# STUDIES ON THE COMPETITIVE ABILITY OF WHITE CLOVER (*TRIFOLIUM REPENS* L.) IN MIXTURES WITH PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.): THE IMPORTANCE OF NON-STRUCTURAL CARBOHYDRATE RESERVES AND PLANT TRAITS

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Mixtures of white clover and perennial ryegrass are important in temperate pasture zones. Proportions of 30-50 percent of white clover on a dry weight basis are required in the sward for a high output of agricultural production

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# Summary

Mixtures of white clover and perennial ryegrass are important in temperate agricultural areas including Australia. In the sward white clover proportions often decline with time, one of the reasons is the higher competitive ability of perennial ryegrass, in the presence of high levels of soil nitrogen. Cultivars of white clover differ in their competitive ability in mixtures with perennial ryegrass. The objectives of the studies reported in this thesis are to investigate whether non-structural carbohydrate (NSC) levels in stolons may affect the competitive ability of white clover cultivars in mixtures with perennial ryegrass. Morphological traits of white clover cultivars in mixtures were also tested for their relationship with competitive ability.

Five glasshouse experiments comprising monocultures and 50:50 mixtures of cultivars of white cover (WC) and perennial ryegrass (PR) were conducted. Competitive ability was measured as competitive ratio (CR), which is the ratio of the relative yields (RY) of the two components, ($CR=RY_{wc}/RY_{pr}$) (de Wit *et al.*, 1966; Willey and Rao, 1980). NSC was separated into soluble sugar and starch in white clover and soluble sugar and fructans in perennial ryegrass. Regression analyses were conducted between CR (Y) and morphological traits and NSC levels (X).

Experiment 1 was conducted to select cultivars of white clover and perennial ryegrass on the basis of the NSC levels in the stolons or stubble and roots, to be used in later experiments. Monocultures of eight cultivars of white clover and four cultivars of perennial ryegrass were examined over two harvests. NSC levels in the storage organs differed between cultivars of white clover and varied between harvests. Thus, at harvest 1 (after seven weeks of growth), Olwen had the highest soluble sugar and total NSC concentration, Huia the lowest and Tahora, Siral, Irrigation, Pitau, Haifa and Kopu intermediate. At harvest 2 (after four weeks of regrowth) Haifa, a large-leaved cultivar, and Pitau, a medium-leaved cultivar had the highest concentration and content of NSC while Olwen, Irrigation and Kopu had intermediate levels and Huia the lowest. Perennial ryegrass cultivars had similar NSC concentration and contents.

Experiment 2 was conducted to select a plant density that would give maximum shoot DM yield, so that an increase in density would not affect RY. The experimental designs (replacement and additive), were also compared. Density had effects on RY of white clover only at harvest 1 in the additive design at total densities of 8 plants pot$^{-1}$ and lower, but not in the replacement design. No effects of density on CR were evident. Shoot DM of perennial ryegrass declined at later harvests presumably due to the decline of nitrogen levels in the soil. The replacement design with the total density in mixtures of 16 plants pot$^{-1}$ was found to be appropriate in the study of white clover-perennial ryegrass competition to ensure that density effects did not confound competition effects. In monocultures, plant density had no effects on the total NSC concentration and content in the stolons of white clover.

Experiment 3 was designed to examine the competitive ability of cultivars of white clover and perennial ryegrass differing in the total NSC levels. Olwen and Irrigation white clovers (intermediate levels of NSC), and Huia white clover (low level of NSC), Ellet perennial ryegrass and Kangaroo Valley perennial ryegrass were planted at 16 plants pot$^{-1}$ in monocultures and in 50:50 mixtures with each other. Irrigation had the highest CR initially, but at harvest 4, it had the lowest CR compared to Huia and Olwen. Huia and Olwen had similar CR initially, but Huia had a higher CR at harvest 3, and at harvest 4 Huia and Olwen had similar CRs. Olwen had higher total NSC levels than Huia in monocultures and mixtures with perennial ryegrass. CR of white clover had a strong positive correlation with specific leaf area ($r^2$=0.86, $P<0.01$) at the same harvest; other traits had non-significant correlations. The relationship between NSC levels and subsequent CR values was non-significant.

Experiment 4 was conducted to study the NSC levels and the competitive ability of cultivars of white clover (Olwen vs. Huia) in mixtures with Ellet, as affected by defoliation (lenient vs. severe) and nitrogen application (nil and 75 kg $ha^{-1}$). Nitrogen applications reduced shoot and stolon DM and CR of white clover. Olwen had a similar CR to Huia in the absence of nitrogen application, but after four weeks regrowth their CRs were similar. In contrast, with nitrogen application CR of Olwen was higher than Huia but after four weeks regrowth, CR of Olwen was lower than Huia. Severe, but not lenient, defoliation in white clover caused a significant decrease in the total NSC concentration in the stolons. Nitrogen applications resulted in similar soluble sugar concentrations at destructive harvest 1 (DH1) in stolons of Olwen and Huia. In the absence of nitrogen application stolons of Olwen had higher ($P<0.05$) soluble sugar levels than Huia, but other forms of carbohydrate were similar in both cultivars. In the absence of nitrogen application, stubble of Ellet had a higher total NSC concentration and content in mixtures with Olwen than with Huia. This was associated with a higher CR for Olwen. The CRs of the white clover cultivars were positively correlated with height. In the absence of nitrogen application increases occurred in NSC levels in both stolons of Olwen and stubble of Ellet, indicating that as in Ellet, Olwen had a high demand for soil nitrogen for its growth.

Experiment 5 was designed to examine the total NSC levels and remobilization in Huia and Olwen in mixtures with Ellet. This was done by frequent cutting of the regrowth in the dark and full light until regrowth ceased, in the presence of different nutrient levels (nil, nitrogen only and complete nutrients). The etiolated regrowth as a measure of the total remobilization of NSC from the stolons and stubble was compared with the laboratory techniques used in the previous experiments. Consistent with the results of previous laboratory techniques, Olwen produced higher total shoot DM in etiolated and full light regrowth than Huia, in the presence of high levels of nutrients. Ellet produced less shoot DM in the regrowth in mixtures with Olwen than

with Huia, indicating a higher competitive ability in Olwen in these conditions. Compared to Olwen, Huia had a higher number of leaves, and longer stolons with higher numbers of nodes, and this could have led to the higher competitive ability of Huia observed in Experiment 3. During the regrowth, Olwen remobilised more total NSC from the roots and less from the stolons than Huia. A high level of soil nitrogen resulted in the accumulation of sugars in the stolons of Olwen in the dark which could be responsible for the tolerance or preference of Olwen to high nitrogen levels in the soil.

The practical implications of these findings are that Olwen white clover may be more suitable in mixtures with perennial ryegrass in areas of high soil fertility or if nitrogen is applied. Nitrogen application combined with defoliation, especially if severe, reduced NSC levels in the stolons; hence the defoliation interval should be long enough to enable white clover to replenish NSC levels in the storage organs. Planting Huia and Olwen white clover together in mixtures with perennial ryegrass may ensure stable DM production, provided management is used to prevent the strong dominance of either cultivar or ryegrass.

# Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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# Chapter I

# General Introduction

Mixtures of white clover and perennial ryegrass are important in temperate zone pasture, especially in more humid areas and areas under irrigation. The benefits of the mixtures from agronomic, economic, environmental and animal production points of view, are well documented in the literature (Curll, 1982; Frame and Newbould, 1986; Frame, 1987). Mixtures of perennial ryegrass and white clover without nitrogen application have been shown to produce a dry matter similar to that of grass monocultures with annual dressings of 140-300 kgha$^{-1}$ nitrogen fertiliser (Curll, 1982). In suitable environments the mixtures can produce sufficient herbage to support approximately 3.3 cattle ha$^{-1}$ from six months of age until slaughter six months later (Stewart, 1985). In Australia, white clover is sown on 6 million hectares of land (Gramshaw *et al.*, 1989) and currently is estimated to contribute $390 million annually to the dairy industry alone (Mason, 1993) and an estimated $10 million in South Australia.

The benefits of perennial ryegrass-white clover mixtures in terms of nitrogen fixation and high feeding value can only be appreciated if a white clover content of 30% on a dry matter basis in the swards is maintained (Martin, 1960; Harris and Thomas, 1973; Curll, 1982; Frame, 1987). However, in mixtures the proportion of white clover generally declines with time resulting in white clover content of much less than 30%, and in some cases as low as 2% (Frame and Newbould, 1986). Various factors have been implicated as the cause of such a decline. White clover is regarded as having lower competitive ability than perennial ryegrass (Grime, 1973; Haynes, 1980; Harris 1987). Perennial ryegrass may harbour fungi (*Acromonium lolii*) which produce allelochemicals affecting germination and growth of white clover (Scott, 1975;

Sutherland and Hoglund, 1989). Increasing soil acidity resulting from a long period of nitrogen fixation (Williams, 1980) and soil compaction by grazing animals disadvantage white clover. In addition, animals which selectively graze white clover over perennial ryegrass enhance the decline of white clover in the swards (Frame, 1987).

Since increasing nitrogen increases the competitive ability of perennial ryegrass, the practice of applying nitrogenous fertilizer to boost grass growth, especially early in the spring when low temperatures limit white clover growth can also reduce clover content (Haynes, 1980; Frame and Boyd, 1987b). However, even in New Zealand where pasture growth relies entirely on symbiotic nitrogen fixation, the swards rarely contain more than 20% white clover (Frame and Newbould, 1986). Thus, pasture management strategies have been designed to maintain a suitable white clover proportion in the swards. Provided that clover content can be raised from 20% to 50% in the swards, the contribution of white clover to the dairy industry alone in Australia could be increased by $107 million annually even without increases in total pasture production (Mason, 1993). More research is clearly justified to achieve this goal.

