how white clover grows under water stress and its ability to adapt to such stress is not available.

Similarly there is a lack of information on the production and persistency of New Zealand white clover under conditions of phosphorus deficiency and with concurrent water stress. Overseas work indicates that some cultivars, such as Dusi, can tolerant water stress as well as low P fertility (Smith and Morrison, 1983). Such cultivars would provide an interesting comparison with New Zealand cultivars of similar growth characteristics. The limitation on fertilizer usage imposed by the current economic conditions makes it clear that for hill country pasture at least, a white clover cultivar tolerant of low fertility, particularly low P fertility, is desirable.

Therefore, the objectives of the present study are:

1. to determine the effects of water stress and P levels on growth, development, and N-fixation of selected white clover cultivars (or selection);
2. to identify and evaluate plant characteristics most effective in distinguishing the cultivars used in the study.

CHAPTER TWO
LITERATURE REVIEW

The importance of white clover in global pastoral industry is such that its physiological and agronomic characteristics have been frequently and extensively reviewed (eg. Erith, 1924; Lowe, 1970; Gibson and Cope, 1985; Frame and Newbould, 1986; Baker and Williams, 1987). Consequently, in the following sections, the general characteristics of white clover growth and development will only be discussed briefly. The main purpose of this review is to elucidate possible limitations to white clover growth, particularly under stress conditions. N-fixation, as an important aspect in white clover research, will also be reviewed in a separate section.

The effects of water deficit stress and phosphorus deficiency on plant growth in general, and white clover growth in particular, will be reviewed in two separate sections. Numerous books and reviews have also been published on these subjects (eg. Hsiao, 1973; Marschner, 1986; Turner, 1986) hence the review here will be brief and will concentrate on pasture plants, particularly white clover.

2.1 Growth and Developmental Characteristics of White Clover

2.1.1 Origin and Distribution of White Clover

White clover is believed to have originated from Eurasia (Erith, 1924; Evans, 1976; Turkington and Burdon, 1983). This belief is disputed by some other authors, such as Zohary (1972), who suggests that white clover is a native of North America. The earliest date of the deliberate cultivation of white clover is not clear but it may have been used regularly in England as early as 1650 since it was introduced or recognized in pastures in the early sixteenth century (Fussell, 1964).

Through European settlers and other means, the cultivated forms of white clover have been brought to many parts of the world, including New Zealand and Australia. Nowadays, white clover is widely spread from the Arctic Circle to cool, temperate sites on tropical mountains (Gibson and Cope, 1985) and subtropical areas, eg. Australia. However, it apparently evolved in areas characterised by fertile soils, adequate soil moisture, and under the influence of grazing animals (Gibson and Cope, 1985). In a survey of unsown grassland in the Sheffield district, UK, Grime and Lloyd (1973) showed that white clover was restricted more or less to flat or gentle sloping areas, where the soil was likely to be deep and moist. Due to the lack of a high density root system (Evans, 1978), white clover is relatively intolerant of drought compared with many other pasture species. It is also susceptible to excessive water or soils that are saline, highly alkaline, or acid (Gibson and Cope, 1985).

2.1.2 White Clover Varieties and Classification

Due to its wide distribution white clover has been used in, and selected for, many types of environments. Consequently, numerous varieties have been developed over the last few hundred years. Its classification is, therefore, complicated.

2.1.2.1 Varieties

Selection and breeding of new white clover varieties is a continuous process. A large number of varieties have been released. In a comprehensive review, Caradus (1986) listed 232 known varieties throughout the world. New varieties are bred to meet specific environmental requirements. Increased emphasis is put on breeding for productivity and persistence under relatively poor growth conditions (Rhodes, 1984, 1985).

2.1.2.2 Classification

a. Taxonomic classification

Erith (1924) mentions that the white clover species (*Trifolium repens*. L.) includes 6 sub-species. They are ssp. *ochranthum*, ssp. *orphanideum*, ssp. *orbelicum*, ssp. *prostratum*, ssp. *repens* and ssp. *nevadense*. This taxonomic classification does not necessarily reflect agronomic usefulness and as such has gained little usage outside the taxonomic circle.

b. Classifying according to leaf size

Currently this method is widely used by agronomists. Leaf size is a parameter often indicative of the genetic potential for productivity of a white clover plant (Davies and Young, 1967). However, it is limited in reflecting the whole set of genetic, physiological or morphological characteristics of a plant. This may account for the variation between various "leaf size" classification methods. Either leaf size is considered as the only criterion as in the UK (Anonymous, 1985) and New Zealand systems, or leaf size as well as plant type are jointly considered, like the system used in Western Europe (Van Bockstaele, 1985).

Generally speaking, small-leaved cultivars are prostrate and of value in closely grazed swards, low fertility soils and dry conditions. Typical examples are the New Zealand variety 'Tahora' or the British 'Kent wild white' clover. Most local unselected ecotypes are also small leaved. Medium-leaved cultivars can persist better under N fertilizer usage and in conserved pastures since they can grow taller by extending their petioles. Their leaf size is variable depending on grazing intensity. 'Huia' is one of the most widely used medium-leaved cultivars in New Zealand as well as in Europe and a number of other countries. Large-leaved cultivars are more productive and are suitable for laxly grazed pastures or for cutting. Large- and very large-leaved cultivars are usually grown under moist conditions (Davies and Young, 1967). Ladino white clover is an example of the large-leaved white clover.

Davies and Young (1967) found a strong correlation between leaflet size and growth potential. For example where conditions impair the expression of potential leaf size, yield will be reduced (Smetham, 1986). The leaflet size will vary within a cultivar depending on environmental factors, through both long term natural selection (Williams, 1987) and short term plant reaction to grazing (Korte and Parsons, 1984).

2.1.3 Growth and Development of White Clover

Despite the variations between varieties or categories of white clover, their basic growth and development processes are the same or similar (Thomas, 1987). These processes include the development of axillary buds into branch stolons or inflorescence, the initiation and growth of leaves from apical meristems, and of adventitious roots from root primordia on nodes.

A mature stolon consists of an apical meristem, leaves, adventitious roots and axillary buds and can be considered as the basic structural unit (Thomas, 1987). Therefore, it is often used by agronomists to describe white clover growth (eg. Chapman, 1983). In the vegetative form, the plant is sustained by the continuous growth of stolon at the apical meristem, through new leaf (and node) initiation and development replacing the aging and dying leaves. It also involves the continuous formation of new branch stolons from axillary buds and continuous development of nodal roots from root primordia on nodes. Under favourable climatic conditions, these processes are active throughout the year (Thomas, 1987), and therefore ensure white clover's perenniality and regrowth after grazing.

2.1.3.1 Stolon Growth

White clover stolon growth and development are discussed in detail by Erith (1924), Chapman (1983), Hay (1985) and Thomas (1987). Following germination, a short primary stem is formed, whose axillary buds give rise to 7-10 primary stolons which extend radially. Thereafter, branch stolons are formed from axillary buds of parental stolons in the same way.

Structurally and functionally, these offspring stolons (loosely called secondary stolons) are like primary stolons except that they are supported by nodal roots after the primary taproot dies, typically after one to one and half years (Chow, 1966). They therefore can survive independently after being severed from the main plant (Erith, 1924; Spedding and Diekmahns, 1972; Chapman, 1983). There is no evidence to suggest that they grow differently from the primary stolons (Haycock, 1981). Thus the continuous growth and survival of white clover is strongly dependent upon secondary stolon development and growth (Knight, 1953a,b; Beinhart *et al.*, 1963; Schillinger and Leffel, 1964; Gibson and Hollowell, 1966; Hollowell, 1966). For example, it has been found that the difference in leaf production was related to the differences in leaf growth on secondary stolons (Beinhart *et al.*, 1963). The number of stolons also influences the ability of the plant to persist. Wild white clover ecotypes, which usually are strongly stoloniferous, have better persistence than less stoloniferous large-leaved types (Davies, 1970; Munro *et al.*, 1975; Baines *et al.*, 1983). Increased length, diameter and weight of stolons are the other factors contributing positively to white clover performance with increasing intervals between defoliations (Wilman and Asiegbu, 1982a,b; Frame, 1985).

Stolon production is influenced by temperature, light and flower initiation (Brougham, 1962, 1965; Beinhart *et al.*, 1963). The mechanisms of the effects can be metabolic, physiological or morphologically based as reviewed by Thomas (1987).

2.1.3.2 Growth of Stolon Components

Apical bud and meristem: The apical growth of white clover largely occurs at microscopic level (Denne, 1966; Thomas, 1987). The number of leaf primordia in the apical meristem is quite constant because leaf initiation is normally at the same rate as leaf appearance. In a natural environment, the rate of initiation varies from nil to approximately 2 leaves per week at latitude 40^{o}S in New Zealand (Thomas, 1979). Leaf initiation is controlled by a complex mechanism of internal and external factors (Thomas, 1987). Light is the single most important factor affecting leaf initiation while low temperature also has a strong influence (Thomas, 1962).

Leaf: The relative growth rate of white clover leaf is highest immediately after initiation and declines rapidly (Denne, 1966). By the time of its emergence, the relative growth rate of leaf was found to be very slow and mainly due to cell expansion, not cell division (Thomas, 1962; Denne, 1966). While leaf expansion can be accelerated by an increase in temperature (Carlson, 1966), moisture stress can severely reduce leaf expansion (Hsiao, 1973). Other environmental factors, such as mineral nutrition, can also affect the growth of white clover leaf. White clover leaf production is a key factor controlling the growth rate of the plant (Brougham, 1960). Beside representing most of the photosynthetic area, leaf also constitutes a large proportion of its harvestable yield. Petiole is actually a part of a leaf and its growth is closely associated with leaf lamina although low light intensity can stimulate petiole growth as an adaptive reaction to shading.

Axillary bud: A vegetative axillary bud develops into a branch. Initially classed as a branch stolon and lateral stolon, it eventually develops into a fully established stolon (Thomas, 1987). Light intensity again may play an important role in the development of the axillary buds. Photoperiod, temperature (Thomas, 1962) and mineral nutrition also affect bud initiation. Outgrowth of axillary buds into lateral stolons does not usually occur until there are several nodes basal to the apical bud; the exact point of such an outgrowth depends on stolon vigour, growing conditions and genotype. It can be reasonably expected that a large proportion of axillary buds may fail to develop (Trautner and Gibson, 1966). A large number of developed axillary buds may die before reaching the full stolon stage. The reasons for such failure to develop, and/or early death, are by no means clear.

Internode: Internode elongation takes place around the time of leaf emergence (Thomas, 1972). Internode extension growth, to a certain extent, represents the stolon extension growth. The growth of internode is correlated with leaf size. Usually, smaller-leaved cultivars have a shorter internode. Similarly the diameter of internode is smaller for smaller-leaved cultivars. Though contributing little to the herbage yield, the internode is the storage part of white clover and it can play a crucial role in regrowth and stress resistance.

Root: For convenience, the primary root system and nodal root system are discussed here together. The primary root system or taproot serves white clover well in the first year after sowing, but it generally survives only one to one and half years after sowing (Chow, 1966). Since most (about 80%) of the photosynthate from mature leaves is translocated to branch stolons and the primary stem tips rather than to the taproot (Ryle *et al.*, 1981), the taproot is likely to be susceptible to external stresses such as diseases, pests etc (eg. Chow, 1966). Also, since the distance from the taproot to the apical meristem of stolons increases as the stolon grows and more branches are supported by their own nodal roots, the importance of the taproot would decrease gradually and be replaced by nodal roots spatially more closely associated with the secondary stolons. Some evidence suggests that nodal roots are very similar to the taproot in their relationship with leaf growth (Haycock, 1982). So the nodal root development would be expected to be related to the growth and persistence of white clover after the first year (Hollowell, 1966). For some cultivars, their nodal root can develop to such size that it resembles the taproot in appearance (Caradus, 1977) and possibly in function. On every node there are 2 nodal root primordia with the potential to develop. However, in field situations, only a very small proportion of these primordia will develop. It is known that moisture level at the soil surface is one of the main factors determining nodal root development (Ueno and Yoshihara, 1967).

2.2 N-Fixation

2.2.1 Introduction

Biological N-fixation is the reduction of atmospheric N_2 to ammonia. There are two basic forms, single organism N-fixation, as in bacterium or algae, and symbiotic N-fixation as in legumes (King, 1983). In white clover and other legumes, the symbiosis with Rhizobium is responsible for N-fixation. Being an important aspect of crop and pasture agronomy and physiology, the N-fixation process in legumes is a widely studied and extensively reviewed subject (Bergersen, 1977, 1982; Postgate, 1982; Sprent, 1979; Havelka *et al.*, 1982; Marschner, 1986; Crush, 1987). The following introduction will be brief and will concentrate on nodulation, nitrogen fixation and factors affecting N-fixation, emphasising the effects of water deficit stress and P deficiency.

2.2.2 Nodulation of White Clover

Nodulation is a process of "infection" by the Rhizobium of legume roots or interaction between the two. However, not all leguminous plants form nodules. For nodulation to occur, the host plant must be susceptible and a compatible infective strain of Rhizobium, capable of multiplying, must be present on the root.

The structure and function of root nodules of legumes have been reviewed by Bergersen (1982). In many legumes, especially temperate species, infection takes place through root hairs. So infection is closely correlated with abundant root hair formation or root hair length (Franco and Munns, 1982; Nambiar *et al.*, 1983). Presence and quantity of root hair, therefore, determines nodulation. Successful infection occurs at defined sites in the zone of elongation near root tips and is usually associated with a particular type of deformation from which an "infection thread" develops. The thread is in fact an invaginated tube of root hair cell-wall within which the rhizobia grow, confined in a chain along the lumen of the tube (Bergersen, 1982). However, in some species, eg. in the peanut (*Arachis hypogaea*), infection threads have not been observed (Dart, 1977). Specific interactions between the host plant and the rhizobia may be expressed at the early stage, and at all subsequent stages of the nodulation process (Bergersen, 1982).

In the developed nodules, the bacteroids are surrounded by a solution of red pigment, leghaemoglobin (Bergersen and Goodchild, 1973) which functions to maintain a rapid flux of O_2 into the bacteroids (Bergersen *et al.*, 1973) and gives the nodules a pinkish colour. The N-fixing enzyme system is contained in the bacteroids.

Nodule initiation and development is a continuous process. Therefore, on the root system of a mature plant, nodules of all ages may present (Hicks, 1978). Between infection and the onset of N-fixation there elapses a period of 3 to 5 weeks, during which time carbohydrates, mineral nutrients, and amino acids are supplied by the host without benefit returned (Marschner, 1986). The mature nodule stage is characterised by high nitrogen-fixing activity which is associated with high leghaemoglobin concentration. The expansion of plant cells and bacteroids may continue but cell division does not. The nodules continue to receive plant carbohydrate and export combined nitrogen in return (Sutton, 1983).

2.2.3 The Process of N-fixation

The N-fixation process in legume nodules is a series of complicated biochemical reactions. This is a process which has been widely reviewed (eg. Bergersen, 1977; Sprent, 1979; Havelka *et al.*, 1982).

In legumes, the N-fixation reaction is catalysed by nitrogenase, an enzyme found in bacteroids of legume root nodules. Nitrogenase is a large molecule with several subunits. They fall into two categories: the so-called iron-protein (fraction 1) consisting of two apparently identical subunits and iron-molybdenum protein (fraction 2) consisting of four subunits. The general structure of the nitrogenase complex is very similar among different nitrogen fixing organisms (Roughley *et al.*, 1983).

A graphical description of how the reduction of nitrogen to ammonia proceeds is presented in Fig. 2.1.

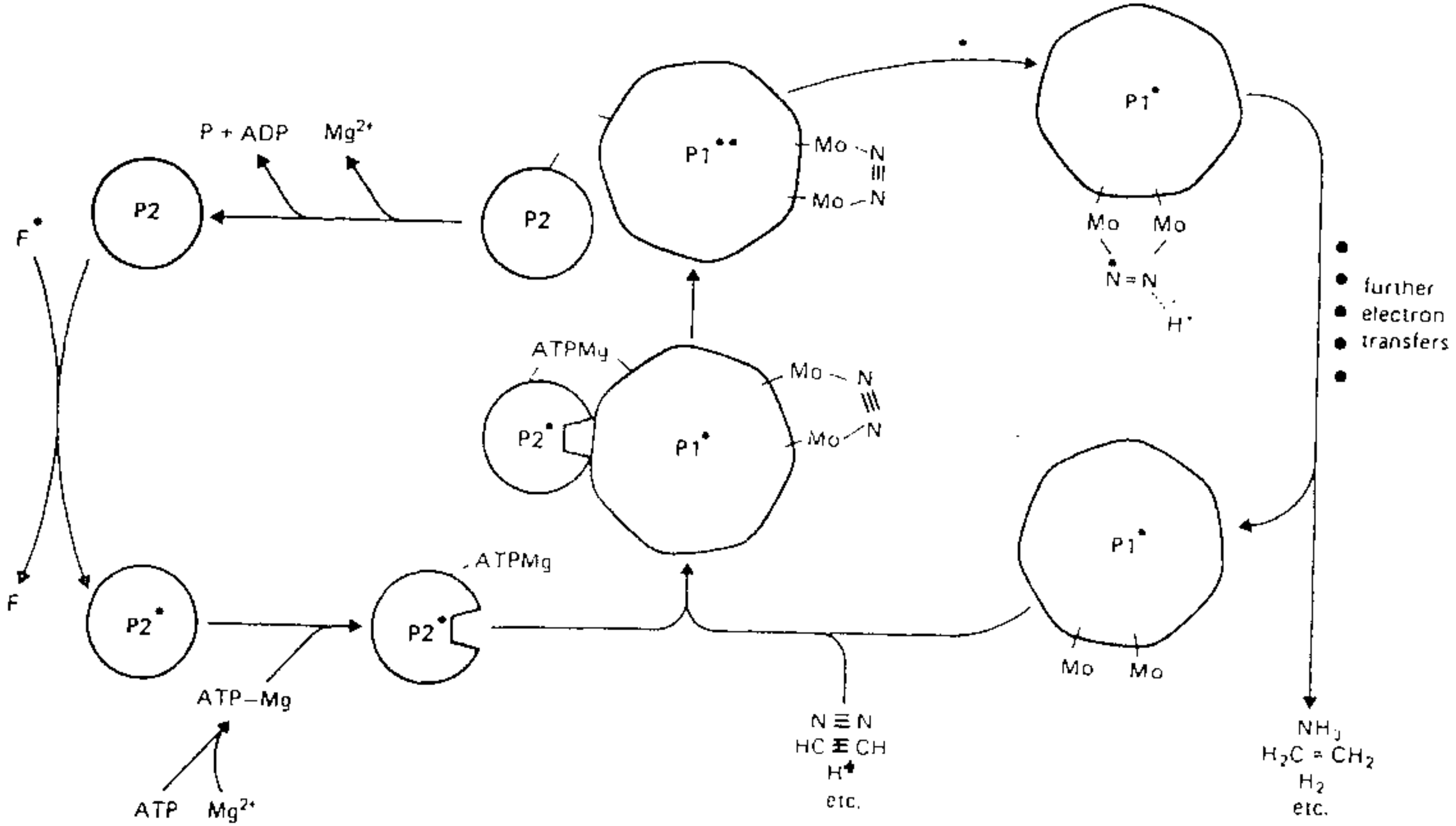


Figure 2.1 A scheme for the action of nitrogenase. P1 is the larger protein containing iron and molybdenum atoms, P2 is the smaller protein with iron atom only. F is an electron-donating substance (ferredoxin or flavodoxin) which donates an electron (a black dot) to iron atom in P2 (From Postgate, 1978).

The requirements for the overall reduction of nitrogen to ammonia are as follows (Roughley *et al.*, 1983):

1. A source of low redox potential reductant (approximately -430 mV). The nature of this reductant on Rhizobium bacteroids is probably a ferredoxin; it donates electrons singly to the nitrogenase reductase;

2. A supply of adenosine triphosphate (ATP). It is likely that nitrogenase consumes about 15 moles of ATP to fix 1 mole of dinitrogen (Postgate, 1982). ATP is produced by oxidative phosphorylation in bacteroids and at least some of it, in the form of its magnesium (Mg) complex, unites with the reduced nitrogenase reductase;

3. A supply of N_2. This is not limited under any known natural conditions. The nitrogen molecule joins with nitrogenase, almost certainly at its molybdenum (Mo)-containing centre. The N_2-nitrogenase then unites with the reduced nitrogenase-ATP-Mg to form the active nitrogenase complex. Electrons flow to N_2 which is reduced in stages until NH_3 is formed.

In addition, in order for the system to function properly, a supply of oxygen and carbon is essential. Oxygen is vital for the production of ATP. Carbon compounds in the nodules are used to produce ATP and also the fixed nitrogen is combined rapidly into amino acids and amides with carbon compounds (Bergersen, 1982). The supply of carbon comes mainly as photosynthates from the leaves, but may be supplemented by CO_2 uptake directly into nodules (Roughley *et al.*, 1983).

Beside the energy required for N_2 reduction, energy in the form of photosynthate is also required for nodule formation and maintenance, hydrogen removal, incorporation and transport of newly fixed nitrogen (LaRue and Patterson, 1981; Havelka *et al.*, 1982).

2.2.4 Methodology of N-fixation Measurement

N-fixation occurs underground and a direct measurement of the process is difficult. On the other hand, based on the mechanism and results of the N-fixation process, several methods have been developed to determine plant N-fixation. A comprehensive comparison of these methods has been reviewed by Bergerson (1980) and LaRue and Patterson (1981). The 3 main methods are briefly discussed as follows:

1. the difference or nitrogen determination method -- Using Kjeldahl determination for nitrogen analysis (Bremner and Malvaney, 1982), the total plant N can be measured. This method is based on the intuitive assumption that the total N is an estimation of N-fixation if no soil N is available. This method usually overestimates N-fixation (LaRue and Patterson, 1981) since it is difficult to get totally N free media. But nevertheless, this method can be used to compare plants or Rhizobia strains under the same conditions (Havelka *et al.*, 1982). By estimating and subtracting the contribution of soil N, a more accurate measure of N-fixation can be obtained (Whitehead, 1970), though the estimation of soil N contribution itself is a complicated process (LaRue and Patterson, 1981).

2. the acetylene reduction method -- Because of the discovery that nitrogenase has the capacity of reducing acetylene to ethylene (Dilworth, 1966; Schollhorn and Burris, 1967) it became possible to measure N-fixation indirectly by using ethylene as a reagent to measure the reductive ability of nodule nitrogenase enzyme. The activity of this enzyme is directly related to the N_2 reduction which is a key procedure in nodule N-fixation (Turner and Gibson, 1980). Many variations of this method have been described (Hardy *et al.*, 1973). This method, as a means of comparative study, is very useful. However, it is of limited use as a quantitative method for the estimation of N-fixation potential of nitrogenase because of the uncertainty about the N_2 fixing potential of nitrogenase within the nodule system (Sims *et al.*, 1983).

3. the ^{15}N isotope method -- Using ^{15}N isotope as a tracer in quantifying N-fixation is about the most definitive method available (Ayanaba, 1980; Chalk, 1985). However, its high cost and requirement of skilled operators has limited its use (LaRue and Patterson, 1981). Less expensive and accurate alternative ways include using ^{15}N fertiliser and the "A value" method (LaRue and Patterson, 1981).

2.2.5 Environmental Factors Affecting N-fixation

For a growing plant, nodulation is occurring continually, but the overall pattern of nitrogen fixation by a population of nodules on a plant resembles that of individual nodules, although over a longer time span (Gibson *et al.*, 1982). Potential N-fixation is difficult to obtain, but it may be estimated on plants nodulated with the most effective Rhizobium strain and grown under the most favourable environmental conditions. Many biological and environmental factors can affect the expression of the full potential of N-fixation of a legume. They can affect it through nodulation and/or the N-fixation process itself. The factors affecting N-fixation have been widely reviewed (Lie, 1981; Havelka *et al.*, 1982; Crush, 1987). Some of the factors relevant to the present study are discussed here.

(1) Moisture Level

Moisture stresses due to either deficiency or excess are detrimental to the legume-Rhizobium symbiosis. Infection is reduced by low soil water potential due to the absence of normal root hairs. The short, stubby root hairs grown under water stress are inadequate for infection by Rhizobium (Worrall and Roughley, 1976). Following successful infection, reduced water supply can retard nodule development (Gallacher and Sprent, 1978). It has been found that soil moisture deficiency led to nodule shedding as it approached permanent wilting point (Diatloff, 1967). The shedding may be due to the mechanical or physiological effects of soil drying. Prolonged desiccation leads to nodule loss with a consequent reduction in the level of nitrogen fixation until new nodules form (Gibson *et al.*, 1982). Slow drying of the soil leads to a progressive decline in white clover nodule activity which is capable of restoration after rewatering (Engin and Sprent, 1973), but the extent of recovery depends on the degree and length of stress. The legume nodule requires water for maintenance of turgidity of its tissue so that it remains functional. Water is also needed to transport the product of N-fixation to the host plant as indicated by Minchin and Pate (1975). Nodules may obtain water by diverting it from root system (Sprent, 1972b).

Excess soil water, beside adversely affecting plant growth, also has a detrimental effect on N-fixation by limiting the oxygen supply to nodules. Water-logging can reduce nodule number and size, as well as increase its water content (Sprent, 1976).

(2) Temperature

During most stages of their formation and development, nodules can be affected by soil temperature. Gibson (1977) has reviewed the effect of soil temperature on N-fixation. In general, the optimum temperature for N-fixation is a reflection of the natural habitat of the host plant (Roughley *et al.*, 1983). Lower than optimum root temperatures are found to retard root hair infection more than nodule initiation and development or N-fixation (Gibson, 1971). A side effect of summer drought is usually the increase of soil temperature. However, the effect of high temperatures on the survival of rhizobia under field conditions is not clear (Gibson *et al.*, 1982). Air temperature may also have effects on nodule activity, more likely through indirect influence on translocation of carbohydrates from leaves to nodules (Brun, 1978).

(3) Nutritional Factors

Nutrient deficiency affecting plant top growth will eventually affect N-fixation. In addition, mineral deficiency may also limit N-fixation by affecting the following three phases: growth and survival of Rhizobia, infection and nodule development, and nodule function (Robson, 1978). Nodules tend to have a higher P concentration than other plant components. N-fixation is also reduced by N fertilizer application (Ham *et al.*, 1975; Child, 1976; Rabie *et al.*, 1979; Manhart and Wong, 1980). However, it is well known that in the seedling stage, the plant benefits from a small supply of nitrogen fertiliser before the nodules start fixing nitrogen.

2.3 Effect of Water Deficit Stress on Plant Growth and Development

Before further discussion of this topic, some common terms used in this thesis are defined as below:

(1) **Drought** - refers to a meteorological condition during which there is no measurable rainfall. The duration is arbitrary, sometimes 15 days (May and Milthorpe, 1962). Here it is used to mean a period of severe plant water deficit.
(2) **Water Deficit** - refers to the lowering of plant water status from that equal to full turgidity at the same temperature.
(3) **Water Stress** - strictly speaking, this can apply to both a deficit or an excess of water, however, more commonly this term implies "deficit stress" (Levitt, 1972). Here it refers to a level of water deficit which is low enough to impair normal plant function.

Water deficiency is probably one of the most common environmental factors limiting plant growth and development in the world pastoral areas. In New Zealand, precipitation is normally medium to high but seasonal and regional water deficits do occur. Sometimes they can be severe enough to cause stress on plant growth and development (Radcliffe, 1982). White clover is more susceptible to water deficit than some grasses (eg. phalaris, Blackie, 1988; ryegrass, Thomas, 1984) and legume species, such as lucerne (Thomas, 1984).

2.3.1 Development of Water Deficiency in Plants

It is well recognized that there is a gradient of water potential from the transpiring leaf surface to the absorbing root surface. This gradient is caused by the 'pulling power' of low water potential in the leaf mesophyll cells. Along this gradient, water moves from the soil through the plant to the atmosphere (i.e., the SPAC, Soil-Plant-Air-Continuum, concept).

A low level of water deficits exists inevitably as a consequence of water loss from the leaf as the stomata open to allow the uptake of CO_2 from the atmosphere for photosynthesis, and/or to reduce leaf temperature.

The water status of a plant is largely dependent upon both ends of the SPAC, ie. upon soil factors as well as atmospheric factors. It is, therefore, a dynamic balance between water absorption and water loss, mainly through transpiration. Plant factors, such as stomatal control, also play important mediating roles (Hsiao, 1973). The amount of water lost to the atmosphere in transpiration is huge compared to the amount of water contained in the plant tissue. For example, rapidly growing and transpiring plants can lose their own weight in water every 2-3 hours. Plant water potential can recover and approach the soil water potential at night when there is no transpiration. But as soil dries, the declining soil water potential sets the upper limit for the plant water potential, also, the rate of recovery in plant water potential becomes slower (Slatyer, 1957; Gardner and Nieman, 1964). Under dry and hot conditions, substantial plant water deficit can still develop even when roots are well supplied with water. Shepherd and Dilley (1970) observed that wilting of leaves occurred when potential evapotranspiration, calculated from meteorological data, exceeded 0.5 mm per hr for several hours, even when the plots were 'well-watered'. Stomatal control of transpiration is the most frequently cited plant mechanism for reducing water loss and maintaining water status under water deficit conditions (Hsiao, 1973). However, it appears to have a different significance in different plant species. For example, paspalum had a higher stomatal resistance than lucerne and white clover (Kerr and McPherson, 1978) and white clover had been found to have poor stomatal control of transpiration (Johns, 1978). Other ways of transpiration control, such as morphological change and leaf movement, are also effective in some plant species.

2.3.2 Plant Water Deficit Measurement

Leaf water status is usually measured to assess the plant's internal water deficit or stress level. Two most commonly used measurements are:

(1) Water potential: This is conventionally regarded as a convenient index of plant water stress level. The concept of water potential and its measurement has been reviewed by Barrs (1968) and Turner (1986). The major method of measuring water potential is by the pressure bomb (Turner, 1986).

(2) Relative water content (RWC) is another common way of expressing water status or stress level in plant tissue. It is defined as:

$$\mathrm{RWC} = \frac{\text{actual water content}}{\text{water content at turgidity}} * 100\% \quad \text{or}$$

$$\mathrm{RWC} = \frac{\text{fresh weight - dry weight}}{\text{turgid weight - dry weight}} * 100\%$$

The technique and precautions in measuring RWC have been described by Barrs (1968) and Turner (1981). Though it is a slightly tedious method, it is widely used because of its simplicity. For white clover, its leaf RWC and water potential are linearly correlated (Cox and Boersma, 1967; Johns, 1978).

In order to compare plant responses to water stress in different experiments, Hsiao (1973) attempted to relate water stress level to plant water status more precisely by defining three classes of stress (in a typical cell):

Mild stress -- cell water status lowered by a few bars or 8-10% RWC;
Moderate stress -- cell water status lowered by more than a few bars but less than 12-15 bars or 10-20% RWC;
Severe stress -- cell water status lowered by > 15 bars or >20% RWC.

Application of these levels may, however, be complicated by changes in the component water status, by changes in plant structure, and by different water potential change in various plant species under similar levels of water deficit.

2.3.3 Plant Responses to Water Stress

The effect of water stress on plants has been extensively reviewed (eg. Hsiao, 1973; Begg and Turner, 1976; Levitt, 1980; Turner and Kramer, 1980; Turner, 1986). In the following section, mechanisms of water stress effects and three physiological aspects of plant growth under water stress will be briefly discussed.

2.3.3.1 Mechanisms of Water Stress Effects on Plants

It is clear from the definition of water stress that it will impair normal metabolic and physiological processes in plants. Because of the numerous important roles water plays in plant metabolism and physiology, the possible mechanisms of water stress effect on plant growth are very complex (Hsiao, 1973). It appears that the reduction of turgor due to water deficiency may be the most important and primary factor causing a series of plant responses to water stress (Hsiao, 1973). In other words, many other responses of a plant to water stress, such as impaired N metabolism, reduced photosynthesis and reduced growing point vitality, may only be secondary (even tertiary) effects of water stress even though consequences of these secondary and tertiary effects can sometimes be more deadly to the plant. For example, some evidence has suggested that, rather than drought itself, the lack of calcium(Ca), or the plant's inability to absorb Ca under water stress, is the real reason for the death of plants under water stress (Troughton, 1977). This is because water stress stops growth of new nodal roots (Troughton, 1978) which are essential for Ca absorption.

2.3.3.2 Transpiration and Stomatal Responses

Stomatal closure and transpiration reduction in response to water deficiency have long been established (Hsiao, 1973). Stomatal closure is the main cause of transpiration reduction. Normally, transpiration is directly proportional to the gradient of water vapour concentration from the internal evaporating surface to the bulk air outside the leaf, and inversely related to the total resistance to water vapour transpiration, including both air boundary layer and leaf resistance. Since stomatal control is only part of the total resistance, their closure will vary in effect with the magnitude of its resistance relative to total resistance (Hsiao, 1973). White clover was shown to be ineffective in reducing transpiration by stomatal closure (Johns, 1978). In addition, increased stomatal resistance may not cause a proportional decrease in transpiration rate because of diminished dissipation of heat by vaporization and the consequent rise in leaf temperature inside the leaf (Raschke, 1956, cited from Hsiao, 1973).

2.3.3.3 Responses in Leaf Growth and Plant Yield

Leaf growth can be viewed as the gross result of leaf cell division and expansion. Cell division is generally considered to be less affected by water stress than cell expansion. However, cell division can be inhibited by long exposure to mild water stress (Hsiao, 1973). In white clover, leaf cell division occurs mainly in the pre-emergence period (Denne, 1966) when the leaf is not exposed to high transpiration loss. In the post-emergence period, leaf growth is mainly due to cell expansion (Thomas, 1987). So it might be expected that the post-emergence growth of white clover leaf will be more severely affected.

Plant yield is dependent on all processes in a plant and directly related to both photosynthesis accumulation and respiration loss. In the case of white clover, nodule consumption of photosynthate would be another factor affecting yield. Water stress is more likely to cause a decrease in the rate of photosynthesis but no change in respiration rate (Foulds and Young, 1977). Such differential responses accelerate the depletion of energy reserves and explain in part the detrimental effect of a prolonged drought. White clover yields are usually related to available moisture (Appadurai and Holmes, 1964; Davidson, 1969; Thomas, 1984).

2.3.3.4 Plant Water Stress Resistance

From the above discussion it may be concluded that plant water stress resistance can be estimated at three levels. First, physiological levels, such as plant water status which represents plant adjustment in water absorption and transpiration to avoid dehydration. Secondly, plant leaf expansion, which, in a way, shows the plant's ability to grow under low turgor. Thirdly, on an integrated level to evaluate overall growth under stress, for example, DM yield or seed yield. The agronomic definition of drought resistance in crop plants involves yield as a major parameter. Drought resistance then can be defined in terms of the yield ratios between stress and non-stress conditions (Blum, 1973). On the other hand, the association between yield and some physiological attributes and morphological (or growth) parameters under water stress constitutes crucial information about the development of physiological and morphological selection criteria. For example, it has been observed that modern high yielding soybean cultivars have a higher leaf water potential than old cultivars (Boyer *et al.*, 1980).

Resistance of plants to drought is attributed to either drought avoidance or drought tolerance or both. The shallow rooting of white clover compared with grass (Evans, 1978) means that it may not be a good stress avoider. The adverse effect of defoliation on grass growth and survival is more severe in dry conditions because new secondary root growth stops (Troughton, 1978). White clover root growth is also dependent upon the moisture level of soil (Ueno and Yoshihara, 1967). There is little direct evidence of drought resistance in clover plants. White clover water resistance may be related to certain growth and morphological characteristics resulting from a certain management practice. For example, Jones (1980) suggests that hard grazing in the previous summer improves white clover's resistance to drought. Jones (1982) found that under subtropical conditions the survival of white clover stolon contributes little to long term persistence. Smith and Morrison (1983) showed that deep rooting is the reason for better persistence of white clover cv. Dusi under water stress. However, there are few studies to compare between white clover cultivars of different characteristics and their water stress resistance.

2.4 Effects of Phosphorus (P) Fertility Level on Plant Growth

The recent increase in the cost of P fertilizer has generated an urgency in understanding the reaction of plants to P fertility in order to improve the efficiency of fertilization. White clover, with its reputation for high P fertility requirements, is attracting much attention. Much work has been carried out both in New Zealand and overseas to determine the P requirements and utilization efficiency by different cultivars (genotypes) of white clover (eg. Caradus and Snaydon, 1986a,b,c).

2.4.1 Function and Content of Phosphorus in Plant

The roles of P in plant nutrition have been widely reviewed (Barrow, 1980; Abbott and Robson, 1982; Colwell, 1983; Nielson, 1983; Marschner, 1987). P is one the most important elements in plant metabolism and composition. The function of P as a constituent of macromolecular structures is most prominent in nucleic acids, which, as units of the DNA and RNA molecule, are the carriers and translators of genetic information. P also plays an important role in metabolic processes for transferring energy as energy-rich phosphates. In actively metabolizing cells, they have an extremely high turnover rate. The effect of P on plant growth is through many aspects of its function in the plant metabolism and as a structural element.

Phosphorus fertilizer is usually taken up as $H_2PO_4^-$ by plants, then it either remains as inorganic P (Pi) or is esterfied. In higher plants two major phosphate pools exist. In the 'metabolic pool', represented by the cytoplasm and including the chloroplasts, phosphate esters dominate, whereas in the 'nonmetabolic pool', or the vacuole, Pi is the dominant fraction. With an interruption of the P supply, the Pi concentration in the vacuoles decreases sharply while the organic P concentration in the cytosol is less reduced.

2.4.2 Effect of P Deficiency on White Clover Growth and Development

Phosphorus concentration of a plant is related to P application regime (Evans *et al.*, 1986). McNaught (1978) has used plant analysis data to indicate 'deficiency', 'just adequate', and 'safe excess' levels of P concentrations in New Zealand as 0.08-0.30%, 0.35-0.40%, and 0.45-0.80% respectively whereas Evans *et al.*, (1986) recommended 0.30% as the critical level, 0.38% as the optimum level, or soil P availability should be 11 ppm, minimum 8 ppm.

P deficiency has detrimental effects on many aspects of plant metabolism and growth, including cell division and expansion, respiration and photosynthesis (Terry and Cerich, 1973). It causes retarded growth of plants and a resultant reddish coloration. The photosynthetic efficiency per unit of chlorophyll of such plants is much lower (Tombes, *et al.*, 1969). Inhibition of leaf cell expansion is particularly expressed during the day-time and caused by decreased root hydraulic conductance in P-deficient plants (Radin and Eidenbock, 1984). The effect of P on partitioning may also be responsible, in part, for the insufficient photosynthate supply to nodulated roots of P-deficient legumes and the occurrence of nitrogen deficiency as a dominant symptom in N fixing legumes suffering P deficiency.

White clover is highly susceptible to deficiencies of the major nutrient elements, except nitrogen (Sears, 1953), partly because it is a poor competitor for these elements when growing in association with grasses in soil low in available phosphate (Mouat and Walker, 1959; Jackman and Mouat, 1970). This results in further disadvantages for clovers in competition for light and other nutrients. Low levels of P would affect the growth and consequently the N-fixation of clover. Although there are no clear demonstrations that P deficiency directly limits nodulation or N-fixation, the possible existence of such effects cannot be ruled out (Munns, 1977).

2.4.3 Factors Affecting P Uptake and Utilization

2.4.3.1 Vesicular-Arbuscular Mycorrhiza (VAM) and P Fertility

VAM, which occur in clover roots in most soils where clover will grow (Crush, 1974), can help plants to absorb P in low P soils (Hall *et al.*, 1977; Hall, 1978; Hayman and Mosse, 1979; Crush and Caradus, 1980). It also has significance for nodulation and N-fixation in legumes in P deficient soils (Barea and Azcon-Aguilar, 1983). The increase in P uptake by mycorrhizal roots is assumed to be primarily due to the greater surface area resulting from the growth of hyphae, which may reach distances of several centimetres from the root surface. This extension permits P uptake outside of the depletion zone in the rhizosphere. But the exact mechanism of the enhanced growth and P uptake in clovers with VAM in low P soil is not entirely clear.

2.4.3.2 Water Stress

There is abundant evidence that uptake of potentially available P by the plant is reduced by soil water deficiency. The availability of some nutrients, particularly P, decreases as soil dries (Dunham and Nye, 1976) and most phosphate is held in the top layer of the soil which dries first in the period of drought. A decline in soil moisture content is associated with a decrease in the diffusion rate of nutrients from the soil matrix to the absorbing root surface (Marais and Weirsma, 1975). So, as the soil and roots dry they shrink so that the roots lose contact with soil particles (Faiz and Weatherby, 1982).

Poor absorption can be partly attributed to poor root growth due to both the low water potential of the plant and the high resistance of dry soil to root growth (Cornish *et al.*, 1984) even though the relative root growth may be increased.

Nutrient uptake by the plant decreases under water stress conditions due to reduced transpiration (O'Toole and Baldia, 1982; Yambao and O'Toole, 1984). Impaired active transport and membrane permeability (Hsiao, 1973) also result in reduced root absorption ability.

2.4.3.3 Genetic Variation in P Responses

From the agronomy point of view, and in an operational sense, genotype differences in the nutrient efficiency of plants are usually defined by the differences in relative growth rate or in the yield when grown in a non-limiting condition and a deficient soil. This definition can be applied to comparisons among genotypes (cultivars or lines) within a species and among species.

Legume nutrient efficiency and intraspecies variation in nutrient efficiency have attracted much attention (Graham, 1982; Caradus and Snaydon, 1986a,b,c). Large differences have been detected among the commonly used varieties in many legumes. For example, Robson and Collins (1981) found that with a low level of phosphate application, one subterranean clover cultivar yielded only 52% of maximum yield while another one produced around 90% of maximum yield.

Root morphology is one of the most important factors affecting plant phosphorus efficiency. Of particular interest is the changes in the roots in response to P declining. Though the relative growth rate of the root generally increases in this situation, the rate is very different in different genotypes. Whitaker *et al.*, (1976) found that under adequate P supply, 2 strains of beans had comparable root:shoot ratios (0.17 vs 0.15), but this changed dramatically (0.16 vs 0.31) under inadequate P supply.

Differential uptake of minerals can be due to physiological variables in the roots. White and red clover have a higher cation exchange capacity than most grasses and this provides the clover with a potential for the accumulation of cations (Mouat and Walker, 1959). But in a mixture of grasses and white clover, the latter is usually a poor competitor for P, K and other minerals. This can be partly attributed to its smaller root system (Evans, 1978).

Snaydon and Bradshaw (1962) concluded that populations of white clover plants that were tolerant of low P levels had a higher rate of P uptake per unit weight of root than intolerant populations. By extensive testing of clover cultivars and ecotypes under various concentrations of minerals, it was concluded that plants of commercially available cultivars or ecotypes from soils with high fertility generally grow larger at all fertility levels than did wild types or plants from low-fertility sites (Kendall and Stringer, 1985). Responses to increased levels of P appear to be affected more by the size of the plant in the lowest treatment level than by an interaction among genotypes (Ozanne *et al.*, 1969; Caradus, 1980; Spencer *et al.*, 1980).

CHAPTER THREE

EFFECTS OF SUMMER WATER DEFICIT STRESS ON GROWTH OF FIVE WHITE CLOVER CULTIVARS UNDER FIELD CONDITIONS

3.1 Introduction

Water deficit stress is one of the key factors affecting plant growth (Hsiao, 1973). Most of the agricultural area in New Zealand has an average annual rainfall between 750 to 2000 mm, which is considered about adequate for temperate pasture (Langer, 1973). However, water deficits may still occur in some areas during summer, mainly due to excessive evapotranspiration (ET). In certain areas, particularly the east coast of both islands, water deficits can be severe enough to affect pasture growth (Radcliffe, 1982) thus limiting the potential for production, while other environmental factors, such as temperature and light intensity, are approaching their optimum for pasture growth.

White clover will complement ryegrass growth by contributing towards summer herbage production under most New Zealand conditions (Brougham, 1966). However, this growth potential will be reduced by water deficit stress which not only adversely affects growth but also reduces the persistence of white clover (Thomas, 1984). For most New Zealand pastures, irrigation, though effective in eliminating water deficit, is not a viable alternative (either unavailable or uneconomical). Therefore white clover cultivars which can withstand water deficit stress are desired, and management strategies that can reduce the detrimental effect of water deficit stress on white clover growth will help to enhance summer production.

Currently there are a number of white clover cultivars available commercially in New Zealand, such as Kopu, Huia, Pitau and Tahora. In addition some local selections are undergoing evaluation, eg. Whatawhata early- flowering (Sheath, per. com.). (Hereafter in this thesis, for convenience, the term, cultivar, will be loosely used to include the Whatawhata early-flowering type, which will be designed as Whatawhata). These cultivars can be categorized into groups according to certain morphological characteristics, such as leaf size (section 2.1.3). Tolerance to environmental stresses is one of the selection criteria receiving more and more attention. It is suggested by many authors that some morphological characteristics can be related to plant stress resistance (Knight, 1953a,b; Beinhart *et al.*, 1963). For example, in lucerne the presence of a large and deep taproot is linked to drought tolerance. Taprooted white clover cultivars are also considered drought tolerant (Smith and Morrison, 1983). Small leaved ecotypes of white clover which have high stolon density persist in less desirable environments (Levy, 1970), and profuse and/or early flowering is an advantage in certain adverse environments, such as severe summer drought (Jones, 1980; MacFarlane and Sheath, 1984). However, for major New Zealand white clover cultivars there is little information available on their growth performance under water stress conditions.

The objective of this experiment was therefore to detect and, if possible, quantify morphological and growth differences between the five selected New Zealand white clover cultivars under two levels of soil moisture conditions in the field.

3.2 Material and Methods

3.2.1 Experimental Site

The experiment was conducted at the Pasture and Crop Research Unit of Massey University, Palmerston North, New Zealand, at grid reference NZMS1, N149/099308. The area was located on the high terrace above the Manawatu River, relatively exposed and nearly flat (sloping slightly to the North East). The soil type is a Tokomaru silt loam (Cowie *et al.*, 1972). It has been characterised in detail by Pollock (1975). This soil is characteristic of large areas of the flat to rolling hill country at the foot of the western Tararua Ranges.

The site was used for a moisture stress experiment on ryegrass during the 1981-1982 summer period (Barker, 1983). Between 1982 and 1985 the area was sown for permanent ryegrass/white clover pasture and grazed by sheep.

Daily rainfall and other climatic information during the trial period was collected at a meteorological station 200 m distant. These data, together with the average monthly rainfall (1928-1980) and other data taken from a meteorological station approximately 1 kilometre away (Grasslands Division, DSIR), are presented in Appendix 3.1.

3.2.2 Establishment of the Trial Plots

Cultivation began in August 1985, when the experimental area was sprayed with herbicide (Paraquat) and then ploughed 10 days later. Lime (3 t/ha) and a NPK (5:10:12) compound fertiliser (500 kg/ha) were incorporated into the soil by rotary cultivation. White clover seedlings (3 weeks old), cvs. "Grasslands Kopu", "Grasslands Pitau", "Grasslands Huia", "Grasslands Tahora" and "Whatawhata" were transplanted into the site

Plate 1: Example of a 'tagged' white clover plant with a plastic ring

as spaced plants one week after cultivation. The plants were about 500 mm apart. The white clover seeds were originally sown in trays in the glasshouse. In order to differentiate the transplanted white clover plants and the inevitable volunteer white clover plants, a yellow plastic ring (diameter 120 mm) was placed around each transplanted seedling (See Plate 1). The ring was fixed by wire on the ground. After transplanting, the areas between spaced white clover plants were sown with "Grasslands Nui" ryegrass (*Lolium perenne* L.). The purpose of sowing ryegrass was to occupy the empty space so as to control the growth of weeds. The plots were then mowed once every 8 weeks until the commencement of the trial.

3.2.3 Design and Treatments

3.2.3.1 Introduction

Before the imposition of treatments, on 29-30 November, 1985, all plots were irrigated to approximately field capacity to ensure that the two treatments started from the same level of soil water status.

3.2.3.2 Summer Moisture Treatments

The imposition of the moisture treatments began on 1 December, 1985 and continued for 75 days during summer, until 1 February, 1986. The two treatments were:

(1) a stress environment, where plots were covered by two automatic rain-out shelters to create a gradually increasing water deficit stress under field conditions (a brief introduction to the principle and function of the rain-out shelter is in Appendix 3.2).

(2) an irrigated environment, where water deficit stress was minimized by irrigating according to the model of Scotter *et al.* (1979).

3.2.3.3 Cultivars

Five white clover cultivars of New Zealand origin were used. These cultivars are widely used in New Zealand and also cover a wide range of leaf size categories. They were:

Large-leaved cultivars: Kopu and Pitau;
Medium-leaved cultivar: Huia;
Small-leaved cultivars: Tahora and Whatawhata.

3.2.3.4 Management during the Treatment Period

Potassium superphosphate fertilizer was applied just before the commencement of the trial at a rate of 250 kg/ha. The fertiliser was bulked with fine pumice and broadcast by hand onto the plots. No fertiliser was applied subsequently.

3.2.4 Measurements

3.2.4.1 Soil Data

Soil in the top 50 cm was sampled with a corer fortnightly and the gravimetric water content (w gH_2O/g dry soil) was determined after drying at 105°C for 72 hours. The soil bulk density (r soil) was determined according to the method described by Scotter *et al.* (1979) and the value, 1.15 ± 0.022, was in agreement with that obtained by Scotter *et al.*, (1979) and Barker (1983). The gravimetric water content (w) was converted to volumetric water content (j cm^3 H_2O/cm^3 dry soil) using the equation:

j=w * r water/r soil.
r water is water bulk density.

3.2.4.2 Plant Data

The plant data collected fell into the following 2 groups:

1). plant/canopy parameters directly or indirectly indicating plant water status or severity of plant water deficit, ie. leaf Relative Water Content (RWC) and canopy temperature;
2). plant growth parameters (described below) as indications of plant responses to water deficit stress.

(1) Canopy Parameters

Leaf water status: Plant water status was measured as leaf relative water content (RWC) by the method described by Barrs (1968). The detailed procedures are described in appendix 3.3. The measurements were made on the youngest fully or almost fully expanded leaf lamina between 12:00 and 13:00 hours once every 15 days. Nine leaflets were taken each time from one plant.

Plant canopy temperature: Plant canopy temperatures were measured with a handheld infrared thermometer (Model IRT) every 90 minutes for a 12-hour period, from 7:00 hours to 19:00 hours. The thermometer probe head was pointed at the centre of the plant to obtain "canopy" temperature.

(2) Plant Growth Parameters

The following plant growth parameters were measured on tagged stolons only. Tagging was carried out at the commencement of the trial. For each plant, three primary stolons with at least 8 nodes were tagged with coloured wires. The position of the tagging was on the internode between the 7th and 8th node. Every effort was made to minimize disturbance during tagging and measurement. In the event of stolon death during the experiment, a similar stolon on the same plant was tagged and further measurement was then made on it. If plant death occurred, then the plant was treated as a missing value.

Leaf appearance: In this thesis leaf appearance is defined to have occurred when leaf lamina first become visible at the tip of the stolon. The increment of leaves was measured once every 2 weeks by counting the leaf number from the tagged position.

Branch number: A branch was defined as a vegetatively developed axillary bud at any stage from the appearance of a first leaf lamina to fully established branch stolon (i.e. covering the stage of axillary bud, lateral branch and lateral stolon development defined by Thomas (1987). The number of branches from the tagged position to the apical bud was counted at the end of the experiment.

Leaf number per stolon: The number of leaves, including the most recent visible leaf, was counted on the tagged stolons. The ageing leaves on the basal end of the stolon were counted as long as they were green and intact.

Leaf size: Leaflet length of fully expanded leaves was measured and used as an index of leaf size. The measurement was taken at the end of the experiment on 12 leaflets from each plant.

Growing point survival: The total number of growing points on the three tagged stolons of each plant were counted every 2 weeks during the last month of the experiment.

Leaf duration: Leaf duration (LD) was derived by multiplying the number of leaves per stolon (NLS) and leaf appearance rate (LAR).

LD = NLS * LAR

3.2.6 Statistical Analyses

The plot layout was predetermined by the location of rain-out shelters and there were four plots; two under rainout-shelters and two with irrigation. Data obtained from the experiment were analysed using the pooled "environment" model (Gordon, 1979). Within each environment the cultivars were arranged in a randomised block design. Thus, the model allows the separate analysis of cultivar effect, treatment effect and their interactions. The data was analysed using ANOVA in SPSSx statistical package (Nie *et al.*, 1983).

3.3 **Results**

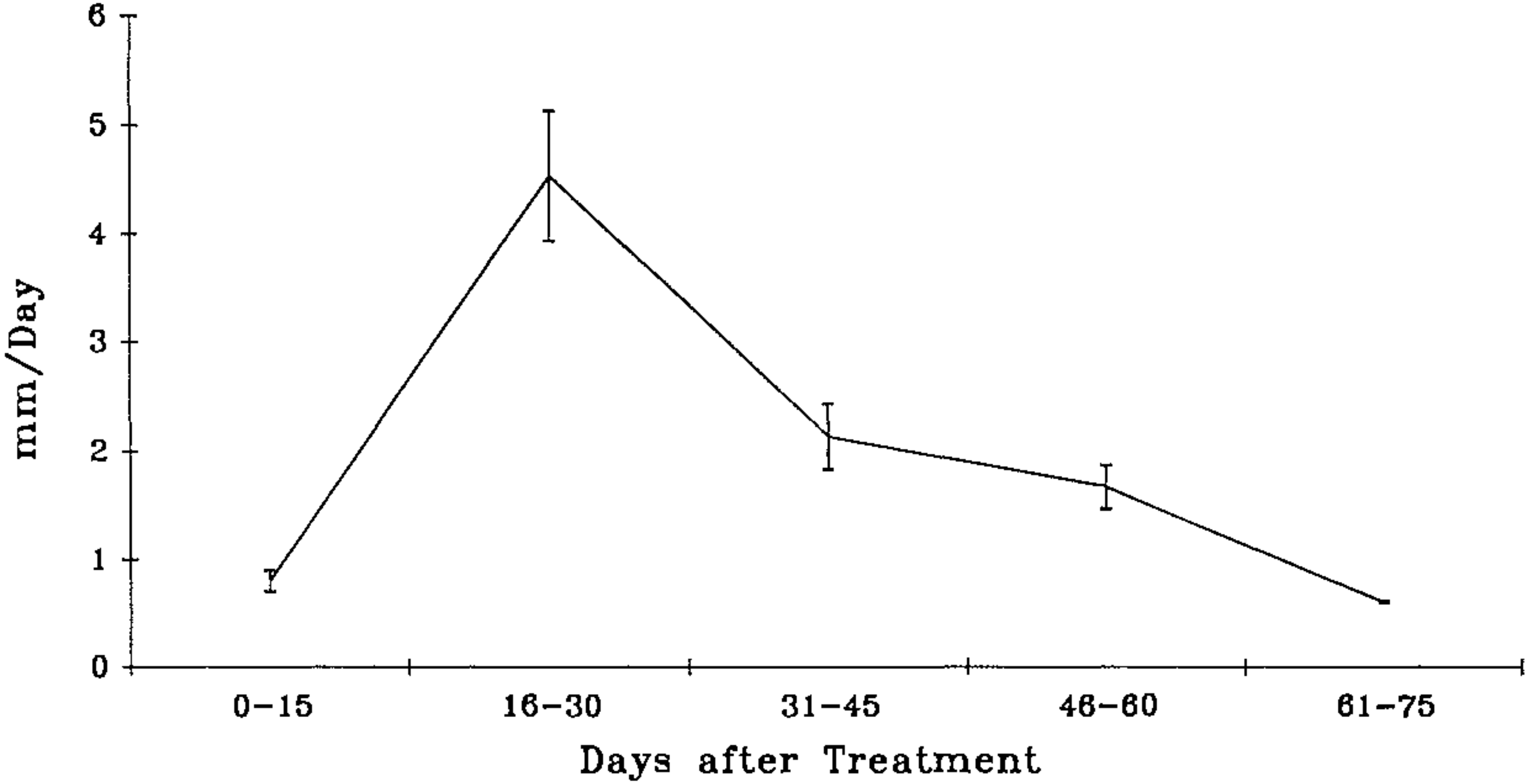
3.3.1 Soil Data

Soil water status, expressed as mm of water deficit from field capacity, is presented in Table 3.1.

Table 3.1 Change of soil water deficit (mm) with time (days)

Environment	Days after water stress treatment							
	0	15	30	45	60	75	Sig.	LSD
Control	23	24	31	26	29	23	ns	--
Water Stress	25	37	105	137	162	171	***	9.5
Sig.	ns	**	***	***	***	***		

Fig 3.1 Rate of soil water deficit development under water stress environment(mean & SEM)



After commencement of the treatment, soil water deficit developed in the water stress environment, so on the first measurement (ie. after 15 days), the difference between the two environments was already significant ($P<0.01$). As expected, the soil water deficit developed further in the water stress environment as the experiment proceeded whereas it was maintained fairly constant at around 25-30 mm below field capacity in the control environment. At day 75, the soil water deficit under water stress treatment was 170 mm below field capacity while under well watered treatment it was around 23 mm. The rate of soil deficit development is presented in Fig. 3.1. The maximum rate of soil water deficit development occurred between day 16 and 30 (4.53 mm/day). The rate declined after 30 days.

Weather changes (see Appendix 3.1) influenced water deficit development. At the commencement of water stress treatment, slow water deficit development was generally related to the wet weather in that period of 15 days. Low temperature may have contributed towards the slow decline of soil water status.

3.3.2 Plant Data

3.3.2.1 Plant Parameters Indicating Water Deficit Level

a. Leaf relative water content (RWC)

Changes in leaf RWC in the two contrasting environments over the experimental period are presented in Table 3.2a.

Table 3.2a Leaf RWC (%) under 2 contrasting environments

Environment	days after water stress treatment							
	0	15	30	45	60	75	Sig.	LSD
Control	91.4	91.8	90.2	92.2	90.0	91.8	ns	
Water Stress	92.2	82.0	73.4	62.2	57.4	54.8	***	7.2
Sig.	ns	**	***	***	***	***		

Like the soil water status, the leaf RWC under water stress treatment was significantly lower throughout the experimental period. Leaf RWC on day 75 was only 55% in the water stress environment while it remained quite constant at about 90 to 92% under well watered control conditions. The rate of leaf RWC reduction is presented in Fig. 3.2. The decrease in leaf RWC under water stress treatment was fast in the first 45 days, averaging 0.67%/day and decreased gradually. It was slower (0.25%/day) between 45 and 75 days.

The time course of leaf RWC change of the 5 cultivars under water stress and the average of these cultivars under control conditions are presented in Table 3.2b.

Table 3.2b Leaf RWC of 5 cultivars under 2 contrasting environments

Environment		Days after water stress treatment							
		0	15	30	45	60	75	Sig.	LSD
control									
mean of 5 cultivars		91	92	90	92	90	92	ns	
water	Kopu	92	81	72	60	58	55	**	7
stress	Pitau	92	80	72	63	57	53	**	6
	Huia	93	84	75	60	56	55	**	7
	Tahora	90	81	73	65	57	54	**	8
	Whatawhata	94	84	75	63	59	57	**	8
Sig.		ns	ns	ns	ns	ns	ns		

Under control conditions the differences among the cultivars were small while under water stress environment, the differences were as large as 5% at any one time (Table 3.2b) but not significant. There were some differences in rates of leaf RWC reduction under water stress conditions in periods from 31 to 60 days but the mean reduction rate of the two periods was not significantly different nor was the mean reduction rate for the whole experiment (Fig. 3.3).

b. Canopy temperature:

Changes in canopy temperature of the 5 cultivars under the two environments over a 12-hour period are presented in Fig. 3.4a and b. Under well watered conditions, there was only up to 2^{o}C difference between cultivars at 13:30 hours (Fig. 3.4a), and it was not

Fig 3.2 Rate of leaf water deficit (RWC%) development under water stress environment(mean & SEM)

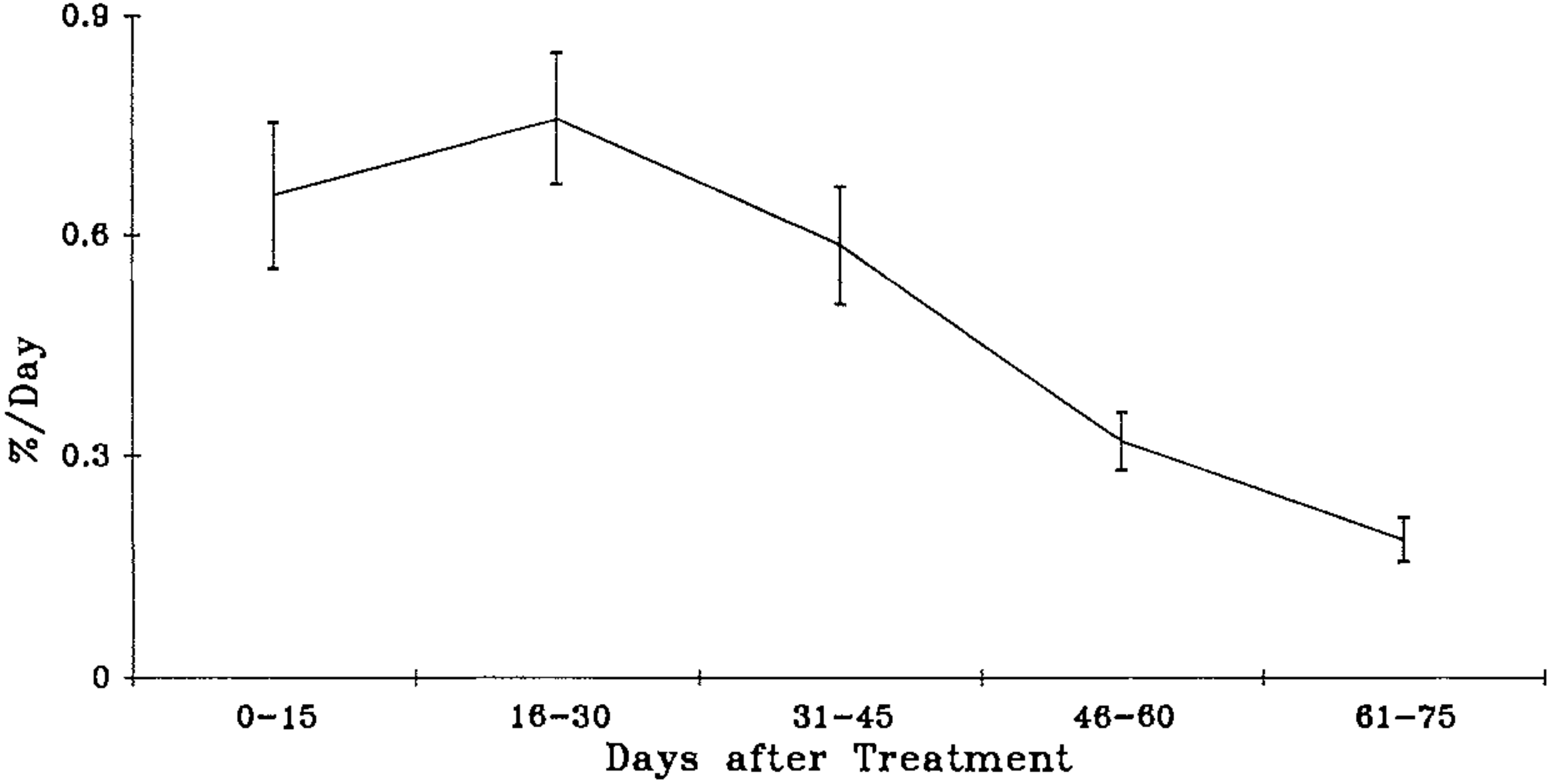


Fig. 3.3 Rate of leaf water deficit development of five cultivars under water stress environment

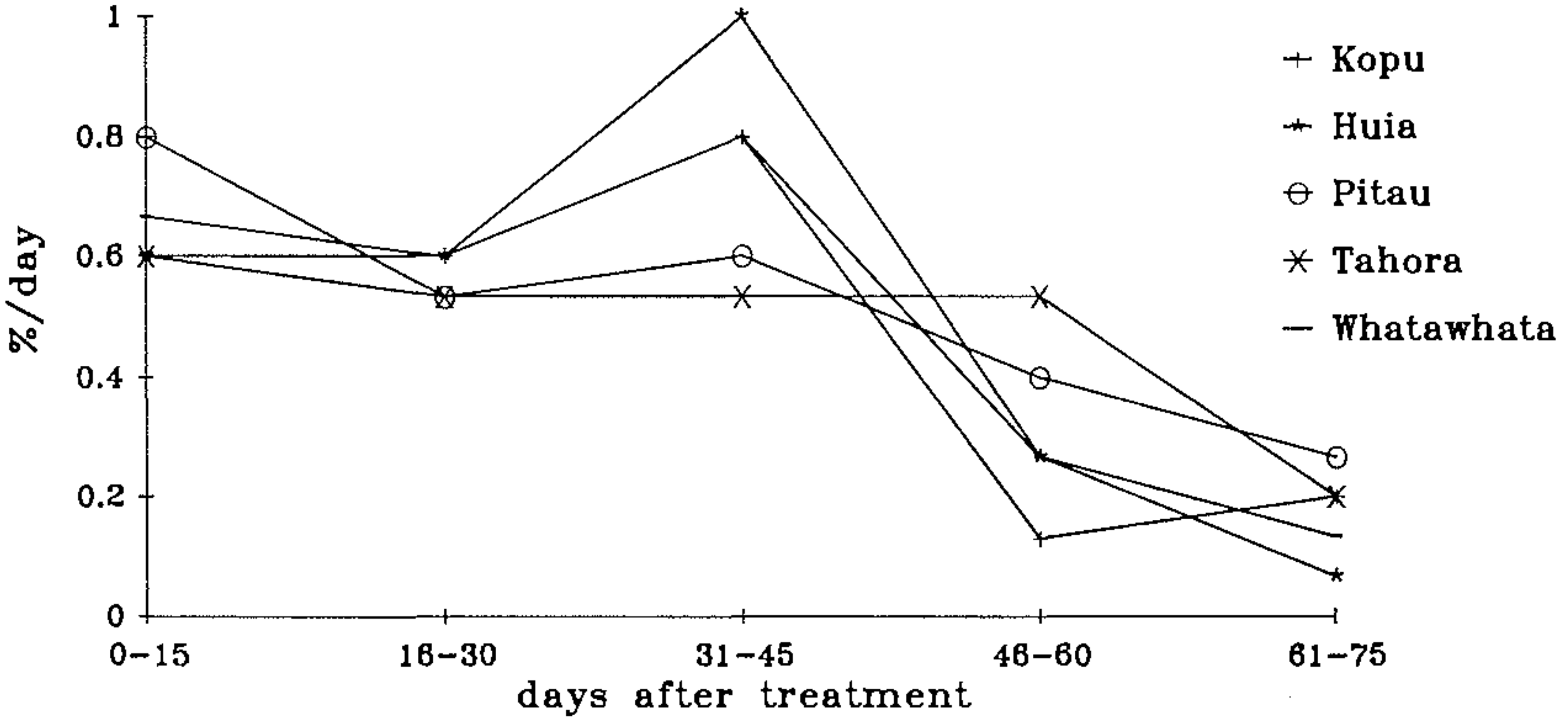


Fig. 3.4a Air and canopy temperatures under control environment

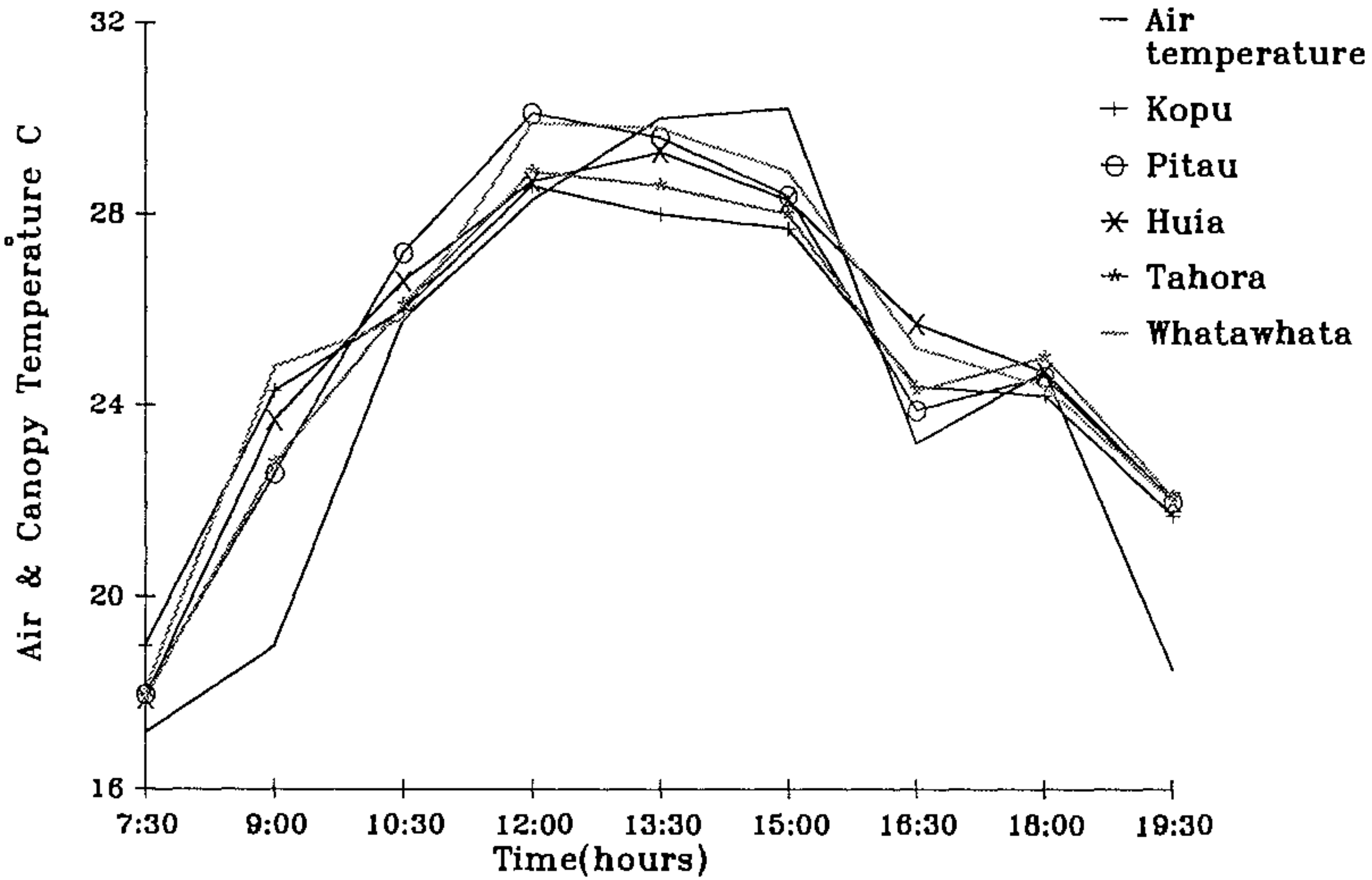
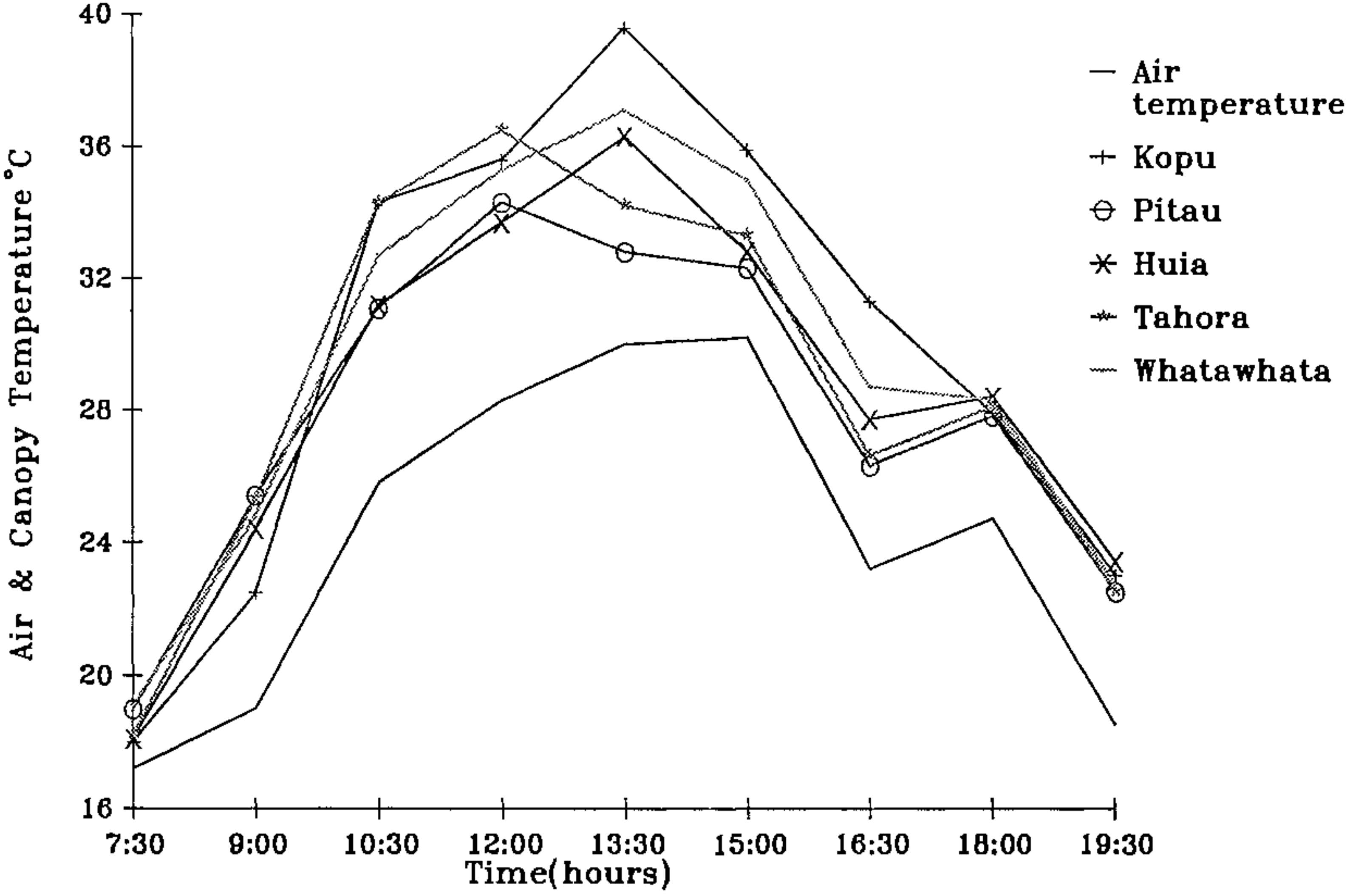


Fig. 3.4b Air and canopy temperatures under water stress environment



statistically significant. But under the water stress environment the differences among cultivars were large. At 13:30 hours, the highest canopy temperature was about 40°C (Kopu), significantly higher than the 33°C recorded for Pitau "canopy" ($P<0.05$). After 13:30 hours, the difference between the two cultivars maintained at about 4°C until 16:30 hours though it failed to reach statistical significance.

The canopy temperature in the water stress environment was usually higher than that of the control environment (Fig. 3.4b). The difference varied from only 1°C (between 7:30 and 9:00, 18:00 and 19:30 hours) ($P>0.05$) to more than 4°C ($P<0.05$) (between 10:30 and 15:00 hours). Between 16:30 and 18:00 hours, the difference was smaller (3°C) but it was still significant ($P<0.05$).

The relation between air and canopy temperature is shown in Fig. 3.5. Canopy temperature under control conditions increased at a similar rate as air temperature while in the water stress environment it increased at a much faster rate, 1.5°C for every 1°C air temperature increase ($P<0.05$). When air temperature was as low as 18°C (7:30 to 9:00 hours and 18:00 to 19:30 hours), the canopy temperatures in both environments were similar. But when air temperature increased to 30°C, the difference between the 2 contrasting environments was as much as 5-7°C.

3.3.2.2 Plant Growth Parameters

a. Leaf appearance rate

Mean leaf appearance rate (LAR), expressed in days leaf^{-1}, was significantly different between the environments (Table 3.3). The average LAR was 6.8 days under control conditions while it increased to 33 days per leaf under water stress environment.

There were significant differences among the cultivars under control conditions. Kopu, Pitau and Tahora had a similar leaf appearance rate, less than 6 days, while Whatawhata took more than 9 days to produce a leaf.

Though the differences in leaf appearance rate among the cultivars were seemingly bigger in water stress environment (maximum 14.6 days), they failed to reach significance because of the large variation within each cultivar (cv. 40-80%). There is a significant interaction between cultivars and environments, suggesting that the leaf growth of the 5 cultivars responded differently to water deficit stress treatments imposed here. It appeared that Kopu and Pitau were more severely affected by stress than Whatawhata and Huia.

Fig. 3.5 Relationship of air and canopy temperature under two environments

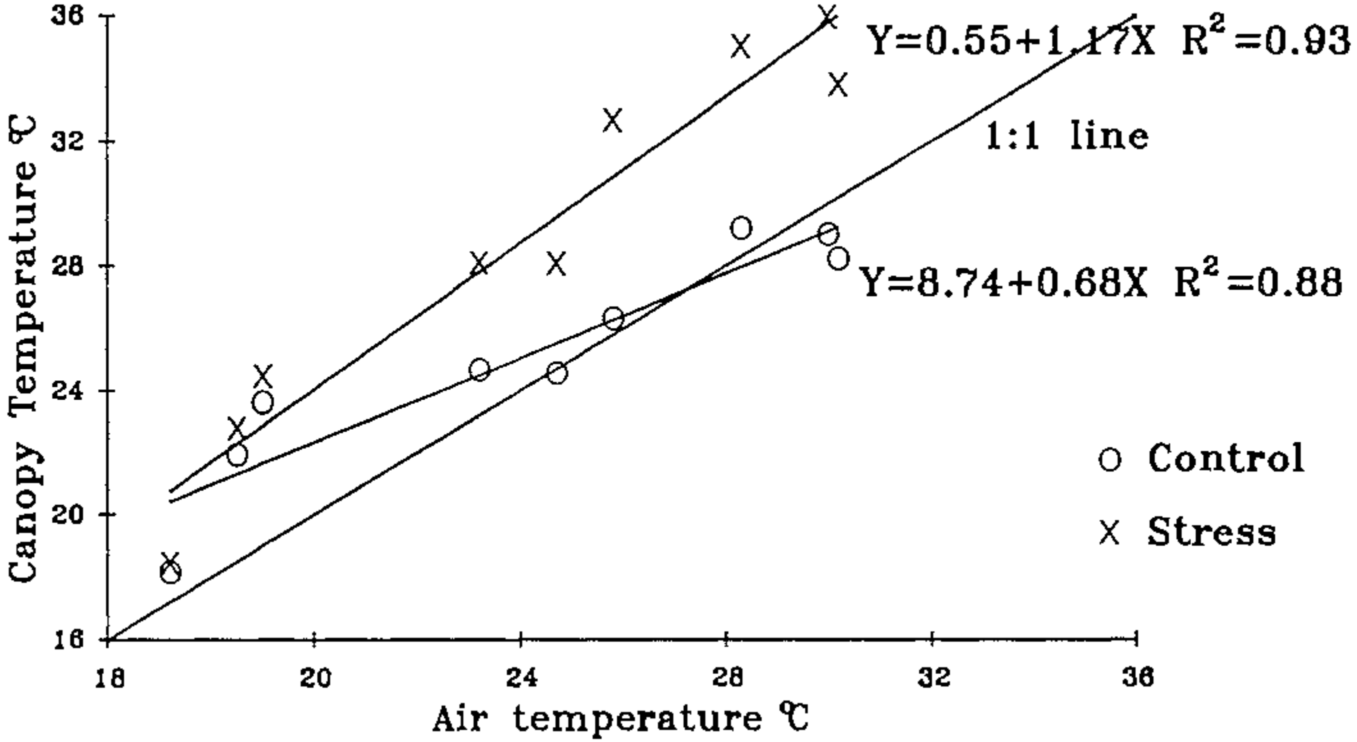


Table 3.3 Leaf appearance rate (days leaf^{-1}) of 5 cultivars under 2 environments

	Environments		
Cultivar	Control	Water stress	Sig.
Kopu	5.95	37.5	**
Pitau	5.88	37.5	**
Huia	7.14	25.0	**
Tahora	5.88	31.3	**
Whatawhata	9.09	22.9	**
Sig.(LSD)	*(2.92)	ns	
Int.(LSD)*	*(14.7)		

int. interaction significant level, LSD for between column comparison

b. Branch number per stolon

Table 3.4 Number of branches per mature stolon under 2 environments

	Environment		
Cultivar	Control	Water stress	Sig.
Kopu	9.87	6.99	**
Pitau	9.32	5.02	**
Huia	10.82	7.29	**
Tahora	11.55	7.89	**
Whatawhata	12.87	7.28	**
Sig.(LSD)	*(0.95)	*(1.99)	
Interaction	ns		

The effect of water stress on branch number per stolon is shown in Table 3.4. The mean number of branches at the end of the experiment was significantly reduced by the stress compared with that in the well watered environment (6.9 vs 10.9, $P<0.05$), representing a 37% reduction.

Under well watered conditions, Whatawhata had more branches than other cultivars, with Huia and Tahora intermediately behind. Kopu and Pitau had the least branches per marked stolon. There was no significant interaction, but the response of the cultivars, expressed simply as the number of branches reduced, was significantly different (eg. 2.9 for Kopu vs 5.5 for Whatawhata). The reduction was correlated with the number of branches under control conditions.

c. Number of leaves per stolon

The results on the number of leaves per stolon are shown in Table 3.5. The mean number of leaves per stolon was markedly reduced by water stress (4.9 vs 1.5, $P<0.05$).

There was a significant difference between cultivars in the number of leaves on a mature stolon under control conditions.

Table 3.5 Number of leaves per stolon under 2 environments

	Environment		
Cultivar	Control	Water stress	Sig.
Kopu	5.67	1.40	**
Pitau	4.47	1.67	**
Huia	4.73	1.73	**
Tahora	5.13	1.20	**
Whatawhata	4.73	1.60	**
Sig. (LSD)	*(0.90)	ns	
Interaction	ns		

However, the number of leaves under water stress treatment was small and the differences between cultivars were non-significant. Across all cultivars the reduction in number of leaves per stolon varied from only 3 for Huia to more than 5 for Kopu but the interaction failed to reach significance (P>0.05). Like leaf appearance rate and branch number, however, the reduction was greater for cultivars with more leaves under control conditions.

d. Leaflet length

Table 3.6 Leaflet length (mm) under 2 environments

	Environment		
Cultivar	Control	Water stress	Sig.
Kopu	19.5	10.9	***
Pitau	15.3	7.2	**
Huia	13.3	8.2	**
Tahora	10.4	8.2	**
Whatawhata	9.2	7.7	*
Sig. (LSD)	** (3.6)	* (2.5)	
Int. (LSD)	* (4.5)		

The mean leaflet length of each cultivar in the two environments is shown in Table 3.6. The reduction for all cultivars under water stress was 60% (P<0.01). Under control conditions, differences between cultivars in leaflet length were significant. The ranking of cultivars in order of their leaflet length was Kopu, Pitau, Huia, Tahora and Whatawhata. However, there were significant differences between the Kopu, Pitau & Huia group and Tahora & Whatawhata group. In the water stress environment, the absolute size was much smaller and the difference between cultivars was also small, though Kopu maintained a slightly larger leaflet size than the other four cultivars, which were similar (P<0.05).

There was significant interaction between environments and cultivars (P<0.05). This arises because of difference in reduction of leaflet size in large and small leaf-sized cultivars. For Kopu and Pitau, leaf size was reduced by 44 - 53%. Huia was reduced nearly 40%, and the small-leaved Whatawhata and Tahora were only reduced by about 16-21%.

e. Survival of growing points

Survival rate of growing point (ranging from branches to fully established stolons) in the two environments for the five cultivars is presented in Table 3.7. The proportion of growing points which survived the trial period was significantly lower under water stress environment, on average 78% compared with 94% under control conditions. Almost all of the growing points survived the trial period under the control environment whereas, under water stress environment, about 25% died.

Table 3.7 Growing point survival rate (%) under 2 environments

	Environment		
Cultivar	Control	Water stress	Sig.
Kopu	95	79	**
Pitau	98	91	**
Huia	92	75	**
Tahora	97	73	**
Whatawhata	89	64	**
Sig. (LSD)	ns	*(8.8)	
Int. (LSD)	*(15)		

Under a well watered condition the differences among the cultivars, by and large, was not significant. On the other hand, under water stress conditions, they were big enough to reach significance ($P<0.05$). Among the cultivars, Whatawhata had the lowest survival rate whereas Pitau maintained a high survival rate and the other 3 cultivars had a similar survival rate. The interaction was significant at 5% level. Whatawhata and Tahora were affected most, the growing point survival rate dropping by about 25%. Pitau was affected least.

f. Leaf duration:

The data on leaf duration of individual cultivars are presented in Table 3.8. Except for Whatawhata, water stress had prolonged leaf duration. Under water stress conditions, the leaf duration was 13.1 days longer than under control conditions.

There were significant differences among the five cultivars under the control conditions. Whatawhata had the longest leaf duration and Pitau the shortest (P<0.05). The differences among the cultivars under water stress treatment were also significant. Pitau had the longest leaf duration and Kopu the second longest. The interaction was significant due to the large difference in response to water stress by Whatawhata in comparison with Pitau and Kopu.

Table 3.8 Leaf duration (days) under 2 environments

	Environment		
Cultivar	Control	Water stress	Sig.
Kopu	33.7	52.5	**
Pitau	26.3	62.6	***
Huia	33.8	43.3	**
Tahora	30.2	37.6	*
Whatawhata	43.0	36.6	**
Sig. (LSD)	*(7.2)	*(9.8)	
Int. (LSD)	*(24.6)		

3.4 Discussion

3.4.1 Water Stress and Plant Water Status

Plant water status was very sensitive to reduction of soil water deficit when RWC was high (see Figs. 3.1 and 2). With further decreases of soil water status, the leaf RWC decrease was smaller. The final RWC of the plant, 55%, was 37% lower than the control. So the final stress level was more severe than the level considered as a severe water stress (Hsiao, 1973). The previous trial, carried out at the site with ryegrass, had created a moderate level of water stress (Barker, 1983). Beside the difference in trial length and possible climatic variation, the shallower and smaller root system of white clover (Evans, 1978) may be responsible for the severer stress, measured by its RWC, it suffered. The further reduction in soil water status between 45 to 75 days did not reduce the leaf RWC much, probably because plant water status had approached a minimal level, though there was no report on the lowest RWC white clover can have.

The differences among the cultivars of their leaf RWC failed to reach significance at any stage of the experiment even though there are differences among them in root type (Caradus, 1977) which may have caused variations in water utilization (Smith and Morrison, 1983). The large variation of RWC of a cultivar within treatment was so large (CV. 40-60%) that it perhaps disguised real differences among the cultivars in plant water status under the same water stress conditions. One possible reason for the large variation observed was microenvironmental variation under the field conditions. The lack of uniformity in the field could also be expected to introduce variation into measurements of plant growth and development.

Changes of the canopy temperature detected here under the two water deficit conditions were consistent with the established theory; namely, that canopy temperature reflects plant water status (Ehrler, 1973). What may be more interesting and probably difficult to explain is the difference of canopy temperature between cultivars, specifically Kopu and Pitau, under water stress conditions, although this difference was not related to their leaf water status. It is probably due to growth or morphological differences between these cultivars, such as the density of leaves in the canopy. Since the plants were grown single spaced and with fewer leaves under the stress conditions, the 'canopy' temperature reading could be affected by the soil temperature. Pitau had short internode and so would be expected to show less soil from its more clustered leaves. Kopu, on the other hand, tended to have long stolons, therefore its high 'canopy' temperature would reflect that more bare ground was being exposed amongst the leaves. The 'canopy' temperature, therefore, may also reflect the higher temperature environment experienced by stolons under moisture stress conditions.

3.4.2 Effect of Water Stress on Plant Growth Parameters

Water stress can cause significant reductions in plant growth (Hsiao, 1973). Results reported here indicated that the parameters measured were reduced significantly. But, in percentage terms, they were affected to different extents (Table 3.9).

Table 3.9 Reductions of Performance under Water Stress Treatment

	Reduction (%)					
Parameter\Cv.	Kopu	Pitau	Huia	Tahora	Whatawhata	mean
Leaf Appearance Rate	84	81	71	81	60	78
#. of Leaves per Stolon	75	63	63	77	66	69
Leaf Size	69	76	62	44	29	63
Number of Branches	29	46	33	32	43	37
Growing Point Survival	17	3	18	25	28	18

Estimating from the reduction percentage (Table 3.9), it seemed that the first 3 parameters which related to the leaf production were more severely affected by water stress than the other two parameters under the conditions of this experiment, mean reduction 70% vs 28%. In order to maintain high water status, it is important that a plant cut its total transpiration loss by reducing leaf area and\or transpiration rate. The water stressed white clover plants in this study have a smaller leaf area by reducing leaf appearance rate, number of leaves per stolon and leaf size while number of branches and growing point survival rate were only slightly reduced. The effect of water deficit stress on a particular parameter is dependent upon many factors. The physiological characteristics of these growth parameters may affect their sensitivity to water deficit stress.

Reduction in leaf appearance represented the reduced vitality of an apical meristem. But the survival rate of the growing point was not so severely affected. This may reflect that though water stress suffered by plants in this experiment may not have been high enough to kill a large number of growing points, it apparently was high enough to cause a large reduction in the growing point vitality.

The sensitivity of the growing point to water stress seemed to increase with number of total branches under control conditions. It can be argued that producing a large number of branches by the small-leaved cultivars is a strategy for foraging nutrients through selecting a desirable microniche in a harsh environment (Hutchinson and Slade, 1988). Therefore, high branching rate and high branch death rate may be two compensating characteristics. Thomas (1984) and Ueno and Yoshihara (1967) concluded from their work that drought had a little effect on stolon number. In the present experiment, the main stolons often persist well while the new branches usually fail to persist through the stress period. Therefore, it appears that the changes that occurred under stress conditions might be adaptive to water stress with a cost on their leaf production.

3.4.3 The Differences among Cultivars in Response to Water Stress

The five cultivars used in the experiment fall into three leaf-size categories, large- (Kopu, Pitau), medium- (Huia), and small-leaved (Tahora and Whatawhata) cultivars. The results clearly showed differences in certain morphological parameters between them under both water deficit levels. Sometimes, the differences between the cultivars of similar leaf size are also significant. Obviously these differences are associated with the normal plant morphological characteristics. Two of 5 parameters measured had significant interactions. Both interactions seemed to be due to different reduction caused by water stress in two or three cultivars, ranking either at the highest and the lowest for the parameter in question. Such unequal reductions in performance apparently were related to the potential performance level under control conditions. Thus the difference between them became smaller or the ranking could even be reversed. When the reduction of a growth parameter caused by water stress was compared with its normal value under the control environment, it was found that the reduction was generally correlated with their performance under control conditions. Such a relationship could be found in almost every parameter measured even though the other 3 parameters did nor record any interactions. It can only be speculated here that this is because in a plant, the most active process at the time of stress occurrence is normally most severely affected by stresses. What seems to be clear is that a better performed cultivar would be more severely affected than a lesser one.