Despite the large number of experiments that have been conducted using different cultivars to suit diverse environments, the phenomenon of decline of white clover with time in the swards has persisted. It is suggested that in the past too much emphasis has been placed on morphological or phenomenological aspects of competition rather than understanding physiological aspects or mechanisms of competition (Rhodes, 1981; Rhodes and Ngah, 1983; Gaudet and Keddy, 1988; Robson *et al.*, 1989). Finally, Rhodes (1981), Woledge (1986) and Robson *et al.* (1989), suggest that there is a need for a better understanding of the physiological mechanisms by which grass and clover compete if reliable methods of management for these mixed swards are to be established.

Non-structural carbohydrates (NSC) are accumulated in plants and are used in maintenance respiration to provide energy in stressful environments (Penning de Vries, 1975 a, b). Begon *et al.* (1990) suggested that levels of NSC reserves in plant storage organs are affected by interspecific competition. These reserves are remobilised and used in regrowth after defoliation (reviewed by Sheard, 1973; and White, 1973), the efficiency of these processes may affect plant persistence and competitive ability (Grime, 1979; Chiariello *et al.*, 1989; Berendse and Elberse, 1990). However, most of the research on carbohydrate partitioning and remobilization during regrowth after defoliation has been conducted using single plants or monocultures. Their effects on competitive ability and the outcome of competition involving perennial ryegrass-white clover mixtures which are normally subjected to repeated defoliation or grazing, are relatively unknown.

This project has been designed to examine the levels of NSC in storage organs of white clover and perennial ryegrass planted in monocultures and mixtures, as affected by cultivar, the nitrogen application and defoliation regime. The effects of those levels on subsequent regrowth and competitive ability of white clover were also studied. Glasshouse experiments were chosen to ensure controlled conditions.

Variations in response to defoliation of NSC reserve levels in some cultivars of perennial ryegrass and white clover were examined, followed by studies on the effects of plant density on competitive relationships and on the accumulation of NSCs. The effects of cultivars with different levels of NSCs combined with the effects of nitrogen application on competitive ability and outcomes of competition were also examined. To quantify the competitive relationships de Wit's model of competition experiments was used. It is hoped that understanding the effects of levels of NSCs in storage organs on the competitive ability of these plants will lead to better managerial decisions and to selection and breeding of cultivars with a pattern of storage (mobilization and replenishment) of NSCs that will confer competitive superiority to white clover and legumes in general. Finally, a clearer insight into management options for maintaining a sufficient percentage of clover in the swards over time, will be achieved.

# Chapter II

# Literature Review

## 2.1 Plant Competition and Competitive Ability

Plant competition has been the subject of extensive studies by agronomists, ecologists and geneticists (Harper, 1961). As a result, voluminous books, journal articles and reviews have been published. Nevertheless, Goldberg (1990) still suggested that factors determining the competitive success of plants (or species) in cultivated fields are still being discussed by agronomists, weed scientists and ecologists.

van den Berg (1968) suggested that botanical composition in permanent grasslands was affected by environmental conditions through their effects on competitive ability of individual species. In sown leys, while the choice of species was under the control of farmers, botanical compositions of pastures were controlled by environments and competitive interferences. Latterly Grime (1979) formulated the Plant Strategy Theory in which plants were viewed to have evolved strategies to cope with "stress" which are environmental phenomena reducing photosynthetic production, i.e. shortages of light, water, mineral nutrition and suboptimal temperatures and "disturbance" which in pasture is physical destruction through grazing, cutting or trampling. The botanical composition of a vegetated area was the result of individual plant responses to stress, disturbance and competition, which according to van den Berg (1968) are determined by competitive ability. Competitive ability in turn is affected by environmental and plant factors viz., morphological and physiological factors which may include non-structural carbohydrate (NSC) reserves in the storage organs (Section 2.1.2).

The effects of morphological factors on competitive ability have been studied, but the notion that NSC reserves in the storage organs also affect competitive ability, especially in white clover-perennial ryegrass mixtures, has never been studied experimentally.

### 2.1.1 Definition

Many definitions of competition and competitive ability have been advanced. The definition of Grime (1979), who defined competition as "the tendency of neighbouring plants to utilize the same quantum of light, ion of a mineral nutrient, molecule of water, or volume of space", received wide support especially for pasture conditions. Competitive ability is defined by Grime (1979) as "the ability to capture growth resources from the environment, which in turn is a function of the area, the activity and the distribution of surface parts of the plants through which different resources are absorbed". Harper (1977), noting that plants modified their environments, which can have positive and negative effects on neighbouring plants, used the term interference to refer to competition for resources, the production of toxins (allelopathy), or changes in conditions such as protection from wind and influences on the behaviour of predators. These terms will be used as they are related to competition indices as used by de Wit (de Wit, 1960; de Wit *et al.*, 1966) (Section 2.1.3). Studies on plant competition become complicated due to the ability of plants to allocate resources to different parts of the plant in response to changing environmental conditions (stress) and disturbance, or allocating more resources to absorb growth factors most limiting (Tilman, 1987). For example, after defoliation some plants have been shown to continue root growth while others gave priority for shoot regrowth (Richards, 1993) making comparison or explanation of competitive ability difficult.

### 2.1.2 Factors Affecting Competitive Ability

Competitive ability in cultivated swards is influenced by a combination of plant features (morphological and physiological), environmental factors and management factors (van den Berg, 1968; Grime, 1979; Berendse and Elberse, 1990; Watschke and Schmidt, 1992). Plant features are usually regarded as conferring competitive ability if there are positive relationships between their values and some measures of success in mixtures such as biomass production (Gaudet and Keddy, 1988; Epp and Aarssen, 1989). These plant factors include features affecting the ability to capture resources, the efficiency of converting captured resources into biomass production, especially into those responsible for capturing more resources, and initial size which may include carbohydrate reserves (Section 2.3.3). Different plant traits may affect competitive ability at different stages of growth (Epp and Aarssen, 1989). Identification of plant traits responsible for superior competitive ability in a particular environment at a specific time is imperative to achieve and maintain the required balance in mixtures of white clover-perennial ryegrass in pastures for high animal productivity.

Environmental factors such as temperature, rainfall and daylength may affect seasonal growth pattern and eventually competitive ability. Soil physical properties (porosity, texture, moisture holding capacity) and chemical properties (pH, nutrient status, and ion exchange capacity) might affect competitive ability of a plant or species in mixtures (Harper, 1977). Probably the most important factors are management factors (the choice of companion genotypes, fertilization, irrigation, defoliation, grazing and pest control) which are thought to have profound effects on the competitive ability of cultivated plants or species in mixtures. It is important to stress that while carbohydrates stored in stolons, stubble and roots were listed as plant features that may contribute to the competitive ability of plants (Grime, 1979; Braakhekke, 1980; Berendse and Elberse, 1990), little research has been conducted to ascertain this notion, especially in perennial ryegrass and white clover mixtures. Some research workers consider that competitive ability has a genetic basis (Harper, 1977; Grime, 1979), but Donald (1963) has cautioned against such a notion, because it tends to defer the identification and analysis of the specific characters that govern the success of plants in competition. It can be concluded that the studies of plant competition are complicated due to the many factors that affect the outcome of competition. Different traits may determine competitive ability at different times which might be due to the plant's abilities to allocate resources to different organs in response to stress and disturbances.

### 2.1.3 Experimental Approaches in Studying and Measuring Competitive Ability in Grass-Legume Association

#### (a) Experimental approach

Generally, depending on the purpose of the study, the experimental approaches are divided to study :

(i) the relative importance of above ground (for light) and below ground (for nutrients and water) competition, and

(ii) the response of a species to competition in the presence of other species.

The relative importance of above and below ground competition has been studied using divided pot techniques, to prevent shoots and/or roots intermingling (Donald, 1963; Wilkinson and Gross, 1964; Martin and Field, 1984; Wilson, 1988). Others have measured leaf area at different strata (Woledge, 1988), or root dry weight and root length at different soil depths (Evans, 1977) and related these measurements to competitive ability and growth of plants (Section 2.1.3b). Root competition has been shown to start earlier than shoot competition (Donald, 1963; Wilson, 1988). The extent of earlier root competition will affect shoot growth and Tilman (1988) suggested that eventually shoot competition will assume more importance especially in fertile habitats where leaf growth is not limited by nutrients, which in turn will affect root growth through the amount of photosynthates translocated to the roots. Others have indicated that habitat richness or fertilizer application shifted competition from below ground to above ground (Donald, 1958). Obviously, superior rates of growth of one component of a mixture prior to achievement of complete ground cover and light interception will confer a competitive advantage (Davidson and Robson, 1985a) in terms of competition for light.

The experimental approaches of examining the response of a species in competition in the presence of other species are of two kinds viz. (a) additive design, and (b) replacement series experiments. In the additive design, the density of one species per unit area is held constant and the density of the companion species varied. Competitive ability of a component is measured as the extent of reduction in yield caused by the presence of companion species compared to their yield in monocultures. Additive design is suggested to give actual conditions as in the field, eg. in crop-weed studies and the results are easy to interpret, however the competitive effects are confounded by density effects i.e. total density of the mixture (Harper, 1977; Snaydon, 1991). In addition no mathematical models are available for quantifying competitive effects (Spitters and van den Berg, 1982).