3.5 Conclusions

1. Leaf water status, expressed as RWC, was affected significantly by soil water status. But by and large, there were no differences among the cultivars in their RWC.

2. Water stress seems to affect leaf area related growth parameters more severely than those related to growing point. The reduction caused by water stress was more than 56% for leaf appearance rate, leaf size and leaf number per mature stolon across all cultivars, whereas branch number and growing point survival rate were reduced only by about 37% (29-46%) and 18% (3-28%).

3. The responses to water stress were generally different between the five cultivars in the growth parameters measured. But no single cultivar consistently showed the largest or smallest changes under water stress conditions for all the parameters measured. Pitau had the largest reduction in 4 out of 5 parameters measured, but it had the smallest change in growing point survival rate. On the other hand, Whatawhata had the smallest changes in leaf appearance rate and leaf size and had the lowest growing point survival rate (68% cf. 93% for Pitau).

4. Quantitatively, the reduction in performance caused by water stress in each cultivar for leaf appearance rate, branch number, leaf size and leaf number were positively correlated with its performance under well watered conditions. In contrast, the growing point survival rate was negatively correlated. So, the sensitivity of a cultivar to water stress, expressed as the reduction of each parameter, was generally negatively correlated to its performance under control conditions.

CHAPTER FOUR
THE EFFECT OF THREE WATER DEFICIT LEVELS ON THE GROWTH OF FIVE NEW ZEALAND WHITE CLOVER CULTIVARS

4.1 Introduction

The detrimental effect of water stress in reducing white clover growth is well documented (for example, Thomas, 1984). In Chapter 3, an experiment comparing the performance of five New Zealand white clover cultivars grown under field conditions with and without water deficit stress was reported. Differences in response to water stress between these cultivars were detected. The main difference was in leaf appearance rate and leaf number per stolon. For example, Whatawhata had the lowest leaf appearance rate under well watered control conditions but the highest under water stress conditions. In contrast, Kopu and Pitau had lower leaf appearance rates under water stress conditions even though they had higher ones under control conditions. Although such differences were detected between cultivars, the differences among the cultivars in the parameters measured were by no means consistent.

The results also suggested that the effect of water stress on a cultivar, expressed as the reduction of a parameter, was positively associated with the cultivar's performance under well watered control conditions (except for growing point survival rate, which was negatively associated). In essence cultivars ranked higher on the performance scale under well watered control conditions tended to be affected more severely by water stress, while cultivars ranked low under normal conditions were less affected. Ecologically, this is an interesting point because it means that the 'better' performing cultivars would be 'harder' hit by water stress.

However, field variation made further detailed analysis of processes underlying these observations difficult. For example, although the mean relative water content (RWC) of each cultivar was similar, the intrinsic variation for each cultivar was large, resulting in coefficients of variation greater than 40%. The previous experiment was also limited to one level of water stress under a rain-out shelter so that it was difficult to construct a water stress response pattern. This chapter reports an experiment designed and conducted in the glasshouse where conditions were more uniform. The objective was to detect the differences in growth responses between the same five New Zealand white clover cultivars under three different soil moisture regimes and confirm the results of the previous experiment in glasshouse conditions.

4.2 Material and Methods

4.2.1 Plants and Experimental Site

The experiment was carried out from March to May, 1986. Five white clover cultivars commonly used in New Zealand (see section 4.2.2) were grown in pots in glasshouse No. 16, Plant Growth Unit, Massey University, Palmerston North, New Zealand. The glasshouse was maintained at between 18 and 25°C with a heating and ventilation system.

Four litre planting bags were used as "pots". The planting medium contained peat, pumice and sand (70:20:10 on volume basis). A mixture of fertilizers, P, K, S, lime and micronutrients was mixed with the medium at 0.45 g/pot (approximately 1500 Kg/ha). The same fertilizer mixture was applied to the plants at 0.3 g/pot (approximately 1000 kg/ha) once again at week 4 after the commencement of the experiment.

The plants were vegetatively propagated from those used in the field experiment reported in Chapter 3. The cuttings were taken on 1 February, 1985 and propagated in the glasshouse. They were transplanted into pots, one in each pot, on 17 February, 1985. After growing in pots for 5 weeks prior to the commencement of the experiment, the plants were defoliated and cut to a uniform size of about 5 main stolons.

4.2.2 Design and Treatments

A completely randomised block design was used in the experiment. The treatments applied were:

a) five white clover cultivars:

Large-leaved cultivars:	Kopu, Pitau
Medium-leaved cultivars:	Huia
Small-leaved cultivars:	Tahora, Whatawhata

b) three water deficit levels:

Control:	30% (w/w) of soil water content
Mild stress:	17-20% of soil water content
Severe stress:	4% of soil water content

The treatment levels were maintained by adding water equivalent to the amount of water loss daily, calculated by weighing 5 sample pots. There were five replicates in each harvest for each combination of the treatments except for harvest one, which had three replicates. There were, in total, 4 harvests at 20 day intervals.

4.2.3 Measurements

4.2.3.1 Non-destructive Measurements

These measurements were carried out in harvest 3 only.

Leaf number: The number of leaves was measured using wire tags around the stolons as a reference point. A tag was put on the internode of the third youngest node of a stolon which had at least 8 nodes. The number of leaves was counted forward (towards the apical bud) every second day and backward at the end of the experiment.

Leaflet, petiole and internode length: Leaflet and petiole length were measured on the most recently emerged leaf every day for five days, whereas internode length was measured every second day for five days, on the youngest visible internode. They were all measured to an accuracy of 0.5mm.

Leaf development stage: The leaf development stage was estimated for the most recently emerged leaf, and periodically estimated until it reached full senescence. A modification of Carlson's scale (Carlson, 1966) was used for the estimation of development. Two more levels of index were used to describe the change after full expansion, i.e. 2 for partial yellow, 3, total yellow or dead.

Leaf relative water content (RWC): Leaf RWC was measured by the same method as described in Chapter 3 (Barrs, 1968, see Appendix 3.3 for detail). Six to nine leaflets were taken at 12:00 - 13:00 hour every 2 days. RWC was measured on 4 replicates in 4 periods, day 0 to 7, day 20 to 27, day 40 to 47 and day 60 to 67.

4.2.3.2 Destructive Measurements

There were 4 destructive harvests, at 20 day intervals subsequent to the harvest just prior to the imposition of the water deficit treatment. Plant and component dry weight (DW) were measured on the plants being destructively harvested. Plants were dissected first into shoots and roots. For 2 replicates in harvest 2, the shoot component was further dissected into leaf, petiole and stolon. The samples were dried at 80°C for 48 hours and then the DW of each component recorded.

4.2.4 Statistical Analysis

The data were analysed with a statistical package, SPSSx (Nie *et al*, 1983). ANOVA was used to test the main treatment effects and interactions between the effects of all parameters measured.

4.3 **Results and Discussion**

4.3.1 Introduction

The selection of water deficit levels used as the water stress treatment is based upon a preliminary trial in which these levels resulted in leaf RWC >90%, 70% to 80% and <70% respectively. The results, apart from those on the soil water status, are presented in sections organized according to the plant parameters, within which the effects of water deficit level, cultivar and their interaction are discussed. After that a brief discussion, bringing these parameters together to indicate the relationship between them, will be presented.

Soil water status under three water deficit levels, measured as water content (w/w), were significantly different. There were no significant differences between the five cultivars and no significant interaction between water deficit level and cultivars. This indicates that the water deficit management in the experiment was carried out successfully with the same water status created for all the cultivars. The result of soil water status measurement and its statistical analysis are presented in Appendix 4.1.

4.3.2 Relative Water Content (RWC)

Mean leaf RWC for four measurement periods at three levels of water deficit treatment for the five cultivars are presented in Fig. 4.1 and Table 4.1 respectively. The significant difference between the three levels of water deficit treatment in all four periods indicated that the plants, for most of the time during the experiment, were at three distinctive internal water deficit levels. Mean RWC at the two water stress levels was 19% and 29% below that of the control plants respectively. Based on Hsiao's water stress level (1973), they were at moderate and severe stress levels respectively. Most plant growth and development processes are affected by such levels of water stress, though the severity of the effect can be different (Hsiao, 1973). The RWC values of the two stress levels also were equivalent to about -1 and -2 bar according to the relationship between white clover leaf RWC and water potential obtained by Johns (1978).

Fig. 4.1 Leaf relative water content (RWC) under 3 levels of water deficit (mean & SEM)

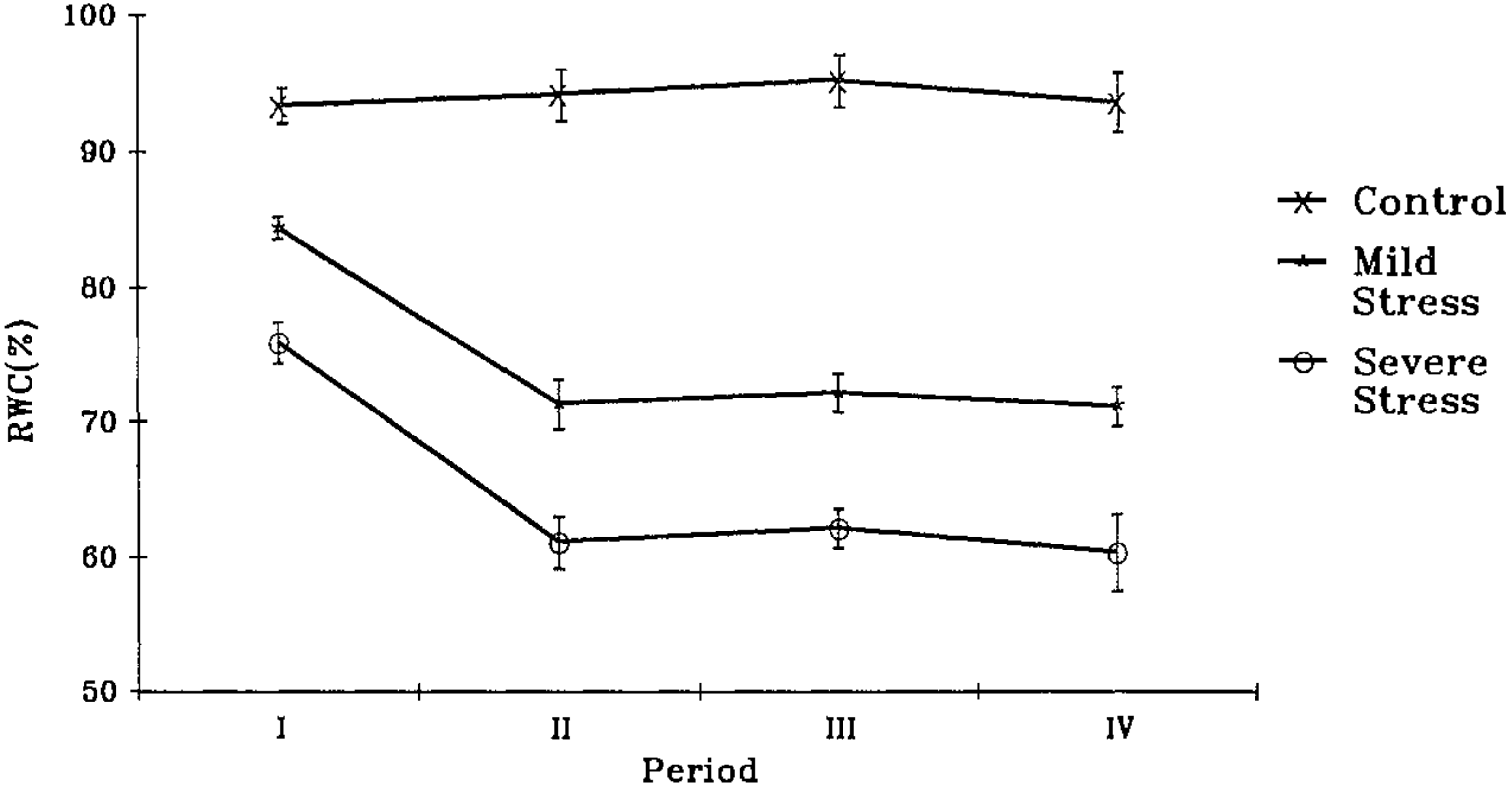


Table 4.1 RWC of 5 cultivars under 3 levels of water deficit

	Water level				
Cultivar	Control	Mild stress	Severe stress	Sig.	LSD
Kopu	93.35	69.34	66.92	**	4.22
Pitau	93.33	76.10	67.10	**	5.13
Huia	91.93	69.00	58.36	**	4.20
Tahora	97.72	75.66	63.35	**	6.21
Whatawhata	93.51	66.19	59.67	**	3.90
Sig.	ns	ns	ns		

The mean RWC of the five cultivars in the experiment did not differ significantly and was fairly consistent for the duration of the experiment (Table 4.1). There was no significant interaction between cultivar and water deficit levels. This is similar to the results for medium water status (Appendix 4.1), indicating that the cultivars responded to the three water deficit levels in a similar manner so that they were at similar levels of internal water deficit with each water deficit treatment. Intraspecies differences in water status are probably difficult to establish, unless one of the cultivars had been selected for dehydration avoidance.

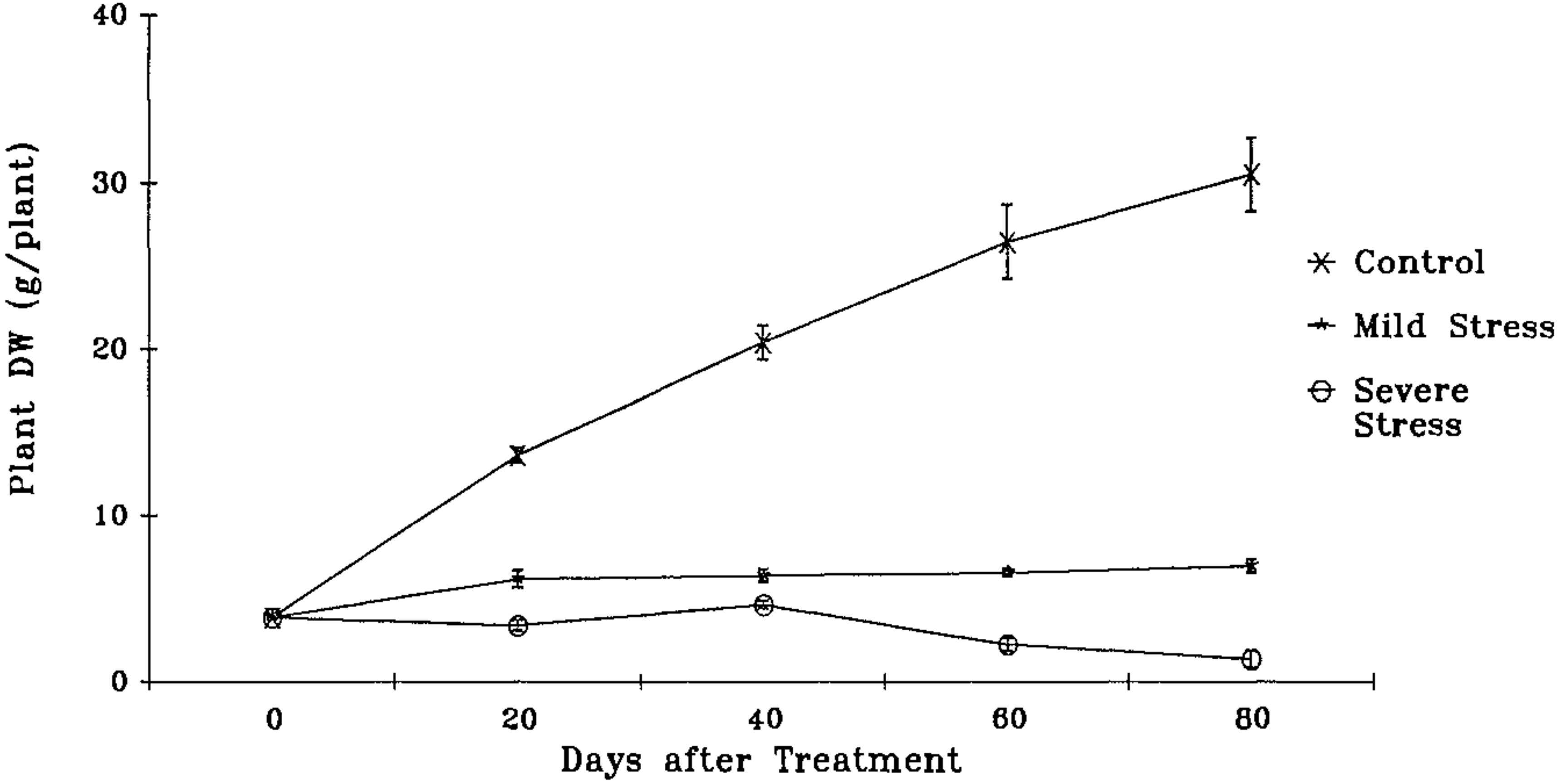
4.3.3 Plant Dry Weight (DW) and DW Partitioning

4.3.3.1 Total Plant DW

The mean plant DW of all cultivars at the three levels of water deficit treatment during the experiment is presented in Fig. 4.2.

Total DW accumulation was reduced significantly by water stress from day 20 (harvest 2). In the final harvest, plant DW at the two stress levels was reduced to 23% and 4% of control plants. This is in agreement with results from other experiments (eg. Chu, 1979). The reduced DM accumulation is often considered to be caused, in the short term, by senescence and, in the long term, by the reduction of both leaf area (LA) and photosynthesis per unit LA (Hsiao, 1973). The difference between the two water stress levels was also significant except at day 40 (harvest 3). Some positive growth occurred from 20 d to 40 d in severely stressed plants, probably as a result of secondary growth. Such secondary growth after possible adaptation to initial water stress has also been observed in tropical legumes (Chu, pers. comm.). Overall, the stressed plants had little or even negative DM accumulation, while results from other sections will show that they still

Fig. 4.2 Effect of water deficit level on white clover DW (mean & SEM)



maintained growth in the apical meristem. The large reduction of DW surely is the main detrimental effect of water stress on white clover.

So cultivar differences in DW were largely non-significant, though, at 80 d (final harvest), the DW of Kopu was slightly higher than Huia and Tahora (Fig. 4.3). As will be shown later, the distribution of dry matter between components (leaf, stolon and root) differed among cultivars. There were no significant differences among the cultivars when they were not water stressed (Fig. 4.4a). The effect of water stress on DW was largely similar except in Harvests 3 and 4 (Fig. 4.4b,c). The interaction between cultivar and water deficit treatment was significant at 60 d. Under severe stress, Huia had a recovery growth from 40 d to 60 d while other cultivars had the such a period of growth from 20 d to 40 d. The significance of the different timing of such recovery growth under severe stress is not clear, but if it really reflects a secondary growth after adaptation, then this may suggest that Huia became adapted to water stress slightly later. Comparing the DW of Huia at harvest 4 with that of the other four cultivars at harvest 3, no significant difference was found, indicating similar levels of DW accumulation at the same stages of adaptation.

4.3.3.2 DW of Plant Components and Their Ratios

According to Davidson (1969) and Thornley (1972), plants can react to environmental stress by maintaining a functional equilibrium between root and shoot. The main functional components in white clover plants are leaf (photosynthesis), stolon (vegetative growth) and root (water and nutrient absorption and N-fixation). Separating the total DW into plant components (leaf lamina and petiole, stolon and root), it becomes clear that though the DW of every component was reduced under water stress conditions in comparison with that of the control plants, the reduction was much larger for leaf and petiole components (75% and 90% at the two stress levels respectively) than for root and stolon components (Table 4.2). Both leaf growth and senescence are very sensitive to water stress (Hsiao, 1973). Plants can avoid water stress by reducing the rate of water loss through reducing leaf area (LA) and transpiration (Turner, 1978; Levitt, 1980). Because the stomatal control of transpiration in white clover under water deficit conditions is not considered sensitive (Johns, 1978; Kerr and McPherson, 1978), the large reduction of leaf and petiole components suggests that transpiration reduction may be achieved primarily through a decrease in LA. The large decrease of leaf and petiole DW, mainly due to senescence, is also affected by the changed DM partitioning within the plant under stress conditions. DW partitioning under water stress conditions is in a direction that favours root growth and activity (supplying water, nutrients and fixed N). This observation fits in with the theory of functional balance within plants (Thornley, 1972). Results from this experiment indicated that the growth of stolon, which was the main part of the shoot, was

Fig. 4.3 Mean Plant DW of Five Cultivars

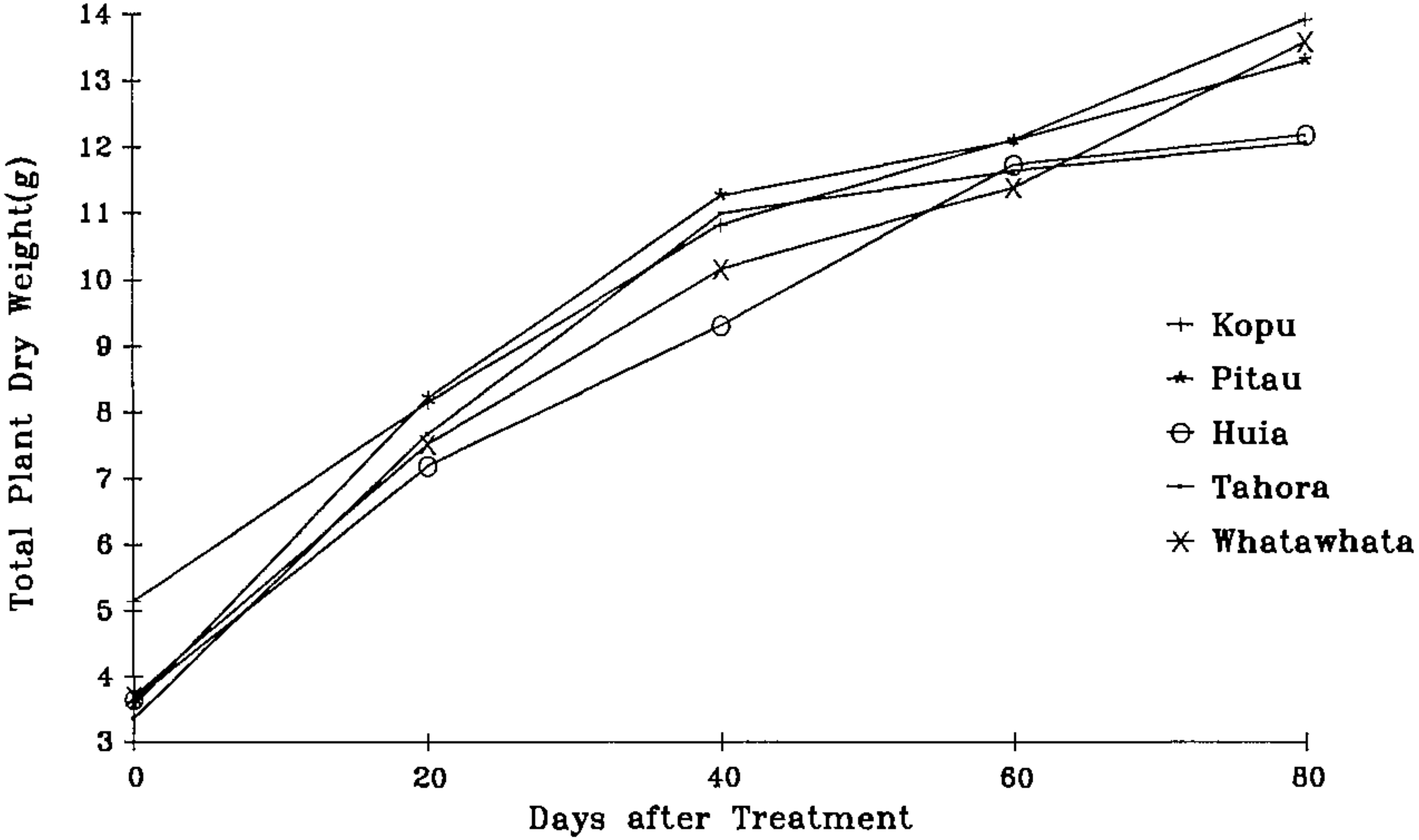


Fig. 4.4a Mean Plant DW of Five Cultivars - Control

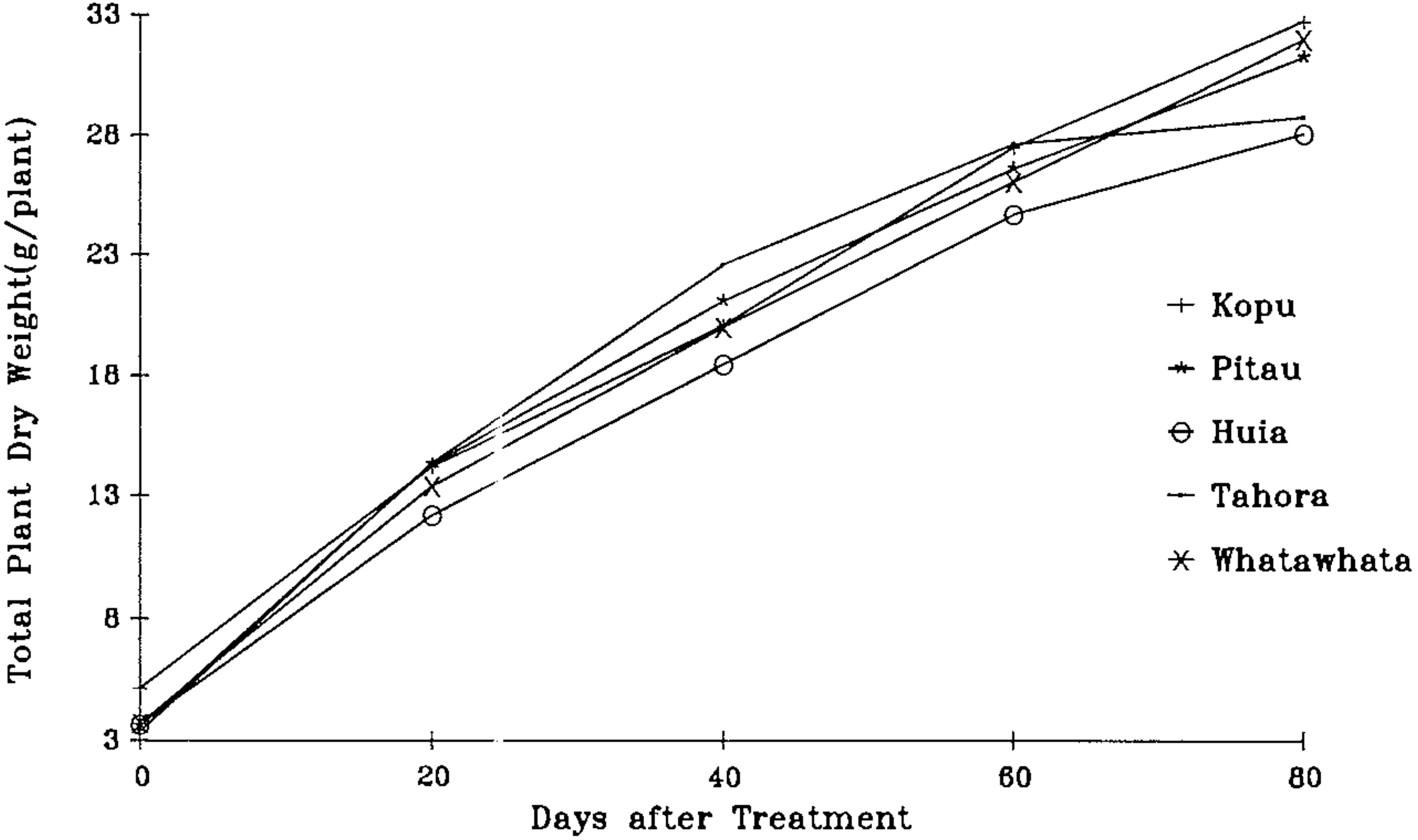


Fig. 4.4b Mean Plant DW of Five Cultivars - Mild Stress

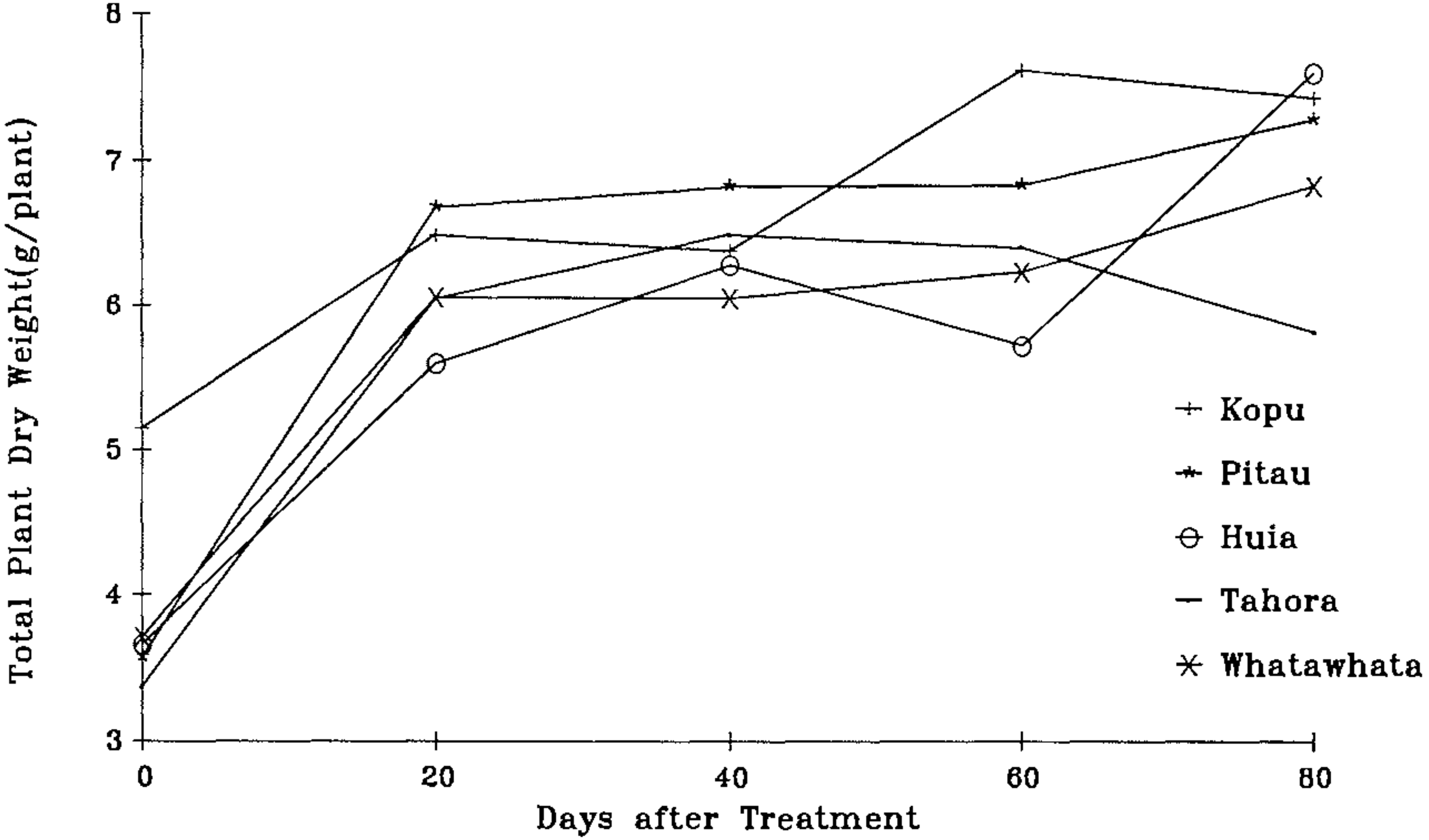
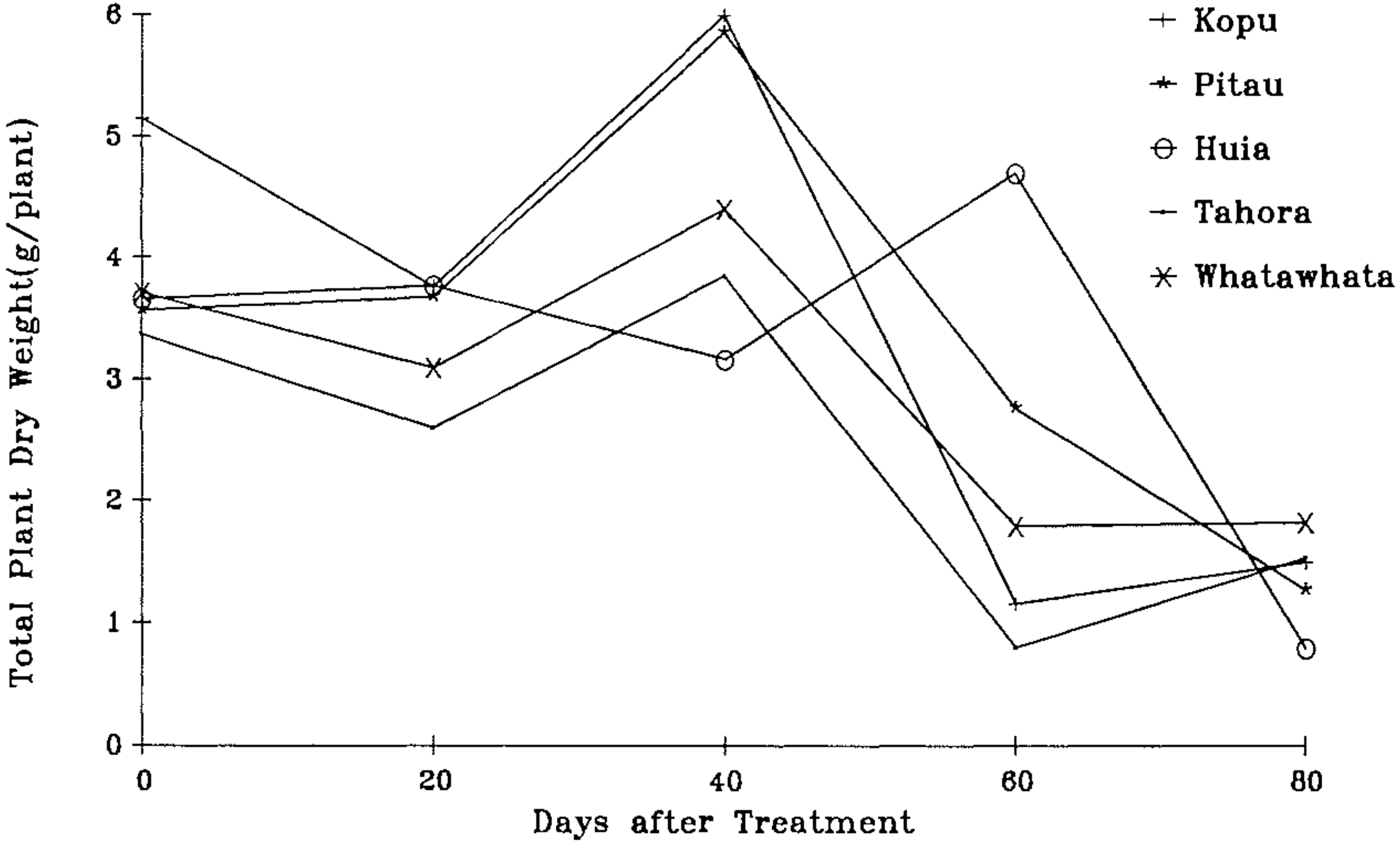


Fig. 4.4c Mean Plant DW of Five Cultivars - Severe Stress



not as severely affected by water stress as leaf or petiole, so that the shoot:root ratio was not significantly changed by water stress treatment (Fig. 4.5a).

Table 4.2 Effect of Water Deficit Levels on Plant Component DM (g)

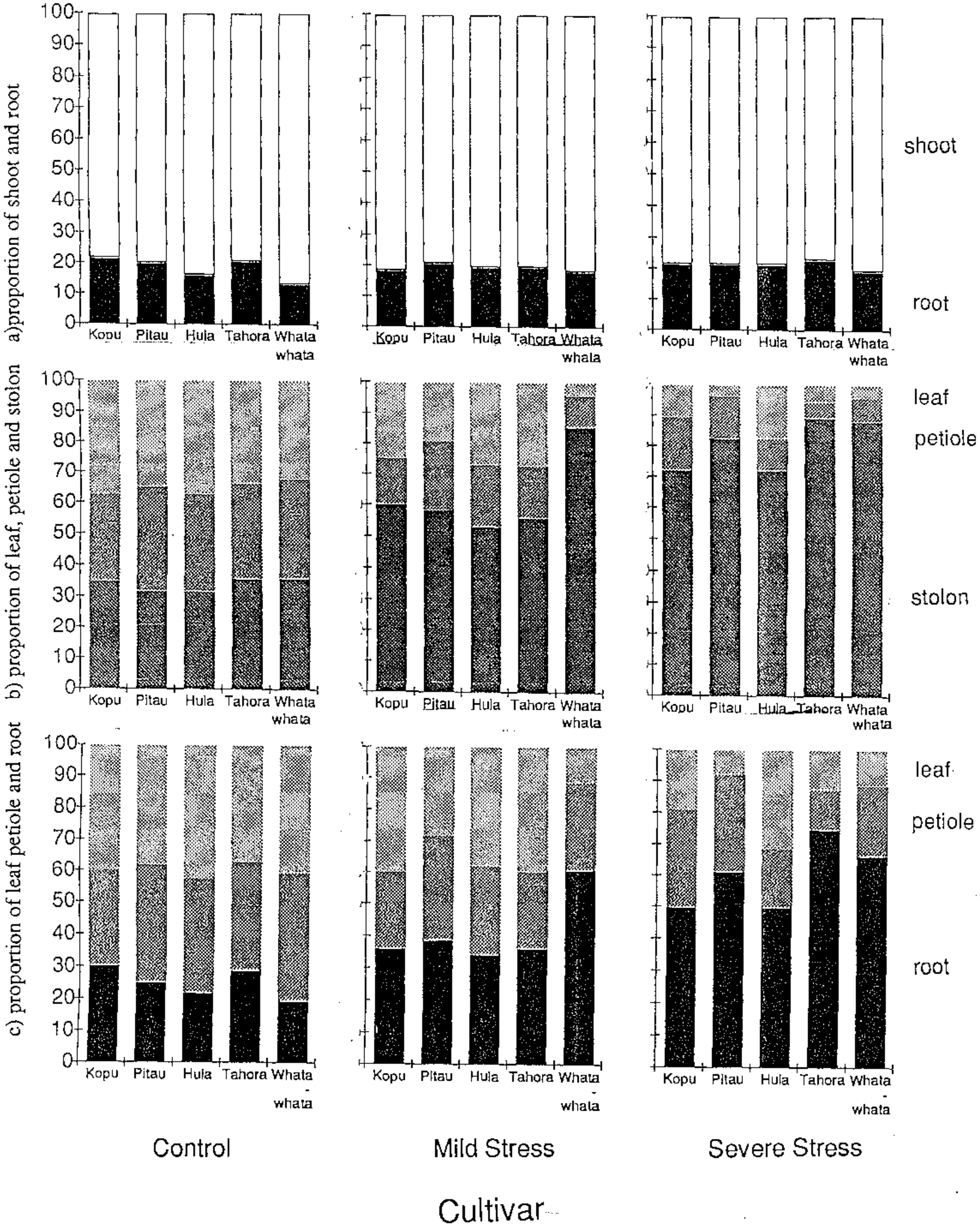
Water Level	Leaf	Petiole	stolon	Shoot(s)	Root(r)	s:r
Control	3.9	3.3	3.9	11.1	2.5	4.44
Mild Stress	1.0	0.8	3.1	4.9	1.2	4.08
Severe Stress	0.2	0.3	2.1	2.6	0.7	3.71
Sig.(LSD)	***(0.5)	***(0.4)	***(0.9)	***(1.8)	**(0.4)	ns

Among the cultivars there were significant differences in most components (Table 4.3). Leaf DW was positively associated with the leaf size category (Table 4.3). For white clover, leaf and petiole components can be considered to constitute the main harvestable yield (Gallagher and Briscoe, 1978), which has been found to be correlated with leaf size (Davies and Young, 1967). Whatawhata and Tahora had a significantly smaller leaf component and a larger stolon component while Huia had a relatively larger leaf component, similar to the two large-leaved cultivars. So Huia has a higher harvestable proportion than Whatawhata and Tahora, confirming its important agronomic role across a range of soil water deficits. At the other extreme, Whatawhata had a high stolon component which should favour its persistence. The analysis here and evidence from other workers (eg. Davies and Young, 1967) shows that the difference in DM partitioning is mainly related to the leaf size category, though selection within leaf size categorise may also be useful in improving yield.

Table 4.3 Mean Plant Component DM Yield of Five Cultivars

Cultivars	Leaf	Petiole	Stolon	Shoot(s)	Root(r)	s:r
Kopu	1.9	1.4	3.1	6.4	1.7	3.76
Pitau	1.8	1.6	3.1	6.5	1.7	3.82
Huia	1.8	1.5	2.6	5.9	1.4	4.21
Tahora	1.5	1.5	3.1	6.1	1.4	4.36
Whatawhata	1.3	1.5	3.5	6.3	1.3	4.85
Sig.(LSD)	**(0.3)	ns	**(0.4)	*(0.5)	*(0.2)	*(0.9)

Fig. 4.5 Proportion of white clover components under three water levels



As for the response of different cultivars to water stress treatment levels, Whatawhata seemed to behave somewhat differently from other cultivars. At mild water stress, the leaf and petiole DW of Whatawhata was most severely reduced, giving that cultivar the lowest proportion of leaf and petiole in relation to stolon and root (Figs. 4.5b,c). Other cultivars had smaller reductions in leaf and petiole DM and their proportion in total plant DM at the mild water stress level. The low proportion of leaf plus petiole of Whatawhata was probably due to the relatively greater reduction of leaf than that of stolon and root, resulting in a smaller ratio of total number of leaves to total number of nodes (Wang, unpublished data). Whatawhata is an early flowering hill country selection and considered to be persistent in drought conditions (MacFarlane and Sheath, 1984). Reducing leaf and petiole proportion at a mild level of water deficit may well be a mechanism of adjustment to resist more severe stress which may follow. The ratio of leaf plus petiole to stolon or root was not significantly different under severe stress among the cultivars, though Tahora and Whatawhata had lower values.

In comparison to the relatively large reduction of leaf and petiole DW, stolon and root DW were more constant under stress conditions (Table 4.2), particularly the stolon weight which was reduced proportionally less than other components. The DW of whole shoot was reduced no more than that of root so that the shoot:root ratio was not significantly reduced in the present water stress treatment. The relative growth rates of root and shoot from harvest 1 to harvest 2 were analysed. Since the plants were still in an early exponential growth period, according to Troughton (1956), the ratio of their relative growth rates could be more useful in comparisons of growth than the ratio of their absolute DW. The ratio of the relative growth rate of shoot and root was obtained with the following allometric formula (Troughton, 1956):

$$DW_{root} = b * DW_{shoot}^{K}$$

In the formula, b is a constant and k is a constant equal to the ratio of the relative growth rates of root and shoot. Troughton (1956) and Gale (1979) have shown that, in constant environmental conditions, k is constant. Its change indicates a change of plant growth pattern. In the early period of the present experiment (0 d to 20 d), k was decreased by water stress (pooled result of the two water stress levels since the difference between them was not significant) (1.53 vs 0.96, se. 0.28), indicating that root growth was slightly more retarded. The causes of poor root growth are not evident, but the trend soon changed. Over a prolonged period, by 40, 60 and 80 d, not only the shoot:root ratio was decreased (4.6, 4.0 and 3.6 for control, mild stress and severe stress respectively, se. 0.02), the relative growth rate of root in relation to shoot, k, has also increased (0.72 vs 1.02, se.

0.13), suggesting that a change of DM partitioning pattern in favour of root growth has occurred (Davidson, 1969; and Thornley, 1972). The change of ratio of shoot and root relative growth may have reflected the process of this DM partitioning pattern change. Authors of several review articles agree that the shoot:root ratio generally decreases in response to water stress, and some regard it as an adaptive change (eg. Levitt, 1972). However, in the early period of stress, the direction of shoot:root ratio change is dependent upon many factors and is a result of combined effects of senescence and increased partitioning (Gale, 1979).

With the decrease of leaf size category, the shoot:root ratio increased (Table 4.3). It appeared that a relatively large stolon component in small-leaved cultivars is responsible for that. Whatawhata had a particularly large shoot component. But it had a small leaf component; so the relatively larger shoot component may not result in advantages in either DM production or harvestable yield in smaller cultivars.

A similar analysis of shoot and root relative growth rate was carried out for a comparison of cultivars. It was found that the ratio of root to shoot relative growth rate, k, was higher for Whatawhata than Kopu and Huia (1.81 vs 1.08, se=0.28), due most likely to a relatively large reduction of leaf and petiole senescence of Whatawhata under stress conditions.

4.3.4 Morphological Parameters

The number of leaves per stolon and the size (length in mm) of mature leaflets, petioles and internodes are shown in Table 4.4. They were reduced significantly by the mild and severe stress treatments (30 to 60% for leaf number and 40 to 73% for organ size respectively). Leaflet and petiole lengths were reduced more than leaf number and internode length, especially under severe stress conditions.

The difference in response among these parameters may be related to the growth patterns of the various organs. Leaf and petiole growth after emergence is predominantly cell expansion (Thomas, 1987) whereas leaf appearance, occurring in the apical region, suffers less influence from reduced water status, though it may be affected by the limited supply of assimilate to the growing point (Haystead, 1983).

The severe reduction of leaf number and leaflet size results in a smaller LA. The general reduction (fewer leaves, smaller leaflet, petiole and internode) is a widely observed stress response (eg. Chu, 1979). This usually results in a more compact plant stature and reduces total water consumption.

Table 4.4 Effect of water stress on no. of leaves per stolon and the size of leaflet, petiole and internode

Water level	Leaves stolon^{-1}	Leaflet length (mm)	Petiole length (mm)	Internode length (mm)
Control	7.8	14.0	59.0	13.9
Mild Stress	4.1	9.6	25.0	8.5
Severe Stress	3.2	7.6	16.2	8.2
Sig. (LSD)	***(1.5)	***(1.7)	***(6.9)	***(3.8)

Significant differences in leaf, petiole and internode size were detected among the five cultivars. There was no difference in number of leaves per stolon (Table 4.5). The ranking of cultivars in size of leaflet, petiole and internode was, by and large, in accordance with the leaf size category, though Huia was ranked lower than expected for its leaflet size. The size of white clover organs is interrelated, indicating a general pattern of growth.

Table 4.5 Differences between cultivars in leaf, petiole, internode size and leaves stolon^{-1}

Cultivar	Leaves stolon^{-1}	Leaflet length (mm)	Petiole length (mm)	Internode length (mm)
Kopu	5.4	13.2	38.2	14.9
Pitau	5.2	10.3	30.9	12.8
Huia	5.2	9.3	36.7	10.2
Tahora	4.5	9.9	30.9	7.8
Whatawhata	5.1	9.2	30.1	9.1
Sig. (LSD)	ns	***(0.6)	**(5.5)	***(2.1)

The significant interactions between cultivar and water level for number of leaves per stolon, leaflet length, petiole length and internode length were an indication of cultivar differences in water stress responses. Looking at the number of leaves/stolon, Kopu had a significantly greater number than Pitau under control conditions, but this difference disappeared at both water stress levels. The significant interaction between water treatment

Fig. 4.6 Relationship between performance under control and Reduction under Two Water Stress Levels

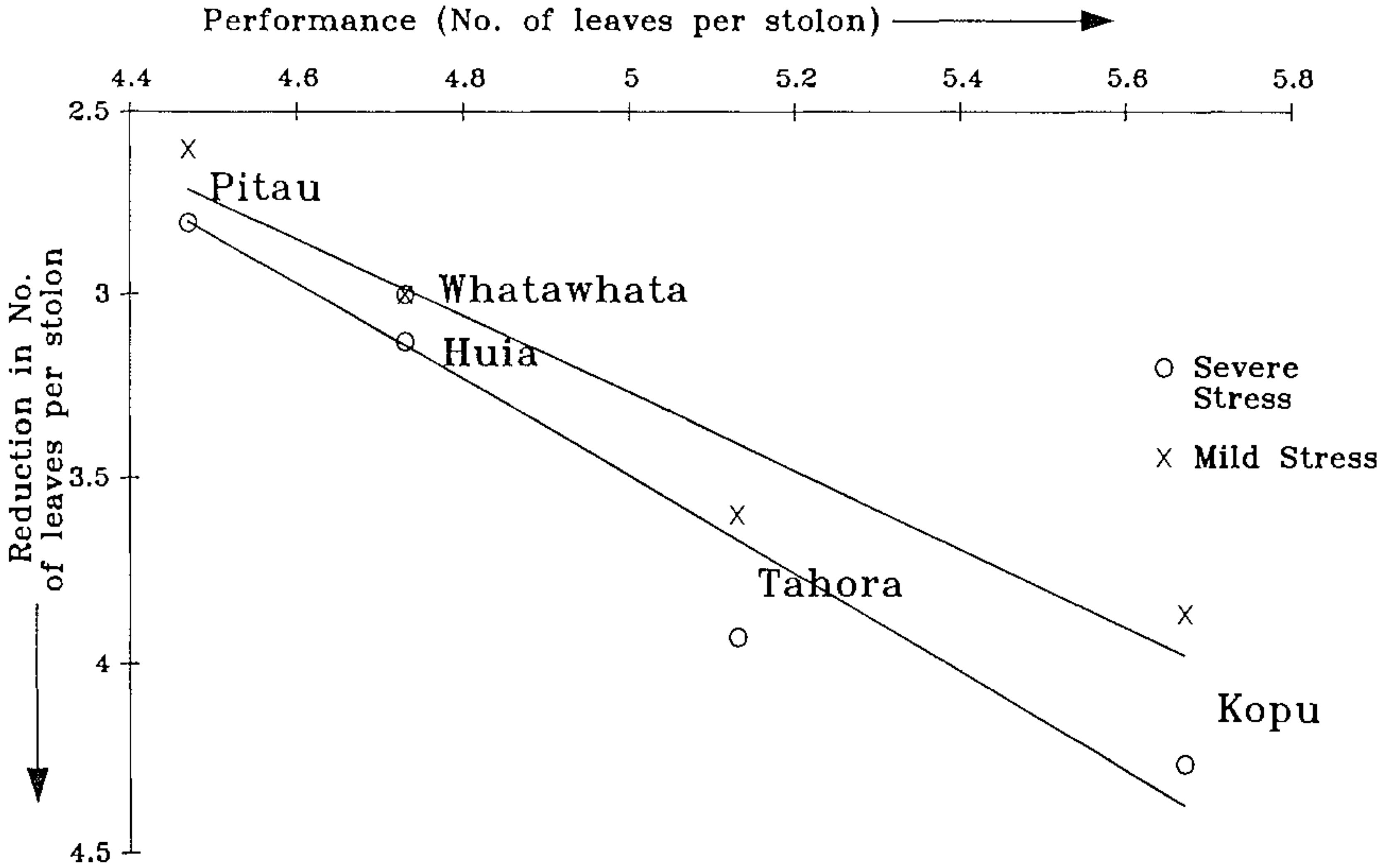


Fig. 4.7 Relationship between performance under control and Reduction under Two Water Stress Levels

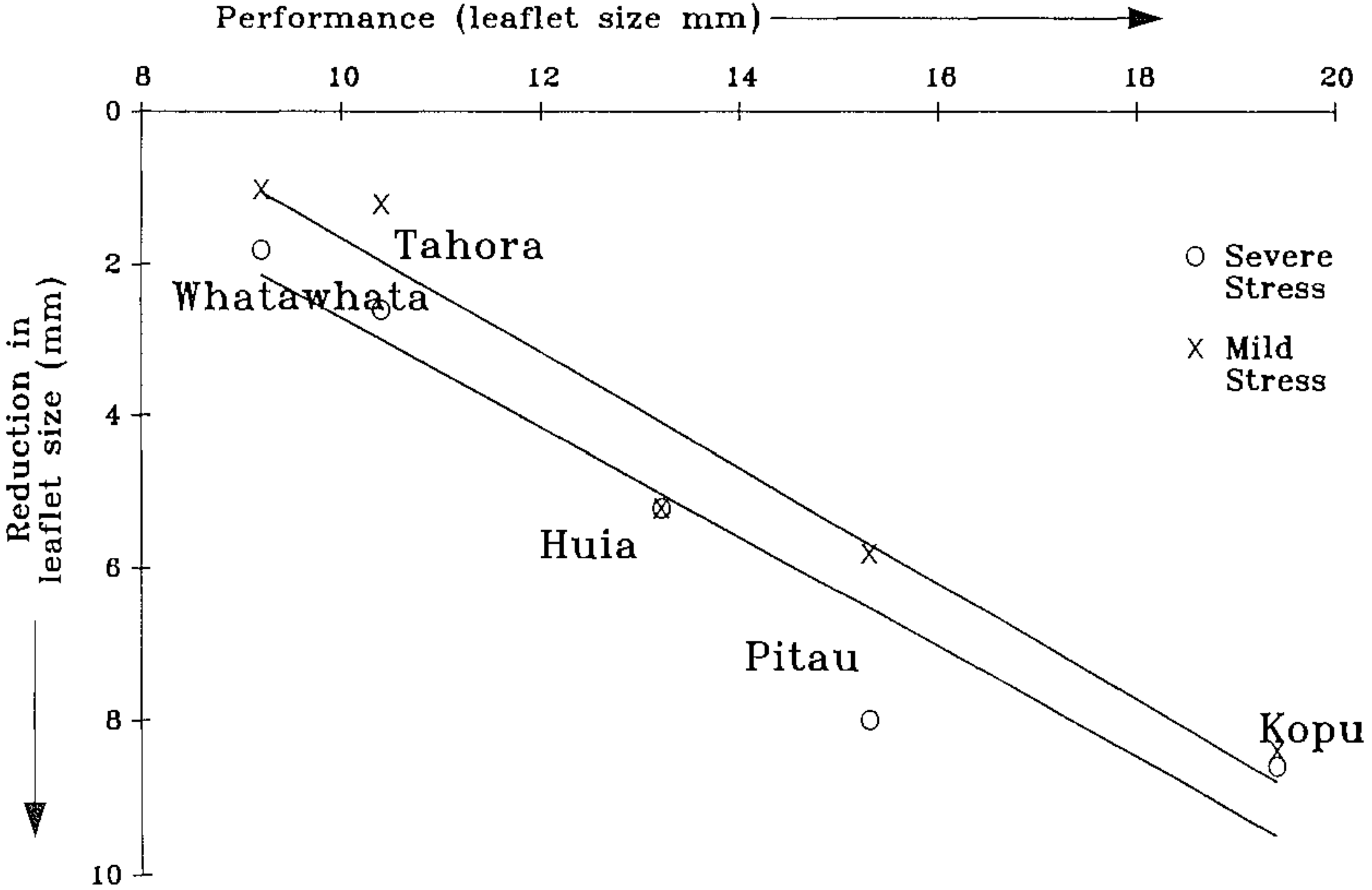


Table 4.6 No. of leaves per stolon, size of leaflet, petiole and internode of 5 cultivars at 3 water deficit levels

Cultivar\Treatment	Control	Mild Stress	Severe Stress
(a)	No. of Leaves Stolon^{-1}		
Kopu	5.67	1.80(31.7%)	1.40(24.7%)
Pitau	4.47	1.87(41.8%)	1.67(37.4%)
Huia	4.73	1.73(36.6%)	1.73(36.6%)
Tahora	5.13	1.53(29.8%)	1.20(23.4%)
Whatawhata	4.73	1.73(36.6%)	1.60(33.8%)
Sig.	ns	ns	ns
(b)	Leaflet Length(mm)		
Kopu	19.5	11.2(57.2%)	10.9 (56.0%)
Pitau	15.3	9.8(64.0%)	7.2 (47.2%)
Huia	13.3	8.2(61.9%)	8.2 (61.9%)
Tahora	11.4	10.2(89.6%)	8.2 (71.7%)
Whatawhata	9.2	8.1(87.5%)	7.7 (83.3%)
Sig.(LSD)	**(3.6)	**(2.9)	*(2.5)
(c)	Petiole Length(mm)		
Kopu	77.2	22.3(28.9%)	15.5(20.1%)
Pitau	61.0	19.5(32.0%)	12.2(20.0%)
Huia	65.1	24.4(37.5%)	20.7(31.8%)
Tahora	48.8	26.8(54.9%)	17.2(35.2%)
Whatawhata	42.9	32.0(74.6%)	15.5(36.1%)
Sig.(LSD)	**(7.9)	**(8.6)	*(7.8)
(d)	Internode Length(mm)		
Kopu	17.0	14.9(87.6%)	7.5(44.1%)
Pitau	19.8	8.6(43.0%)	4.6(23.2%)
Huia	11.7	6.7(57.3%)	4.4(37.6%)
Tahora	11.3	6.8(60.2%)	3.9(34.5%)
Whatawhata	9.1	8.3(91.2%)	6.5(71.4%)
Sig.(LSD)	**(2.6)	*(4.9)	*(1.8)

(value in quotation are % of control)

and cultivars was mainly due to the extremely different response between Kopu and Pitau, and the responses of other cultivars fell between them. There is a positive correlation between leaf number under control conditions and the reduction caused by water stress (Fig. 4.6). This relationship is an indication that the effect of water stress on a cultivar is linked to its growth potential. Overall, cultivars with more leaves, larger leaf and longer petiole and internode are more severely affected (Fig. 4.6 and Fig. 4.7). Therefore the differences between the cultivars under stress conditions were diminished and became non-significant. In all four parameters measured, Whatawhata was recorded lower, consequently, it was less affected by water stress. This pattern of response to water deficit stress was the same as the previous results showed (Chapter 3), indicating an inverse relationship between growth potential and susceptibility to water stress. The existence of such a relationship, however, could pose difficulties for the simultaneous improvement of growth potential and stress resistance.

4.3.5 Growth Parameters

4.3.5.1 Leaf Appearance Rate and Leaf, Petiole and Internode Extension Rates

Means of these dynamic parameters at the three levels of water deficit treatment are presented in Table 4.7. Water stress significantly reduced the rate of leaf appearance and leaf, petiole and internode extension rates. Among the characters, leaflet and petiole extension rates were more severely affected than internode extension. It took 6.5 and 15 more days (or 140% and 330% respectively) for a leaf to appear at the two water stress levels. The extension growth of leaflet, petiole and internode was reduced by about 50 to 62% under mild stress and 85 to 95% under severe stress.

Table 4.7 Water stress effect on leaf appearance rate(LAR) (days leaf^{-1}) and leaf, petiole and internode extension rate (mm day^{-1})

Water Level	LAR	leaflet	petiole	internode
Control	4.5	0.89	7.45	0.72
Mild Stress	11.0	0.34	3.03	0.36
Severe Stress	19.5	0.13	0.34	0.06
Sig.(LSD)	***(3.7)	***(0.20)	***(2.37)	***(0.28)

Considering leaf appearance and leaflet, petiole and internode extension rates together, it appeared that the leaf area production of white clover was severely affected by water stress. Leaf extension, in particular, was sensitive to water stress. The mechanism by which water deficit stress affects leaf extension growth is extensively discussed by Hsiao (1973). Turgor pressure is often regarded as the main factor causing plant growth, particularly expansion growth (Hsiao, 1973) though more recent work showed that leaf expansion can be directly affected through plant hormone without reducing leaf water potential (Passioura, 1988). The level of water stress in the present experiment, indicated by RWC reductions, was great enough to cause detrimental effect on plant leaf growth.

The reduction of leaf growth under water stress can directly lead to plant growth reduction as shown in perennial ryegrass and prairie grass (Chu, 1979) and in legumes (Tudsri, 1986). It can be expected to be similar in white clover.

Table 4.8 Leaf appearance rate(LAR)(days leaf^{-1}) and leaf, petiole and internode extension rate (mm day^{-1})

Cultivar	LAR	leaflet	petiole	internode
Kopu	9.33	0.61	5.28	0.65
Pitau	12.53	0.55	5.95	0.78
Huia	16.73	0.41	2.45	0.23
Tahora	12.88	0.40	2.37	0.27
Whatawhata	7.15	0.30	1.99	0.37
Sig.(LSD)	***(3.1)	***(0.05)	***(2.3)	***(0.27)

Petiole extension was also severely reduced by water stress (Table 4.7). Though the reduction means that petiole will make a smaller contribution to the total herbage yield, its influence may be limited to that. Under water stress conditions, the shading from other plants in the sward is likely to be reduced so that short petiole would not compromise white clover's competition for light. While leaf duration may have been prolonged, the reduction in leaf appearance rate and slower leaf expansion, and a low photosynthetic rate would have resulted in reduced total photosynthesis. In the present experiment, new growth in apical meristem or axillary buds was recorded under stress conditions as the continued leaf appearance, and leaf extension growth, but overall net DM accumulation was minimal under mild stress and negative under severe water stress (Fig. 4.2), indicating an equal and greater senescence than growth at the two stress levels.

The overall effect of cultivars on leaf appearance rate and the extension rates of leaflet, petiole and internode was significant, indicating a general genetic variation between these cultivars. Since there were also significant interactions between cultivars and water deficit levels the discussion will concentrate on differences in their responses to water stress.

The effect of water stress on white clover growth is similar to that on the static parameters discussed above. The leaf appearance rate of Pitau was affected more than other cultivars while Whatawhata and Kopu were least affected (Table 4.9). Altogether the reduction of the parameter under water stress conditions was positively correlated with performance under control. The cultivars with large value in a parameter were affected more severely in that parameter (Table 4.9). Pitau seemed always to be affected more than Kopu. This extra sensitivity of Pitau to water stress may be related to the effect of high temperature. It is known that Pitau is a winter-active cultivar, so it probably is relatively more sensitive to the high internal temperatures associated with water stress. Under severe water stress conditions Whatawhata and Kopu were less affected. This is in agreement with Ledgard *et al.*'s (1988) result in field work, using yield to evaluate performance.

Internode extension rate was more variable than leaflet and petiole growth. This was probably due to its small extension rate under stress conditions and as such relatively large measurement error but the general trend of the interaction between cultivar and water deficit levels was similar to that of the other parameters in its reduction under stress conditions.

The magnitude of organ size (or growth rate) reduction under stress conditions may reflect the impact of stress on plant growth, but can be taken as an indicator of stress resistance of the plants. Therefore the inverse relationship between plant stature and the magnitude of organ size (or growth rate) reduction suggests that small stature may be considered advantageous for plant survival under stress conditions. The results also indicate that total DW production has been affected more in cultivars with higher growth potential. The pattern of the relationship between DM under control conditions and its reduction under mild and severe stress is similar to patterns of leaf number or leaflet growth rate change as shown in Fig. 4.6 and Fig. 4.7.

Table 4.9 Leaf appearance rate(LAR)(days leaf^{-1}) and leaf, petiole and internode extension rate (mm day^{-1})

Cultivar\Treatment	Control	Mild Stress	Severe Stress
(a)		Leaf Appearance Rate	
Kopu	3.70	10.00	14.29
Pitau	4.35	12.50	33.33
Huia	4.55	12.50	20.00
Tahora	4.35	14.29	20.00
Whatawhata	5.56	5.88	10.00
Sig.(LSD)	*(1.75)	*(2.99)	**(8.99)
(b)		Leaf Extension Rate	
Kopu	1.25	0.32(25.9%)	0.25(20.1%)
Pitau	0.97	0.64(66.3%)	0.04(4.1%)
Huia	0.83	0.32(38.4%)	0.08(9.9%)
Tahora	0.83	0.23(28.5%)	0.13(15.2%)
Whatawhata	0.58	0.18(31.8%)	0.13(23.1%)
Sig.(LSD)	***(0.37)	*(0.30)	*(0.15)
(c)		Petiole Extension Rate	
Kopu	11.35	3.53(31.1%)	0.97 (8.5%)
Pitau	12.18	5.57(45.7%)	0.09 (0.7%)
Huia	5.42	1.77(32.7%)	0.17 (3.1%)
Tahora	4.88	2.22(45.5%)	0.00 (0.0%)
Whatawhata	3.43	2.06(60.1%)	0.47(13.7%)
Sig.(LSD)	***(2.77)	**(3.20)	*(0.49)
(d)		Internode Extension Rate	
Kopu	0.68	0.54(79.4%)	0.05(7.9%)
Pitau	1.65	0.68(41.2%)	0.02(1.2%)
Huia	0.39	0.22(56.4%)	0.07(17.9%)
Tahora	0.60	0.15(25.0%)	0.06(10.0%)
Whatawhata	0.69	0.28(40.6%)	0.13(18.8%)
Sig.(LSD)	**(0.89)	*(0.25)	ns

(value in quotation are % of control)

4.3.5.2 Leaf Development

The morphological development of leaves at different phytochron ages at 3 levels of water deficit and of five cultivars are presented in Figs. 4.8a,b,c,d,e. For all treatments and cultivars, leaf development was generally synchronized with leaf appearance, so leaves reached full expansion at a similar phytochron age, age 2, ie. by node 2, regardless of cultivar and water level differences. In other words, leaf appearance and development were affected by water stress and genetic makeup of a cultivar to a similar extent. It can be argued that assimilate availability had a decisive and equally limiting effect on both leaf appearance and its development, therefore, the ratio between them is not changed by water stress. Generally, the leaf is considered the plant organ most sensitive to water stress. The present results indicate that in white clover, leaf development may not be more sensitive to water stress than node appearance. The absolute effect of environmental factors, such as moisture availability, upon leaf morphological development of some tropical plants has been shown by Higgins *et al.* (1964). The present study confirmed this in white clover. Such absolute reduction is partly responsible for the reduced plant photosynthesis. Water stress prolongs the physiological age of leaves (Troughton, 1978) on a chronological time scale. Being equally affected means that a white clover leaf would reach its full expansion at a similar position on a stolon regardless of its genetic make-up or environmental conditions. In other words, the development of leaf and the appearance of leaf from the apical bud are synchronized. Thus expressed in phytochron age scale, leaf physiological age would probably be less affected by water stress. There appeared to be little interaction between cultivar and water level in leaf development (Figs. 4.8a,b,c,d,e).

In contrast to leaf development, its ageing process, in relation to leaf appearance, was significantly accelerated by water stress treatment (Figs. 4.8a,b,c,d,e). Therefore, leaves of stressed plants started senescence about one to two phytochron ages earlier, and reached partial death 2 or 3 phytochron ages earlier, than those under control conditions. This resulted in the smaller number of leaves per stolon under stress treatments and smaller leaf area per plant. But water stress levels caused little difference in the leaf ageing rate. Among the cultivars, senescence started earlier in Tahora and Whatawhata, particularly under stress conditions, so that their leaves reached partial senescence about 1 to 2 phytochron ages earlier than those of other cultivars (Figs. 4.8a,b,c,d,e). This indicated an acceleration in leaf death for Tahora and Whatawhata and was reflected in their slightly, though non-significant, smaller number of leaves per stolon (Table 4.6a). So only a small difference, if any, of leaf relative development and ageing in response to water stress may exist between cultivars, based on the results discussed above.

Fig. 4.8a Effect of water deficit levels on leaf development on a scal of phytochron age

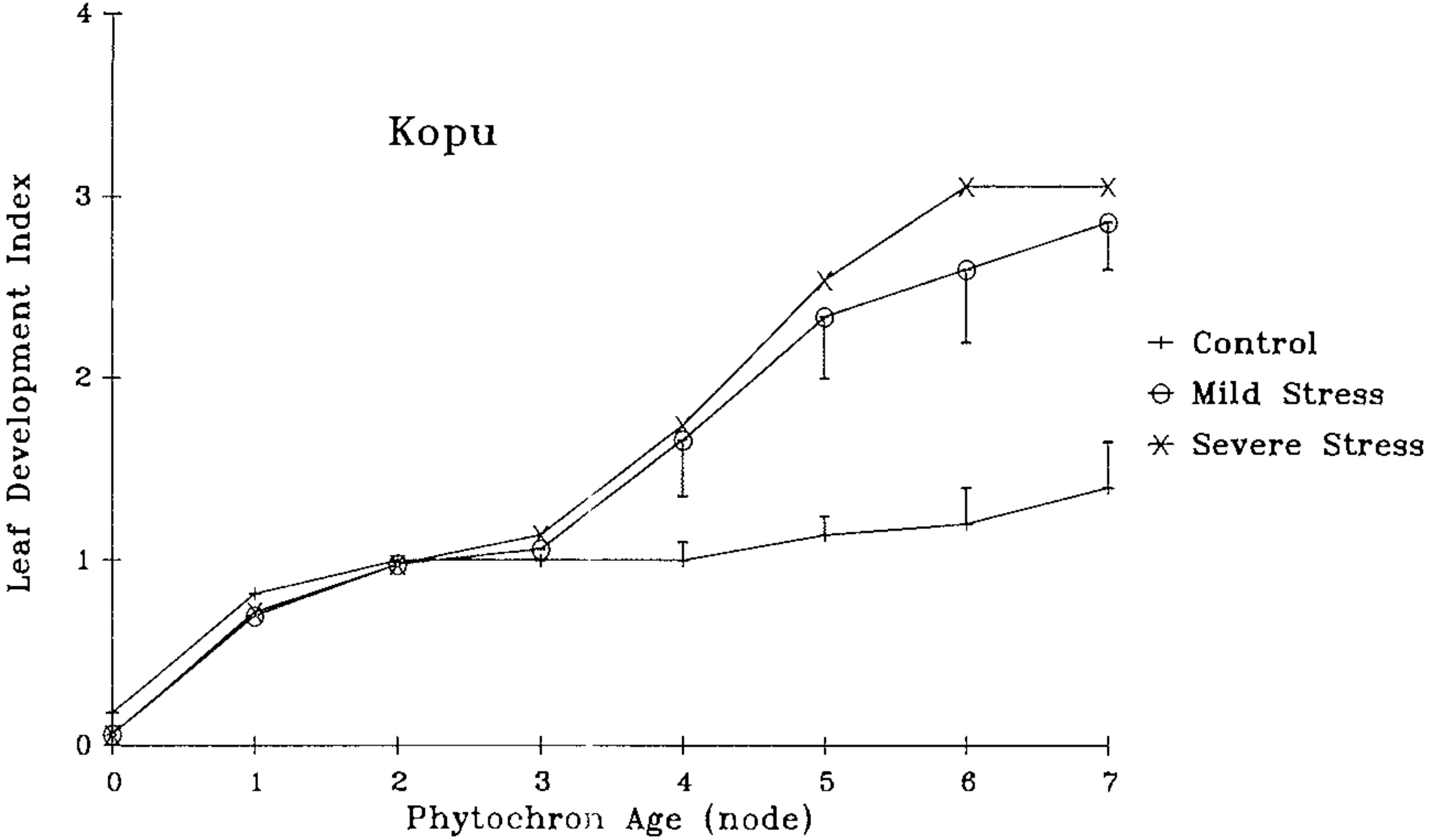


Fig. 4.8b Effect of water deficit levels on leaf development on a scale of phytochron age (mean & SEM)

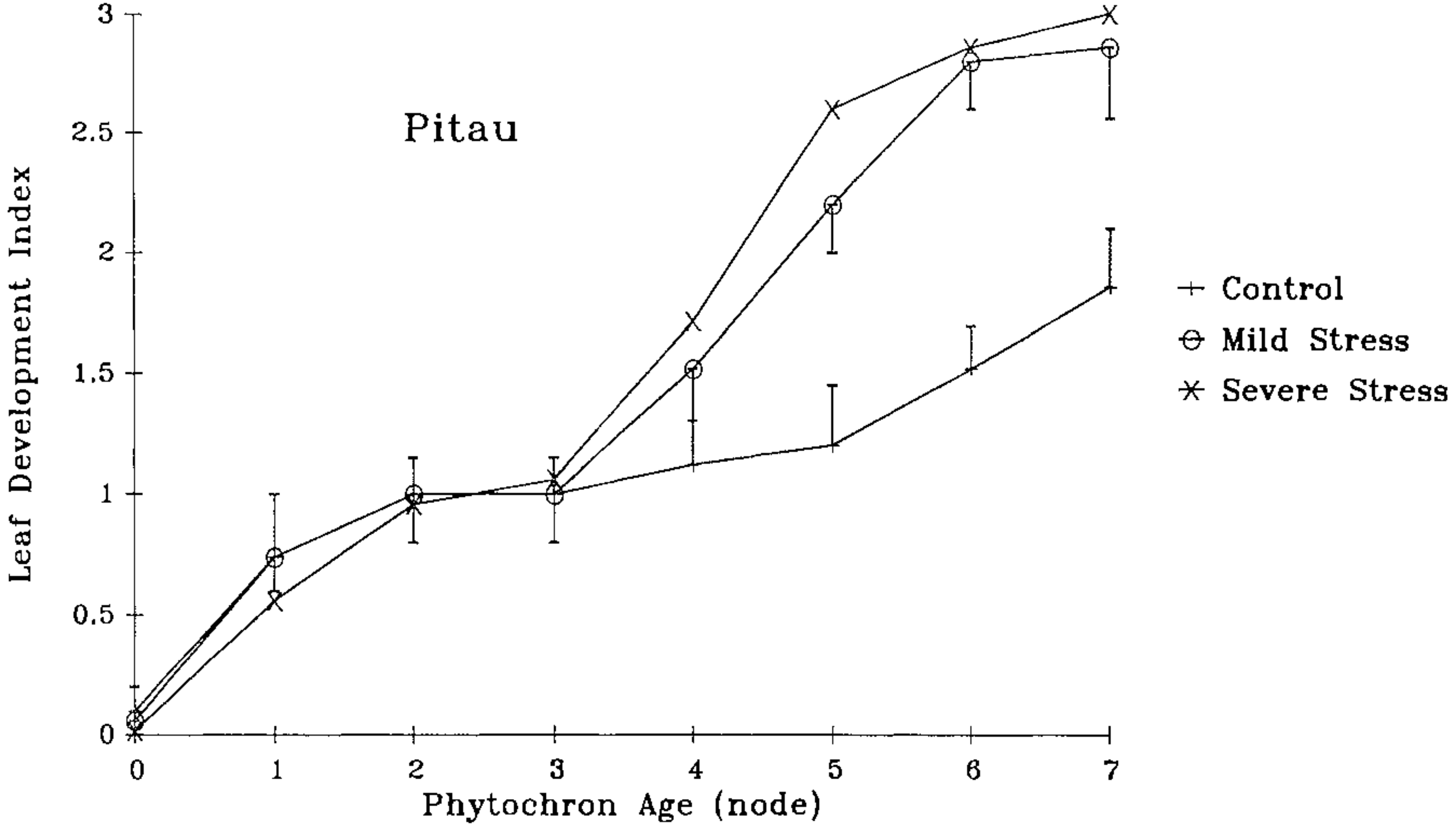


Fig. 4.8c Effect of water deficit levels on leaf development on a scale of phytochron age (mean & SEM)

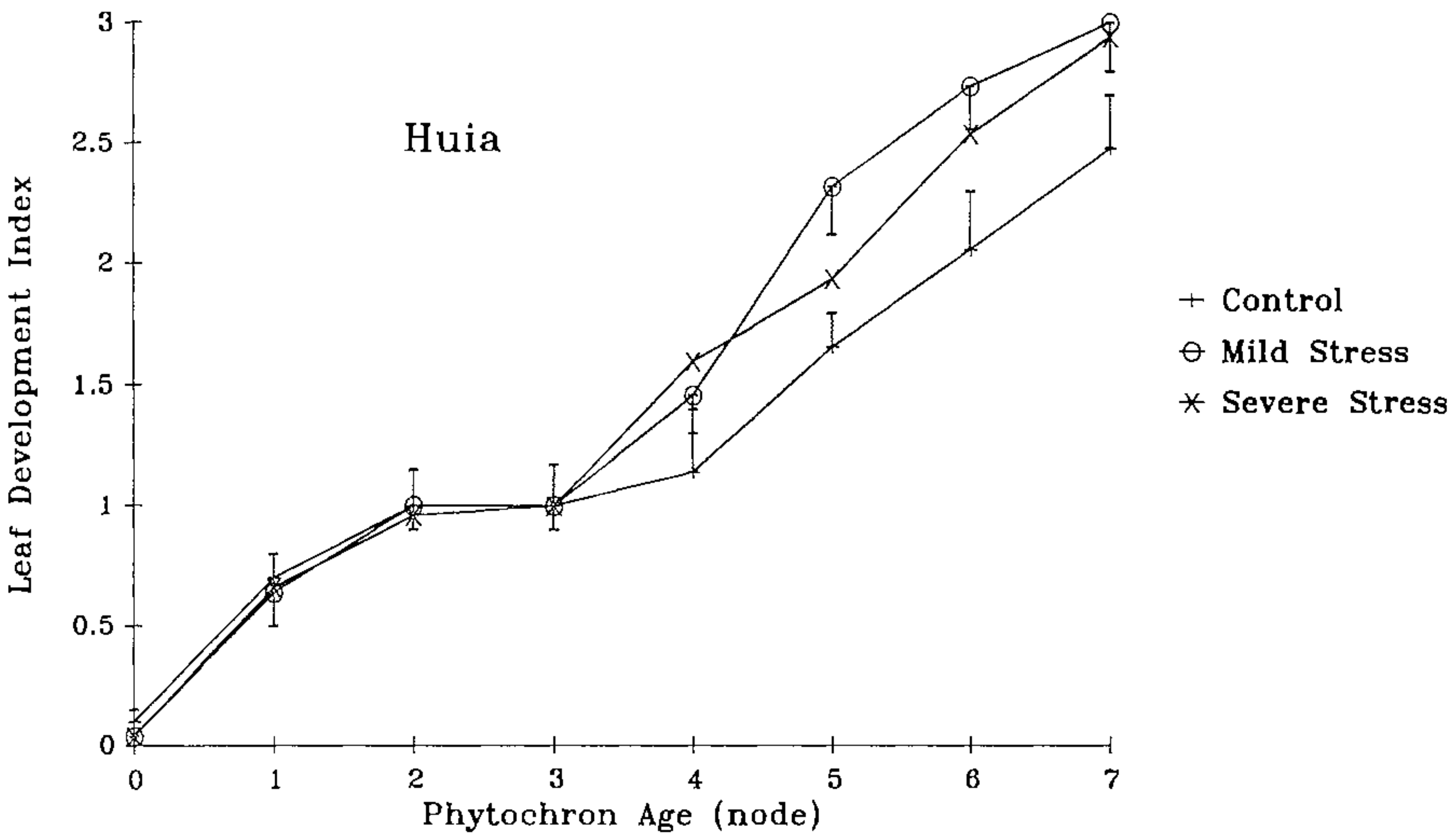


Fig. 4.8d Effect of water deficit levels on leaf development on a scale of phytochron age (mean & SEM)

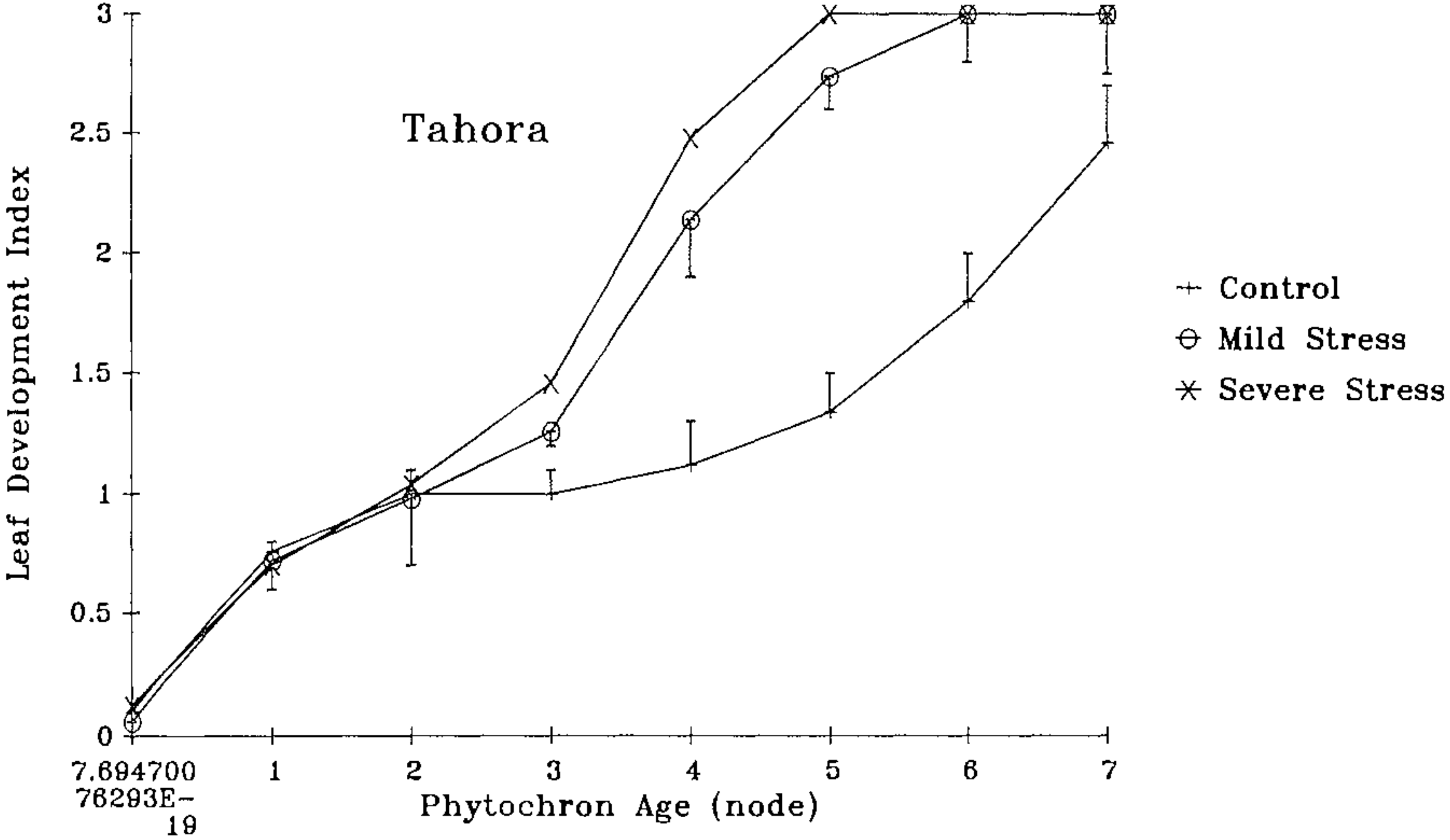
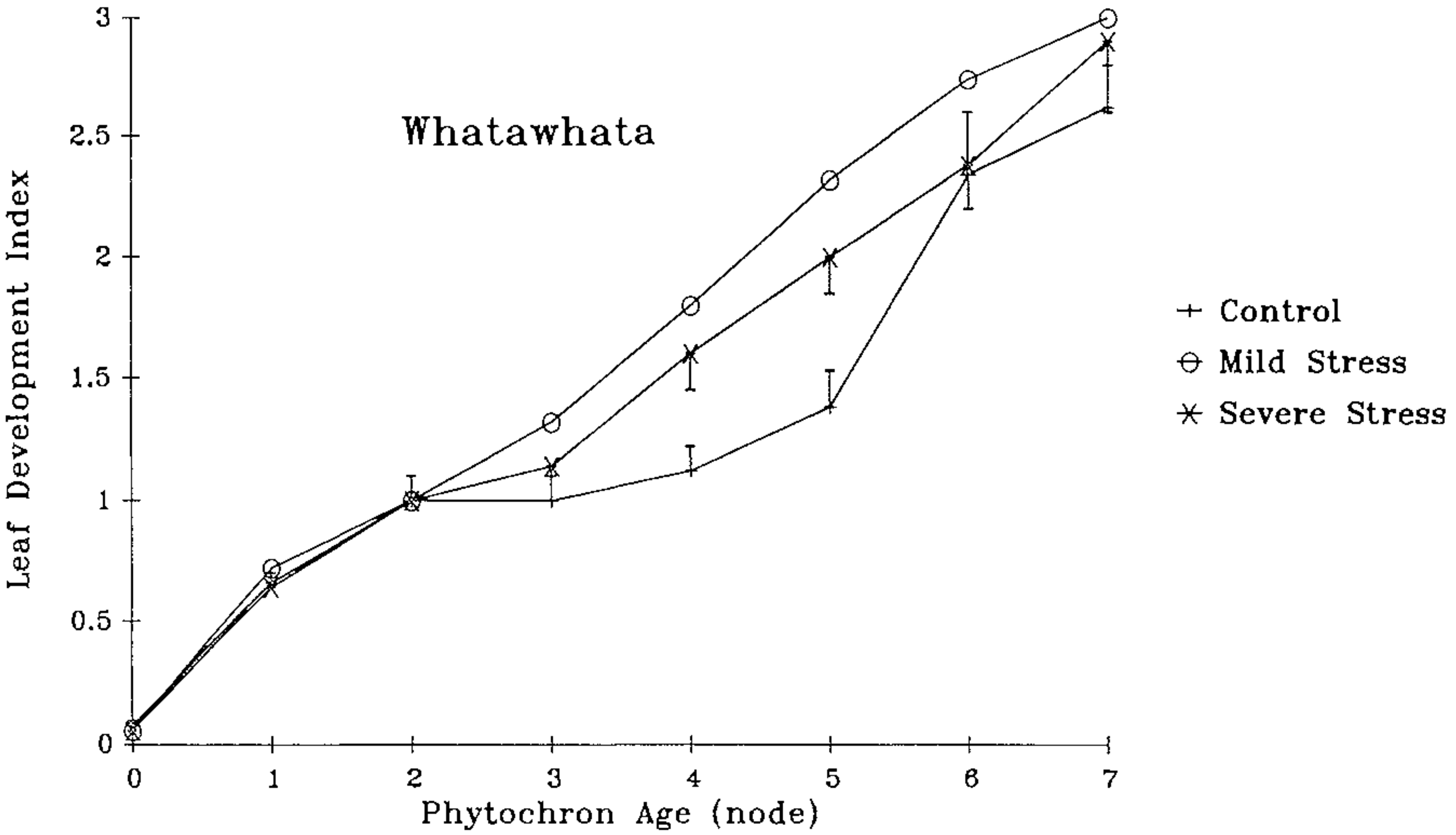


Fig. 4.8e Effect of water deficit levels on leaf development on a scale of phytochron age (mean & SEM)



4.4 Conclusions

1. Water stress caused large reductions in growth and DW accumulation. The reduction of total DW, DW of every component and most growth parameters increased with increasing water deficit levels.

2. There were no consistent differences among the cultivars in their total DW under the three levels of water deficit, though large-leaved cultivars and Huia had a higher leaf and petiole DW and smaller stolon or root DW. Therefore, they had a higher harvestable DW and harvest index than the small-leaved cultivars, Tahora and Whatawhata.

3. The partitioning of DW towards leaf and petiole was reduced more by water stress in comparison with stolon and root. Such unequal reduction may help plants to reduce water loss and persist better under stress conditions. Whatawhata was more sensitive than other cultivars in this aspect. It had the lowest leaf and petiole DW proportion under both mild and severe water stress treatments. Whatawhata appeared to be more suitable for pasture persistence under stress conditions than for high production under optimum growth conditions.

4. Water stress treatment accelerated leaf ageing, but did not do so to leaf development. There was no difference between the two levels of water stress in causing leaf accelerated ageing. Among the five cultivars, leaves of Tahora and Whatawhata started to age slightly earlier and/or faster compared with those of the other three cultivars under stress conditions.

5. Overall, there were significant differences among the cultivars in their response to water stress, evaluated from the growth parameters measured. Generally speaking, smaller leaved cultivars, particularly Whatawhata, were less affected than large leaved cultivars. From the results of both this and the previous experiment, it can be concluded that a cultivar's performance reduction, caused by water stress, is positively linked with its performance levels under well watered control conditions.

CHAPTER FIVE
THE RELATIONSHIP BETWEEN SOIL WATER STATUS, PLANT WATER STATUS AND LEAF EXTENSION GROWTH OF FIVE WHITE CLOVER CULTIVARS

5.1 **Introduction**

White clover leaf water status is reduced significantly under even mild water deficit conditions, as shown in the previous experiments (Chapters 3 & 4). Leaf water status, estimated as leaf water potential or leaf relative water content (RWC), can be used as an indicator of the plant's internal water deficit level and has been shown to be closely related to leaf extension growth (Hsiao, 1973). The maintenance of relatively higher leaf water status and leaf growth under water stress conditions are considered to be two important drought adaptive attributes (Blum, 1983). Information on the quantitative relationship between soil water status and plant water status of different white clover cultivars may help to interpret the differences in their responses to water deficit stress. At the same time, the relationship between plant water status and leaf extension growth under water stress conditions may help to differentiate among cultivars for stress tolerance.

Significant differences among cultivars in leaf (leaflet and petiole) extension growth at three water deficit levels were observed in the previous experiment (Chapter 4). The study reported in this chapter, on the relationship between leaf extension growth and plant water status over a wide and continuous range of soil water deficit levels, was aimed at shedding light on possible reasons for the observed significant interaction between water deficit level and cultivar.

Under water deficit conditions, plant water status is reduced; so is plant transpiration rate. Comparisons of transpiration among various pasture plant species showed that white clover had poor transpiration control under water deficit conditions (Johns, 1978; Kerr and McPherson, 1978). This could be one of the reasons for the susceptibility of white clover to water stress. However, it was not clear why higher transpiration loss occurred when the earlier evidence showed that white clover stomatal closure did occur even under mild stress (Cox and Boersma, 1967). It is also not known whether there are differences in transpiration rate among the cultivars of contrasting morphological characters and leaf size categories, although such information would be useful for plant breeders. Because of the high accuracy required for such measurements, the experiment was carried out in a growth room. The objectives of the present experiment were:

1. to detect changes in leaf water status for the five cultivars under progressive water deficit stress;

2. to establish the relationship between plant water status and leaf extension growth, transpiration, plant relative growth, and plant water use efficiency.

5.2 **Material and Methods**

5.2.1 Plants and Experiment Site

The experiment was carried out in growth cabinet No.1, Agronomy Department, Massey University, from 15 October to 15 December, 1986. The plants were germinated in 2 litre (15cm deep) pots four weeks prior to the commencement of the experiment. The planting medium contained peat, pumice and sand (70:20:10 on volume basis). To prevent evaporation loss, the soil surface was sealed by a layer of plastic chips 2 cm deep. No nutrients were added to the medium but the plants were watered with "Lush" solution before and during the experiment. The "Lush" solution contained 10% N, 3% P and 6% K, plus other trace elements, Mg, Fe, Cu, Zn, Mn, in a chelated form.