Replacement series experiments were initially designed by de Wit (1960) where the total density of plants in mixtures was held constant but the proportion of respective species progressively varied. For example, in studying the competitive relationship between panicum (*Panicum maximum*) and glycine (*Glycine javanica*), de Wit *et al.* (1966) used five treatments with the proportion of 100:0, 75:25, 50:50, 25:75, and 0:100 for panicum and glycine, respectively, resulting in relative frequency of 1.0, 0.75, 0.50, 0.25, 0.0 for panicum and 0.0, 0.25, 0.50, 0.75, and 1.0 for glycine. The yield in terms of the dry mass was measured both in monocultures and mixtures, and relative yields were calculated as :

$$RY_g = \frac{G_m}{G_o} \qquad RY_l = \frac{L_m}{L_o}$$

$$RYT = RY_g + RY_l$$

where RY = relative yield
L, l = legume, G, g = grass
m = DM yield in mixtures,
o = DM yield in monocultures
RYT= relative yield total

Hall (1978); Trenbath (1978) and Fisher and Thornton (1989) noted possible outcomes of competitive relationships of mixtures in replacement series as in Figure 2.1.

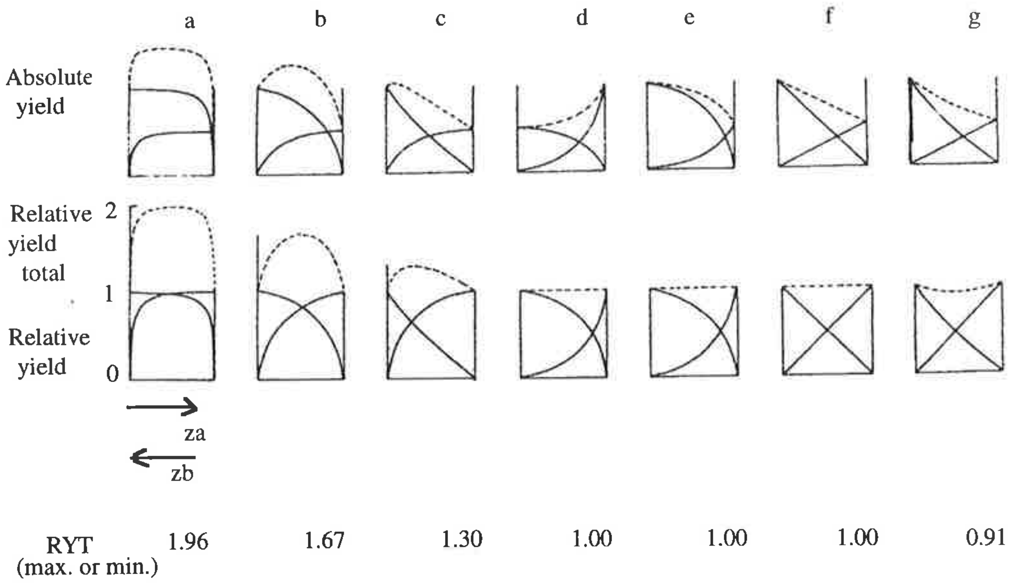



**Figure 2.1** Replacement diagrams illustrating the range of situations (a-g) that may arise in competition experiments using replacement series. Both absolute and relative yields of species a and b (including RYTs) are plotted against their relative frequency (z) in the mixtures (after Hall, 1978).

| | | |
|---|---|---|
| In a, b and c | RYT > 1 | Indicating coexistence (complementary) |
| In d, e and f | RYT=1 | species a and b are said to be mutually exclusive, in d and e species b is gaining and a is losing, and in f competition is balanced, neither species is gaining or loosing. |
| In g | RYT <1 | Allelopathic effects exist, one species having poisoned the other |

Plant relations such as in a, b, and c are ideal conditions usually sought by pasture agronomists (Fisher and Thornton, 1989) (Section 2.2.1). They commonly occur with grass-legume mixtures.

In grass-legume mixtures, RYT is usually greater than unity because legumes have an independent source of nitrogen. In the absence of *Rhizobium*, or if environmental conditions do not permit nitrogen fixation, RYT in the mixtures of grasses and legumes is often equal to one (de Wit *et al.*, 1966). In the presence of *Rhizobium* and if soil nitrogen levels are low, legumes become more competitive than grasses. However, eventually grasses benefit from nitrogen transfer and the legumes suffers from the more competitive grass.

Because of the use of several proportions, Silvertown (1982) pointed out that replacement diagrams are an extremely effective means of determining the yielding performances of species in mixtures. In addition de Wit's model makes it possible to differentiate the response of plants due to competitive interference, non-competitive interference (coexistence and allelopathy) (de Wit, 1960; Hall, 1978). The designs can also be used for studying competition for a single factor i.e. nutrient (Hall, 1974 a,b). However, their implementation is laborious and as such they are not widely used in actual field conditions (Fisher and Thornton, 1989) or in competition involving more than two components.

There are continuing debates in the literature on the appropriateness of the use of the additive design compared to the replacement series design for studying competition (Connolly, 1986; Snaydon, 1991, 1994; Sackville Hamilton, 1994) especially in terms of density effects, and ease of interpretation (Connolly, 1986; Taylor and Aarssen, 1989; Snaydon, 1991). In order to overcome the confounding of competitive effects with those of density effects, Snaydon and Satore (1989) suggested the use of several total densities to allow their effects on competition to be compared. The use of this so called bivariate design would lead to the experiment becoming too big, and beyond the resources of the experimenter. Austin et al. (1988) compared the effects on competition between two thistle species of varying total density, design (substitutive vs replacement) and nutrient concentration. On the basis that the yield-plant density relationship is asymptotic, they concluded that in order to ascertain that the observed effects are due to competition and not density in replacement experiments, the density of each species in both monoculture and mixture should be on the horizontal section of the response curve, ie where yield is a maximum. This would require prior knowledge of density effects in monocultures. However, most of the experiments in which confounding of density effects and competitive effects were observed have used mixtures of similar species (grass versus grass) which use similar resources from the environment, or they have used growth only during establishment to quantify competitive ability (e.g. Austin *et al.* 1988). Competition has been shown to be more intense between plants with similar resource requirements (eg. grasses) compared to white clover-ryegrass mixtures in which white clover has an independent source of nitrogen. Furthermore, if plants in mixtures are allowed to establish and to reach complete ground cover, population density effects might be small. Thus, despite the criticisms, the replacement design has been regarded as the only sure way to describe competition between two species in mixtures (Hall, 1978; Trenbath, 1978). The choice of the design to be used will depend on prior information of the component under study, the purpose of the experiment and the resources available. If the experiment is designed to determine factors causing competitive superiority of a component, de Wit's replacement technique is preferable due to the availability of mathematical models to describe the competitive relationship (Sackville Hamilton, 1994).

### (b) Measuring competitive ability

Competitive ability is always measured in terms of one plant relative to another. In mixtures of grasses and legumes, competitive ability is measured by some as the degree of reduction in growth (shoot dry matter production) in mixtures compared to their yield in monocultures (Rhodes, 1981; Rhodes and Ngah, 1983; Snaydon, 1991). Others have measured competitive ability as the relative ability of plants to suppress the growth of a common indicator species or phytometer (Gaudet and Keddy, 1988). The term competitive intensity is sometimes used as a measure of the competitive effect (Grace, 1995).

Competitive ability is also measured, in relation to the de Wit model of competition, as relative replacement rate (van den Berg, 1968), relative crowding coefficient (de Wit, 1960, Harper, 1977). However, these measures only apply to plants which are mutually exclusive. Others have measured aggressivity (McGilchrist and Trenbath, 1971) competitive ratio (Willey and Rao, 1980), and relative resource total (RRT) which is the total area required to produce the same output in a pure stand of the component species as was produced in a unit area of the mixture (Connolly, 1986; Menchaca and Connolly, 1990).

McGilchrist and Trenbath (1971), based on de Wit's model, introduced aggressivity (based on yield per plant) as :

Aggressivity (A) of grass towards legumes = 1/2 ($RY_g$-$RY_l$).

Willey and Rao (1980) noted that because aggressivity was calculated by subtraction, it was possible to have two different mixtures with different values of relative yield, yet they have similar aggressivity. For example :

if $RYg_1$=0.8 and $RYl_1$=0.6 ;

and $RYg_2$=0.6 and $RYl_2$=0.4;

both have A=0.1, which means that the grass in both mixtures has similar aggressivity and hence similar competitive ability with respect to the legume which might not be true. To overcome that problem, Willey and Rao (1980) proposed the competitive ratio which is the ratio between the relative yields of the species to each other :

$$CR = \frac{RY_l}{RY_g}$$

Willey and Rao (1980) then calculated regression analysis between competitive ratio (Y) and different plant characteristics (X) and claimed that competitive ratio could be used in identifying which plant characters are associated with competitive ability. They showed that in pigeon pea/sorghum intercropping, competitive ratio had a positive correlation with height ($r^2 = 0.54$) and with time of maturity (days) of the sorghum ($r^2 = 0.32$).

Others have calculated the regression between plant performance and the performance of associated species in mixtures (Breese and Hill, 1973; Mather *et al.*, 1982) in order to identify plant traits that contributed to higher competitive ability. Mather *et al.* (1982) provided evidence that linear regression after square root transformation gave better fits compared to curvilinear regression. Gaudet and Keddy (1988) calculated multilinear and linear regression between plant traits and competitive ability defined as the ability of a species to suppress the growth of a common indicator species, or phytometer (Table 2.1). A total of 44 test species were grown in 1-litre pots in mixtures with the phytometer. Four individuals of test species were planted around the phytometer. Plants were grown over one growing season and harvested once. With water and nutrients not limiting they found a strong multilinear relationship between plant traits of the test species and the suppression of the phytometer as a measure of competitive ability ($r^2=0.74$), where 63% of the variation in the competitive ability can be assigned to plant biomass. Measurements on test plants grown in the absence of the phytomer showed that the traits correlated with competitive ability were inherent characteristics and not dependent on competition from the phytomer. Simple linear regression which was also conducted showed that above ground biomass had a strong significant ($P<0.01$) correlation ($r^2=-0.791$), while other traits had less strong relationships. The higher simple linear correlation coefficient for above ground biomass with competitive ability compared to that of multiple regression suggested that above ground biomass was the most important factor affecting competitive ability in that experiment. The strong correlations between above ground biomass and height with competitive ability suggested that competition for light might be a stronger factor than competition for nutrients or water. Their data also show that canopy diameter and area were more important than leaf characters i.e. leaf length, width, area, and leaf number in determining competitive abilities. This might indicate that complete surface cover has been achieved in the experiment and self shading occurred among strata of leaves causing the lower leaves to receive reduced light and contribute little advantage in competition for light. In addition, the age of the leaves and the physiological development of the leaves sampled were not considered. For example in white clover it has been shown that when leaf stage is 0.6, i.e. when the leaflets start expanding (Carlson, 1966) they start to become net exporters of assimilates, and these leaves are subsequently elevated above the canopy (Woledge, 1988) and hence would contribute to the ability to compete for light. Therefore, the use of morphological traits of leaves stage 0.6 or higher might give a better regression with competitive ability for light.

**Table 2.1** Correlation (*r*) between traits of 44 test species and biomass of the phytometer (*Lytrum salicaria*). (After Gaudet and Keddy, 1988)

| Plant traits of test species | Correlation with phytometer biomass |
|---|---|
| Biomass, total (g) | -0.775** |
| Biomass, above ground (g) | -0.791** |
| Biomass, below ground (g) | -0.710** |
| Height (cm) | -0.659** |
| Leaf length (cm) | -0.084 |
| Leaf width (cm) | -0.179 |
| Leaf area ($cm^2$) | -0.302 |
| Leaf number | -0.244 |
| Leaf shape (length:width) | 0.356* |
| Canopy diameter (cm) | -0.455* |
| Canopy area ($cm^2$) | -0.593** |
| Shoot to root ratio (g/g) | -0.016 |

*P<0.05; **P<0.01. Correlations are simple linear correlations with phytometer biomass

Grace (1995) suggested the relative competition intensity (RCI) to measure competition intensities. This is calculated as:

$$RCI = \frac{P_{mono} - P_{mix}}{P_{mono}}$$

Where $P_{mono}$ = Yield in monoculture

$P_{mix}$ = Yield in mixture

Because RCI=1-RY, if the yield in the mixture is half of that in the monoculture, then RCI is equal to RY. The difference between the two indices is that as the yield in mixtures declines RY values become smaller, but RCI values become larger. In addition, if the yield in mixture exceeds that in monoculture, as when grasses benefit from nitrogen transfer in grass-legume mixtures, RCI become negative while RY values exceed unity. Based on this ease of interpretation the combination of RY and RYT in describing plant interference in grass-legume associations is preferable.

Direct comparisons as to the appropriateness and advantages (or disadvantages) of different indices to identify plant traits that conferred competitive abilities are lacking in the literature. Hence, selection of the index used depends entirely on the choice of the experimenter. In this thesis, the competitive ratio (de Wit *et al.*, 1966; Willey and Rao 1980) will be used, because competitive ratio is based on replacement techniques (de Wit, 1960; de Wit *et al.*, 1966) and has been used in studying grass-legume mixtures. Thus any observed response can be related to previous published work.

In summary, the de Wit replacement design makes it possible to distinguish among competitive exclusion, coexistence, stimulation or non-competitive inhibition. Competitive ratio in conjunction with de Wit replacement design, can be used to identify plant traits that relate to higher competitive ability. Competitive ability is affected by plant factors (morphology and physiology), environmental factors and management factors, which cause problems in the identification of plant factors that relate to competitive ability.

## 2.2 Plant Relations in White Clover-Perennial Ryegrass Mixtures

In pastures, white clover adds nitrogen to the soil, the mixture is nutritionally superior to perennial ryegrass, highly acceptable to the animals, enhances forage intake, and improves the efficiency of feed conversion (Curll, 1982; Frame, 1987). Hence, high clover content up to 50% in the swards (Mason, 1993) is regarded as a sign of productive pastures and good management.

White clover is best suited to grow in temperate regions with adequate rainfall in warmer months. In such an environment, different temperature optima for growth viz. 21-25 $^{O}C$ for white clover and 18-21 $^{O}C$ for perennial ryegrass (Mitchell, 1956), lead to perennial ryegrass making the bulk of dry matter (DM) production early in the spring, and provide some degree of protection to white clover stolons against grazing and low temperature damage. Later in summer and autumn when the temperatures are warmer and available nitrogen in the soil declines, white clover grows vigorously and fixes nitrogen which is later released for the growth of the grass (Haycock, 1981; Harris, 1987). A dynamic equilibrium might be achieved, in which no marked suppression of the components occurs and white clover and perennial ryegrass growth oscillate depending on the nitrogen contribution of white clover to the system (Harris and Thomas, 1973), hence some degree of coexistence is achieved. It is difficult to maintain this equilibrium, because as soil nitrogen increases resulting from nitrogen contribution by white clover, ryegrass becomes more competitive. However, achieving and maintaining this dynamic equilibrium is the core of studies on competition between grass and legumes.

White clover dominance in the sward, except in some circumstances (i.e. seed production), is rarely an objective in pasture management. However, in Australia the maintenance of a high clover content of up to 50% on the DM basis is suggested should be an objective of every farm manager (Mason, 1993) if higher animal output per unit of land is to be achieved. This objective seems to be difficult to achieve, as perennial ryegrass-white clover swards on most properties contain only 20% or less clover content in the swards (Frame and Newbould, 1986; Harris, 1987). Perennial ryegrass is regarded as more competitive than white clover which is also more prone to setbacks from extreme environmental conditions (low or high temperatures, water stress, low soil pH), the use of aggressive grasses, selective grazing by animals, nitrogen application and disease (Haynes, 1980; Frame and Newbould, 1986; Harris, 1987; Mason, 1993). Because of the interdependence of white clover and perennial ryegrass their relationships can be in the form of coexistence (complementarity), competitive interaction, and allelopathy. These aspects will be examined in the following sections.

### 2.2.1 Coexistence

The long use of perennial ryegrass and white clover in managed grasslands, through spatial and temporal compatibility (Rhodes, 1981), has led to some degree of coexistence (Evans *et al.,* 1985), defined as living together in the same habitat such that neither tends to be eliminated by the other (Begon *et al.,* 1990). For example Turkington and Harper (1979) and Simpson *et al.* (1987) found that white clover has a positive association with perennial ryegrass and mostly a negative association with other grasses i.e. *Agrostis tenuis* Sibth. Simpson *et al.* (1987) attributed this positive association between white clover and perennial ryegrass to their similarity in response to changes in soil pH. In the de Wit model of competition, coexistence in mixtures is usually associated with a relative yield total greater than unity (Hall, 1978) (Section 2.1.3) and possibly with a tendency towards establishing equilibrium.

Some researchers found that the yields of white clover and perennial ryegrass species selected from coexisting components in the field were superior to species without such prior association (Turkington and Harper, 1979; Evans *et al.*, 1985; Turkington, 1989), and had a higher yield of 20 per cent or more. Consequently, the use of species with a history of coexistence is suggested to be one way to improve yield from pastures under varying levels of nitrogen application. However, until the mechanisms related to competitive ability leading to coexistence can be identified, doubt on the selection of species based on coexistence remains. Coexistence may just reflect the diversification of the use of resources or differential requirements for resources at different times or seasons.

Clearly identifying traits related to competitive ability of white clover is imperative to achieve coexistence and dynamic equilibrium in white clover-perennial ryegrass mixtures.

### 2.2.2 Competitive Relationships

Competitive relationships in perennial ryegrass-white clover mixtures have been extensively studied and reviewed by Brougham *et al.* (1978), Curll (1982), Harris (1987), Gramshaw *et al.* (1989). Plant, environment and management factors will all affect relative competitive abilities of perennial ryegrass-white clover associations (Section 2.1.2), however the relative importance of those factors may vary for different plants in different intensities of competition and defoliation regimes. Also different plant traits may affect competitive ability at different times and stages of growth (Epp and Aarssen, 1989).

Evidence has accumulated suggesting that nitrogen availability was the most important factor determining the relative performance of perennial ryegrass and white clover in mixtures (Turkington and Harper, 1979; Harris, 1987) while Rhodes and Ngah (1983) have suggested that genotype, defoliation (cutting and grazing) and the application of nitrogenous fertilizer are the most important factors under management control in affecting performance of perennial ryegrass-white clover mixtures.

Experiments on competition in mixtures of white clover-perennial ryegrass generally pointed to the initially superior competitive ability of the grass, but this superiority declines over time (Bakhuis and Kleter, 1965; Harris and Thomas, 1973; Davidson *et al.*, 1986; Davidson and Robson, 1990; Menchaca and Connolly, 1990) presumably due to the decline in soil nitrogen levels. At the same time the competitive ability of white clover increases resulting from its ability to fix nitrogen. In the following discussion, plant morphological traits affecting competitive ability of perennial ryegrass and white clover in mixtures during vegetative growth and after defoliation will be discussed with emphasis on the effects of genotype and the application of nitrogenous fertilizers.

### (a) Plant morphological traits affecting competitive ability

Plant features that are claimed to affect competitive ability include growth rates, size, shoot:root ratio, leaf area ratio and specific leaf area (Grime, 1973, 1979; Berendse and Elberse, 1990). Poorter and Remkes (1990) examined relative growth rates and other growth parameters in some species during vegetative growth at establishment (less than 8 weeks after planting), and their data for perennial ryegrass and white clover are presented in Table 2.2. Perennial ryegrass had a higher relative growth rate, leaf area ratio, leaf weight ratio and root weight ratio, while white clover had a higher net assimilation rate, specific leaf area and stem weight ratio.

**In shoot competition**. Poorter and Remkes (1990) planted 24 species of plants including white clover and perennial ryegrass in a growth room for a period of 17 days. The plants were planted in monoculture in solution cultures supplied with full strength standard nutrients. They found a strong correlation between LAR (total leaf area:total plant weight) and RGR, indicating the more the plant invests in leaf area, the higher the total carbon gain and the faster the growth.