The conditions of the growth cabinet were:

Photoperiod:	12 hour light/12 hour dark
Room temperature:	18°C(night)/22°C(day)
Relative humidity:	80%

5.2.2 Treatments

Five white clover cultivars of New Zealand origin were used in this experiment. They were:

Large-leaved cultivars:	Kopu, Pitau
Medium-leaved cultivars:	Huia
Small-leaved cultivars:	Tahora, Whatawhata

The water deficit treatment was imposed in an extended "dry-down" period lasting one month by gradually reducing the watering level. The rate of drying down was monitored using leaf water status as an indicator with the objective of having similar rates of water stress development (around 1.5% reduction of RWC day^{-1}). There were 7 plants for each cultivar, three replicates for well watered control treatment, and four for water stress treatment.

5.2.3 Measurements

Soil water status (SWS): The soil gravimetric water content (GWC) at field capacity level at the commencement of the experiment, and during the experiment, was determined indirectly by the following equation:

GWC=(water weight/pot medium weight) * 100.

While pot medium weight was constant, water weight could be determined by subtracting the dry pot weight from total pot weight. The change of plant weight, being relatively small compared with the total pot weight, was ignored. The soil water status (SWS) was then expressed as a percentage of GWC at field capacity.

Transpiration loss and rate: The total water loss was determined by calculating the pot weight difference between two successive days plus the amount of water added during the period. Transpiration rate was determined by dividing the total water loss by the total leaf area estimated at the same time.

Leaf RWC: The same method described in Chapter 3 and detailed in Appendix 3.3 was used. Leaf RWC was measured on each plant every two days at 1200 to 1300 hours.

Plant leaf area: Total plant leaf area was determined indirectly by multiplying the mean leaf size of a particular cultivar measured separately and the number of leaves on the plant, as counted every two days.

5.2.4 Statistical Analysis

The results were analysed by NLIN procedure in SAS statistical package (SAS, 1985). The relationships between leaf RWC and soil water status, plant transpiration etc. were fitted with both linear and non-linear procedures or a combination of them fitted simultaneously, where the relation dictated it.

5.3 Results and Discussion

5.3.1 Relationship between Soil Water Status (SWS) and Leaf RWC

5.3.1.1 The General Pattern of the Relationship

In the range of water deficit conditions studied, there was a positive correlation between the soil water status (SWS), expressed as percentage of field capacity (%FC), and plant water status, measured as leaf RWC. Regardless of the cultivar differences, the general pattern of the relationship can be best described as a combination of a quadratic function and a straight line, once the leaf RWC reached its plateau (Fig. 5.1). The function is shown as follows:

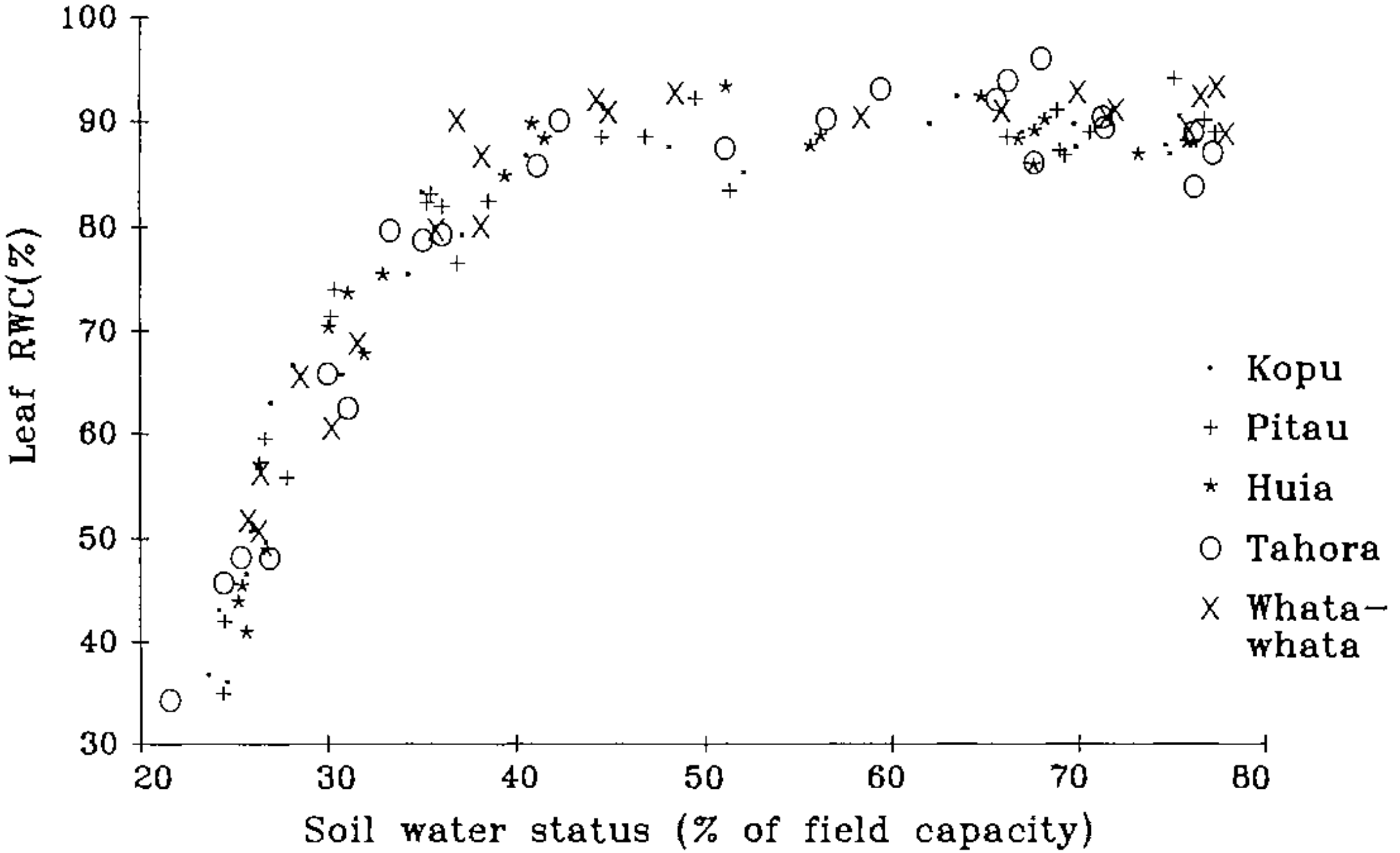
$$\mathrm{RWC} = \mathbf{a} - \mathbf{b}(\mathrm{SWS} - \mathrm{SWSc})^2 \quad \text{if SWS} < \text{SWSc, critical SWS}$$
$$\mathrm{RWC} = \mathbf{a} \qquad \text{if soil water status} \geq \text{SWSc}$$

By definition, **a** indicates the highest level of RWC that white clover plants can reach with an adequate water supply; **b** the rate of RWC reduction with the power of soil water status reduction from a certain level (SWSc); while SWSc represents the critical soil water status below which the leaf RWC will be affected. Overall, for different cultivars, the **a** values varied from 87.7 to 90.2, SWSc from 0.409 to 0.458. These values suggested that leaf RWC started to drop sharply at about 41-46% FC under the conditions of this experiment. Above this level the plants maintained leaf RWC at 87 to 90%. Leaf RWC dropped to about 35% at the lowest soil water status of about 25% FC in this experiment. Though this may not be the lowest RWC white clover plants can reach, it is already much lower (20 to 25% lower) than those recorded in other experiments (eg. Cox and Boersma, 1967; Johns, 1978). It is well established that leaf water status changes with soil water status (Lawlor and Lake, 1976). Though more often leaf water potential instead of RWC was used to express the relationship, leaf RWC and water potential are linearly related in white clover (Cox and Boersma, 1967; Johns, 1978). The sharp reduction of leaf RWC below the critical soil water status suggested that there was no significant osmotic adjustment in the leaves. Turgor maintenance through osmotic adjustment is apparently rare in legumes (Turner, 1978).

5.3.1.2 Cultivar Difference

Estimates of **a**, **b** and SWSc for the five cultivars are presented in Table 5.1. Comparing the five cultivars, the pattern of RWC changes with soil water status is largely similar though some differences can be noted here. The maximum level of RWC in

Fig. 5.1 Relationship between leaf RWC (%) and soil water status (% of field capacity)



Whatawhata at the high soil water status, **a**, was nearly 3% higher than that of Kopu ($P<0.05$) while the other cultivars had intermediate RWC. RWC at lower soil water levels were similar for all cultivars. A higher plant water status under water stress conditions is often taken as an indication of dehydration avoidance (Blum, 1983). So the lack of general difference between cultivars under water stress may indicate that there is no significant difference among the cultivars for this attribute. This is not a surprising result since none of these cultivars has been selected for water stress resistance nor for dehydration avoidance. Whatawhata, which originated from a dryland area (MacFarlane and Sheath, 1984), may have some potential worth exploring in this respect, but it only showed higher RWC at an adequate water level in the present experiment. The difference in RWC, as will be discussed in the following section, is probably related to the plant transpiration rate.

Table 5.1 Relationship between SWS and RWC (%)

Cultivar	**a**	**b**	SWS_c	R^2	Sig.
Kopu	87.71	-1675.91	0.409	0.96	****
Pitau	88.71	-1446.79	0.424	0.93	****
Huia	88.25	-1554.96	0.419	0.96	****
Tahora	88.59	-968.82	0.458	0.94	****
Whatawhata	90.21	-1385.03	0.427	0.93	****
Sig. (LSD)	** (2.02)	** (515.52)	** (0.033)		

The significant differences in SWS_c and **b** values (Table 5.1) indicated that the cultivars responded differently to decreasing water status. Kopu maintained the ceiling leaf RWC at a lower SWS than Tahora ($P<0.05$) but its leaf RWC reduced faster in response to further stress than Tahora ($P<0.05$). The other cultivars were intermediate. Overall all cultivars had similar leaf RWC at the highest stress level tested.

The results suggested that the white clover cultivars used in the present experiment differed slightly under well watered conditions but at high levels of water stress they had similar levels of RWC. So the differences in white clover growth and development response to water stress are probably mainly due to adaptation in morphological characters. For example, visual observation showed that leaf wilting of Whatawhata and Tahora occurred at a slightly lower RWC than other cultivars, suggesting that wilting is affected by both the level of RWC and leaf size. The more sensitive morphological reaction of Whatawhata in leaf component weight is also advantageous for reducing water loss, while the large-leaved cultivars usually have a larger root system and can avoid stress for an extended period by exploiting soil water deeper in the profile (Smith and Morrison, 1983).

5.3.2 Relationship between Leaf RWC and Leaf Extension Growth

Leaflet extension rate was correlated with leaf RWC (Fig. 5.2a,b,c,d,e). The general pattern of the relationship for the five cultivars is similar, although the value of extension rate varied with absolute leaf size. The relationship fitted to the data is a quadratic function;

$$\text{Leaflet Extension Rate} = \mathbf{a} - \mathbf{b}^*\text{RWC} + \mathbf{c}^*\text{RWC}^2$$

Leaf extension rate declined rapidly as leaf RWC decreased below 85 to 90%, falling to zero at approximately 70% RWC (Fig. 5.2a,b,c,d,e). This pattern agrees with the well-documented relationship between leaflet extension growth and leaf water status (eg. Hsiao, 1973; Chu, 1979) even though most of the reported work was carried out with grasses. However, as shown in other species, the extension rates of leaves at any specific water status varied greatly (Ong *et al.*, 1985).

There was some intraspecies variation in the relationship between leaf water status and leaflet extension rate (Table 5.2 and Fig. 5.2a,b,c,d,e). Small-leaved cultivars tended to have a sharper reduction in leaf extension growth with the reduction of RWC while Pitau seemed to decline more gradually. At least for Whatawhata, the large variation in leaf extension at higher RWC was partially responsible for the pattern of this relationship. So the reduction of mean leaf extension from higher RWC (approximately 90%) to medium RWC (80 - 85%) was less dramatic. Pitau maintained a low level of growth under stress conditions while other cultivars almost stopped extension growth at 60-70% RWC. The maintenance of leaf extension growth, though important as an indicator of water stress resistance, may be too small to make a big difference in growth under stress conditions, as observed in a previous experiment. The large variation in leaf extension rate at any one level of RWC, except low RWC, can be due to many factors. Recent work showed that root factors can also affect leaf transpiration rate independent of leaf water status (Passioura, 1988).

Fig. 5.2a Relationship between RWC and Leaflet Extension Rate

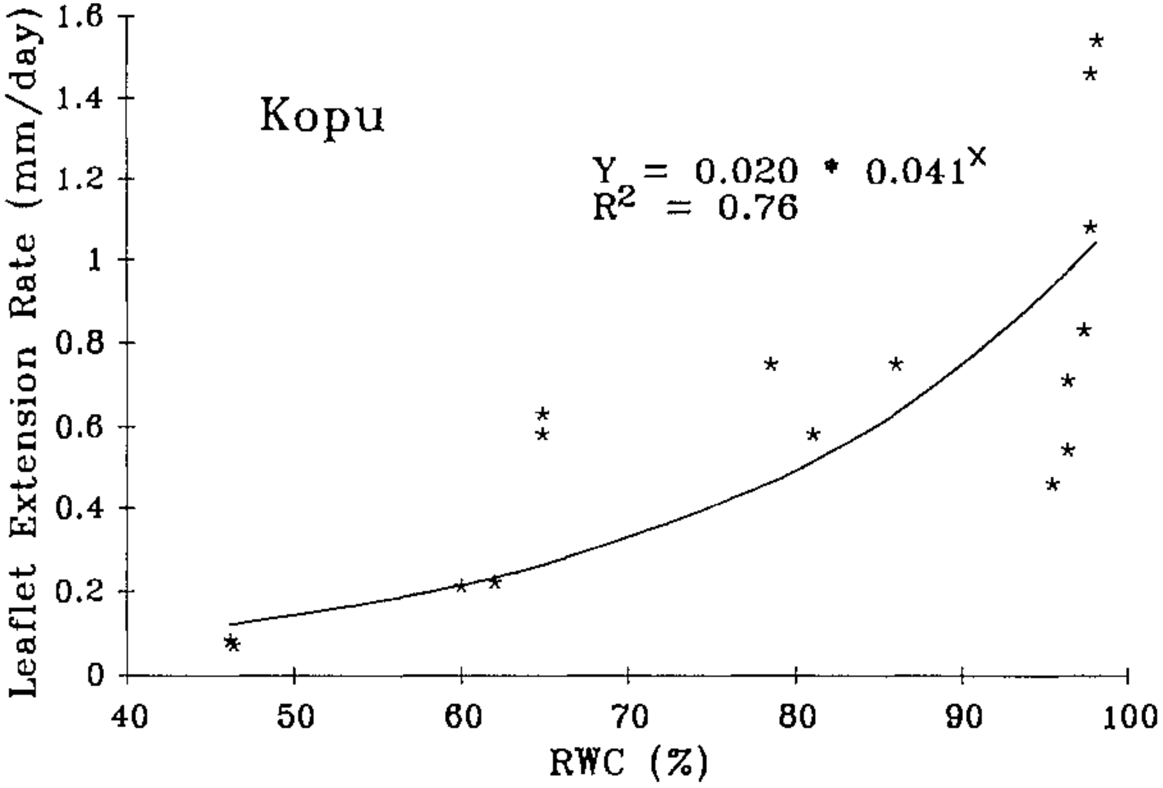


Fig. 5.2b Relationship between RWC and Leaflet Extension Rate

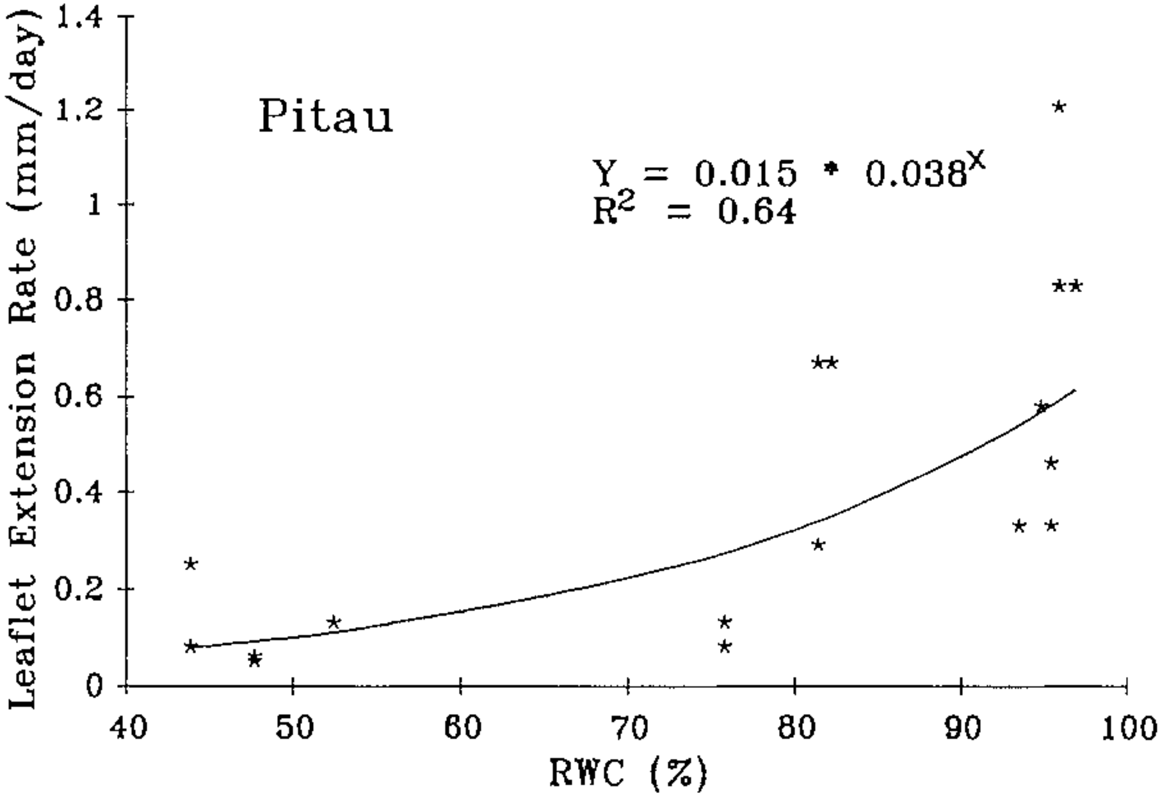


Fig. 5.2c Relationship between RWC and Leaflet Extension Rate

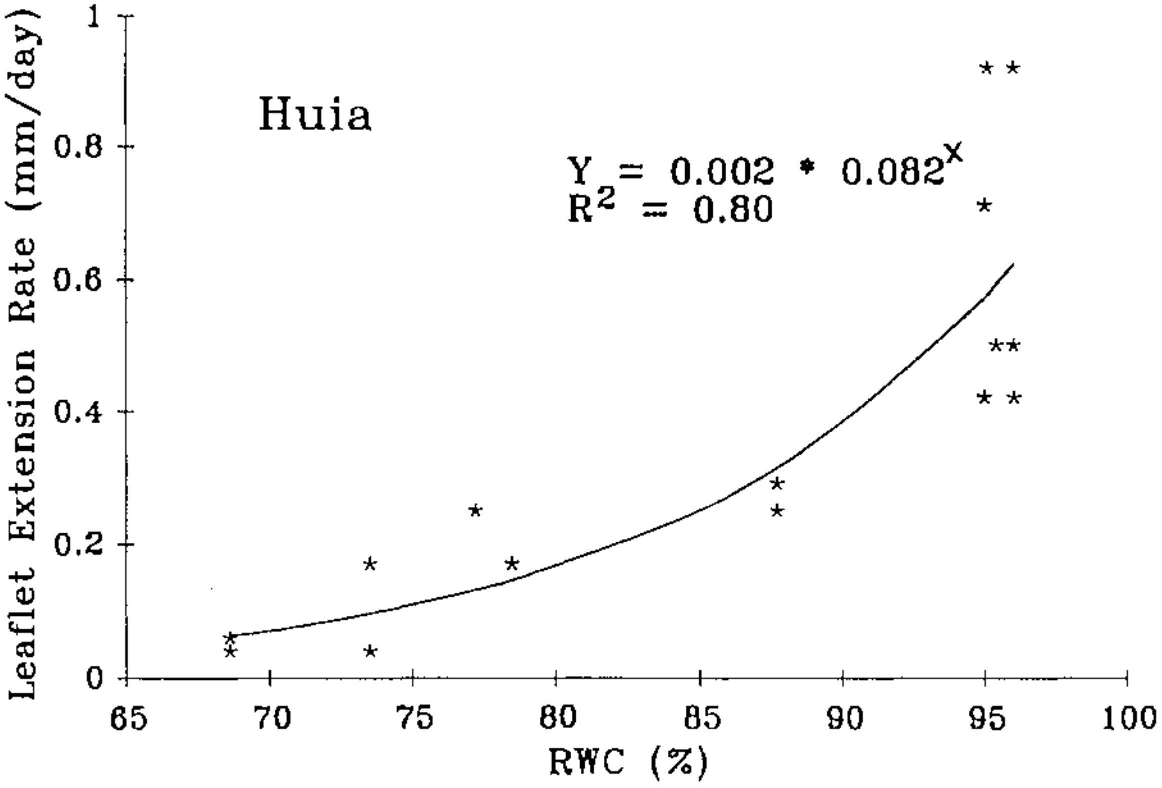


Fig. 5.2d Relationship between RWC and Leaflet Extension Rate

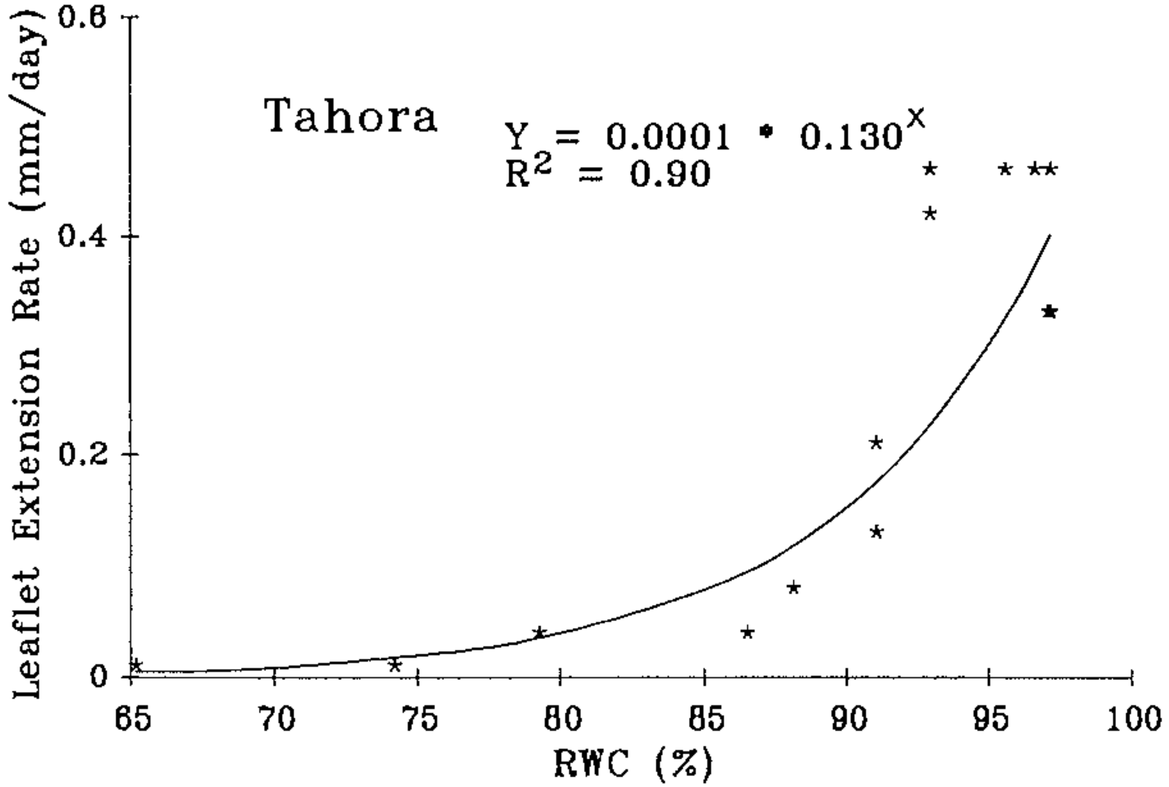


Fig. 5.2e Relationship between RWC and Leaflet Extension Rate

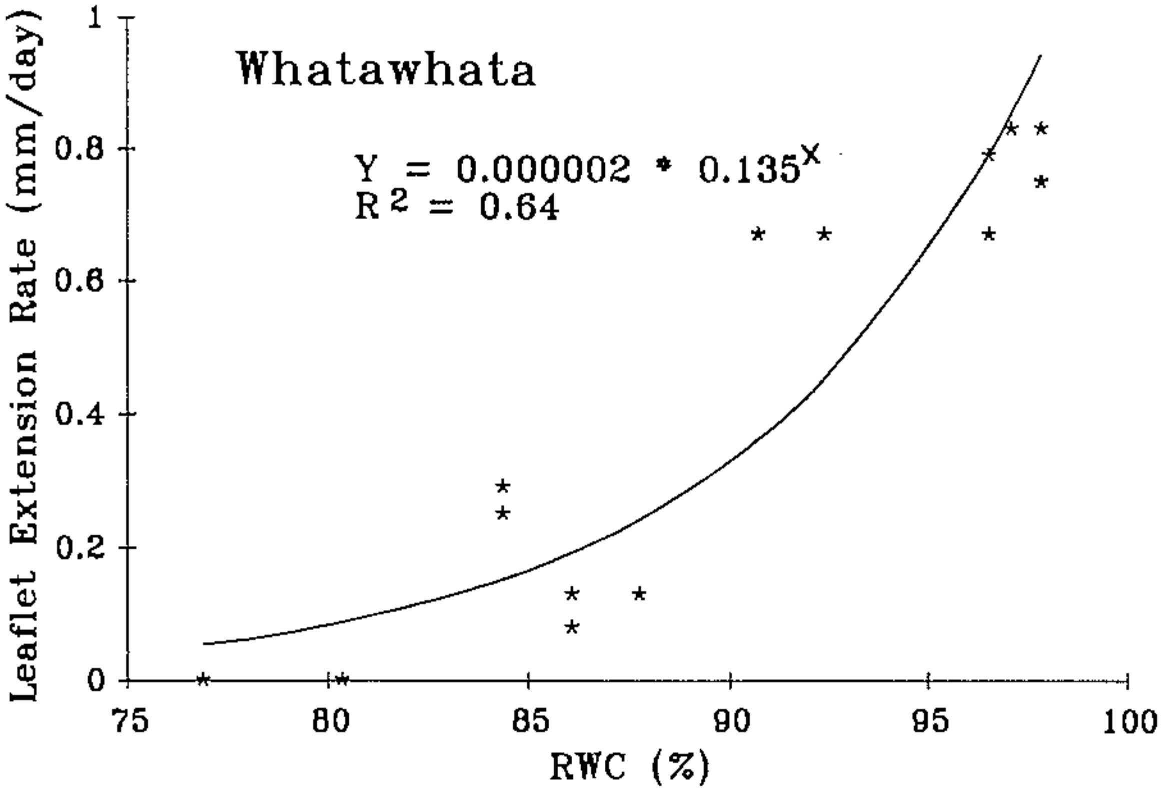


Table 5.2 Relationship between leaf RWC and leaflet extension rate

Cultivar	a	b	c	R^2	Sig.
Kopu	23.04	-0.598	0.0039	0.66	***
Pitau	0.45	-0.018	0.0002	0.80	****
Huia	5.87	-0.185	0.0015	0.91	****
Tahora	9.12	-0.260	0.0018	0.75	***
Whatawhata	5.78	-0.183	0.0014	0.70	***
LSD.	7.00	0.361	0.0022		

5.3.3 Relationship between Leaf RWC and Plant Transpiration Rate

The relationship between leaf RWC and transpiration is shown in Figs. 5.3a,b,c,d,e. It is not at all clear physiologically what function would best fit the relationship; therefore an empirical function has been used here to describe the relationship. Estimating from the R^2 value, it is found that the relationship can be best described by a logarithmic linear function:

$$\log_e(\text{transpiration}) = \mathbf{a} + \mathbf{b} * \text{RWC}$$

When leaf RWC was about 90% or higher, transpiration seemed to be unrelated to leaf RWC. The decrease of transpiration when RWC dropped below 90% under the present experimental conditions fits Cox and Boersma's (1967) observation that white clover stomatal closure occurred at a relatively high leaf water potential. In contrary some workers showed that white clover had poor stomatal control (eg. Johns, 1978; Kerr and McPherson, 1978). Under the field conditions stomatal adjustment is usually more likely to occur because of the slow induction of water deficit stress. But because of the effect of vapour pressure difference in determining transpiration (Cox and Boersma, 1967), transpiration rate and the status of stomata cannot always be linked directly. Caution is also needed when the results presented here are related to a field situation since the work was carried out in the growth room. Jarvis and McNaughton (1985) have pointed out that under a drought condition, covering a large area, the vapour pressure difference would be bigger than in a growth room or a small area experiment, so transpiration will correspondingly increase. Also, transpiration here was measured over a 24 hour period which included time of stomatal closure at night. This made it difficult to compare with other experiments where the transpiration rate was measured over a short period of time.

Fig. 5.3a Relationship between RWC and Plant Transpiration Rate

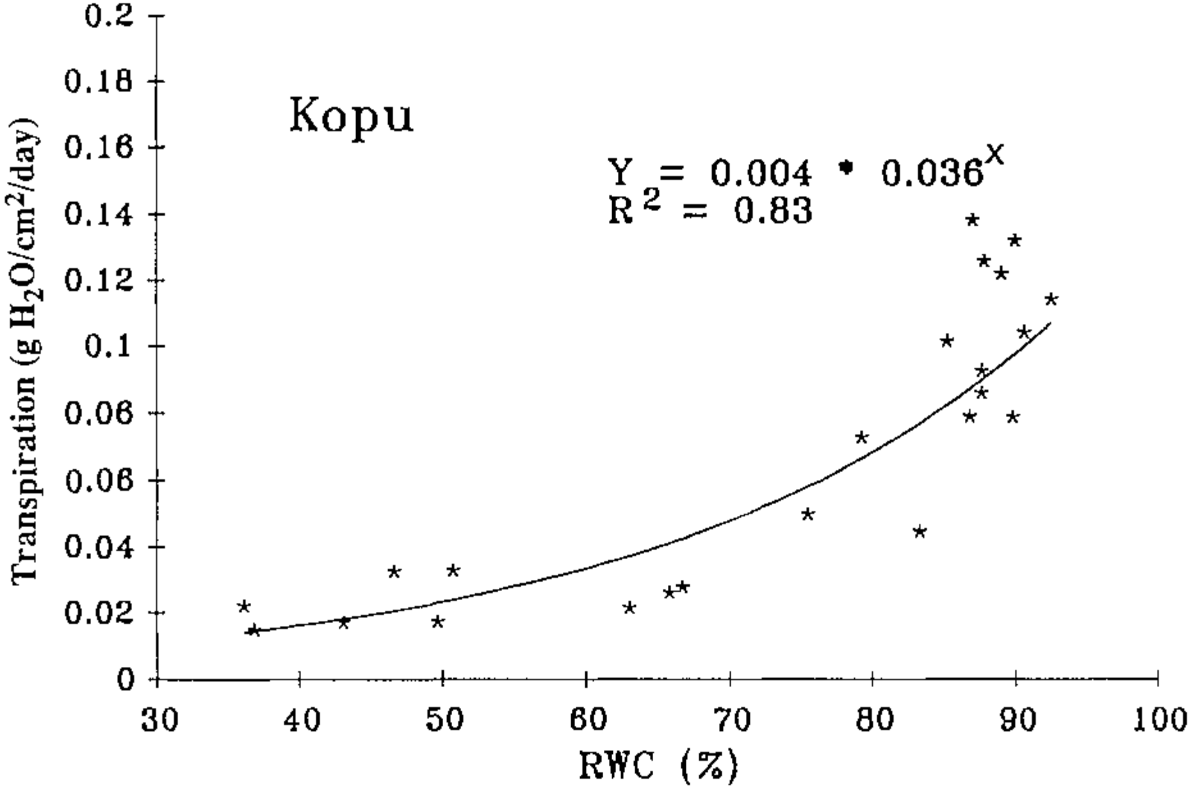


Fig. 5.3b Relationship between RWC and Plant Transpiration Rate

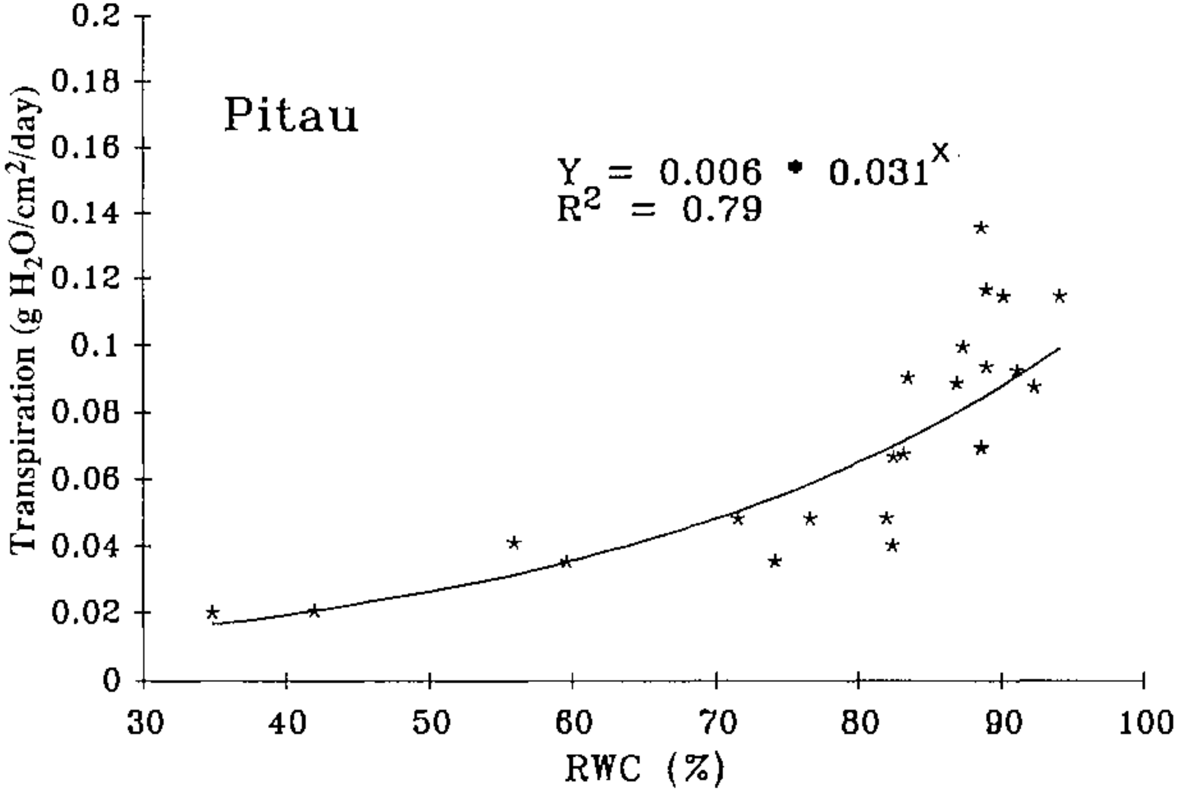


Fig. 5.3c Relationship between RWC and Plant Transpiration Rate

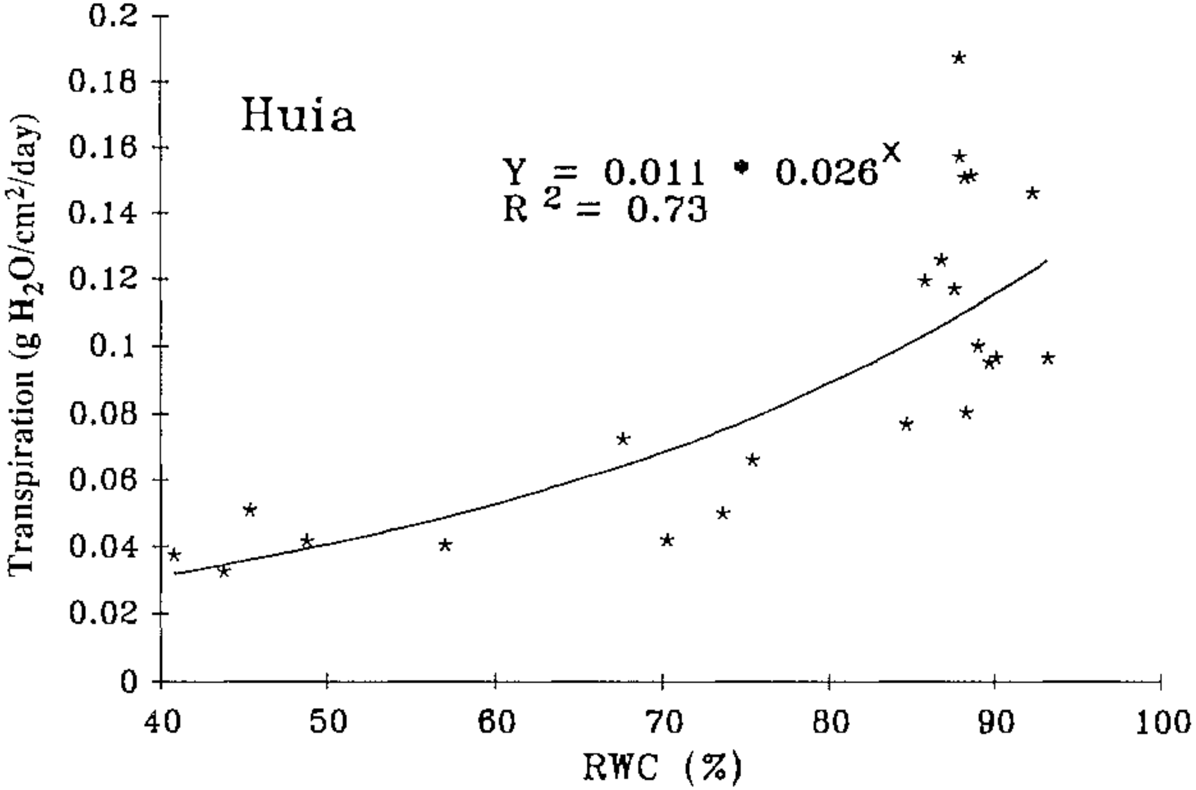


Fig. 5.3d Relationship between RWC and Plant Transpiration Rate

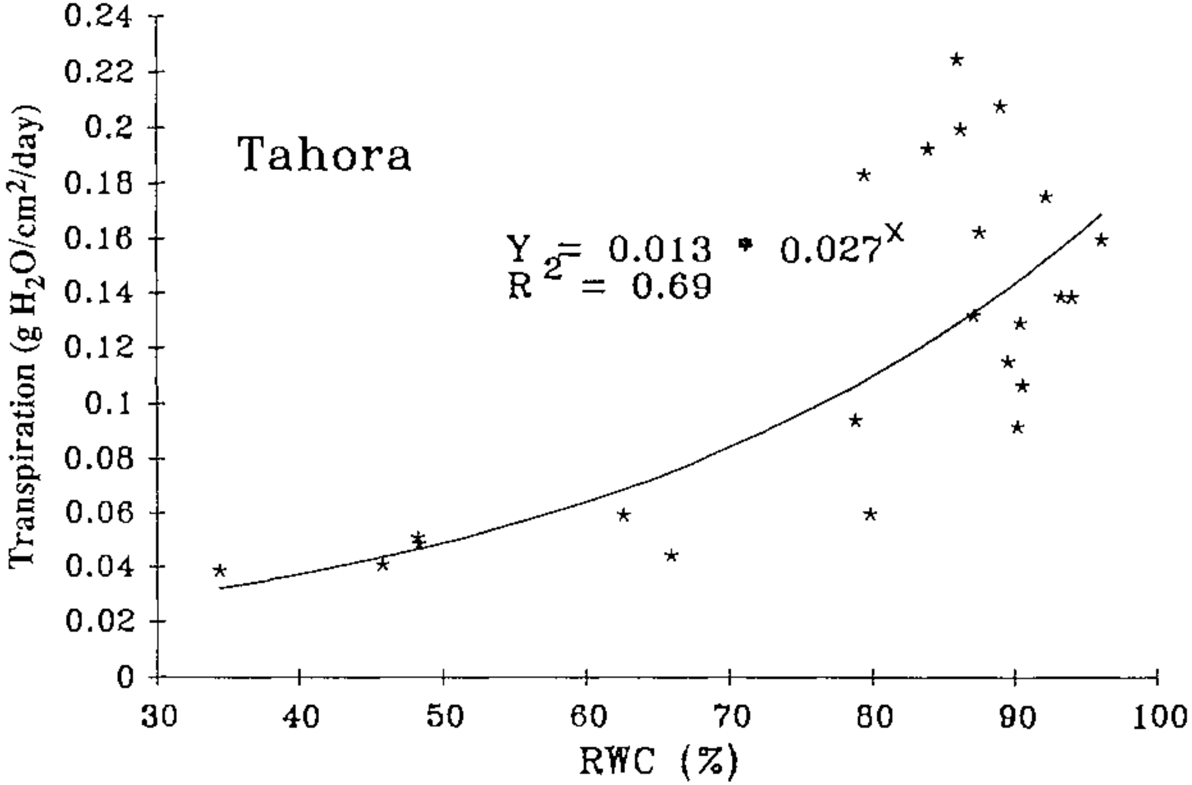
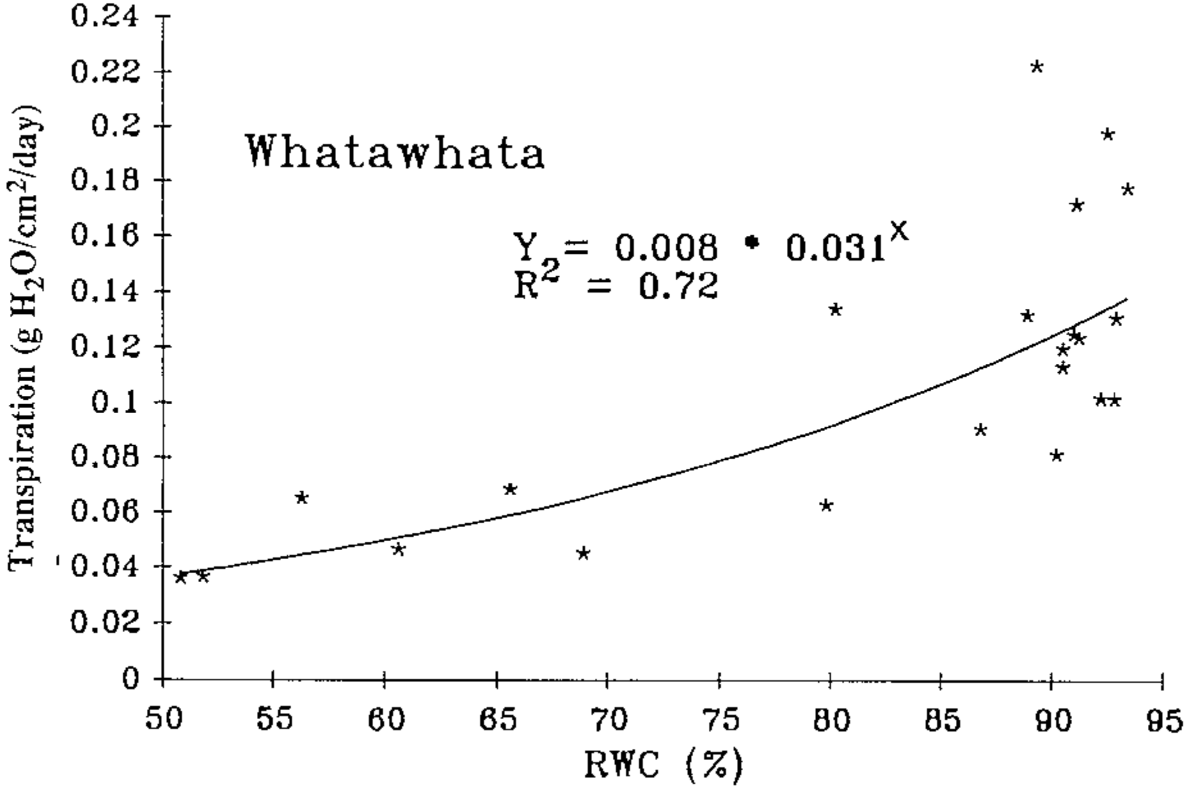


Fig. 5.3e Relationship between RWC and Plant Transpiration Rate



But for the purpose of comparing the cultivars in their relationship between water deficit level and transpiration the location of the trial may not have be very critical.

The leaf transpiration rate at high RWC levels tended to vary a lot but there was also a wide range of soil water status over which plants maintained a high RWC (Fig. 5.1). The relationship between leaf transpiration rate and soil water status was a linear one (Fig. 5.4a,b,c,d,e). So while RWC remained more or less constant between 45% to 90% of field capacity, leaf transpiration was nearly halved over the same range of soil water status. It might be suggested that the relative high RWC in this range of soil water deficit was possibly maintained by the progressive reduction of transpiration rate.

The levelling off of transpiration rate at low RWC might be due to the total closure of stomata which would mean that the low levels of transpiration would be an indication of transpiration through cutical layer.