While the differences between white clover and perennial ryegrass in morphological traits were mostly small (Table 2.2) and may vary in relation to habitat productivity and over time, they may be important in determining initial competitive ability. Leaves of perennial ryegrass have been shown to contain a higher percentage of water soluble carbohydrates on a DM basis than white clover (10.5 vs 6.1 % of DM) (Wilman and Riley, 1993). Higher leaf area ratio in perennial ryegrass means more light will be absorbed per unit plant weight, and a higher leaf weight ratio means a lower weight of plant tissue maintained per unit of leaf biomass.

**Table 2.2.** Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), leaf weight ratio (LWR), stem weight ratio (SWR) and root weight ratio (RWR) of perennial ryegrass and white clover. (After Poorter and Remkes, 1990).

| Growth parameter | Perennial ryegrass | White clover |
|---|---|---|
| RGR ($mgg^{-1}day^{-1}$) | 214 | 206 |
| NAR ($gm^{-2}day^{-1}$) | 11.5 | 12.1 |
| LAR ($m^2kg^{-1}$) | 19.5 | 17.1 |
| SLA ($m^2kg^{-1}$) | 38.8 | 40.4 |
| LWR ($gg^{-1}$) | 0.50 | 0.44 |
| SWR ($gg^{-1}$) | 0.20 | 0.28 |
| RWR ($gg^{-1}$) | 0.31 | 0.28 |

Hence, Snaydon and Baynes (1981) concluded that shoot competition from grasses affects the performance of white clover (i.e. white clover content) rather more than root competition. However, the relative importance of shoot and root competition in clover-ryegrass association will vary with nutrient availability. Thompson and Harper (1988) observed that perennial ryegrass canopies caused less reduction in red:far red ratio compared to the canopy of other plants. However, reduction in red:far red ratio caused a reduction in internode length, mean number of nodes, number of branches and rooted nodes, but increased greatly the petiole length of white clover. This feature enables white clover to position its leaves in the best lit sections of the canopy as suggested by Davidson *et al.* (1981); Davidson and Robson (1985 a,b); Dennis and Woledge (1985); Woledge (1988), and Woledge *et al.* (1992) who showed that even in well fertilized mixtures, white clover, regardless of leaf size, can position the leaves in the canopy to absorb more light per unit ground area, and fix more carbon than perennial ryegrass. Many workers also have provided evidence that photosynthetic capacity of white clover leaves grown in mixed swards was comparable to that in perennial ryegrass (Dennis and Woledge, 1981). They pointed out that in white clover the successive petioles were longer, hence are able to position the younger leaves in better lit positions, while in perennial ryegrass the photosynthetic capacity of successive newly expanded leaves generally declines. If plants are defoliated by cutting or grazing, reserves in storage organs will be remobilized and used for regrowth (Section 2.3). However, positioning the leaves in the best lit area incurs expenditure of resources to form longer petioles, resulting in less resource accumulated as reserves in the stolons (Tesar and Ahlgren, 1950) which might affect future growth and competitive ability.

Higher SLA in white clover compared to perennial ryegrass (Poorter and Remkes, 1990) was also observed in mixtures (Davidson and Robson, 1985a; Parsons *et al.*, 1991; Barthram and Grant, 1994) which may mean higher energy absorption per unit of leaf weight. Barthram and Grant (1994) pointed out that higher specific leaf areas in white clover would mean greater return per unit of carbon invested in new leaf area, which is an advantage in grazed swards. They further suggested that the relationship between specific leaf area and proportion of white clover in mixtures might support the hypothesis of relationships between specific leaf area and the success of white clover (measured as the proportion of the total population). However, higher stem weight ratio and a lower leaf weight ratio mean the maintenance of more tissues, resulting in a higher energy requirement for maintenance respiration. This is in line with the finding of Shinano *et al.* (1993) who pointed out that compared to *Gramineae*, *Leguminaceae* had a larger respiratory loss under both dark and light conditions in the shoot. However, no comparable research has been conducted to examine specific leaf areas and maintenance requirements of different cultivars of white clover and perennial ryegrass.

**In root competition**. Perennial ryegrass also had a higher root weight ratio (RWR) (Pooter and Remkes, 1990) or root:top ratio (Ozanne *et al.*, 1964) which might be related to generally a higher competitive ability of perennial ryegrass for nutrients and water over white clover reported in the literature. Evans (1978) examined root length at different soil depths of perennial ryegrass and white clover in monocultures and mixtures (Table 2.3). In monocultures in the top 0-20 cm of soil, roots of perennial ryegrass were longer than white clover. This is the part of the soil where most root activities in terms of nutrient absorption exist (Ozanne *et al.,* 1964), hence where competition for nutrients is most severe. At all other soil depths roots of perennial ryegrass were longer except at the depth of 100-120 cm and120-140 cm when

**Table 2.3.** Means root length (cm) of perennial ryegrass, white clover and mixtures, per 1000 g dry soil at various depth (cm). (After Evans, 1978).

| Soil depth | Perennial ryegrass | White clover | Mixture |
|---|---|---|---|
| 0-20 | 14490 | 3310 | 8060 |
| 20-40 | 2180 | 630 | 1310 |
| 40-60 | 540 | 220 | 470 |
| 60-80 | 340 | 130 | 230 |
| 80-100 | 190 | 90 | 130 |
| 100-120 | 110 | 110 | 110 |
| 120-140 | 90 | 90 | 130 |

perennial ryegrass and white clover have similar root lengths. In mixtures a similar trend applies but the proportion of root length of each component was not provided.

It can be concluded that perennial ryegrass has a stronger competitive ability for nutrients and water due to their higher rootlength:weight ratio, root:top ratio, and root biomass especially at the top 0-20 cm soil depth compared to white clover.

**(b) Environmental, cultivar and management factors affecting competitive ability**

Regrowth after defoliation and hence, competitive abilities depend on the ability to reconstruct and maintain the activity of tissues (shoots and roots) that are directly responsible for the absorption of resources. This in turn will be affected by several factors viz.: (i) residual photosynthetic areas, (ii) the amount of reserves available if residual photosynthetic areas are low, (iii) the number of growing points, and (iv) favourable environments to facilitate regrowth (Caldwell, 1986; Briske, 1986). Points (i), (ii), and (iii) will be influenced largely by management i.e. initial choice of cultivars, frequency, intensity and timing of defoliation, nutrient applications, and degree of pest and disease prevention.

Plants may avoid or tolerate grazing (Trlica and Rittenhouse, 1993). Avoidance reduces the probability and severity of defoliation by reducing growth form (Silvertown, 1982). Tolerance, that is facilitating regrowth after defoliation, is achieved through physiological plasticity. For example, Thomas (1984) showed that defoliation during drought in small-leaved white clover cultivars resulted in much reduced leaf size and short petioles that almost completely avoided defoliation. At the end of drought periods these plants had higher stolon DM and larger regrowth compared to the control (sufficient water). The preferential allocation of current photosynthates after defoliation for shoot growth (Culvenor *et al.*, 1989a; Chapman *et al.*, 1991a), storage formations and reserve filling (Steinlein *et al.*, 1993) enables the plants to replenish their reserves, which might be expected to influence subsequent competitive ability. On the other hand, Chapman *et al.* (1990b) provided evidence that the immediate response of white clover to defoliation was an increase in SLA which will increase competitive ability for light.

It can be concluded that environmental and management factors are the main factors affecting competitive ability and physiological plasticity eg. reduced leaf size in response to defoliation, increases in SLA and preferential allocation of resources are responses of plants to changes in those factors and will affect their competitive ability.

**Season**. Seasonal climatic conditions affect the growth of plants and subsequently their competitive ability. Brock *et al.* (1988) sampled white clover from perennial ryegrass-white clover swards over several seasons. Stolon dry weight, stolon length and numbers of stolons and leaves per plant declined significantly in spring, with recovery the following summer. Number of roots per plant increased over winter, to peak in early spring, followed by a decline in the following summer-autumn.

Clover content declined when temperature was low, and total leaf area index was low (Davidson and Robson, 1990). The competitive ability of perennial ryegrass which has a growth temperature optimum of 18-21 °C (Mitchell, 1956), will be severely reduced by more frequent hard grazing during late autumn, winter and early spring. A longer rest period in late spring will increase the growth and competitive ability of white clover, which has a temperature growth optimum of 24 °C (20-30 °C) (Hart, 1987). For example, in mixtures with perennial ryegrass, Olwen white clover, a summer active cultivar, produced higher DM and hence was more competitive in summer, but less in winter than winter active Haifa (Fulkerson and Slack, 1994).

Cultivars with better growth in winter caused a reduction in the growth of associated grasses in winter (Brock, 1971). This prompted Ennik (1981) to suggest that the use of clover varieties with higher competitive ability might increase N yield, and hence the quality of forage on offer to the grazing animals. However, whether that same clover would increase total DM production was in doubt because the increase in clover yields were usually achieved at the expense of the companion grass yield (Ingram and Aldrich, 1981).

In summary, it can be concluded that seasonal climatic conditions affect competitive ability of cultivars of white clover through the effect on their growth. White clover increases in growth and competitive ability when air temperature is higher (20-30 °C) compared to lower temperature, while competitive ability of perennial ryegrass which has a growth temperature optimum of 18-21 °C, will be severely reduced by more frequent hard grazing during late autumn, winter and early spring. A longer rest period in late spring will increase the growth and competitive ability of white clover.

**Moisture**. Literature on competition between white clover and perennial ryegrass points to the intolerance of white clover to drought compared to perennial ryegrass. Part of the reason might be due to the larger, longer and deeper root system of perennial ryegrass, as indicated by Evans (1978). Thus, Turner (1991) showed that white clover was very sensitive to drought, especially when dependent on nitrogen from symbiotic fixation, with stolon growth most affected. A rapid decline of white clover occurred if defoliation was accompanied by water stress, especially in summer (Archer and Robinson, 1989). Drought reduced the growth of white clover more than that of perennial ryegrass (Thomas, 1984). In small-leaved cultivars this resulted in much reduced leaf size and short petioles that almost completely avoided defoliation. At the end of drought periods these plants had higher numbers of stolons and larger regrowth compared to the control plants. Hence, more frequent defoliation during drought maintained clover content in the swards (Thomas, 1984).

It is concluded that white clover is less tolerant to drought than perennial ryegrass, hence, more frequent defoliation during drought to reduce the growth of perennial ryegrass will maintain the white clover content in the swards.

**Nitrogen and other nutrients.** Soil nitrogen and other nutrients are important in determining the growth and competitive ability of plants. Thus, Ennik and Hofman (1983) found that soil nitrogen levels were positively related to the competitive ability of perennial ryegrass, through increasing growth rates and root biomass. However, if high soil nitrogen levels were combined with more frequent defoliation, their root biomass decreased and subsequently their competitive ability might also decline.

Nitrogen application to newly cut grass has been shown to increase leaf area and the photosynthetic capacity of the next leaf to expand (Woledge and Pearse, 1985), and to increase the competitive ability of perennial ryegrass for other nutrients (Mouat and Walker, 1959; Rhodes, 1981). Clover percentage was higher in unfertilized swards (receiving no nitrogen application) and defoliated swards compared to fertilized and undefoliated swards (Davies and Evans, 1990b). White clover also showed a decrease in nitrogen fixation in the presence of high nitrate levels in the soil and in low temperatures. Nonetheless, white clover cultivars differed in their response to the presence of high nitrogen concentrations in the soil. The large-leaved cultivars, for example Olwen, are more adapted to the presence of high nitrates in the soil than medium- or smaller-leaved cultivars (Wilman and Asiegbu, 1982a,b; Goodman and Collison, 1986). The cause of this adaptation is not well known.

Similarly, Brock (1971) and Brock and Hoglund (1974) found that in mixtures with the perennial ryegrass 'Grasslands Ariki' in the field, white clover 'Grasslands 4700' was more responsive to nitrogen application than Huia, and reduced the DM production of the associated grass by 20 per cent. It was suggested that 4700 had stronger root growth and/or better competitive ability for nitrogen and other nutrients.

Shoot growth of perennial ryegrass is stimulated by the presence of high nitrate in the soil. Defoliation during this time may encourage tiller formation, as found in *Sporobolus kentrophyllus* (Ruess, 1988) and its associated adventitious roots may increase the competitive ability of perennial ryegrass for above- and below-ground growth resources.

In mixture with perennial ryegrass, defoliation and nitrogen application cause a decline in biomass, stolon length, stolon weight and water soluble carbohydrate content of white clover, and the decline is greater with infrequent defoliation (Harris *et al.*, 1983). Nitrogen application resulted in a reduction in white clover content which was due to a larger leaf area as well as larger photosynthesis rates per unit area of grass (Woledge and Pearse, 1985) at a height unattainable by white clover (Boller and Nösberger, 1985). Fewer stolon growing points exist as a result of the nitrogen application. Other workers reported that white clover content declines with increasing nitrogen application (Frame and Boyd, 1987a; Davies and Evans, 1990b). Hence, the white clover proportion was higher if no nitrogen was applied compared to the application of 100 kg N $ha^{-1}$, or after 2 years when nitrogen has been depleted by ryegrass (Hopkins *et al*, 1990). Inhibition of stolon branching and the reduction of white clover leaf area are the major factors in the adverse effects of nitrogen fertilizer on clover (Dennis and Woledge, 1987).

White clover and perennial ryegrass differ in their response to the presence of high soil nitrogen availability. Application of nitrogen to mixtures of white clover-perennial ryegrass resulted in an increase in grass height, size of grass leaves, and yields (Wilman and Asiegbu, 1982a). Cultivars of white clover differed in their response to applied nitrogen, with medium-large leaved varieties being more tolerant than small-leaved varieties (Wilman and Asiegbu, 1982a). Stolon diameter was reduced by 6%, but stolon length was less adversely affected in the medium and large-leaved than in the small-leaved varieties. Small-leaved varieties had thinner stolons and in the absence of nitrogen application, had twice the stolon length and a greater proportion of leaves which escaped defoliation (Wilman and Asiegbu, 1982b).

In mixtures, larger-leaved white clover varieties had higher competitive ability and were more productive than the smaller-leaved varieties at all nitrogen rates. No interaction was found between cultivars and cutting height (Frame and Boyd, 1987a). In the presence of applied nitrogen white clover cv. Kopu (large-leaved cultivar) and cv. Huia (medium-leaved cultivar) absorbed similar amounts of nitrogen from the soil, however Kopu produced 15% more DM and fixed 35% more nitrogen than Huia (Eltilib and Ledgard, 1988). Kopu had superior winter production and increased the winter production of the pasture (Ledgard *et al.*, 1990). Similarly, Olwen (medium-large leaved) produced more DM and fixed more nitrogen than S184 white clover (small-medium leaved) under mowing, and the associate grass took up more nitrogen when grown with S184 than with cv. Olwen (Goodman and Collison, 1986).

Nutrient additions other than nitrogen can alter the end results of competition: these have been reviewed by Harris (1987). Generally, irrigation and the addition of phosphorus, potassium, magnesium, sulphur and micro nutrients have favourable effects on white clover, especially in conditions where water and these nutrients are limiting.

In summary, soil nitrogen and other nutrients affect competitive ability of white clover in mixture with perennial ryegrass. Medium large-leaved cultivars have higher competitive ability in lenient and infrequent defoliation and in the presence of high soil nitrogen levels than smaller leaved cultivars. Nitrogen application increases grass growth and its competitive ability, and at the same time decreases those of white clover. In white clover-perennial ryegrass mixtures, perennial ryegrass is usually more competitive than white clover initially, but as growth continues and nitrogen in the soil declines, white clover becomes more competitive.

**Cultivars**. White clover cultivars are grouped into small-, medium- and large-leaved cultivars. In monocultures, differences in herbage production among cultivars of white clover have been observed (Caradus, 1986) and these were attributed to leaf size and petiole length (reviewed by Harris, 1987). Others have grouped white clover populations into a summer active group, a winter active group and a group which shows autumn activities (Jahufer *et al.*, 1994). They further noted that morphological traits viz. stolon density, stolon branching, plant spread, plant height, stolon thickness and leaf length had a strong correlation to herbage yield over seven seasons. Differences in herbage production in response to defoliation among perennial ryegrass cultivars (Fulkerson *et al.*, 1994) have also been observed.

In mixtures, low yielding perennial ryegrass cultivars resulted in the highest proportion of white clover (Frame, 1990), and the reverse is also true (Widdup and Turner, 1983). Under grazing, cultivars of white clover differing in leaf-size have differential persistence, defined as the ability to maintain density (Sheath and Hay, 1989). Thus, Caradus and Chapman (1991) found that under frequent hard grazing Tahora, a low growing, small-leaved cultivar, was more persistent than Kopu, a tall, medium large-leaved high producing cultivar. The reverse is also true under infrequent lenient grazing. Tahora also produces branches earlier, higher node production rates, smaller leaves, shorter petioles, thinner and longer stolons than Kopu, and a lower proportion of nodes with flowers.

In mixtures, cultivars of white clover differ in their ability to absorb soil nitrogen and phosphorus (Brock and Hoglund, 1974; Goodman and Collison, 1986), eg. the larger-leaved cultivar Olwen is able to absorb more nitrogen from the soil and possibly compete more strongly with perennial ryegrass in the presence of high nitrogen in the soil, than the smaller-leaved cultivar S 184 (Goodman and Collison, 1986). Eltilib and Ledgard (1988) found that in mixtures with Ellet perennial ryegrass, Kopu white clover (a large-leaved cultivar) absorbed a similar amount of nitrogen from the soil as Huia (a medium-leaved cultivar), but Kopu produced more DM (15%) and fixed more nitrogen (35%) than Huia. The reasons for these variations are not clear, and little research seems to be directed towards this end.

Caradus *et al.* (1991) evaluated 158 different cultivars and breeding lines of white clover in mixed swards with perennial ryegrass cv. Grasslands Nui for persistence under grazing. The swards were grazed by sheep to a height of 1-2 cm for 1-3 days at intervals of 24 days in spring and 60 days in winter, 6-10 times per year. They found that over four years Huia and Irrigation gave the highest clover content, followed by the other group, i.e. Haifa, Olwen, and Pitau, and the third group which includes Kopu and the fourth group viz. Siral and Tahora (Table 2.4).

The abundance of a cultivar in the swards will depend on its morphological and physiological traits (Grime, 1979). In addition there appears to be trade off between leaf size, clover content and stolon density. As shown in Table 2.4, the larger the leaf-size, the higher was clover content (shoot DM) but the stolon density was lower. In white clover in the presence of competition and defoliation, genotypic traits that are commonly regarded as conferring high persistence and possibly competitive ability are: high stolon density, continued stolon growth (Archer and Robinson, 1989) as the original tap root is short-lived (Jones, 1980), high stolon branching, and a high stolon:total shoot ratio (a low harvest index) (Caradus and Chapman, 1991). Highly stoloniferous cultivars with high levels of stored reserves overwinter better and have more persistence (Harris *et al.,* 1983; Gramshaw *et al.,* 1989). This is because of higher number of growing points in highly stoloniferous cultivars and higher amounts of non-structural carbohydrate reserves for regrowth than less stoloniferous cultivars.