5.3.3.2 Cultivar Differences

Table 5.3 Relationship between Transpiration (g $H_2Ocm^{-2}day^{-1}$) and RWC(%)

Cultivar	**a**	**b**	R^2	Sig.
Kopu	-5.46	0.034	0.82	****
Pitau	-5.01	0.028	0.77	****
Huia	-4.40	0.024	0.75	****
Tahora	-4.37	0.027	0.72	****
Whatawhata	-4.59	0.027	0.74	****
LSD.	0.70	0.009		

The relationship fitted well for all cultivars (R^2 from 0.72 to 0.82) and revealed certain differences among the cultivars. Kopu had a lower intercept value, **a**, than Huia, Tahora and Whatawhata. It would mean that at extremely low RWC Kopu would have a lower transpiration rate. However, its higher **b** value, the regression coefficient, suggested a greater increase of transpiration rate per unit RWC increase (Table 5.3). This pattern was reflected in Figs 5.3. Kopu generally had a lower transpiration rate than any other cultivars except Pitau. On the other hand, small-leaved cultivars tended to have higher transpiration rates at lower RWC and increased their transpiration slower than Kopu. The lower transpiration rate of Kopu was in agreement with its better performance even under water

Fig. 5.4a Relationship between soil water status and transpiration

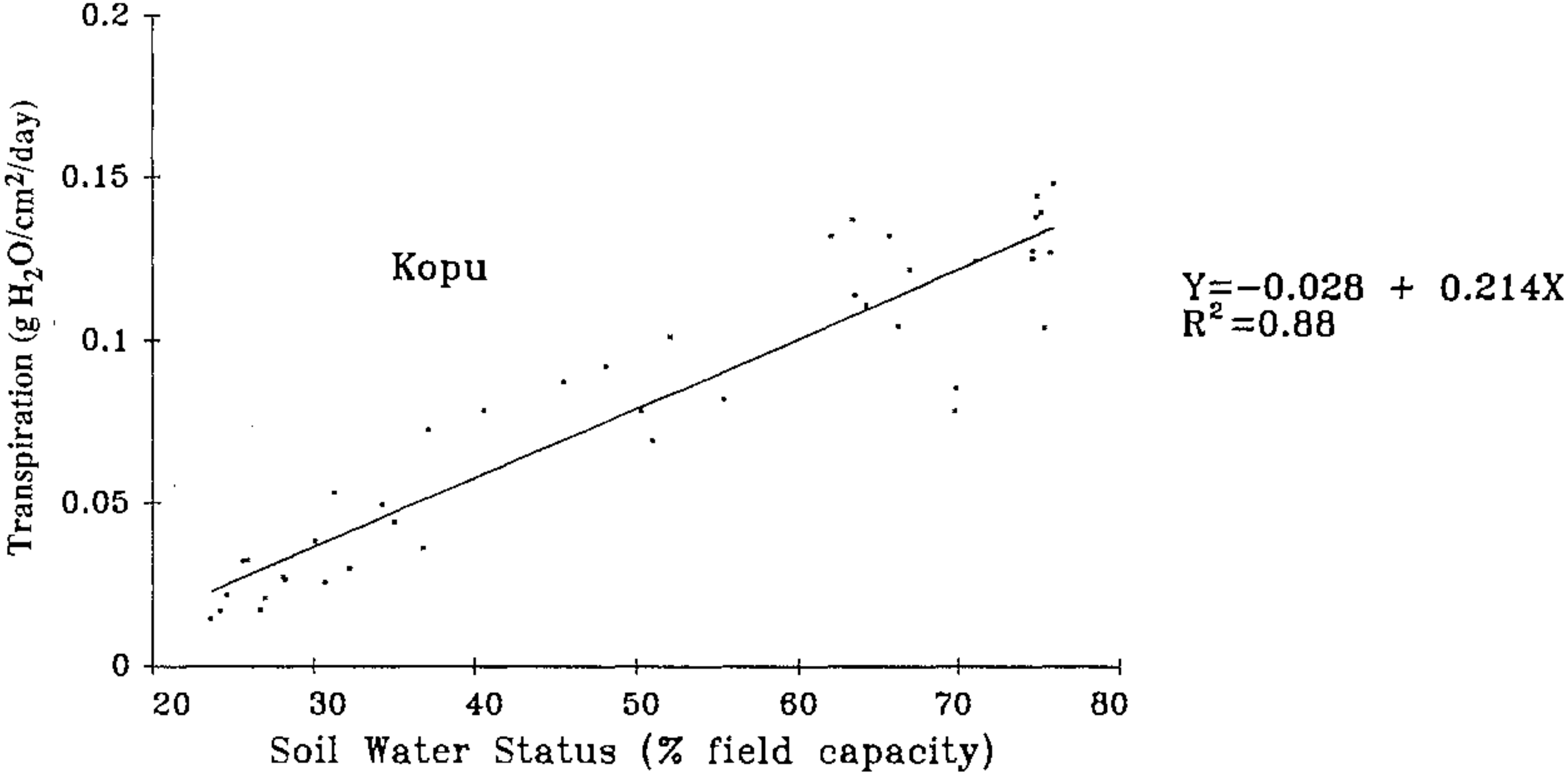
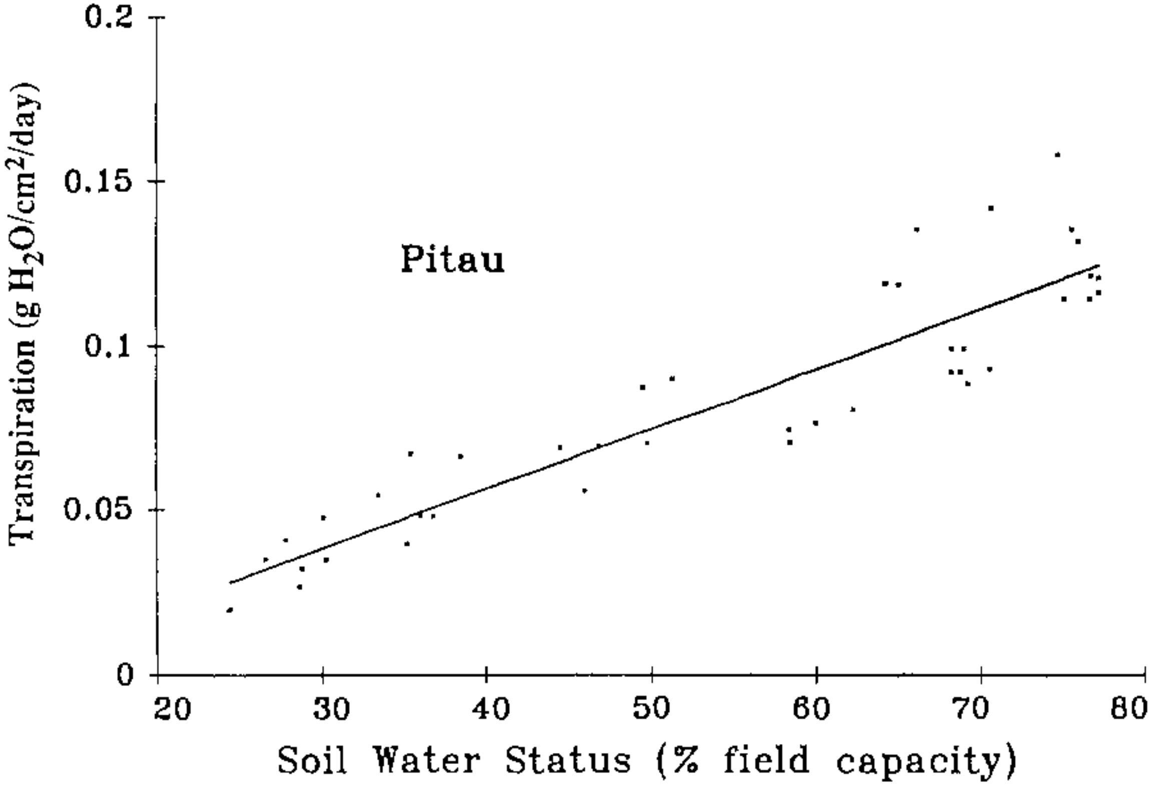
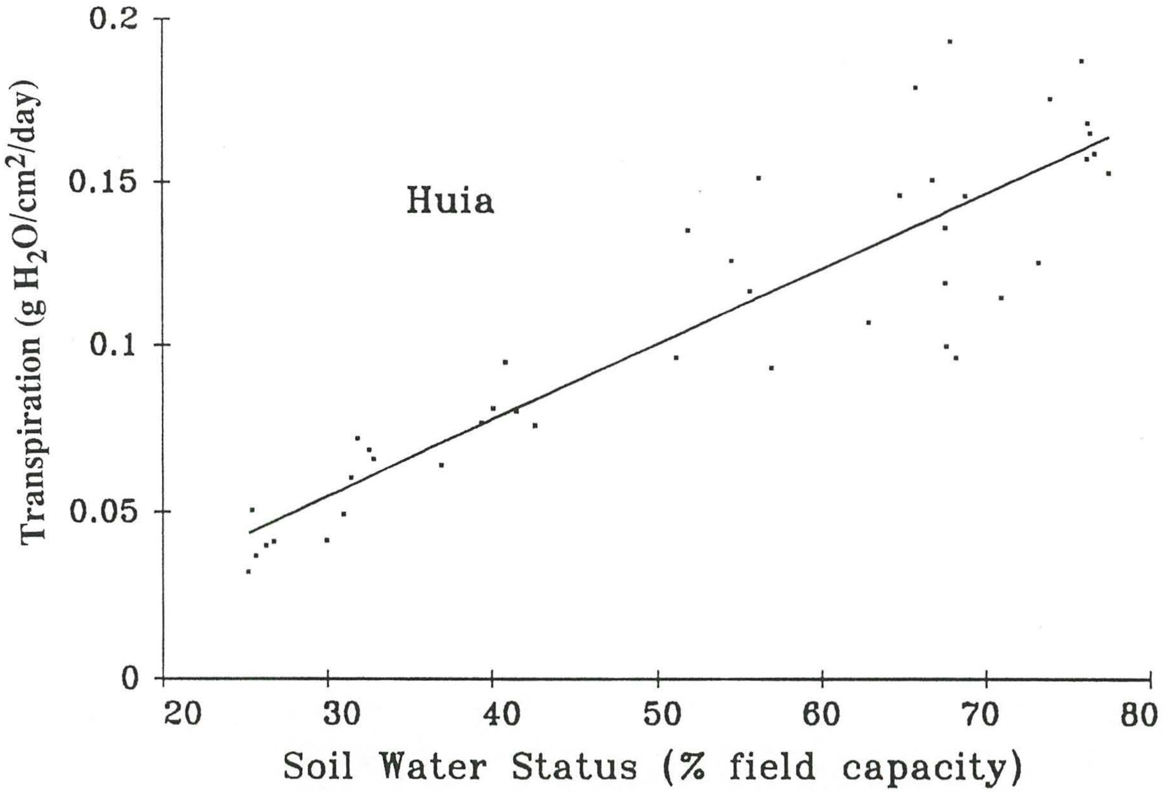


Fig. 5.4b Relationship between soil water status and transpiration



$Y=-0.016 + 0.182X$
$R^2=0.84$

Fig. 5.4c Relationship between soil water status and transpiration



Y=−0.014 + 0.230X
R^2 =0.81

Fig. 5.4d Relationship between soil water status and transpiration

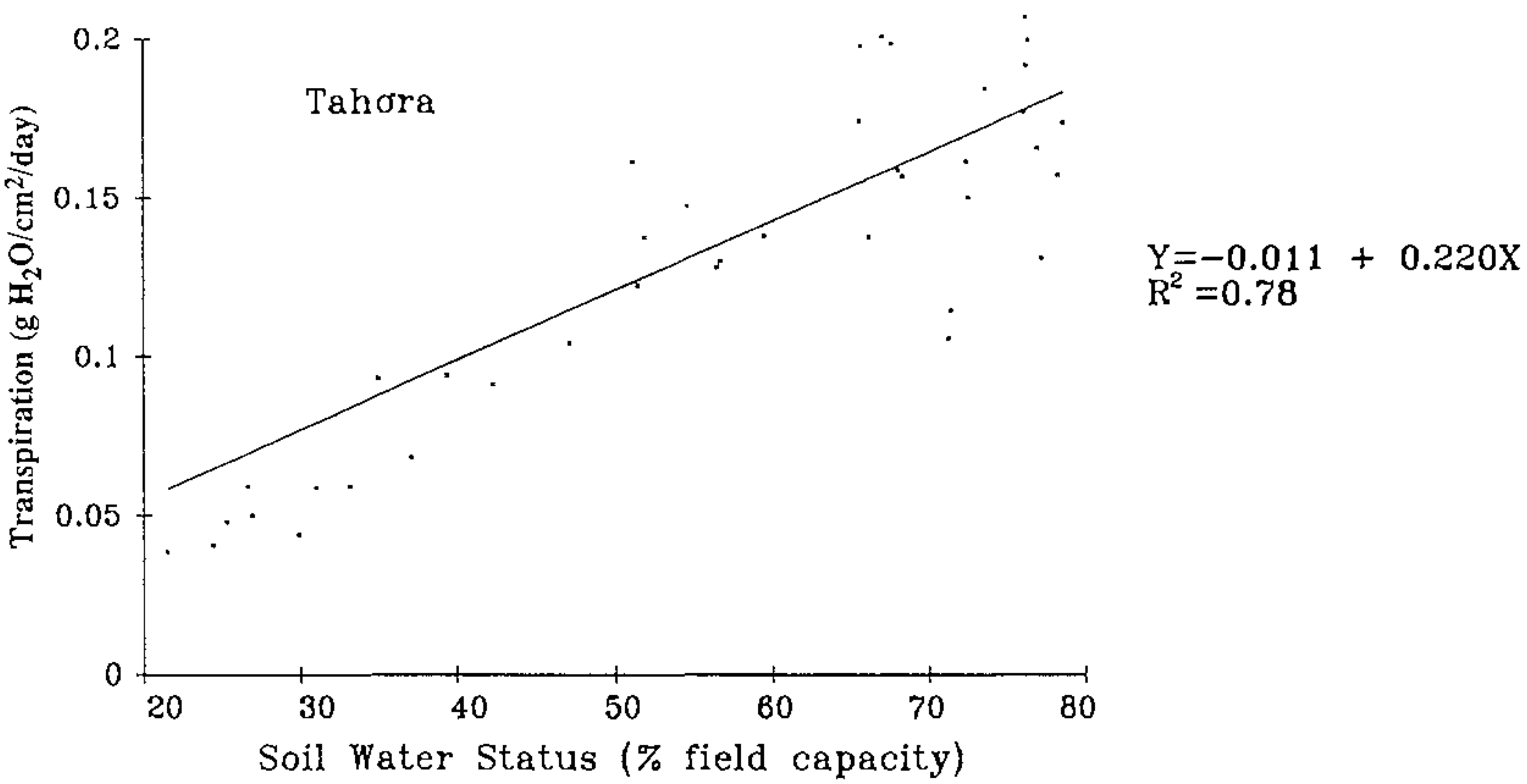
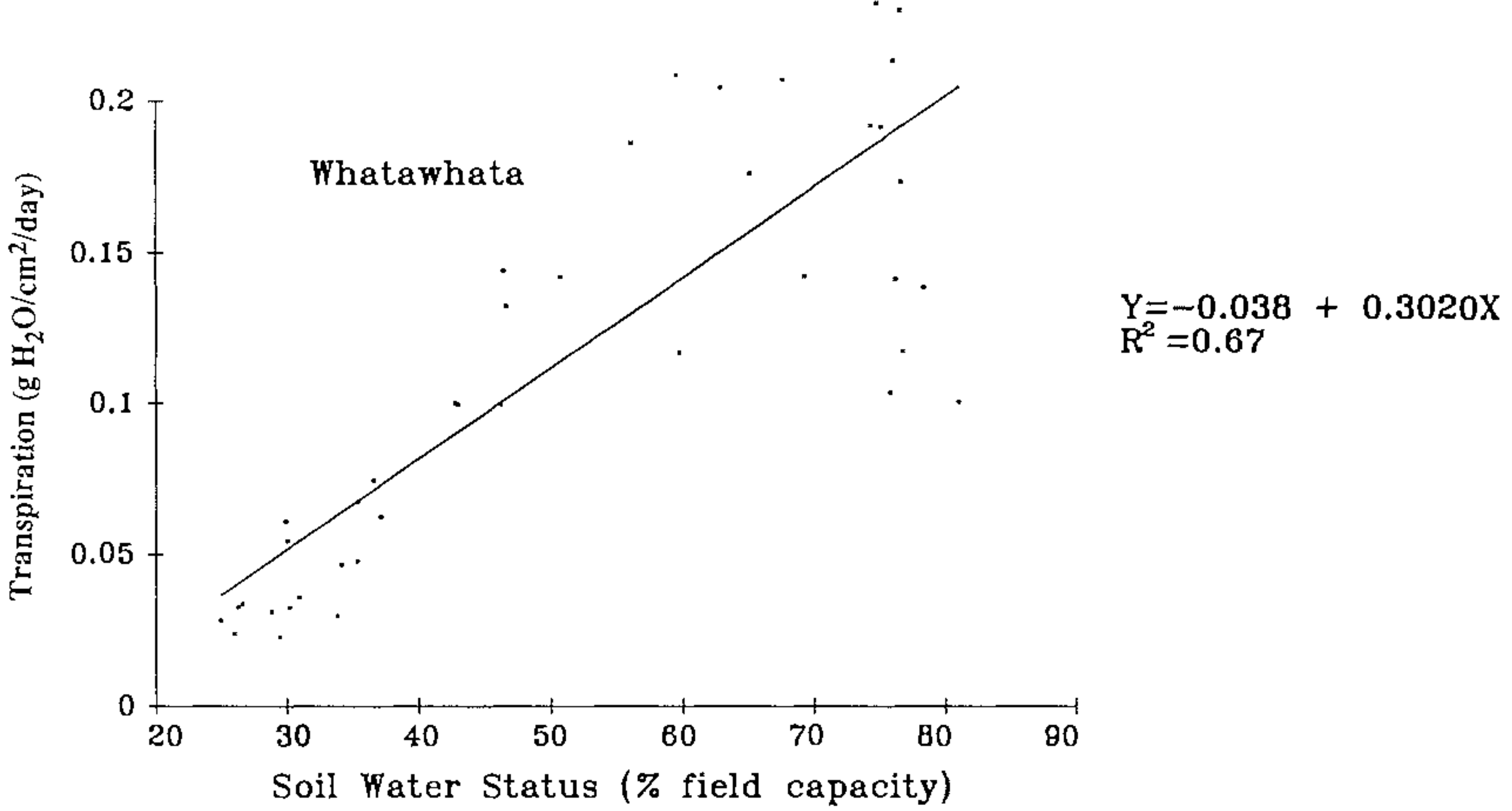


Fig. 5.4e Relationship between soil water status and transpiration



stress conditions (Ledgard *et al.*, 1988). It may even have a better control of transpiration rate under stress conditions compared with other cultivars.

5.4 Conclusions

1. There were few differences between the cultivars in their RWC response to a range of water deficit levels. The most distinctive was a tendency for Whatawhata to have a slightly higher RWC under adequate water supply than Kopu, while Kopu maintained a higher RWC, except at a very low soil water status, than other cultivars. So at high levels of water deficit, the cultivars had a similar leaf water status;

2. Leaf extension responded in a similar fashion between the cultivars although Pitau seemed to maintain a slightly higher level of growth than other cultivars at a low RWC;

3. Leaf transpiration (water loss) continued even where leaf extension had stopped, although a reduction of transpiration rate was also evident from 90% RWC to as low as about 50% RWC. The reduction was exponential. Kopu had a higher transpiration rate than other cultivars in the range of water deficit measured.

CHAPTER SIX
RESPONSES OF GROWTH AND NITROGEN-FIXATION TO WATER DEFICIT AND PHOSPHORUS LEVELS OF SEVEN WHITE CLOVER CULTIVARS

6.1 **Introduction**

The detrimental effect of water stress on white clover growth and development is well documented (Johns, 1978; Aparicio-Tejo *et at.*, 1980; Thomas, 1984) and has also been clearly shown in the previous experiments (Chapters 3,4,5). But given the variation in the vast number of white clover varieties, difference in water stress resistance may well exist. In previous experiments, it was shown that there were significant differences among the cultivars studied in their responses to water deficit. It was concluded that such differences are significant particularly in parameters such as leaf size and total leaf DW (Chapter 4). Plant leaf plays important roles in growth; the change of leaf size and total leaf area in white clover is significant in keeping the balance between resistance to water stress and maintaining productivity. Leaf size and total leaf area are the parameters that have influence on transpiration, so with the imposition of water stress, it is possible that leaf area reduction is an adaptive mechanism to reduce water loss (Hsiao, 1973). On the other hand, reduction of leaf area may well be a general plant adaptive reaction to stress by reducing its growth and changing DM partitioning between top and root growth.

With the downturn in agricultural receipts, phosphorus (P) fertilizer application, particularly in hill country pasture, has declined dramatically. However, most white clover cultivars released in New Zealand in the past have been selected for high yielding ability under medium to high fertility levels so it might be expected that they will be poorly adapted to low P levels. Whatawhata was selected from low fertility, dry hill country sites (MacFarlane and Sheath, 1984), and may be better adapted to infertile soils. Therefore P level was selected as a second treatment factor in the present experiment. The purpose of this treatment is to evaluate white clover responses to P fertility deficiency, in addition to water stress.

In order to test the effects of treatments on white clover growth patterns which are represented by many variables, the multivariate analysis techniques are used. With proper design and analysis techniques, it is possible to test whether the growth pattern or the DW partitioning pattern as a whole is responsive to the treatment stresses, and what the difference is between cultivars in their general responses to these stresses. Details of the statistical method are presented in Appendix 6.1.

One of the important contributions of white clover is its ability to fix atmospheric dinitrogen (N_2) (Low and Armitage, 1970). In New Zealand, it is estimated that approximately 1 million tons of N_2 is fixed annually (Ball and Field, 1985). The transfer of fixed N from white clover to grasses promotes their growth while the high N content in the white clover herbage improves pasture quality. However, N-fixation is known to be affected by environmental stresses, including water deficit stress (Sprent, 1972a,b; Aparicio-Tejo *et al.*, 1980) and low P fertility (Palmer and Iverson, 1983). The N-fixing ability of white clover is a key parameter in evaluating a cultivar's merit. It is generally considered that N-fixation is positively correlated with dry matter production so during cultivar evaluation N-fixation assessment is not normally carried out. In some legumes, such as the common bean, there is evidence of genetic differences for N-fixation at low levels of P availability (Graham and Rosas, 1979). Such information would be useful in interpreting the plant growth response but it is not available for white clover.

The objectives of the present experiment were:

1. to evaluate the effects of water deficit on the growth, development, dry matter partitioning, and N-fixation of 7 white clover cultivars under different P levels; and to estimate the relative sensitivity of various characteristics to water stress and low P levels;

2. to study the relationships between growth, development, dry matter partitioning, morphological characteristics and N-fixation;

3. to evaluate the plant characteristics that can effectively differentiate the cultivars used.

6.2 Material and Methods

6.2.1 Site and Medium Preparation

The experiment was carried out in Glasshouse No. 16, at the plant Growth Unit, Massey University. The glasshouse was maintained at between 18^{o} and $25^{o}C$ with a heater and ventilation system. The planting medium contained pumice and unsterilised Egmont sandy loam (50:50, v/v) and two-litre planting pots were used. The soil is naturally P deficient with a P retention of 98%. The nutrients, $Ca(NO_3)_2.4H_2O$ at 221 mg/l dry medium, $MgSO_4.7H_2O$ at 663 mg/l, K_2SO_4 at 891 mg/l, $MnSO_4.4H_2O$ at 13mg/l, H_3BO_3

at 4.3 mg/l, $FeSO_4.7H_2O$ at 1.8 mg/l, and $NH_4Mo_7O_2.4H_2O$ at 0.14 mg/l were mixed with the medium. The plants were germinated from seed 3 weeks prior to the experiment. Inoculation with *Rhizobium trifolii* was carried out twice at two weeks after sowing and again just prior to the commencement of the experiment.

6.2.2 Treatments

Phosphorus was added as phosphoric acid at five levels viz. 50, 200, 400, 800 and 1600 ppm into the medium, equivalent to Olsen P values of 7, 8, 12, 15 and 32 ppm measured respectively after equilibrating for two weeks. Moisture level was maintained by adjusting pot water content daily to a predetermined weight so as to maintain the plant leaf relative water content (RWC) relatively constant at 90% (well watered control) and 80% moisture stress) respectively. (See Appendix 3.3 for RWC measurement procedure).

Five New Zealand cultivars and two overseas cultivars (Dusi and Haifa) were used in this experiment. They were:

Large-leaved cultivars:	Dusi, Kopu, Haifa, Pitau
Medium-leaved cultivar:	Huia
Small-leaved cultivars:	Tahora, Whatawhata

6.2.3 Measurements

6.2.3.1 Non-destructive Measurements

Leaf parameters: Leaf number per stolon, leaflet length and leaf appearance rate were measured using the same method as described in Chapter 4.

Branch: Number of branches per stolon was measured as follows: a wire tag was put on the internode of the third youngest node of a stolon which had at least 7 nodes. The number of branches was counted forward (towards the apical bud) at harvests 2 and 4.

6.2.3.2 Destructive Measurements

Plant component DM: DW measurements were made at four harvests, each with four replicates, at 20-day intervals. The samples were dissected into shoot and root in all harvests and into leaf, stolon, root and senescent material in harvest 2. The samples were dried at $80^{o}C$ for 48 hours and the DW of each component then recorded.

Nitrogen (N) and Phosphorus (P) concentration: N and P concentrations in plant components were determined on a DM basis, using an automatic analyser with the method as described by Twine and Williams (1971).

Nitrogen fixation: Nitrogen fixation was estimated by the Acetylene reduction activity (ARA) measurement. It was made on plants grown at three P levels (viz. 50, 200 and 800 ppm). The omission of the other two P levels was to reduce the workload to a practical level. The ARA assay was carried out at harvest two, 40 day (d) and harvest four, 80 day (d). Harvesting and ARA assay were carried out in the morning from 8:00-10:00 hours on four successive days (one replicate per day). After shaking off soil from the roots, the whole plant was put in a one-litre air-tight jar. Ten percent (100ml) of the air in the jar was sucked out with a syringe and the same volume of C_2H_2 was injected as replacement. After incubation of one hour, gas samples were taken with 0.4 ml syringes and kept on rubber bungs to stop leaking before analysis. All gas samples were analysed for their ethylene (C_2H_4) content within three hours after sampling on a Pye Unicam 203 gas chromatograph. The gas chromatograph was calibrated for each run (daily) with an ethylene standard drawn into 4 ml Vacutainer tubes at the time that the incubation vessels were sampled. The ethylene concentrations obtained from the gas chromatograph analysis were then transformed into acetylene reduction activity of the nodules by a standard program on an Apple II computer (Robertson, pers. comm).

6.2.4 Statistical Analysis

The data were analysed using a split-split plot design with the ANOVA procedure in SAS (1985) to test the effects of main treatments and interactions in all the variables measured.

Canonical correlation analysis and discriminant analysis were then carried out on the data from harvest 2 with the CANCORR and CANDISC procedures in SAS (1985). The main purpose of using these analytical techniques was to study the variables on a joint basis so that the effect of treatments on a group of variables (for example, all the morphological characteristics) could be assessed together.

6.3 Results and Discussion

6.3.1 Growth Responses

6.3.1.1 Plant Component Dry Weight (DW)

DW of plants was significantly reduced by both water stress (Table 6.1) and low P fertility (Fig. 6.1). At the end of the experiment, total DW of water stressed plants was only 60% of well watered plants. DW of shoot (leaf and stolon) and root, and the amount of dead material were all affected in a similar pattern, though to different extents. The reduction of the leaf component was not as severe as observed in the previous experiment reported in Chapter 4 (section 4.3.3.2), probably due to the difference in water stress level. In this experiment leaf RWC of plants under the stress treatment was 80% compared with 70-75% reported in Chapter 4. Leaf was again more severely affected than stolon and root components. A similar result was obtained by Johnson and Raguse (1985) in white clover, and in ryegrass by Chu (1979). Root dry weight was least affected.

The relationship between P levels and DW of leaf, shoot and total plant is shown in Fig. 6.1. Obviously, the response of the shoot component was much greater than that of the root. So the decrease of total DM under low P level was mainly due to the reduction of shoot growth. DM response per unit P declined rapidly as P level increased (Fig. 6.2). There were differences between the cultivars. The differences between 50, 200 and 400 ppm P levels in shoot DW, root DW and total DW were generally significant (Fig. 6.1) while the increase in DW from P level 400 ppm to 800 ppm and 1600 ppm was non-significant, thus indicating that for this experiment, the maximum P level of white clover response was about 800 ppm (Olsen P 15 ppm).

The interaction between cultivar and water level was significant, arising mainly from the greater reductions of Haifa, Pitau and Dusi under stress conditions compared with Tahora and Whatawhata (Table 6.1). Again, cultivars with a larger DW under control conditions tended to be more severely affected by water stress than those with smaller DW. Huia was performing slightly better than the other cultivars at both water levels (Table 6.1) with its relatively large DW and small reduction. The decrease in productivity with decrease in P levels started at 800 ppm, most of the reduction occurring at 400 down to 50 ppm stage, but Whatawhata did not decrease its DW any further below 200 ppm level (Fig. 6.3). Overall, large leaved cultivars were still more severely affected by low P fertility.

Table 6.1 Plant Leaf, Stolon, Root and Total DW of 7 Cultivars under 2 Water Deficit Levels (Harvest 2)

Water Level	Cultivar							mean	Sig.	LSD.
	Dusi	Kopu	Haifa	Pitau	Huia	Tahora	Whatawhata			
Leaf										
Control	4.19	3.88	5.04	4.23	3.86	3.40	2.88	3.93	**	1.20
Water stress	1.71	1.56	1.80	1.48	1.70	1.36	1.20	1.55	**	0.48
Stolon										
Control	2.22	2.00	2.78	2.32	2.30	1.85	1.64	2.09	**	0.59
Water stress	1.64	1.81	2.04	1.42	1.86	1.74	1.13	1.66	**	0.38
Root										
Control	1.93	1.85	2.29	2.33	2.02	1.61	1.08	1.87	**	0.62
Water stress	1.35	1.28	1.71	1.80	1.65	1.43	0.89	1.45	**	0.57
Total DW										
Control	8.35	7.73	10.11	8.88	8.18	6.86	5.60	7.89	**	2.19
Water stress	4.70	4.65	5.55	4.70	5.21	4.54	3.22	4.66	*	1.58

Fig. 6.1 Effect of P levels on plant shoot, root and total DW (mean & SEM of seven cvs)

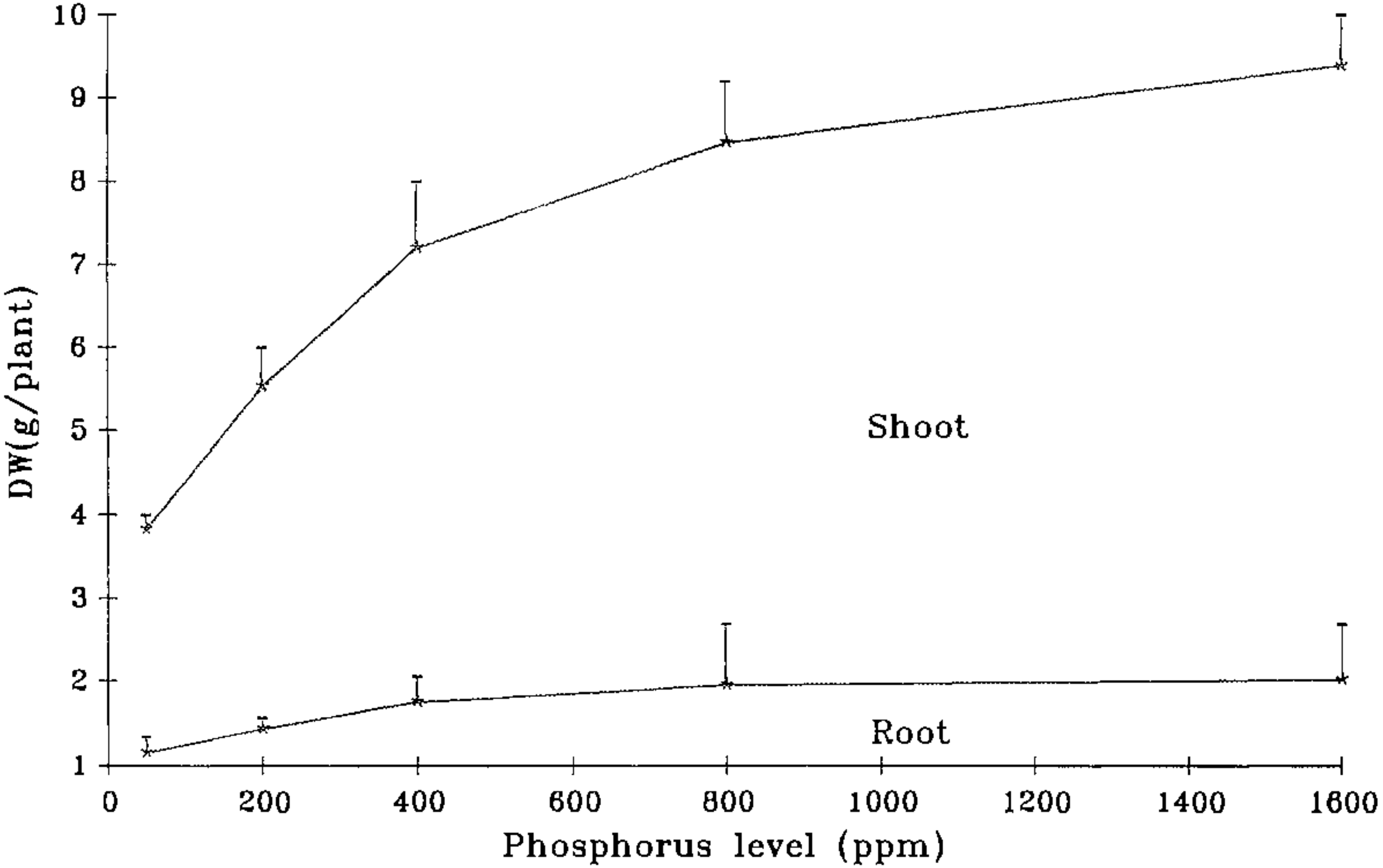
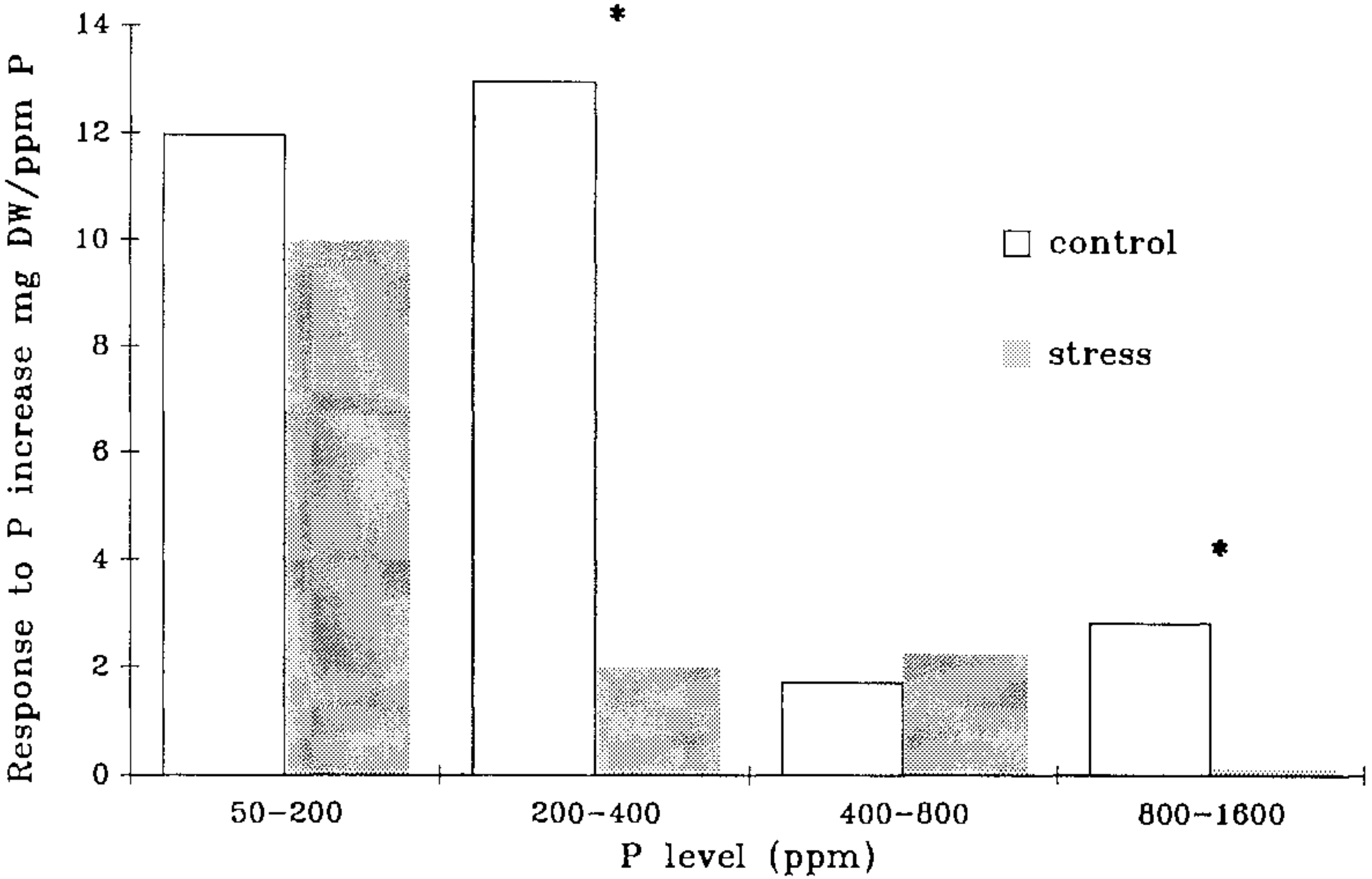
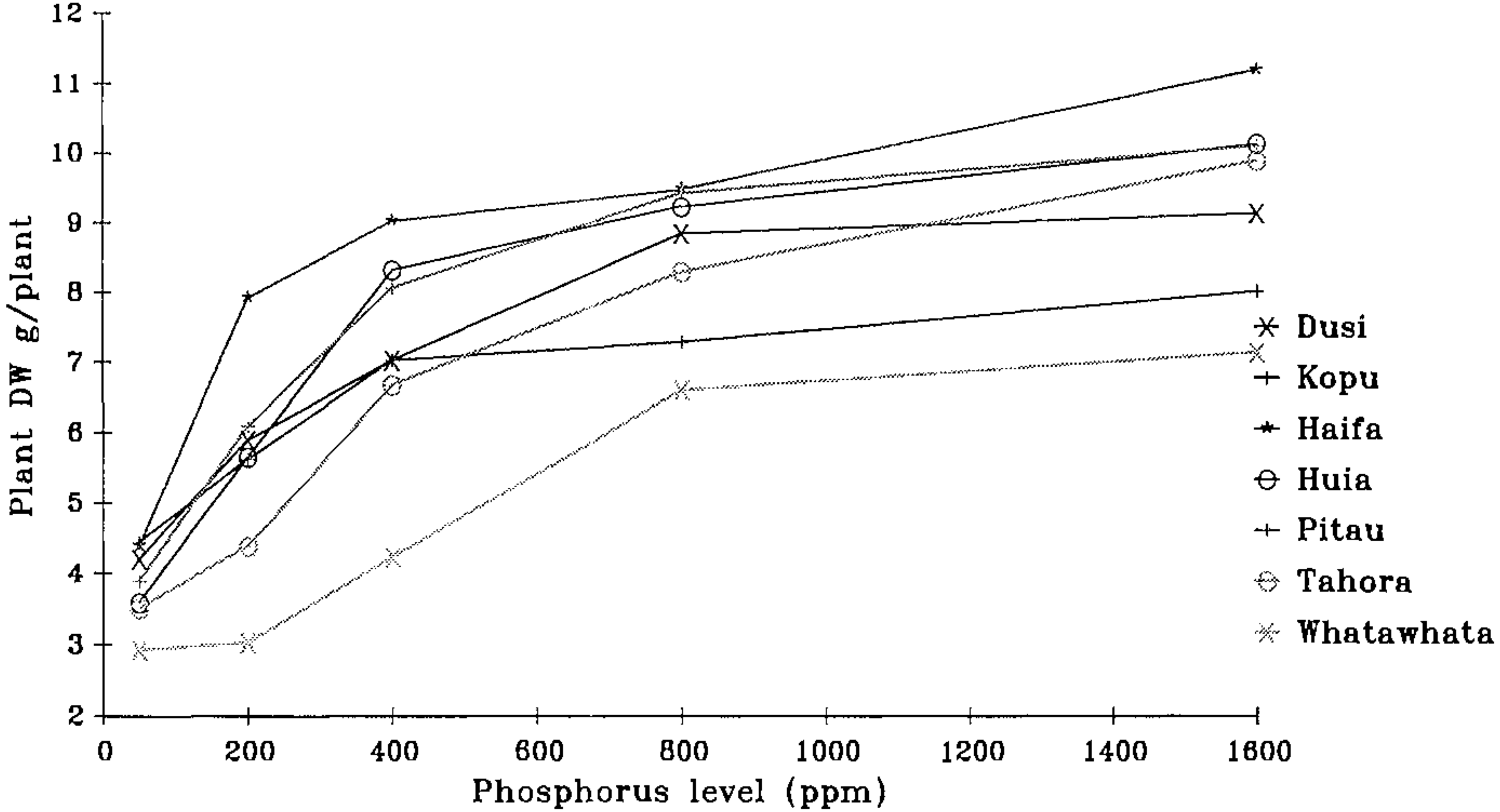


Fig. 6.2 Relationship between DW response of white clover and P levels (harvest 4)



* different at $p<0.05$ level.

Fig. 6.3 Effect of P levels on total DM of seven cultivars



af DW differed significantly between cultivars under control conditions, but not under stress conditions. Discriminant analysis (Appendix 6.2a) showed that root DW was an important variable for distinguishing between cultivars, because differences between cultivars persisted under stress conditions whereas differences in top growth were reduced sharply.

6.3.1.2 Component DW Partitioning

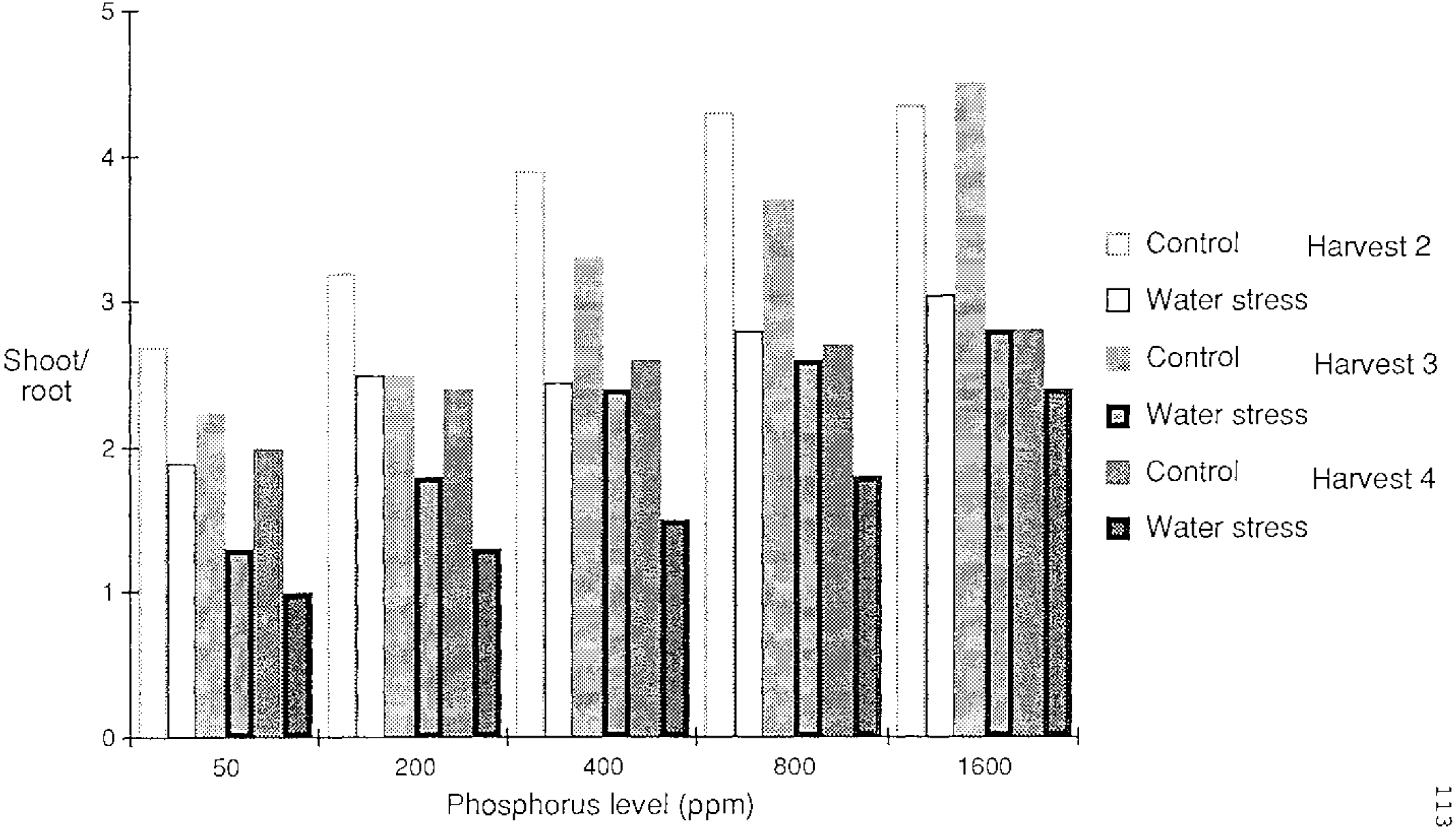
Shoot:root ratio of plants, calculated as the mean of harvest 2 to 4, was reduced by water stress from 3.68 to 2.56 (Table 6.2). Water stress significantly reduced the shoot:root ratio in every cultivar, but there was no interaction between the cultivars and water level. Over the four harvests, the shoot:root ratio declined (Fig. 6.4), mainly due to gradually changing growth pattern under water stress conditions.