**Table 2.4.** Leaf size, visual clover content score, stolon growing point density, and number of flower heads relative to Huia (Score 1) of some cultivars of white clover. (After Caradus *et al.*, 1991).

| Cultivars | Leaf-size | Clover content | Stolon density | Flower number |
|---|---|---|---|---|
| Huia | 1.0 | 1.0 | 1.0 | 1.0 |
| Kopu | 1.30-1.31 | 0.96-1.11 | 0.48-0.71 | 0.80-1.55 |
| Pitau | 1.24-1.55 | 0.90-1.35 | 0.73-0.89 | 0.54-0.69 |
| Tahora | 0.56-0.77 | 0.69-0.91 | 0.87-1.51 | 1.34-1.70 |
| Haifa | 0.89 | 0.98 | 0.64 | 0.88 |
| Irrigation | 0.97 | 1.04 | 1.11 | 1.98 |
| Olwen | 1.15 | 1.0 | 0.49 | 0.83 |
| Siral | 1.10 | 0.71 | 0.49 | 0.39 |

In cultivars of white clover, the number of growing points and the amount of organic reserves in stolons available to initiate regrowth following defoliation have been held to account for the success of plants in the sward. At a later stage, the numbers of leaves per apex and leaf dry weight determine the yield of white clover (Haycock, 1981). Varietal difference in yield is determined by stolon density (amount of reserves) and the number of growing points. For example Rhodes and Evans (1993) have shown that the success of white clover in over wintering depends on the amount of reserves (abundance of stolons) and number of growing points. Any factors that reduce their abundance prior to winter will generate damaging effects on white clover in spring.

Larger pools of organic reserves and the availability of more abundant growing points make regrowth following defoliation faster which might lead to a better competitive ability (Section 2.3). Large- and small-leaved cultivars of white clover, in mixtures with perennial ryegrass generally yield similar above ground biomass (Rhodes, 1981), however larger-leaved cultivars have lower stolon biomass, with the consequence of less stolons and organic reserves available during overwintering and in early spring to initiate new regrowth (Tesar and Ahlgren, 1950).

In summary, cultivars of white clover differ in their competitive ability in mixture with perennial ryegrass. This difference is affected by stolon length (amount of organic reserves) and number of growing points to initiate growth after defoliation. Low-growing small-leaved cultivars of white clover have longer stolons and more biomass that escape grazing, and thus have higher ability to regrow and are more persistent under hard grazing than tall-growing large leaved cultivars. Smaller-leaved white clover cultivars also produce branches earlier, have higher node production rates, shorter petioles, and thinner and longer stolons than larger-leaved cultivar. Cultivars of white clover differ in their ability to absorb nitrogen in the presence of perennial ryegrass, but the mechanisms of this difference are not known.

**Effects of frequency, intensity and timing of defoliation on competition between white clover and perennial ryegrass**. Harris (1987) stressed the importance of frequency, intensity and timing of defoliation in affecting the outcome of competition between white clover and perennial ryegress and the relative proportion of white clover in the swards. In general, small-leaved, low growing cultivars of white clover were more competitive in frequent grazing or set stocking compared to larger-leaved, tall and erect cultivars, due to higher stolon density and higher numbers of leaves that escape grazing or defoliation.

Medium and large-leaved cultivars became more competitive by less frequent defoliation than small-leaved cultivars (Wilman and Asiegbu, 1982a). Small-leaved and more stoloniferous cultivars e.g. Tahora were generally more tolerant to set stocking, or to frequent or hard cutting (Wilman and Asiegbu, 1982b) compared to large-leaved cultivars (Brock *et al.*, 1988). Defoliation reduced nodule number (Marriott and Haystead, 1990), nitrogen fixation and nitrogenase-linked respiration, which declined by 80% within 3 hours and by 100% within 24 hours (Gordon *et al.*, 1990). If the interval between defoliation was sufficiently long, white clover began to fix nitrogen at a very low level 3 days later and return to control level after a further 15 days. Hence, when the soil nitrogen is low, a time lag in the growth of white clover exists until nitrogen from fixation is sufficient for growth (Brock and Hoglund, 1974).

In perennial ryegrass, defoliation especially if severe and frequent, reduced reserves in the stubble (Davies, 1966; Pilbeam *et al.*, 1986), and reduced root growth (Evans, 1973, 1978), which are the major causes of slow growth of the swards after defoliation (Dovrat *et al.*, 1980).

Most studies on the effects of grazing and/or defoliation have examined only the above-ground components, overlooking the impacts on below-ground components (Rhodes, 1981; Trlica and Rittenhouse, 1993). Evans (1973) provided evidence that defoliation caused a complete cessation of root elongation in grasses, including perennial ryegrass, while in white clover it reduced root elongation to the level of approximately five percent of that of undefoliated plants.

Leaf appearance rates are similar in grasses and clover, but slower in clover under tall grass due to shading than short grass swards (Parsons *et al.*, 1991), suggesting the effect of intensity of interspecific competition. Under grazing, clover leaves escape by expanding close to the soil surface (morphological plasticity). Hence small-leaved, short petioles and more stoloniferous cultivars viz. Tahora were generally able to maintain biomass better under set stocking than under rotational grazing, compared to large-leaved cultivars (Brock *et al.*, 1988).

Maintaining sward height of 6 cm in perennial ryegrass cv. S23 and white clover cv. Huia when continuously stocked by sheep led to optimum performance in terms of animal live weight gain and clover content (Orr *et al.*, 1990). While frequent defoliation in suitable environments increases the contribution of white clover (Hill, 1991) medium to large-leaved cultivars benefit more from less frequent defoliation than small-leaved cultivars (Wilman and Asiegbu, 1982a).

If swards are cut at 4-5 cm height, increasing the harvest interval from 3-4 to 8-12 weeks increases competitive ability and yield of white clover, and reduces the grass tiller number (Wilman and Asiegbu, 1982a). Increasing height of cutting to 10 cm almost eliminates white clover over 3 years (Acuna and Wilman, 1993) and leads to 50% reduction of herbage yield compared to close cutting, indicating higher competitive ability of perennial ryegrass with lenient defoliation. The decline in white clover growth resulted in lower nitrogen contribution of white clover leading to reduction in herbage yields.

In ryegrass higher cutting height (more than 4 cm) results in higher residual green material and higher water soluble carbohydrates and increased regrowth, but mutual shading reduces net canopy photosynthesis and yield (King *et al.*, 1979). In white clover, the effects of increasing harvest interval are to increase stolon length per unit area of ground, stolon diameter, petiole length, number of leaves and weight per leaf, while reducing leaf emergence (Wilman and Asiegbu, 1982b).

In summary, it can be concluded that frequency, intensity and timing of defoliation affect the outcome of competition between white clover and perennial ryegrass. Small-leaved, low growing cultivars are more tolerant to frequent defoliation than medium large-leaved cultivars due to higher stolon density and higher number of leaves that escape grazing or defoliation. Thus smaller-leaved white clover cultivars have higher competitive ability in such conditions. Defoliation causes reduction in nodule number, nitrogen fixing ability and reduced root elongation. In perennial ryegrass higher cutting height results in higher regrowth due to higher residual material and higher water soluble carbohydrates than lower cutting height.

### 2.2.3 Allelopathy

Allelopathy is caused by chemical compounds liberated by one plant and affecting the growth of neighbouring plants. McGowan (1990) suspected an allelopathic effect when it was observed that the growth of white clover was less on soil taken from beneath vegetation with several years of improved pasture, compared to that on adjacent soil devoid of vegetation, while perennial ryegrass growth was not affected. However, others attributed such declines to increasing soil acidity, low available phosphorus and plant diseases (Burnett *et al.* 1994).

Perennial ryegrass may be infected by clavicipitaceous fungal endophytes (Clay, 1988; Sutherland and Hoglund, 1989). Toxins that were subsequently produced might cause a decline in ryegrass palatability which in turn leads to selective grazing of white clover (Sutherland and Hoglund, 1989), an increased competitive ability of perennial ryegrass through increase in relative growth rates and net assimilation rate (Marks and Clay, 1990), and an increase in survival and resistance to herbivory (Clay, 1988; Marks and Clay, 1990; Hill *et al.*, 1991). Sutherland and Hoglund (1989) also provided evidence that fewer white clover seedlings survived and white clover growth was less in mixtures with endophyte-infected compared to endophyte-free perennial ryegrass. This led to less nitrogen fixation by white clover and lower growth of wheat plants on soils previously occupied by mixtures of endophyte-infected perennial ryegrass and white clover.

It can be concluded that endophyte-infected perennial ryegrass has higher competitive ability in mixture with white clover compared than non-infected perennial ryegrass.

## 2.3 Non-Structural Carbohydrate Reserves in White Clover and Perennial Ryegrass

Non-structural carbohydrates (NSC) in plants are consumed in respiration and provide energy and carbon skeletons for the construction of tissues, and are the major forms of carbon for reserve storage in vegetative parts of plants (Trlica, 1977; Chiariello *et al.*, 1989; Chapin *et al.*, 1990). Chapin *et al.* (1990) defined storage as resources that build up in the plant that can be mobilized in the future to support biosynthesis for growth or other plant functions. The major storage organs in white clover are stolons and roots, and in perennial ryegrass the lower stem (stubbles) and roots (May, 1960).

Non-structural carbohydrate storage and current assimilates play important roles in plant regrowth after defoliation (Section 2.3.4), hence are important in white clover and perennial ryegrass mixtures which are consistently subjected to defoliation or grazing. Surprisingly, little information is available in the literature on the effects of these reserves on the regrowth and survival of the plants in competitive situations. The reasons might lie in the notion that in mixtures the responses of plants in terms of carbohydrate partitioning and remobilization for regrowth are similar to those in monocultures (Robson *et al.*, 1989). In addition, the analyses of NSC are laborious and time consuming. Hence, the suggestions that NSC reserves affect the competitive ability of mixtures after defoliation have never been proven by experimentation. In the following discussions, aspects of NSC in herbage grasses and legumes will be discussed, with emphasis on their roles in regrowth of perennial ryegrass and white clover after defoliation.