Table 6.2 Shoot:Root Ratios of 7 Cultivars under 2 Water Deficit Levels (Harvest 2)

Water level	Cultivar							mean/LSD	Sig.
	Dusi	Kopu	Haifa	Pitau	Huia	Tahora	Whata.		
Control	3.80	3.56	3.65	3.02	3.48	3.86	4.43	3.68/0.58	**
Water Stress	2.66	2.66	2.57	2.05	2.49	2.54	2.91	2.56/0.49	**

The shoot:root ratio increased with P levels (Fig. 6.4) corresponding with the observation that the stimulating effect of P on shoot growth is greater than on root growth (Pereira and Bliss, 1987). On the other hand, the root proportion increased under the low level of P fertility to adapt to the conditions (Davidson, 1969; Thornley, 1972). When the root function is limited (ie. by H_2O or P stress) the root has first access to the scarce resource with excess transported to the top. Also photosynthesis is less affected under water stress than leaf extension, so the excess carbohydrate (CHO) can be sent down for root growth. The growth and function of root, therefore, would be less affected than those of the shoot. The reduced vitality of growing points, either apical meristem or axillary buds, may be a direct cause of changing DW partitioning. The relationship between the morphological characteristics and plant DW partitioning will be further discussed in section 6.3.4.3.

Fig. 6.4 Relationships between shoot:root ratio and P levels under 2 water levels



6.3.2 Morphological Characteristics of White Clover Cultivars

6.3.2.1 The Static Characteristics

Anova tests of the static characteristics (viz. leaf size as expressed by leaflet length, leaf number and weight per stolon, number of branches per stolon) showed significant treatment effect and cultivar differences (Table 6.3 and Fig. 6.5). The overall trend was the reduction of these variables by both water stress and low P fertility. For example, leaf size was reduced about 23% by water stress and 24% by low P levels (50 ppm).

The reduction of leaf size and leaf number per stolon caused by water stress were larger in Haifa than in Tahora, Kopu and Whatawhata (Table 6.3). Therefore its leaf DW per stolon was reduced more than the other cultivars. Compared with the large-leaved cultivars, Tahora and Whatawhata were less affected.

For both leaf number and leaf weight per stolon, the interactions between cultivar and water deficit level were significant. Cultivars with more leaves and higher leaf DW per stolon tended to have a greater reduction in both parameters.

Leaf number per stolon was reduced 47% ($p<0.05$) by P stress but the number of branches was not significantly reduced (Fig.6.5). This may be attributed to the different sink strength of different growing points. Interactions between cultivar and P fertility were also significant, due to the inverse relationship between cultivar performance at adequate P level and the reduction under low P fertility. This response is similar to the water stress relationship discussed in the previous chapter.

6.3.2.2 The Dynamic Characteristics

The leaf appearance rate (for analysis purpose, it is expressed by its inverse, or interval, days/leaf, so is the branching rate) (Fig. 6.6) and branching rate (days/branch) of white clover were significantly affected by both water deficit stress and low P fertility (Table 6.4). Both were slowed by the stress treatments. The reduction in leaf appearance rate was nearly 50% for all cultivars pooled together, with little difference between cultivars and no interaction between cultivar and water level. It appeared that the highest leaf appearance rate occurred at medium P level, 200 to 400 ppm.

For branching rate the differences amongst the cultivars were significant with Whatawhata having the shortest interval between the formation of two branches, while Huia and Haifa were also shorter than Dusi. Branch formation was also higher at the three medium levels of P fertility, probably related to the higher leaf appearance rate at the medium P levels. Branch development is primarily determined by the number of leaves, hence the number of available axillary buds (Thomas, 1987). Branch development is also dependent upon the supply of assimilates (Haystead, 1983). The absence of large differences in leaf appearance rate and branching rate between P levels here could not be conclusively regarded as an indicator of insensitivity of these parameters to P fertility level; rather it was probably due to the strong sink strength of growing points for P which made the effect of low P fertility less detrimental to leaf emergence and axillary bud development.

6.3.3 N-fixation

6.3.3.1 Effects of Main Treatments on Acetylene Reduction Activity (ARA)

The effect of moisture stress on ARA was highly significant on a per plant basis, but not always so on a unit plant DW basis. Under stress conditions, ARA per unit leaf DW was significantly increased ($p<0.01$) (Table 6.5). At extreme levels, moisture stress can affect N-fixation directly by reducing nodule size and number (Gibson *et al.*, 1982), however, here the supply of carbohydrate from photosynthesis may be a key factor affecting nodule function (Huang *et al.*, 1975; Haystead *et al.*, 1979). ARA is usually lower because of the effects of stresses on leaf production and photosynthesis. But the partitioning of photosynthate towards shoot or root also has an important influence on root growth and function, including N-fixation. ARA measurement estimates N-fixation rate in a short time period but is influenced by the current environmental and plant effects on nodule number and mass, and photosynthate supply.

Table 6.3 Leaf size (leaflet length in mm), number of leaves and leaf weight (g) per stolon and leaf specific weight (cm^{2}g^{-1}) of 7 cultivars under 2 water deficit levels

Water level	Cultivar							mean	Sig.	LSD.
	Dusi	Kopu	Haifa	Pitau	Huia	Tahora	Whatawhata			
Leaf size										
Control	15.17	13.00	14.67	14.00	12.67	8.00	7.17	12.10	***	1.80
Water stress	12.17	11.00	9.33	10.83	9.67	6.33	5.83	9.31	***	0.69
No. leaves stolon^{-1}										
Control	7.28	5.97	10.19	8.50	6.40	7.96	6.14	7.99	**	2.30
Water stress	4.27	4.77	6.13	4.42	4.60	4.87	5.08	4.88	*	1.05
Leaf DW stolon^{-1}										
Control	0.49	0.43	0.48	0.37	0.34	0.13	0.14	0.34	**	0.19
Water stress	0.27	0.30	0.28	0.21	0.19	0.09	0.10	0.19	*	0.15
Leaf specific weight										
Control	380.9	409.6	380.7	377.9	416.6	374.9	531.8	410.3	**	80.2
Water stress	250.6	263.2	243.8	238.9	270.3	232.8	281.8	254.5	**	43.7

Fig. 6.5 Effect of P levels on number of leaves and branches per stolon (mean & SEM)

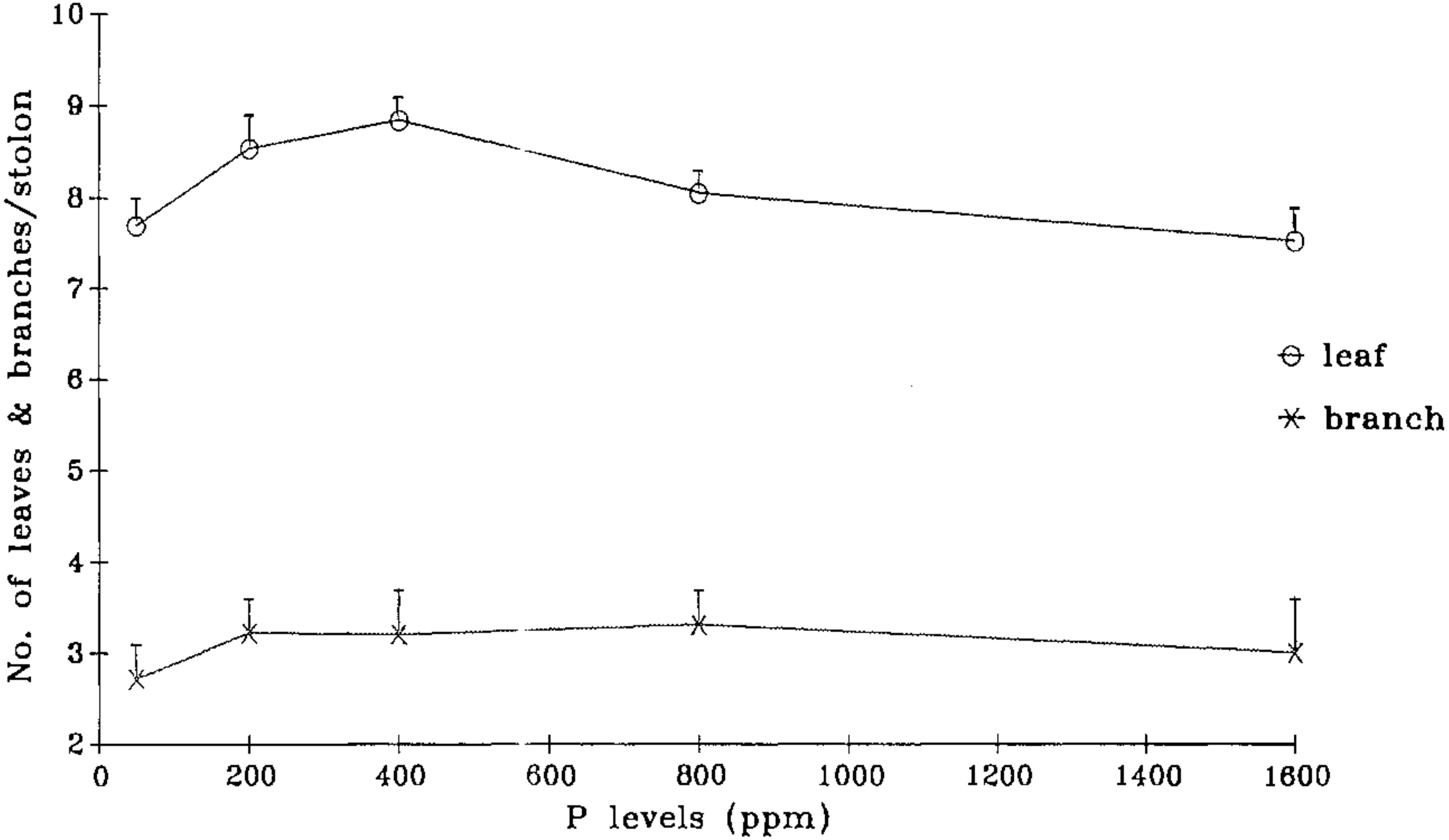


Fig. 6.6 Leaf appearance rate of 7 white clover cultivars under 5 P levels

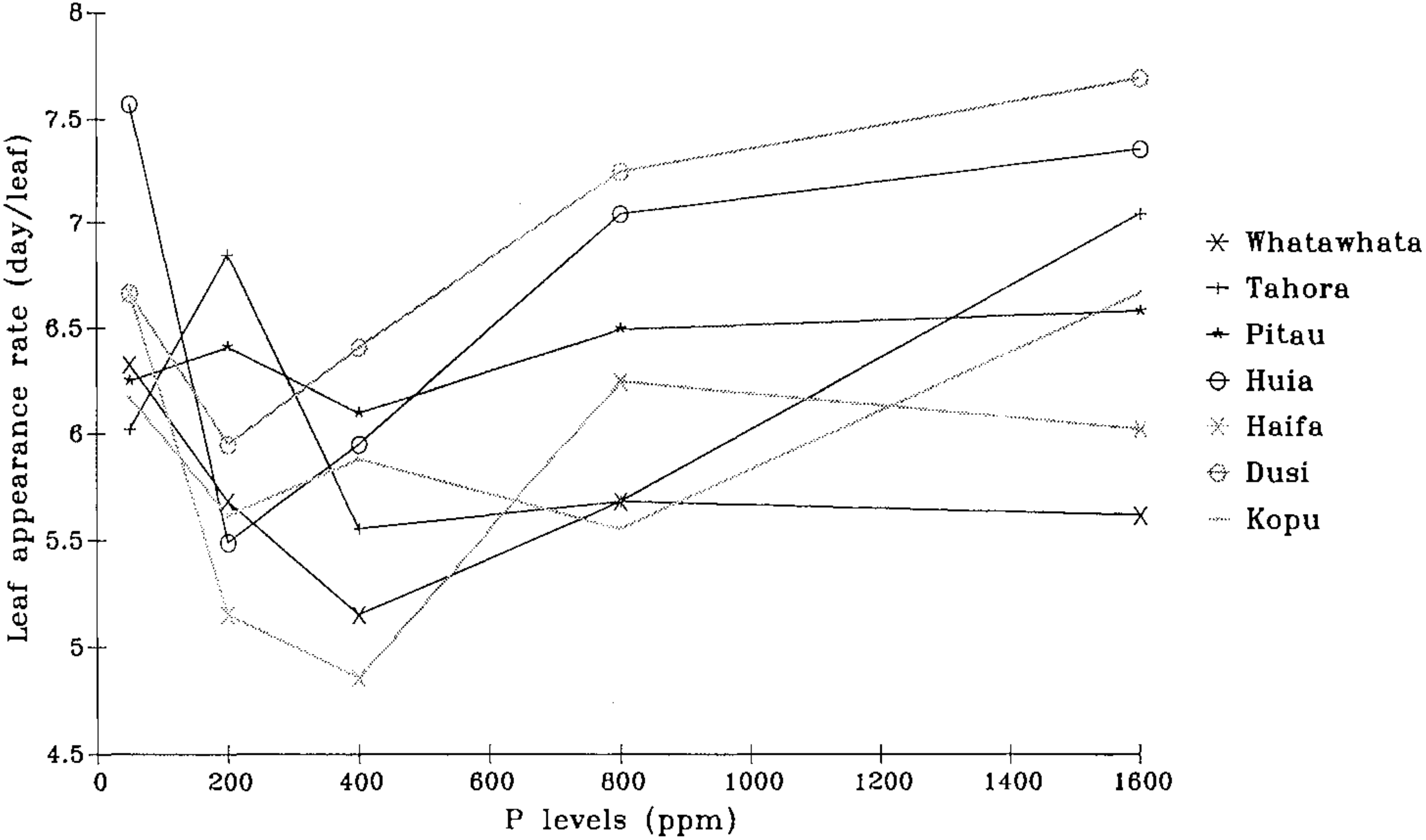


Table 6.4 Leaf appearance rate (days node^{-1}) and branching rate (days branch^{-1}) and leaflet extention rate (mm day^{-1}) of 7 cultivars under 2 water deficit levels

Water level				Cultivar				mean	Sig.	LSD.
	Dusi	Kopu	Haifa	Pitau	Huia	Tahora	Whatawhata			
Leaf appearance rate										
Control	5.05	4.61	4.24	4.81	4.81	4.61	4.39	4.63	*	0.69
water stress	10.20	8.40	8.70	9.35	10.20	9.43	8.06	9.09	ns	
Branching rate										
Control	13.04	11.05	9.59	8.88	10.53	10.61	7.92	10.00	*	2.65
Water stress	35.00	28.38	19.35	24.28	20.29	17.28	14.33	21.00	**	5.87
Leaflet extension rate										
Control	1.03	0.62	1.20	0.63	0.62	0.44	0.44	0.72	*	0.38
Water stress	0.37	0.12	0.50	0.28	0.26	0.12	0.18	0.27	*	0.16

note: for analysing purpose, both the leaf appearance rate and branching rate are expressed as the inverse of the rate, or interval.

Table 6.5 Effect of water deficit stress on ARA ($umC_2H_4hr^{-1}$) at harvest 2 (40 d) and harvest 4 (80 d)

	Moisture Deficit Level		
	Control	Stress	Sig.
Harvest 2			
plant^{-1}	2.73	1.33	***
g^{-1} total DM	0.31	0.26	ns
g^{-1} leaf	0.91	1.48	**
Harvest 4			
plant^{-1}	2.58	1.45	**
g^{-1} total DM	0.28	0.41	ns

(values mean of 3 P levels and 7 cultivars)

The results of the current experiment agree with the well documented evidence that moisture stress affects N-fixation (eg. Huang *et al.*, 1975; Sprent, 1975). But under the water stress treatment in this experiment ARA was reduced by about 50%, which was slightly less than other reported results where ARA was reduced by about 67% (Engin and Sprent, 1973; Jonhson and Raguse, 1985). Furthermore, ARA per unit DM was not reduced, indicating that N-fixation was affected by moisture stress to a similar extent as DM production.

It may be argued that ARA should be correlated with the amount of leaf tissue under control conditions. But under stress conditions, the results here showed that ARA per unit leaf DM was greater than that of the control. It was suspected that under stress conditions the carbohydrate supply to the nodules may actually be improved by the increased partitioning of photosynthate towards stolon and root components and so increase their CHO level. In some other work where severe and quick stress was imposed (Huang *et al.*, 1975; Haystead *et al.*, 1979) photosynthate partitioning might not have changed. The theory of functional equilibrium between shoot and root also showed that with stresses limiting root growth and function, such as under water stress or low P fertility, the partitioning towards root can be increased (Davidson, 1969; Thornley, 1972). So for an extended stress period, the functions of roots (nitrogen fixation) might be less affected than those of the shoot component (photosynthesis).

From harvest 2 to harvest 4, though the control plants continued to grow, their total ARA decreased slightly while for water stressed plants, their AR activity increased but again only slightly. The net result of these changes was the increase of ARA per unit DM in harvest 4 under stress conditions. The control plants were defoliated once at 60 d, and probably because of an incomplete recovery from defoliation their total ARA decreased slightly. Ryle *et al.* (1985) have shown that defoliation can reduce N-fixation. On the other hand, ARA of the stressed plants, which were not defoliated, increased slightly.

Effect of P levels: High P level (800 ppm or Olsen P 16 ppm) significantly increased ARA per plant by more than 100% while the two low P levels (Olsen P 6 and 7 ppm respectively) differed significantly only at 40 d (harvest 2) (Table 6.6). The two low P levels differed little in plant N fixation at the later harvest date. This lack of difference between the two low P levels in harvest 4 was probably mainly due to the small difference in the P availability, either prior to or at the end of the experiment ($p>0.05$). On a total DM basis, ARA did not differ significantly between the three P levels at both dates. This again showed that ARA was affected by low P availability proportionally in a manner similar to that of the DW accumulation, pointing to the common influence of photosynthesis on both ARA and DM accumulation. It was unexpected that ARA per unit leaf DM decreased significantly with increasing P level from 200 to 800 ppm. It might be speculated that the higher P availability had caused a plant partitioning pattern in favour of shoot growth, hence significantly reduced the ability of the leaf tissue to support N-fixation even though it would be expected to have a higher photosynthetic rate with an adequate P supply.

Table 6.6 Effect of phosphorus level on ARA (um$C_2H_4hr^{-1}$)

	Phosphorus Level (Olsen p)				
	50(7)ppm	200(8)ppm	800(16)ppm	Sig.	LSD.
Harvest 2 (40 d)					
plant^{-1}	1.09c	1.81b	3.22a	***	0.70
g^{-1} leaf	1.22ab	1.38a	0.96b	*	0.40
g^{-1} total DM	0.57	0.73	0.74	ns	--
Harvest 4 (80 d)					
plant^{-1}	1.32b	1.55b	3.25a	***	0.88
g^{-1} total DM	0.59	0.48	0.53	ns	--

(values mean of 2 water treatments and 7 cultivars)

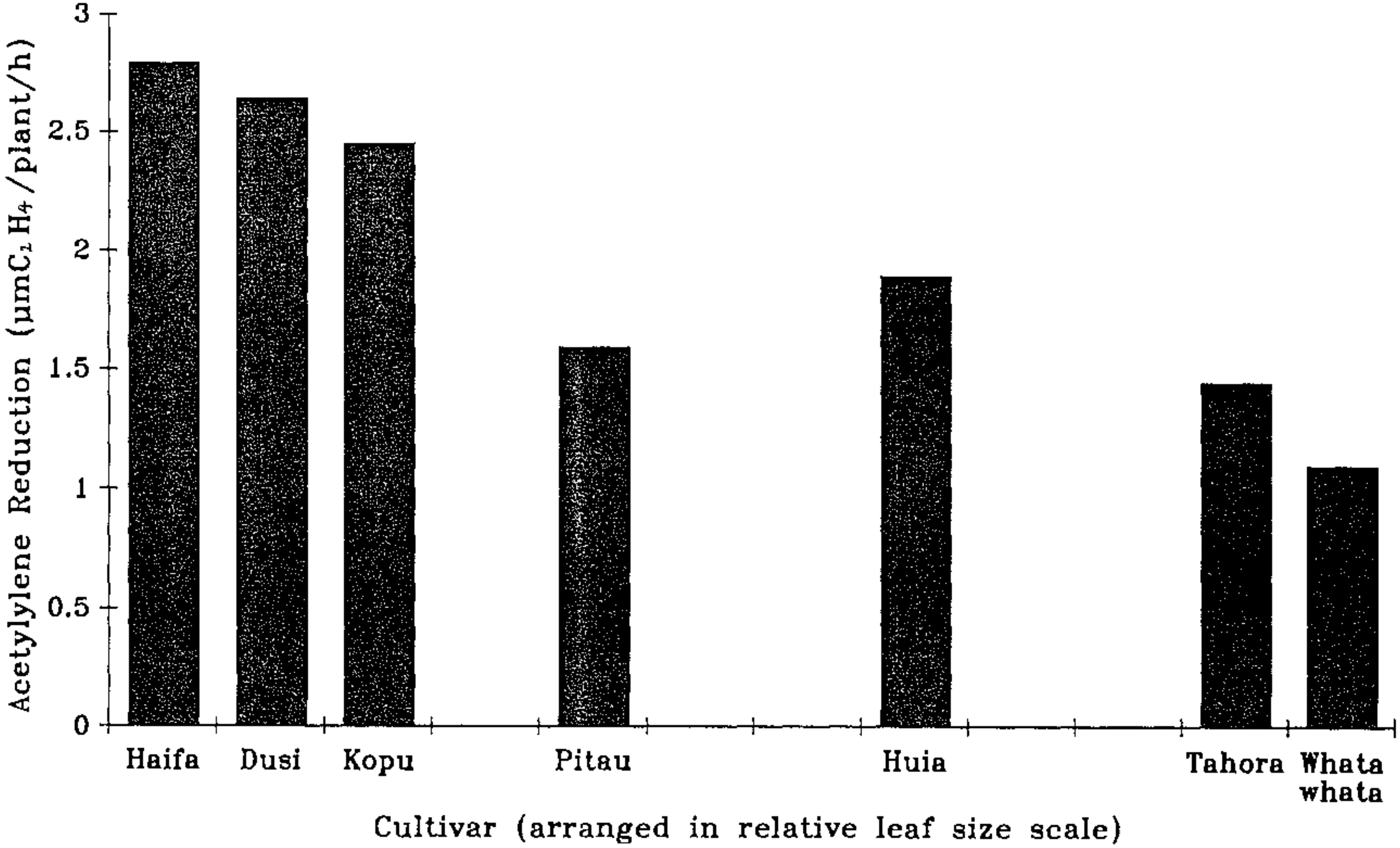
Table 6.7 Differences between cultivars in ARA ($umC_2H_4hr^{-1}$)

Cultivar	Dusi	Kopu	Haifa	Huia	Pitau	Tahora	Whata.	Sig.	LSD
Harvest 2									
$plant^{-1}$	2.36	2.08	2.09	2.04	2.43	1.79	1.48	ns	--
g^{-1}total DM	0.72	0.70	0.53	0.67	0.76	0.68	0.70	ns	--
g^{-1} leaf	1.30	1.38	0.73	1.11	1.86	1.24	0.65	*	0.47
Harvest 4									
$plant^{-1}$	2.95	2.93	3.56	1.80	0.90	1.16	0.62	**	0.60
g^{-1}total DM	0.91	1.22	0.83	0.25	0.13	0.18	0.14	**	0.59

(values mean of 2 water levels and 3 P levels)

Cultivar differences: At 40 d, differences among cultivars for ARA on both per plant and per unit DM basis were not significant except for ARA per unit leaf weight, for which Pitau was the highest while Dusi and Kopu were significantly higher than Haifa and Whatawhata (Table 6.7). At 80 d, ARA of Tahora, Whatawhata and Pitau was significantly less than that of Dusi, Kopu, Haifa and Huia. It seemed that ARA was largely correlated with the leaf size category of a cultivar with the exception of Pitau (Fig. 6.7). This tendency was apparent in both harvests even though at 40 d the differences between the cultivars were not significant. This relationship was probably caused by the differences in leaf size. The greater proportion of leaf DM in the large-leaved cultivars as well as in Huia (Chapter 4) may have been a contributing factor in their higher ARA value, while the low ARA value recorded for Whatawhata may be linked to its adaptation to stress by reducing or even stopping growth activity. Besides, indigenous white clover populations are usually less vigorous than the bred varieties (Davies and Young, 1967). It has long been observed that the DM yield of a white clover genotype is positively correlated with its leaf size, at least initially (Davies and Young, 1967). Though N-fixation may be positively associated with DM yield, the efficiency of N-fixation (N fixed per unit DW) showed a big variation at 80 d. This indicates that there might be some differences between the cultivars in addition to their absolute leaf size variation. One explanation is the possible difference in photosynthate partitioning. The relatively poorer performance of Pitau, on the other hand, may not be very surprising since it is a winter active cultivar.

Fig. 6.7 Relationship between cultivar leaf size and AR (mean of H2 and H4)



6.3.3.2 Interactions Between Cultivar, Moisture Level and phosphorus Level

Table 6.8 ARA(um C_2H_4plant^{-1}hr^{-1}) of 7 Cultivars under 2 Water Levels

Water Level	Cultivar Dusi	Kopu	Haifa	Huia	Pitau	Tahora	Whatawhata
Control	3.42	2.66	2.76	2.49	3.15	2.16	2.48
Water stress	1.22	1.51	1.36	1.59	1.70	1.41	0.49

LSD: cultivar comparison:0.89; moisture level comparison:0.66
(values are mean of 2 harvest and 3 P Levels)

Dusi and Whatawhata were affected more severely by moisture stress than the other cultivars (Table 6.8). Their ARA was reduced by around 2 um C_2H_4plant^{-1}hr^{-1}, or 64% and 80% respectively. Kopu, Haifa and Pitau were reduced by 43-50% while Huia and Tahora were reduced by less than 1 um C_2H_4plant^{-1}hr^{-1}. Apart from Whatawhata, the relative reduction of ARA is apparently related the leaf size or plant size of a cultivar. The large reduction of ARA in Whatawhata is probably related to its large reduction in leaf component under water stress. Ledgard *et al.* (1988) found that N-fixation by Kopu was less affected by summer drought than was the case in Pitau or Huia. In this experiment, however, the ARA of Kopu fell between the other 2 cultivars in response to water stress (Fig. 6.8). Differences in the severity of stress applied and the location of the trials (field vs. glasshouse) would make direct comparisons difficult. But it is possible that where water is available from a deep soil profile under dry field conditions, Kopu, which has a larger taproot system (Caradus, 1977), is likely to be less affected by drought than in the pot trial reported here.

Fig. 6.8 Relationship between ARA under control conditions and ARA reduction under water stress

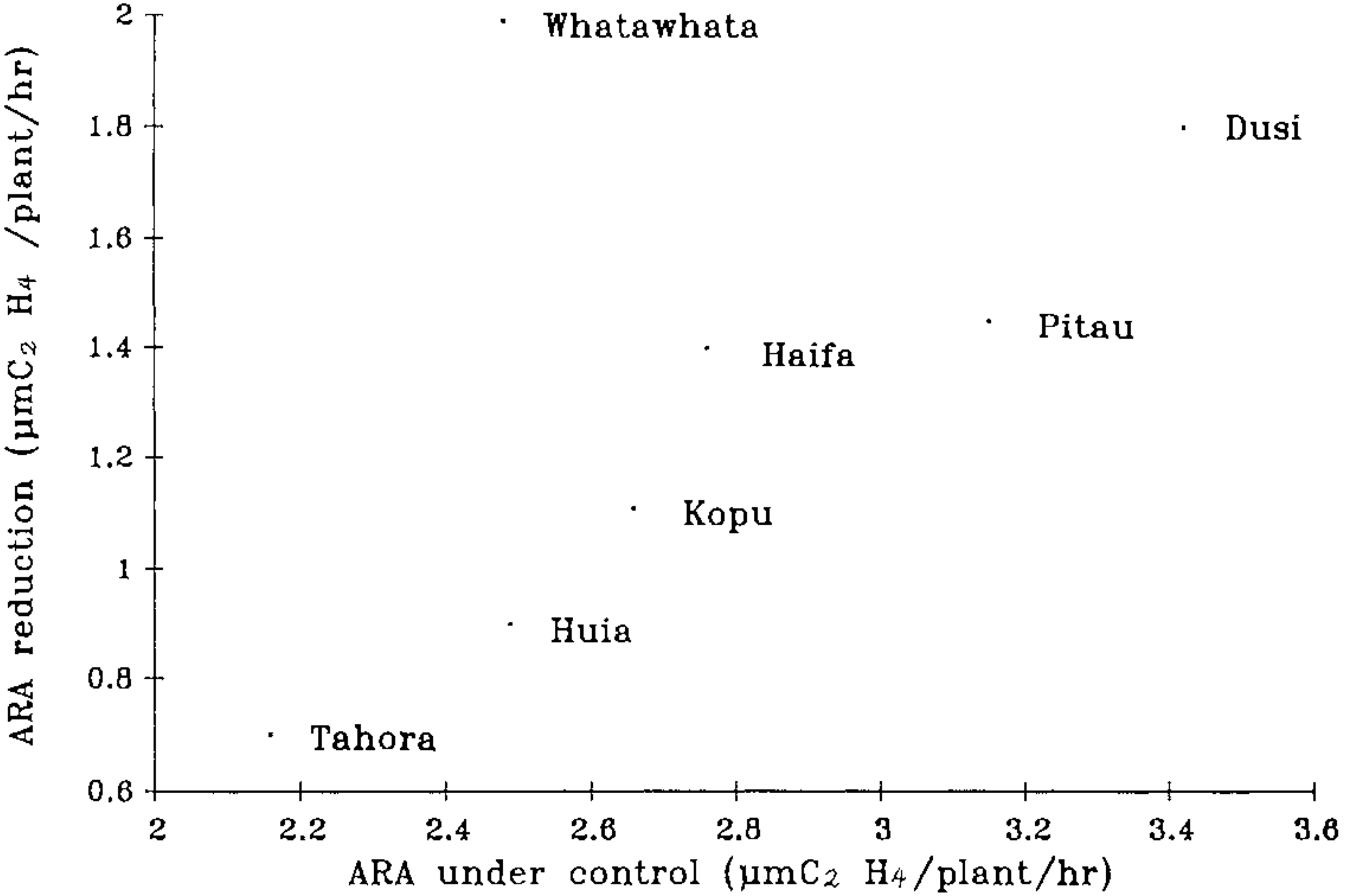


Table 6.9 ARA (um C_2H_4plant^{-1}hr^{-1}) of 7 Cultivars under 3 P Levels

P Level	Dusi	Kopu	Haifa	Huia	Pitau	Tahora	Whatawhata
50 ppm	1.64	1.52	1.13	1.13	0.83	0.68	0.70
200 ppm	2.05	1.70	1.66	2.16	2.34	1.54	1.19
800 ppm	3.55	3.03	3.35	2.84	4.11	3.14	2.56

lsd:cultivar comparison:0.82;phosphorus level comparison:0.46
(values mean of 2 harvests and 2 water treatments)

In general ARA was reduced by low P levels for all cultivars in a similar pattern. However, Pitau, Huia and Haifa suffered a greater loss than other cultivars when the P level was reduced from 800 ppm to 200 ppm, whereas Pitau and Huia were more severely affected by the P level decreasing from 200 ppm to 50 ppm compared with other cultivars (Table 6.9). It was also apparent that in general cultivars with a higher ARA under the high P levels suffered a larger loss under the low P levels. Overall, Pitau, Huia and Tahora were more severely affected by the low P levels, but Pitau, which had the highest unstressed ARA, showed the most extreme response to low P fertility and was different from the rest (Table 6.9). This might be expected from Pitau's requirement for a generous P supply in order to achieve its production potential.

6.3.4 The Canonical Correlations between Plant Parameter Groups

Many of the plant parameters measured can be expected to be correlated with each other, for instance, leaf size and total leaf DW. Furthermore, a group of parameters of similar characteristics may be related to another group of parameters, such as the morphological parameters and DW of the plant components. But generally speaking they are only moderately related in simple correlation. Such problems of finding correlation between two groups of variables can be solved effectively with the canonical correlation analysis in which one or more canonical variables are generated with the largest possible correlation for the whole set of variables (Cooley and Lohnes, 1971).

6.3.4.1 Plant Component DW and Their P & N Concentrations

The between-set simple correlations of these variables are only moderate, with the highest value (0.60) occurring between total leaf DW and stolon N concentration (Appendix 6.3a). But there are some higher within-set correlations, such as between leaf DW and stolon DW ($r = 0.70$), between leaf N and P concentration ($r = 0.77$) and between stolon P and leaf P concentration ($r = 0.81$). Between the two groups of variables, the first (and the most important) canonical correlation is 0.80 ($p<0.0001$) which is substantially larger than the between-set correlations (Appendix 6.3b). The second canonical variable is also significant, however, it can only explain a much smaller proportion of the total dispersion while the first one explains 81% of the total dispersion.

The results also showed that leaf DW was more closely associated with plant N & P concentrations than any other component ($r = 0.74$) (Appendix 6.3b). This is not surprising, since, based on the theory of a functional balance (Thornley, 1972) between leaf and root, the leaf is more sensitive to the change of plant nutrient status, reflected here by their N and P concentrations.

6.3.4.2 Plant Component DW and Plant Morphological Variables

The effect of plant morphological variables, viz. leaf size, leaf appearance rate, branch number and branch appearance rate, leaf number and weight per stolon on plant component DW is predictable but the actual simple correlation between any one morphological character and one component DW is only moderate, eg. $r = 0.62$ for leaf weight per stolon and total leaf DW, $r = 0.53$ for leaf size and total leaf DW (Appendix 6.4a). This is in contrast to some close correlations within both sets of variables, suggesting that the relationship between them was possibly confounded by the within-set correlation. The results of canonical correlation analysis show that the two groups of variables are better related (canonical correlation = 0.78, $p<0.0001$) (Appendix 6.4b) than any single pair of variables from both morphological characteristics and component DW.

Further examination of the analysis result shows that leaf DW is the parameter most closely related to the morphological variables, whereas the stolon DW is less sensitive to the change of morphological parameters. This might explain the large reduction of leaf and relatively stable DW of stolon under stress conditions with the changed morphological parameters. It seems that none of the morphological variables is quite outstanding in determining any component DW. This may seem to be in contrast with the general belief that leaf size of white clover is a key factor determining plant yield. But it can be argued that leaf size probably is an obvious indicator of a growth pattern which consists of a whole set of morphological variables. Therefore, all the morphological variables together would be reasonably good in predicting plant leaf DW ($r = 0.72$) and plant shoot DW ($r = 0.78$). Eagles and Othman (1988) observed that the yield variation of white clover between seasons was related to leaf size as well as to petiole and internode length. The result of this analysis also indicated that branching only had a moderate influence on plant DW and stolon DW ($r = 0.56$).

6.3.4.3 Plant DW Partitioning and Morphological Variables

One important finding in the previous experiment was the seemingly high partitioning of DW to the leaf component in large-leaved cultivars. Plant morphological parameters would have important influences on plant DW partitioning and a whole set of plant morphological variables may have more influence than any one parameter since partitioning is an integrated result of activity of the various parts. Relating the plant DW partitioning and morphological variables as two groups of variables, the canonical correlation coefficient is high, $r = 0.77$ ($p<0.0001$) (Appendix 6.5b). This is a large improvement over simple correlation analysis (Appendix 6.5a). Further examination of the canonical correlations indicates that among all the morphological parameters both leaf number and leaf size were very strongly correlated with leaf:root ratio, $r = 0.77$ ($p<0.0001$) but shoot:root and leaf:stolon ratios were little affected by morphological parameters measured here.

6.3.4.4 Plant DW Partitioning and N & P Concentrations

These two groups of variables, though poorly to moderately correlated on a one-to-one basis (Appendix 6.6a), are reasonably correlated canonically, with canonical correlation coefficient $r=0.71$ ($p<0.0001$) (Appendix 6.6b), indicating that there is a strong association between the two groups of variables. The N & P concentrations in the plant and plant components can be viewed as indicators of the plant nutrient status, particularly the shoot P level. So it is not surprising that the ratios of plant components, particularly the shoot:root ratio, were most closely related to shoot P concentration (Appendix 6.6b).

6.3.5 The Differentiation of Cultivars and Relative Sensitivity of the Variables to the Treatments

One of the objectives of the present experiment is to distinguish the difference between the cultivars in their response to water deficit and low P stresses and to determine which plant variable(s) can be used most effectively to explain cultivar differences. Multiple discriminant analysis is used here to statistically distinguish the cultivars and to identify the variables which contribute most in distinguishing between the cultivars. The plant parameters measured are grouped into four groups according to the characteristics of these parameters. They are: plant DW variables - leaf DW, stolon DW, root DW and dead DW; plant morphological variables - node appearance rate, branch number, leaf number and leaf weight per stolon, leaflet size and leaf extension; plant DW ratios - leaf:stolon, leaf:root, stolon:root, shoot:root, dead:leaf, dead:stolon, dead:root; and N and P variables - total acetylene reduction activity and N, P concentrations of plant components. The analyses were carried out with each group of plant variables with three main effects, ie. cultivar, water level and P level.

The interpretation of the result is largely similar to that of an ANOVA result except that with multivariate analysis, the difference between treatments or cultivars can be attributed to many variables and they can be ranked according to their contribution to the difference (estimated from its canonical correlation coefficient with discriminant function). Therefore in cultivar differentiation the rank of a variable represents its importance in grouping the cultivars while in treatment differentiation the rank of a variable would represent its relative sensitivity to the treatment (Laohasiriwong, 1986).

Only the first discriminant function is used since the cumulative explanation for the total dispersion is usually greater than 60% (Tables 6.10 to 6.15).

6.3.5.1 DW Variables

The discriminant analysis result showed that the cultivars had overall different DW components (Table 6.10a). Examining the DW group closely, it was found that the important parameters contributing to the cultivar differences were root DW, dead DW and leaf DW (Table 6.10b), suggesting that the cultivars differ mainly in their root component over all the treatments.

The ranking of sensitivity to water stress is as follows: leaf DW, stolon DW, dead DW and root DW (Table 6.10b). The fact that leaf DW is the most sensitive component is not surprising while the lack of sensitivity in root weight can also be explained by the relative increase in root DW under water stress. The relative sensitivity to low P fertility of the plant component DW is similarly ranked to water stress though less extreme. This shows that white clover would generally respond to both water stress and low P fertility by reducing its leaf production for the benefit of root production, but the effect of low P fertility on leaf production is less severe than that of water deficit. Reducing leaf and stolon components is in the interest of maintaining a functional equilibrium (Davidson, 1969; Thornley, 1972).

Table 6.10 a) Discriminant analysis with component DW

Effect	Canonical correlation	Eigen value	Cumulative explanation(%)	F	Sig.
Cultivar	0.695	0.93	68.4	6.33	****
Water level	0.927	6.11	100.0	188.01	****
P level	0.890	3.80	97.7	39.61	****

b) Structure matrix/canonical correlation coefficient

Characteristics	Cultivar	Water level	P level
Stolon DW	0.63/-0.41	1.00/-2.03	0.97/-1.42
Leaf DW	0.82/0.65	1.00/4.19	1.00/1.86
Dead DW	0.68/0.93	-1.00/0.23	0.99/0.62
Root DW	0.90/1.72	1.00/-0.03	0.98/0.65

6.3.5.2 Plant Morphological Characteristics

Some of the morphological characteristics are different amongst the cultivars in the present study as shown clearly in Chapters 3 and 4. This fact is also shown in the highly significant discriminant function in Table 6.11. Among the characteristics, leaf size and leaf weight per stolon are the two most important factors to separate the cultivars. The results also revealed that leaf appearance rate had the smallest contribution to the discriminant function, indicating that this parameter has the smallest variation among the cultivars in the present study. Wilman and Asiegbu (1982a,b) also found that leaf appearance rate is little affected by genotype.

The morphological changes under stress conditions were generally big as discussed in section 6.3.2. Among them, the relative sensitivity to water stress is ranked as follows: leaf appearance rate > leaf size > leaf weight per stolon > leaf extension growth > leaf number per stolon > branch formation. The relative sensitivity to low P fertility is: leaf size > leaf weight per stolon > leaf number per stolon > leaf appearance rate > leaf extension growth > branch formation. It is clear that while leaf size and leaf weight per stolon are more sensitive to both stresses, the branch formation is always the least affected. This indicates that leaf production is more sensitive than axillary bud development.

Table 6.11 Discriminant analysis with some morphological variables

Effect	Canonical correlation	Eigen value	Cumulative explanation (%)	F	Sig.
Cultivar	0.904	4.50	85.0	11.36	****
Water level	0.958	11.07	100.0	238.09	****
P level	0.767	1.43	90.7	14.37	****

b) Structure matrix/canonical correlation coefficient

Variables	Cultivar	Water level	P level
Node	0.10/0.36	1.00/1.52	-0.80/-0.33
Branch	-0.69/-0.24	1.00/0.13	1.00/0.06
leaf no/stolon	0.15/-0.05	1.00/0.28	-0.92/-0.68
leaf g/stolon	0.97/1.75	1.00/0.89	0.99/1.20
leaf extension	0.62/0.27	1.00/0.53	0.98/0.32
leaflet length	0.96/1.66	1.00/1.46	1.00/1.73

Furthermore, the sensitivity of different morphological characteristics to water stress is different from that to low P fertility. Water stress mainly caused greater leaf appearance reduction than leaf size reduction while under low P levels, leaf size and leaf weight per stolon were both reduced severely.

6.3.5.3 Component DW Ratios

The ratios of leaf components to other plant components were usually important in distinguishing the cultivars, particularly leaf:root ratio (Table 6.12).

Leaf:root and leaf:stolon contributed to the differentiation of cultivars positively while dead:leaf ratio had a negative effect. This again confirms the importance of leaf DW in differentiating the cultivars, not only in absolute terms but as a result of the partitioning of

DW in different cultivars. So when leaf DW increased its proportion in the plant, leaf:root and leaf:stolon ratios also increased. Stolon:root, shoot:root and dead:stolon ratios contributed mainly to the second discriminant function, suggesting that they differentiated the cultivars in a different dimension, and less effective as the other ratios discussed above by the definition of the discriminant analysis.

Amongst component DW ratios, dead:leaf, shoot:root and leaf:root were more sensitive to water stress. Again it seemed that leaf DW change was the main contributor to the sensitivity of these parameters. The most sensitive ratio to low P fertility is shoot:root. Both have indicated that shoot:root ratio is a sensitive character while under water stress conditions, leaf DW changed.

Table 6.12. Discriminant analysis with component DW ratios

Effect	Canonical Correlation	Eigen Value	Cumulative Explanation(%)	F	Sig.
Cultivar	0.768	1.43	59.2	5.74	****
Water level	0.808	1.88	100.0	30.82	****
P level	0.675	0.84	89.7	6.89	****

b) Structure matrix/canonical correlation coefficient

Variables	Cultivar	Water level	P level
leaf:stolon	0.78/-0.19	1.00/-0.22	0.96/0.64
leaf:root	0.87/0.39	1.00/0.52	0.95/0.13
stolon:root	0.20/-0.21	-1.00/-0.11	-1.00/-0.24
shoot:root	0.11/0.03	1.00/0.86	-0.98/-1.21
dead:leaf	-0.83/-2.16	-1.00/-1.53	-0.72/-0.34
dead:stolon	0.25/0.37	-1.00/0.28	0.97/0.10
dead:root	0.29/0.97	-1.00/0.10	0.85/0.13

6.3.5.4 N and P Characteristics of White Clover

For the group of acetylene reduction, N and P concentrations, except for root N concentration, all variables contributed about equally to the first discriminant function, though leaf P% contributed relatively more than other variables. So the cultivar differences were not clearly related to few variables. What was notable was the negative sign for ARA suggesting that for cultivars having higher acetylene reduction activities, their N and P% tended to be low. Across the cultivars, this finding is quite logical since cultivars with a high ARA, and large leaf tended to grow faster and had lower N% and P% in their tissues, probably due to a dilution effect.

It seemed that stolon P and N concentrations were sensitive to both stresses whereas ARA was usually much less sensitive than component N and P concentration ratios. Both water stress and P deficiency would have caused additional partitioning of assimilates to the root which may have helped maintaining ARA. Stolon can be considered as a storage organ for white clover, so its P and N content would change first and most when the supply of nutrients changes under stress conditions.

As discussed above, all groups of variables were significantly different between cultivars and treatments. But they are probably different in "discriminatory power", that is, the cultivars are 'more different' from each other in some variables than others. When the mean discriminant function one and two were plotted for all seven cultivars (Figs. 6.9, 6.10, 6.11 and 6.12), the grouping of cultivars could be seen on the relative location surface. On the main (first) discriminant function (X axis) basis, it can be seen that the morphological characteristics distinguish the cultivars clearly into two groups; Tahora and Whatawhata in one group, the remaining ones in another group (Fig. 6.9). This pattern was still traceable when the cultivars were distinguished by their component DW (Fig. 6.10). But as a whole, DW partitioning and plant N and P concentration did not group the cultivars so well.

Fig. 6.9 The relative position of seven white clover cultivars based on discriminant functions of their morphological characters

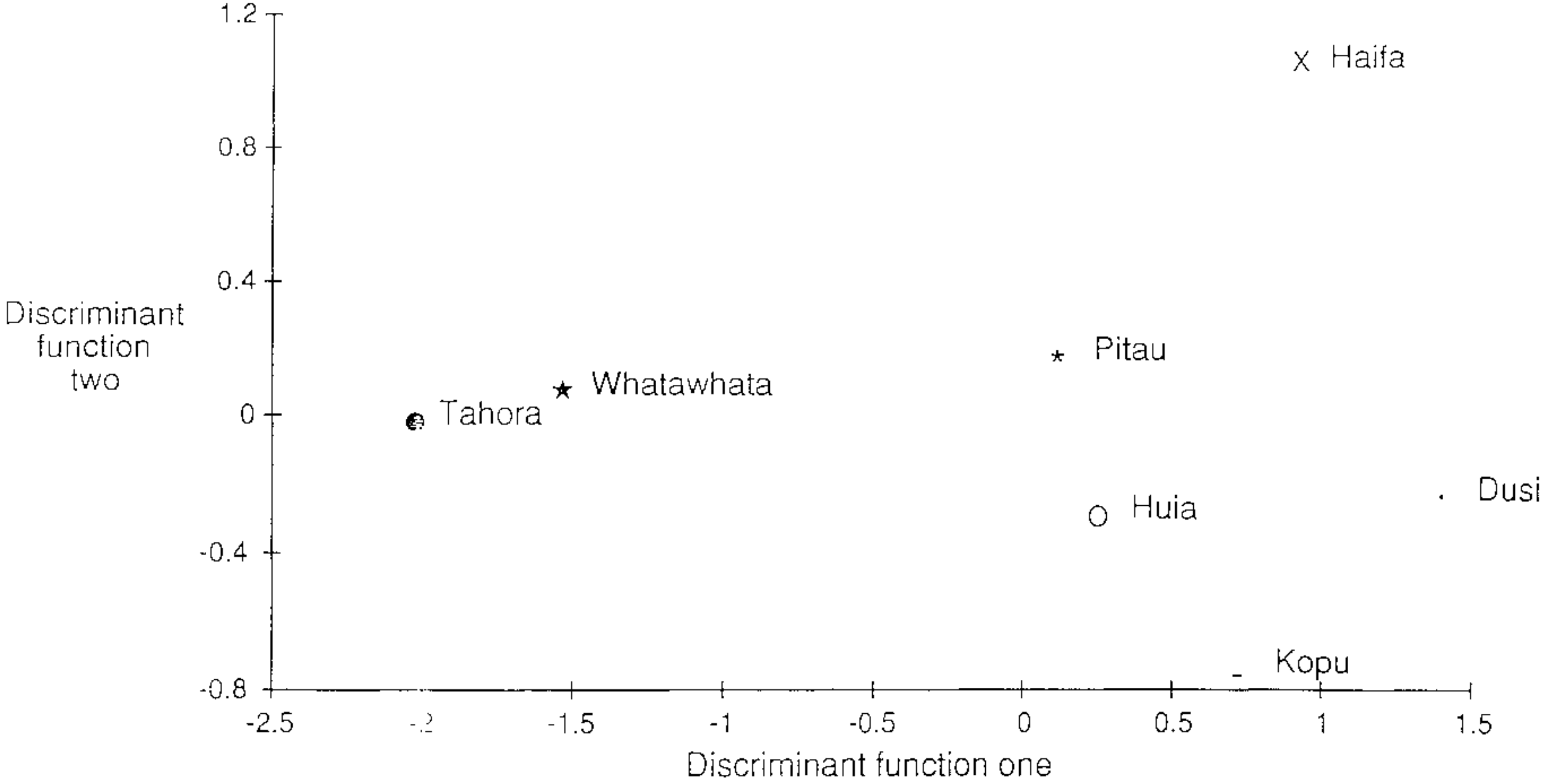


Fig. 6.10 The relative position of seven white clover cultivars based on discriminant functions of their DW variables

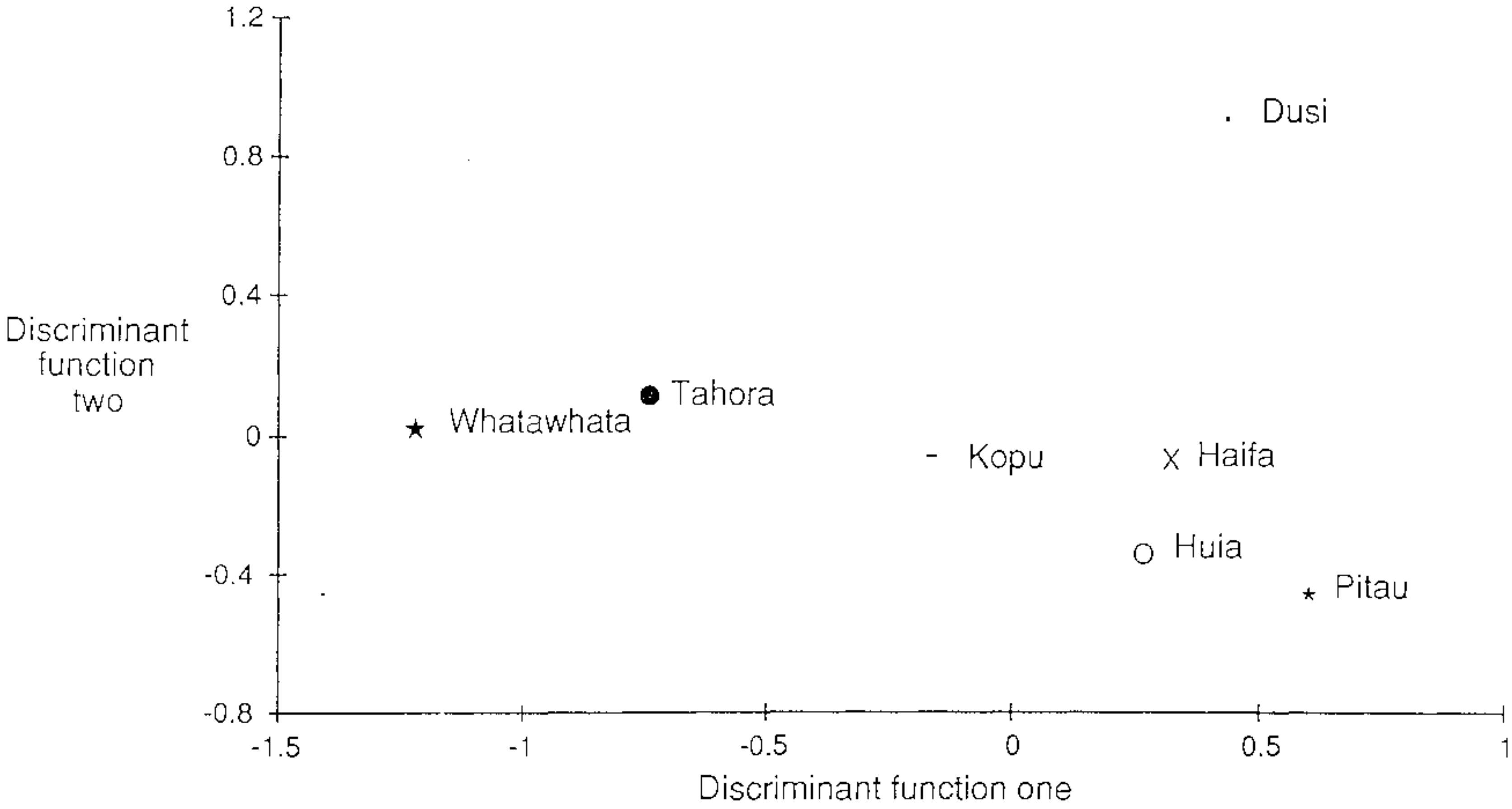


Fig. 6.11 The relative position of seven white clover cultivars based on discriminant functions of their DW partitioning variables

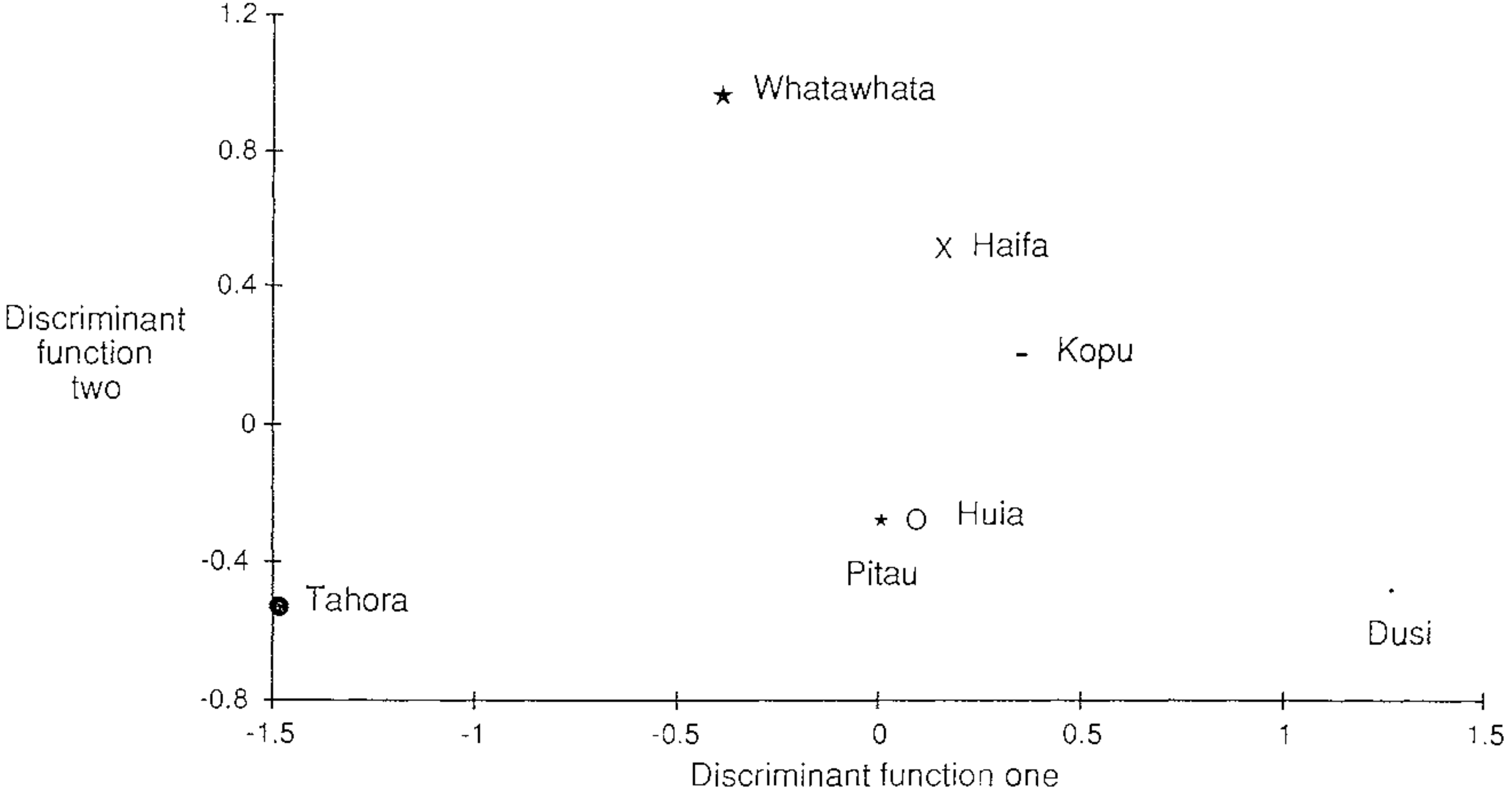


Fig. 6.12 The relative position of seven white clover cultivars based on discriminant functions of their N and P concentration variables

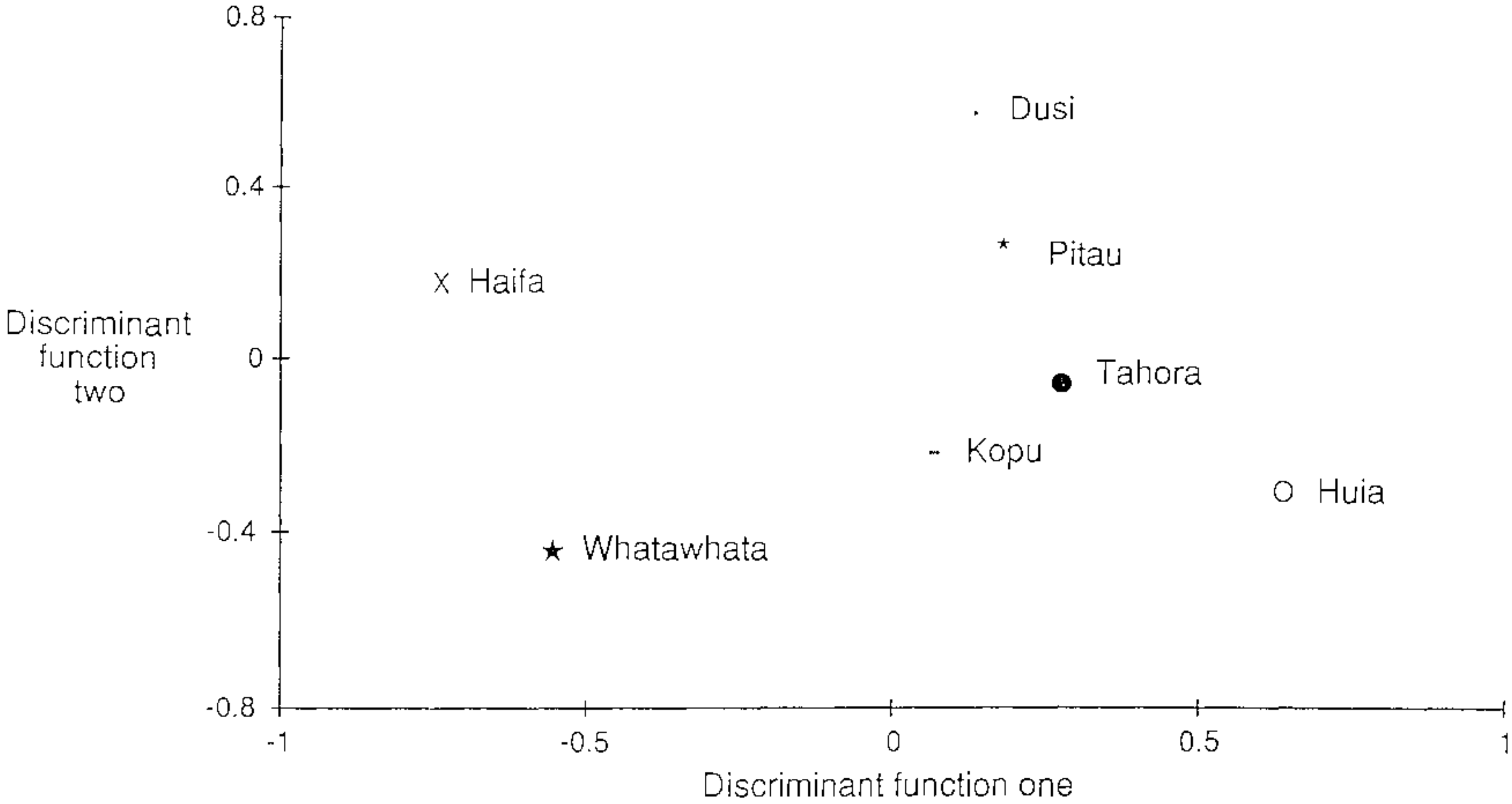


Table 6.13. a) Discriminant Analysis with N and P Concentrations

Effect	Canonical correlation	Eigen value	Cumulative explanation(%)	F	Sig.
Cultivar	0.665	0.79	44.8	2.87	****
	0.574	0.49	72.5	2.20	****
Water Level	0.864	2.95	100.0	30.86	****
P Level	0.942	7.81	96.8	24.28	****

b) Structure matrix/Canonical Correlation Coefficient

Variables	Cultivar	Water level	P level
ARA	-0.67/-0.42	1.00/0.44	1.00/0.25
root P%	0.52/0.23	1.00/0.19	1.00/0.32
root N%	0.05/-0.40	-1.00/-0.55	1.00/-0.15
leaf P%	0.84/0.63	1.00/-0.13	1.00/0.29
leaf N%	0.72/0.75	-1.00/-0.17	0.96/0.58
stolon P%	0.75/-0.39	1.00/1.03	1.00/0.87
stolon N%	0.78/1.09	1.00/0.23	1.00/0.47
shoot P%	0.72/0.76	1.00/0.57	0.99/0.57
shoot N%	0.60/0.27	1.00/1.17	1.00/0.57

6.4 Conclusions

1. The effects of treatments in the present experiment are highly significant, indicating that as a whole white clover is highly sensitive to the change of both water deficit stress (RWC = 80%) and low P fertility (from 50 to 400 ppm). The responses are shown in almost all parameters measured, suggesting the importance of these two environmental factors to white clover growth and development. Large differences of plant component DW are observed and correlation analysis indicates that many morphological variables, particularly leaf size, determine the leaf DW.

2. The results of the present experiment show that there are some differences between the cultivars. The main difference under the control conditions is their leaf size and consequently their leaf weight per stolon and total leaf DW.

3. Under stress conditions, the differences between the cultivars generally become smaller and non-significant, largely because of the unequal reduction caused by stresses, particularly water stress. But the differences in plant root DW and branch number are still large enough to distinguish between the cultivars.

4. The results showed that N-fixation (measured by acetylene reduction assay) of white clover was significantly affected by moisture deficit stress as well as low P levels, while the cultivar differences in N-fixation ability appeared to be associated with their leaf size category. Significant interactions between cultivar and moisture deficit level, cultivar and phosphorus level indicated the existence of variation between cultivars in response to moisture stress and low P fertility. This fact might be also useful for the further breeding programmes.

5. When expressed as N fixed per unit plant dry weight these differences were non-significant.

CHAPTER SEVEN
GENERAL DISCUSSION AND CONCLUSIONS

7.1 Introduction

The main objectives of the present study were to evaluate the responses of several white clover cultivars with different morphological characteristics to water deficit stress and low P fertility, and to identify the plant characteristics most effective in differentiating between the cultivars. In this chapter, the results and conclusions presented in the previous chapters will be discussed in relation to these objectives.

7.2 Differences in Cultivar Responses

The findings of all the experiments reported in the previous chapters showed that there were significant differences in growth and developmental responses between the cultivars studied. There were also small variations among the cultivars in water relations and N-fixation performance. In the following section these three aspects will be discussed separately.

7.2.1 Growth and Development

7.2.1.1 DW and DW partitioning

Water deficit stress has two effects on a plant; first, it restricts photosynthetic production due to the general slow-down of the plant metabolism (Hsiao, 1973) and secondly, it can cause the partial or total destruction of the plant biomass due to accelerated senescence. Thus plant DW changes under water deficit stress clearly involve both effects though in this discussion no attempt will be made to distinguish them.

Total DW, including shoot and root, of white clover was weakly correlated to the leaf size of the cultivars. This finding is in general agreement with some earlier work (Davies and Young, 1967; Aldrich, 1970; Laidlaw, 1980 and Frame *et al.*, 1982). However, it is also known that the relationship is by no means consistent (Eagles and Othman, 1988). Such variation can be due to the limitation of growth space in pot trials, seasonal variation and other factors affecting leaf size expression (Smetham, 1986), thereby limiting the growth potential. The correlation was relatively clear in early harvests and under control conditions, ie. with adequate watering and P fertility, thus suggesting that only under fertile conditions will the advantages of large leaf size be expressed.

Under both types of stress studied here, leaf size and total leaf weight per stolon were often the most sensitive to changes in stress levels; so cultivars with large leaf and great leaf component, ie. large-leaved cultivars were more severely affected. In addition to leaf size, the results from canonical correlation analysis (Chapter 6) showed that yield of white clover was more closely correlated with a group of morphological characteristics including leaf size, leaf appearance rate and branch formation rate though, among them, leaf size was relatively more important.

These findings suggest that leaf size probably is a characteristic largely representing a group of morphological characteristics, together forming a type of "growth pattern" and it is this growth pattern, rather than any individual characteristic, that determines the plant growth potential. Eagles and Othman (1988) observe that the increase in productivity of white clover is correlated with the increase in size of leaf, petiole and internode. This study showed that the dynamic parameters of white clover are also important, especially branching rate.

The importance of leaf area and leaf expansion rate for white clover growth is widely reported (Haycock, 1981). On the other hand, under less favourable conditions, when survival and competition for favourable sites become important, increased growing points, even with leaf growth sacrificed, may be vital for a plant to forage for nutrients and soil moisture (Hutchson, 1988). This characteristic was more clearly shown in the small-leaved cultivars, particularly Whatawhata, under stress conditions. A flexibility of the two aspects of growth, leaf growth and axillary bud growth, and a flexibility in vegetative production and reproductive growth as MacFarlane and Sheath (1984) observed in Whatawhata, is very useful for plants to survive stressful environments as in a dry hill country situation. A large reduction of leaf component by Whatawhata under relative severe water stress, observed in the present study, may be considered as an indication of flexibility in changing growth direction. But it occurs only under relatively severe stress conditions (leaf RWC < 80%). Though there is a price to pay for such flexibility in DM yield it would probably be worth the loss for long term persistence under certain conditions. Branch number can partially offset the leaf size effect in DM production since they have been observed to be negatively correlated both in previous work and in the present study. Korte and Parsons (1984) showed that the smaller leaf size of white clover under hard grazing can be compensated by a large number of leaves.

Looking at the responses of cultivars to water stress or low P fertility, when the response was defined as the reduction of DW, or other parameters positively contributing to DW production, then a cultivar's responses, expressed as reductions, were often positively correlated with its performance level under control conditions. This relationship was most clearly shown in some of the morphological characteristics, such as leaf extension rate and leaf size (Chapter 4). In other words, the better performed cultivars would always be more severely influenced by stresses. It might be argued that this is an indication that better performed cultivars usually are less prepared for stresses. But even taking the reduction into account, such cultivars would often still have a large leaf, or more leaves on a stolon. In crop breeding, similar phenomena are also reported (Blum, 1983). Some authors suggested that given the reductions caused by water stress, selections of higher yields under adequate watering conditions would still yield more.

Selection for high yield under drought conditions is sometimes carried out under normal environmental conditions on an assumption that the breed would still perform relatively better under drought conditions (Blum, 1983). The results of this study give some support to this assertion. For example, the ranking of total DW, N-fixation etc. was almost unchanged under the stress treatment though, as has been discussed, the relative reduction was often higher for the top-ranking cultivars. But in the field experiment, a reverse of this trend was observed in one character, viz. growing point survival rate, where the low survival rate of Whatawhata under control conditions was even more reduced by water stress. It may be speculated that growing point survival is in fact also an indication of growing point susceptibility to stress even under control conditions where growing point death would be triggered by some external or internal factors. For a cultivar from a harsher environment, such as Whatawhata, its profuse branching may reflect both its selectivity for microenvironment and a tendency to occupy the most favourable site. To accomplish the gaol, it has to have many growing prints and they would have to have a sensitive reaction to external environment and not waste too much photosynthetic product on a less promising site. The results of the present study seemed to support this hypothesis. This may also explain the higher branch death rate of Tahora, a cultivar for relatively harsher environment. Though growing points of other cultivars may have the same function, they would be less sensitive because in a fertile environment growing direction may not be as critical as in a less fertile environment. They also have to avoid being shaded by other plants but this can be accomplished by growing upward through extending the petiole.

Good and Bell (1980) showed that the total growth of a plant depends almost equally on the rate of photosynthesis and on the proportion of the photosynthetic products devoted to the synthesis of new photosynthetic area. The slightly higher proportion of leaf in total DW for large leaved cultivars, and Huia, therefore, is a factor contributing towards their higher yield. White clover harvestable yield is mainly constituted by the leaf and parts of the stolon, so greater partitioning of photosynthate to the leaf would also mean higher economical yields. But the advantage of a high proportion of leaf may be partly offset by the high senescence rate of leaf as shown by the close correlation between leaf DW and total dead weight (Chapter 6), though, in grazed swards, senescence can be reduced by more frequent grazing.

The performance of the two small leaved cultivars, Tahora and Whatawhata, was very comparable. The slightly better performance of Tahora over Whatawhata may reflect the selecting effect within a similar range of leaf size. Davies and Young (1967) showed that as a whole, bred varieties perform better than the local selections. Selection on high yielding ability in white clover is usually carried out under moderate to high fertility conditions with adequate soil moisture and phosphate supply (Williams, 1987). So the relatively better performance of the selected cultivars could well be associated with more efficient utilization of nutrients. The higher P concentration of Whatawhata in comparison to other cultivars, particularly Dusi and Haifa, may indirectly suggest that Whatawhata had a poor phosphorus utilization efficiency although a better P uptake is also possible.

The change of DW partitioning under stress conditions is part of the plant's reaction to maintain a balanced functional equilibrium (Davidson, 1969; Thornley, 1972). So under both water stress conditions and low P fertility the general trend was that root proportion increased while shoot component, mainly leaf, reduced. The severity and length of the stress and cultivar variation affect the shoot:root ratio. Water stress accelerated the leaf senescence in relation to leaf appearance and leaf expansion consequently reduced leaf component (Chapter 4). Compared with leaf components, stolon and root components are relatively stable.

Some of the large-leaved cultivars used in the study are considered as drought resistant, such as Dusi (Smith and Morrison, 1983) and Kopu (Ledgard *et al.*, 1988), but the findings of the present study did not show any real superiority of these cultivars over other cultivars. It may be because their drought tolerance ability is related to their large taproot which can absorb water from the deep soil profile (Smith and Morrison, 1983), thus, in a short term pot trial, the conditions might be closer to hill-country conditions. They may also have a high P utilization efficiency which can be shown by the low P concentrations in their plant components.

7.2.1.2 Plant Morphological Characteristics

The reduction of DW in all white clover components can be viewed as an integrated result of reduced leaf, stolon and root growth and branch formation, and increased senescence of these components. All these parameters were significantly affected by water stress and low P fertility. The mature size of leaflet, petiole and internode was decreased by 50 to 80% under water stress conditions. Leaf production characteristics, including leaf appearance rate, leaflet extension rate and final leaf size were more severely affected by water stress than characteristics relating to growing point production, branch formation and growing point survival rate. This could be an effect of internal water status. Growing points, be they axillary buds or apical meristems, are less exposed, therefore may suffer less water loss and possibly lower water deficit. Also, cell division is the main activity of growing point growth and this process is less sensitive to water stress than cell expansion (Hsiao, 1973). Denne (1966) and Thomas (1987) concluded that leaf cell division in white clover mainly occurs before its emergence. It is also known that, within a given plant, the younger tissues, such as growing point, are the most hardy, due in part at least to the greater ease with which water can move into them. Leaf appearance rate was usually little affected by many other factors, such as grazing management (Chapman, 1983) or genotype (Wilman and Asiegbu, 1982(a, b)). In the present study, however, the differences among the cultivars in leaf appearance rate were always significant. In two out of three experiments, Whatawhata had a lower leaf appearance rate while large leaved cultivars usually had a higher leaf appearance rate. This may compensate for the fewer growing points, on a per plant and per unit area basis, of these cultivars under control conditions.

Direct comparison of morphological characteristics of different cultivars seemed to be inappropriate since these cultivars are different in the size of their individual parts. The concept of phytomer and the use of phytomer growth to describe white clover growth (White, 1979) was incorporated by using the parameters reflecting phytomer development, such as the leaf appearance rate and branch formation. However, it was found that generally they played a relatively smaller role in either indicating plant DW differences or differentiating cultivar differences and they were correlated with growth only moderately. These parameters may be effective in describing plant growth potential, but their contribution in describing plant growth was inevitably confounded by the unit size.

Beside there are relationships between plant unit size and plant leaf appearance rate, and branch formation rate. One of the interesting relations is the negative correlation between leaf size and branch number formation shown both by the simple correlation analysis and by the discriminant analysis (Chapter 6). It conforms with the general observation that small-leaved cultivars would have more growing points on a per unit area basis (Chapman, 1983). It may be suggested that it is the assimilate partitioning which affects the relative activity of leaf and growing points. However, from the present results it can be suggested that none of the parameters measured is outstanding or exceptionally effective in distinguishing the cultivars studied, and even less so under stress conditions, mainly because the stress reduced the differences between the cultivars by relatively more severe effects on better performed cultivars. Nevertheless, leaf size and branch number jointly are relatively more important characteristics in distinguishing cultivars, especially under control conditions.

White clover persistence can also associate with early flowering. Though flowering behaviour was not studied in this study, its effect on vegetative growth might be estimated from the branch formation characteristic. Whatawhata was selected or noted for its early flowering characteristic, but this seemed to have little detrimental effect on branching rate, as shown in the study where Whatawhata still had a higher branching rate. Early flowering favours a summer-dry situation to avoid water stress (MacFarlane and Sheath, 1984). It appears that under a prolonged drought, white clover populations with smaller-leaf would have a bigger chance of survival.

7.2.2 Effect of Soil Water Level on Leaf RWC, Extension and Transpiration

Change of plant water status, either RWC or water potential, under water deficit conditions is commonly used to show plant dehydration avoidance ability which is an important drought adapting attribute (Blum, 1983). The relationship found here with white clover as a whole did show that it can avoid the drop of RWC till about 45% of field capacity (FC) even though its transpiration started to drop at 80% of FC or even higher. In other words, white clover maintains a high level of RWC by reducing transpiration loss as soil water status decreases from more than 80% to 45% FC.

There is, however, little difference between the cultivars in their dehydration avoidance ability, estimated from their RWC level under the continuous reduction of soil water status. Whatawhata had a slightly higher RWC than Kopu at high water level while Kopu maintained a high RWC at slightly lower FC. However, these differences occurred at adequate water level or low water deficit level, with RWC at or near maximum, above 90%, so the differences may not cause much difference in their water stress resistance ability. With the increase of water deficit, the differences between cultivars disappeared.

The lack of difference between RWC of cultivars under high levels of water stress may be taken as indirect evidence that the white clover cultivars used in this study had little variation in dehydration avoidance. This, in a sense, is not a surprising result because none of the cultivars studied here was intentionally selected for dehydration avoidance. The difference in this aspect between species is usually considered to be very small (Blum, 1983). Probably except for Whatawhata, the cultivars studied were not selected for drought tolerance. Dusi, Haifa and Kopu are found to grow under drought conditions by some workers (Smith and Morrison, 1983; Ledgard *et al.*, 1988) but reports showed that their drought tolerance ability is associated with their deep taproot which can explore and absorb soil moisture in the deep soil profile (Smith and Morrison, 1983; Caradus, 1977). This characteristic may be useful in a short term drought or in situations where the soil is deep, however, in hill country where the soil is often shallow, it is doubtful that such deep rooting characteristic would be a real advantage.

Though there were no large differences in dehydration avoidance ability between the cultivars the result showed that white clover could maintain high RWC by reducing its transpiration until field capacity dropped to about 40-45%. Findings from earlier work indicated that white clover, like many other plants, closes its stomata under mild stress (Cox and Boersma, 1967). So the reduction of transpiration can be a result of stomotal closure, though much of the later work, by measuring stomatal conductance, showed that white clover has poor stomatal control under water stress (Johns, 1978; Kerr and McPherson, 1978). It is known that transpiration is determined by both stomatal resistance and vapour pressure difference (Cox and Boersma, 1967) or saturation deficit (Jarvis and McNaughton, 1985). Since the present work was carried out in a growth room where the conditions of light intensity, relative humidity and temperature were kept constant, there would be little variation in the saturation deficit.

The relationship between leaf extension and RWC was similar to the findings of other experiments (eg. Chu, 1979) and in agreement with the general conclusion of Hsiao (1973). Loss of turgor is usually considered to be responsible for the reduced leaf extension growth (Hsiao, 1973). There seemed to be large variation in leaf extension rate at high levels of RWC. Such variation may indicate that other factors, which have not been well controlled here, could exert considerable influence on leaf extension. Of course, the difficulty in accurately measuring the leaf extension of white clover is another possible source of variation.

The cultivar differences in leaf extension rate also were not large. Obviously the small size of the white clover leaf has contributed somewhat to this. Compared with grass leaves, the extension rate of white clover is minute even in a time scale of 24 hours. While petiole extension rate can be more accurately measured, its variation was relatively large, probably because its extension rate is affected by some other factors, eg. light intensity.

7.2.3 N-fixation

N-fixation of white clover has long been shown to be related to the supply of plant photosynthate (Huang *et al.*, 1975; Haystead *et al.*, 1979) and so presumably to DM production. Detailed study with simultaneous measurement of photosynthesis and acetylene reduction activity (ARA) also shows the importance of leaf photosynthesis supply to nodule activity (Huang *et al.*, 1975; Haystead *et al.*, 1979). The finding in this study, that total ARA was correlated to leaf size and leaf DW, suggested that, generally, white clover ARA was largely determined by the supply of photosynthate.

On the other hand, the change of plant growth pattern and the balance of shoot (more likely leaf) and root function caused by water stress and low P fertility also made the N-fixation less affected than total plant DW. So ARA per unit DW did not decrease under water stress conditions, or even increased slightly. As Thornley (1972) and Davidson (1969) show, root activity will be enhanced under conditions where root function is to be limited by external factors, such as water deficit or low nutrient availability. Such enhancement of root function may mean a favourable partitioning of CHO to root. This will also benefit N-fixation. There were significant interactions between cultivars, water stress and P level. The interaction, like plant growth parameters, also seemed to arise from the unequal reduction of total ARA under both water stress and low P fertility. Once again the reduction was negatively associated with the ARA level at control conditions with adequate P supply. This fact might be useful for future breeding programmes.

7.2.4 The Important Characteristics Differentiating the Cultivars

The findings that significant differences exist between the cultivars in almost all of the parameters measured prompted the question of which parameter was the key parameter or the most different one between the cultivars. Findings from discriminant analysis ranked the parameters used in the analysis according to their contribution to the discriminant functions, particularly the first discriminant function. This also indicates the relative difference of these parameters between the cultivars.

Leaf weight per stolon and leaf size are the two most important characteristics distinguishing the cultivars. In other words, the cultivars are most different in their leaf characteristics. This conformed with the general usage of leaf size as a key classifying parameter (see section 2.1.2.2). The importance of leaf weight per stolon was about equivalent to leaf area.

As a whole, morphological characteristics are more effective in distinguishing the cultivars than in any other group of plant parameters. This can be interpreted in two ways. On the one hand, these results suggested the important role the plant morphological characteristics played in distinguishing between the cultivars. On the other hand, the large differences in morphological characteristics of white clover were not totally reflected in other aspects of their growth. So the importance of the morphological difference between the cultivars should not be over-emphasized.

Another trend can be seen from the discriminant analysis based on various variable groups, that white clover cultivars usually differed slightly under either water stress or low P fertility. This fits with the observation reported in Chapters 3 and 4 that cultivars of better performance tended to be more severely affected by stress, therefore the difference among the cultivars would diminish under stress conditions.

7.3 Conclusions

7.3.1 Growth and Development

1. The findings from the present study indicated that there were differences in growth and development between the cultivars studied.

2. The differences were larger in the morphological characteristics and relatively large in total plant dry weight (DW) and the dry weight of plant components, mainly leaf and root.

3. Cultivars were less different in the ratios of their component DW as a whole. But under stress conditions, the leaf:root ratio became an important factor for differentiation between the cultivars.

4. The large-leaved cultivars generally had higher total DW and total leaf DW. Huia tended to be closer to the large-leaved cultivars like Pitau. The favourable distribution of DW to leaf was probably the main reason for the high yielding performance of the large-leaved cultivars and Huia.

5. In response to water stress, cultivars with high DW or high values in growth parameters are usually affected most severely. Therefore, the difference between the cultivars under stress conditions became smaller.

6. Low P fertility caused reduction in growth and plant DW. Cultivars with large leaf were affected more severely by low P fertility and so their DW was similar to that of the smaller-leaved cultivars under low P fertility conditions.

7.3.2 Plant Water Relations

Most of the cultivars are basically similar in their water status and transpiration under water deficit conditions.

1. RWC: Whatawhata seems to maintain a higher RWC than Kopu when the soil water supply is adequate.

2. The dehydration avoidance of the cultivars is similar once the soil water status is below 45% of field capacity.

3. White clover can maintain a high RWC by reducing its transpiration before its RWC starts to drop quickly.

4. Leaf extension growth was positively correlated with leaf RWC in all cultivars.

5. Cultivars with a smaller extension rate under control conditions tended to maintain a higher level of leaf extension under lower RWC.

6. Kopu had a higher transpiration rate under both high and low water deficit level than Tahora and Whatawhata.

7.3.3 N-fixation

1. N-fixation or ARA of cultivars was positively correlated with their leaf size.

2. N-fixation of white clover was significantly affected by moisture deficit stress as well as low P levels.

3. Significant interactions between cultivar and moisture deficit level, cultivar and phosphorus level indicated the existence of variation between cultivars in response to moisture stress and low P fertility.

7.3.4 Variables Useful in Differentiating between Cultivars

1. Among the plant morphological characteristics, leaf weight per stolon and leaf size are the two most important characteristics distinguishing the cultivars used while leaf appearance rate is not so different between the cultivars so it has the smallest contribution in distinguishing between them.

2. Plant morphological characteristics are more effective in distinguishing the cultivars than any other plant characteristics measured.

Appendix 3.1 Comparison of Long Term Climatic Information (DSIR) with that on the Site (Pasture & Crop Unit) during the Trial

Monthly rainfall (mm)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
DSIR	77	58	72	75	93	87	98	86	74	87	82	102	991
Site	74	52	--	--	--	--	--	--	--	--	81	98	---
Monthly mean Temperature (°C)													
DSIR	17	17	16	14	11	8	7	9	10	13	14	16	--
Site	19	18	--	--	--	-	-	-	--	--	16	17	--

Appendix 3.2 Information on Rainout Shelter

The rain-out shelters used in the present experiment had first been used by Barker (1983) and proven to be effective in creating drought. They consisted of two 10 m X 5 m aluminium frames, each supporting a clear polythene cover. Each frame was mounted on rails and could be pushed manually or driven by a pair of small electric motors on or off the plots. Automation of the rain-out shelters was achieved by means of a mains driven microprocessor and rain sensor. The latter could detect a single raindrop, and through the microprocessor the motors can be activated to move over the water stressed plots. Fifteen minutes after the last raindrop is detected the covers will move back off the plots to the rest area. Another feature of the rain-out shelters was that they had an alternative battery power which can used whenever the main power supply is cut.

Appendix 3.3 Procedures on RWC Measurement

1. Three to six leaves are taken and stored in air tight bottle and weighed before being saturated in distilled water.

2. The leaves are placed in petri dish and water is added to submerge them. A piece of tissue is placed on top to ensure a total submergence.

3. After 4 hours, the leaves are dried thoroughly with soft tissue and then dried at 80°C for 24 hours and their dry weight is then measured.

4. Then the leaf RWC is calculated with the following formula:

$$\text{RWC}(\%) = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Saturated Weight} - \text{Dry Weight}} \times 100$$

Appendix 4.1 Soil Water Content under Three Levels and for the Five Cultivars

Soil Water Content under Three Levels of Water Deficit

Water Level	Soil Water (%)
Control	33.0
Mild Stress	17.8
Severe Stress	9.6
Sig.	***

Soil Water Content for the Five Cultivars

Cultivar	Soil Water (%)
Kopu	20.7
Pitau	20.0
Huia	19.0
Tahora	19.7
Whatawhata	21.3
Sig.	ns

Appendix 4.2 Modified Carlson' Scale:

Observation	scale
leaf just appeared	0.1
trifoliate leaf separated	0.3
leaf unfolding	0.6
leaf fully expanded	1.0
part leaf showing yellow	2.0
half or more showing yellow	3.0
leaf dead	4.0

Appendix 6.1 Introduction of Canonical Analysis

The plant growth, development, and other aspects of plant activity often have complicated relationships which can not be properly described by simple correlation analysis. So these relationships have been analysed with a canonical correlation analysis procedure in SAS (Statistics Analysis for Social Science) (1985). This analysis is a technique for analysing the relationship between two groups of variables on a joint basis of several variables in each group (Cooley and Lohnes, 1971).

A multiple discriminant analysis has been carried out using the MANOVA procedure in SAS (1985). This analysis combines many plant variables into one index of cultivar or treatment performance. It aims to differentiate both cultivars and treatments on a joint basis of several or more plant variables. The total dispersion (ie. all variance and covariance) is partitioned into treatment and error components and discrimination is made on the basis of maximizing the ratio of treatment to error dispersion (as if in F-test). The model of this analysis is similar to that described by Cooley and Lohnes (1971) and used by Laohasiriwong (1986). With the derived discriminant functions, it may also be possible to obtain satisfactory discrimination with only one or two of them, by using only those which take into account a large proportion of total dispersion of the original data (say 80%). The discrimination or differeniation between both cultivars and treatments was achieved by interpretation of 'structure matrix', which is the matrix of between-group correlations between the discriminant functions and discriminant variables, and the canonical coefficient for each variable to find the variables which contribute most to differentiation along the particular function.

Appendix 6.2 a) Relative sensitivity of plant characters to water stress

Water level	Control		Water stress	
Characters	Discriminant Function 1	Discriminant Function 2	Discriminant Function 1	Discriminant Function 2
stolon DW	0.7105	0.1119	0.2934	0.7848
leaf DW	0.7886	0.4031	0.6463	-0.2123
dead DW	0.7122	-0.6817	0.8780	0.2568
root DW	0.8622	0.4752	0.8671	0.3370
Explanation%	65.9%	11.6%	62.9%	24.2%
sig.	****	***	****	****

Appendix 6.2b Relative sensitivity of plant characters to low P fertility

P level	50 ppm		800 ppm	
Characters	Discriminant Function 1	Discriminant Function 2	Discriminant Function 1	Discriminant Function 2
stolon DW	0.2592	0.9246	0.1861	0.6386
leaf DW	0.4220	0.7374	0.7904	-0.0213
dead DW	0.9863	0.1498	0.6161	0.5385
root DW	0.4128	-0.1903	0.8913	0.1305
Explaination%	85.5%	6.4%	64.3%	26.9%
sig.	****	ns	****	**

Appendix 6.3a).Correlation matrix between DW and plant N and P variables

	ARA	root P%	root N%	leaf P%	leaf N%	stem P%	stem N%	shoot P%	shoot N%
stem DW	.39	.12	.29	.22	.28	.22	.41	.24	.21
leaf DW	.54	.27	.25	.44	.36	.58	.60	.51	.59
dead DW	.28	.12	.23	.15	.12	.27	.31	.11	.05
root DW	.45	.16	.29	.31	.33	.35	.43	.27	.27

b).Canonical correlations between DW variables and N & P concentration variables

canonical correlation	approx. standard error	eigen value	proprotion of variation explained(%)	sig.
0.80	0.03	1.78	81	****

Appendix 6.4a).Correlation matrix between DW and plant morphological charaters

	node No.	branch No.	leaf No.	leaf DW/stolon	leaf ext.	petiole ext.	leaf size
stem DW	.14	.05	.03	.43	.16	.15	.30
leaf DW	.42	.31	.05	.62	.47	.41	.53
dead DW	-.12	-.14	-.12	.24	.15	-.12	.22
root DW	.12	.11	-.04	.46	.23	.18	.47

b).Canonical correlations between DW variables and plant morphological characters

canonical correlation	approx. standard error	eigen value	proprotion of variation explained(%)	sig.
0.78	0.03	1.55	74	****

Appendix 6.5a).Correlation matrix between DW partitioning and plant morphological charaters

	node No.	branch No.	leaf No.	leaf DW/stolon	leaf ext.	petiole ext.	leaf size
leaf/stem	.19	.22	.23	.30	.11	.15	.15
leaf/root	.37	.22	.41	.55	.26	.31	.27
stem/root	.06	-.02	.13	.02	-.02	-.01	-.11
shoot/root	.32	.33	-.16	.31	.27	.30	.21
dead/leaf	-.48	-.39	-.30	-.50	-.31	-.47	-.47
dead/stem	-.27	-.30	-.15	-.03	-.02	-.22	-.04
dead/root	-.24	-.28	-.11	-.05	-.03	-.24	-.10

b).Canonical correlations between DW partitioning and plant morphological characters

canonical correlation	approx. standard error	eigen value	proprotion of variation explained(%)	sig.
0.77	0.03	1.42	63	****

Appendix 6.6a). Correlation matrix between DW partitioning and plant N & P variables

	ARA	root P%	root N%	leaf P%	leaf N%	stem P%	stem N%	shoot P%	shoot N%
leaf/stem	-.06	.09	-.13	-.04	-.12	.04	-.09	.05	.10
leaf/root	-.11	-.09	-.23	-.16	-.24	-.09	-.17	-.06	.07
stem/root	-.14	-.05	-.07	-.05	-.02	-.09	-.02	-.08	-.09
shoot/root	.27	.28	.34	.40	.26	.45	.24	.60	.42
dead/leaf	-.09	.01	.04	.07	.14	-.08	-.06	-.11	-.30
dead/stem	.11	.01	.05	.03	-.01	.06	.00	-.07	-.15
dead/root	-.01	-.01	.02	.04	.02	.00	-.04	-.13	-.19

b).Canonical correlations between DW partitioning and plant N & P concentration variables

canonical correlation	approx. standard error	eigen value	proprotion of variation explained(%)	sig.
0.71	0.04	1.04	62	****

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