### 2.3.1 Forms and Methods of Analysis of Non-structural Carbohydrates

#### (a) Forms

Various forms of NSC are found in the vegetative parts of grasses, including monosaccharides (glucose, fructose), disaccharides (sucrose, maltose), polysaccharides (glucosans, starches) and fructans (fructose polymers) (McIlroy, 1967; Smith, 1973b; White, 1973). Fructans are generally accumulated by cool-season grasses while sugar and starch accumulate in grasses adapted to warmer climates. In white clover sugars and starch are the major reserve carbohydrates (Baur-Höch *et al.* 1990), while in perennial ryegrass glucose, fructose, sucrose and fructans are the main NSC (Prud'homme *et al.*, 1992).

In many articles and reviews about NSC in herbage plants, different terms for carbohydrates viz. reducing sugars, non-reducing sugars, sucrose, ethanol soluble sugars, free sugars, labile sugars, starch, fructans, total alcohol soluble carbohydrates (Noble and Lowe, 1974), water soluble carbohydrates (Ross *et al.*, 1978; King *et al.*, 1979; Humphreys, 1989a,b; Griffith, 1992), total soluble carbohydrates (Davidson, 1969b; Hunter *et al.,* 1970), total NSC, total available carbohydrates (Weinmann, 1961; Davidson, 1969b), etc., are used depending on the method(s) used for their extraction and analysis. Different analyses and different extractants are required to measure specific sugars and the different forms of carbohydrate. It should be noted that no extraction technique perfectly extracts any particular carbohydrate or discriminates between different carbohydrates and Richards (1986) pointed out that the choice of extraction and analysis techniques could cause large differences not only in the amount of carbon measured but also in the amounts of the different forms of carbohydrate. In the following discussion the procedures for extraction and the choice of techniques to quantify carbohydrates that will be used later in the experiments, are presented.

Reducing sugars (glucose and fructose) have ketone or aldehyde groups on their molecules. These groups reduce $Cu^{+2}$ to $Cu^{+1}$ in Benedict's or Fehling's solution. In non-reducing sugars (sucrose) or related compounds, these groups have been changed to a non-reducing alcohol or are blocked by combining with other groups from other molecules (Salisbury and Ross, 1978). Free sugars of plants consist of monosaccharides such as glucose and fructose; disaccharides such as sucrose, oligosaccharides and the smaller (lower molecular weight) fructans (Chiariello *et al.,* 1989). These classes together with most high molecular weight fructans are soluble in hot water and in some articles are called water soluble carbohydrates (WSC). Total non-structural carbohydrates (TNC) consist of all of the above classes of sugars together with starch (amylose and amylopectin) and any other readily metabolized carbohydrates that are available for plant function. They are also called total soluble carbohydrates or total available carbohydrates (TAC) (White, 1973). Structural carbohydrates, hemicellulose (pentosans and hexosans) and cellulose are not considered to provide significant reserves available for metabolism (Weinmann, 1948).

### (b) Methods of analysis

Various procedures are reported in the literature to extract carbohydrates in grasses and legumes. Generally, the methods of analysis involves three steps: (1) preserving of samples; (2) extraction from the plant and (3) laboratory analysis. Smith (1973a) examined the effects of drying (freeze and heat drying) and boiling with 80% ethanol to preserve samples before laboratory analysis for NSC. The main purpose of preservation is to prevent the breakdown of carbohydrates by respiratory enzymes during periods between sampling and laboratory analysis. Freeze drying and drying at 100 $^{o}$C for 24 hours followed by drying at 70 $^{o}$C until constant weight is achieved, kills enzyme activities. However, changes in NSC can occur during storage prior to processing so samples should be processed as soon as possible. Boiling fresh samples in 80% ethanol kills the enzymes and results in least change during storage. The time between sampling and dropping samples into boiling ethanol should be kept as short as possible.

Laboratory analysis used to quantify NSC also includes simple extraction by cold and/or hot water (Ross *et al.*, 1978) or the use of acid extractant to convert carbohydrate to simple sugars (monomers). The monomers are subsequently quantified by the phenol test (Dubois *et al.*, 1956) and reading in the photometer or other methods. Chromatography (paper, gas, liquid) have also been used to quantify different form of carbohydrates.

Monosaccharides and disaccharides dissolve in boiling 80% ethanol and this procedure in combination with assays specific for mono- and disaccharides is used to measure these sugars in tissues containing starch or fructan. Fructans and starch are quantified by various methods. Fructans dissolve in boiling water, hence extraction in boiling water has been used to quantify water soluble carbohydrates in perennial ryegrass which consist mostly of fructan. Starch, the main form of reserve polysaccharide in white clover, does not dissolve completely in boiling water, and various methods are used to extract and quantify starch.

Rose *et al.* (1991) compared enzymatic methods with a modified method of perchloric acid extraction without KI precipitation, and the AOAC standard method of perchloric acid extraction with KI precipitation to quantify starch (AOAC, 1984). They found that enzymatic methods using commercial enzyme preparations without additional purification, gave slightly higher values than the standard method due to contamination by other enzymes such as cellulase and hemicellulase. Elaborate and laborious procedures are required for purification. Perchloric acid methods present problems with the extraction of pectic substances to form glucose. This can be reduced by precipitating starch fragments with iodine (Pucher *et al.*, 1948). However, perchloric acid can hydrolyse starch fragments to glucose which will not precipitate with iodine. Hydrolysis can be decreased by reducing the exposure time of the samples to the acid. This is done by quickly drip-percolating perchloric acid through the tissue sample and immediately diluting the eluate in water. Following precipitation of starch with iodine the anthrone reagent or the phenol test (Dubois *et al.*, 1956) is used to measure the glucose which is produced by the hydrolysis of starch in the concentrated acid of these procedures. Others have used weak acid (for example Bowen and Pate, 1993) to break starch into simple sugars followed by the phenol test (Dubois *et al.*, 1956).

From the foregoing it will be realized that there is no simple or single method for the extraction and analysis of carbohydrates; that the procedures selected are a compromise between specificity and ease of assay at the one hand and discrimination between different classes of carbohydrate and the number of steps acceptable at the other. Thus the actual method(s) used will depend upon the types of carbohydrates present in the tissue and the resources available to measure them. Also, the results will reflect the methods used more than the presumed chemical components, so except for starch, classes of carbohydrate are referred to in this thesis on the basis of the method of extraction and analyses. Starch is extracted by a specific method, so is referred to as starch.

The quantities of NSC are usually reported as a percentage or $mgg^{-1}$ of DM in the literature. Richards (1993) argued that the concentration of NSC reserves may appear to decrease due to the increase in growth of storage organs, hence the total content (mg plant$^{-1}$ or mg.unit area$^{-1}$) should also be reported. The total content of NSC in the plant is obtained by multiplying the concentration by plant mass. The disadvantages of these laboratory techniques in measuring the NSC in competitive situations are the uncertainty in the amounts that will actually be available for the formation of new photosynthetic materials, some being used in maintenance respiration. Hence, others have measured the amount of etiolated regrowth to measure the NSC pool actually available for regrowth (McKendrick and Sharp, 1970; Sheard, 1973; Richards and Caldwell, 1985; Rechel 1993). In such experiments, the plants were usually put in the dark in controlled environments, or the plants were covered by inverted black plastic pots which were painted white to prevent the increase of temperature around the plants. The regrowths were then cut regularly until regrowth ceased. Using this technique, Richards and Caldwell (1985), for example, were able to show that the more grazing tolerant *Agropyron desertorium* consistently produced more growth in the absence of photosynthesis than *A. spicatum*.

### 2.3.2 Partitioning and Storage of Non-structural Carbohydrates and Other Reserves in White Clover and Perennial Ryegrass During Vegetative Growth

It has been generally accepted that the surpluses of current photosynthates in excess of that used for maintenance and growth, are accumulated in storage organs and reused to support future activities (McIlroy, 1967).

In white clover, Woledge (1988) and Chapman *et al.* (1990a) found that the newly formed leaves start becoming net exporters of carbohydrates at stage 0.6 in Carlson's scale, that is, the stage when leaflets are about 10% unfolded (Carlson, 1966) (Appendix 2.1), or when the leaves have reached about 35% of their maximum surface area and 50% of their maximum weight (Chapman *et al.*, 1990a), while in perennial ryegrass the newly formed leaves become net exporters of photosynthates when they have reached two thirds of their mature size (Robson *et al.*, 1989).

During the day the rates of photosynthesis exceed the rate of transport of carbohydrates to various sinks. Some of these excess carbohydrates are temporarily accumulated in the leaves as starch or fructan which, during the night periods, are transformed back to sucrose and eventually translocated to various sinks throughout the plant. Transport of NSC occurs through phloem from actively photosynthesizing leaves to growing parts nearby to form new leaves and to supply storage organs, or in the absence of current photosynthesis, from storage organs to adjacent growing points (Wardlaw, 1990).

It has been suggested that reserve storage competes with growth for a share of current assimilates (Millard, 1988; Chapin *et al.,* 1990). These reserves, will be used for future growth, or shed as litter, as might well be the case in a productive habitat with little disturbance or stress (Section 2.1.). In pasture, where plants are regularly subjected to disturbance and stress, NSC might be depleted to levels that impair regrowth (May, 1960). Accordingly only a small amount of NSC is shed as litter.

Others have suggested that the formation of the tissues of storage organs, as distinct from reserve storage filling, also competes with growth for resources (Steinlein *et al.,* 1993). Chapin *et al.* (1990) further divide storage into three classes : (i) accumulation which occurs when the provision of resources exceeds demand for growth and maintenance (Millard, 1988); (ii) reserve formation which is the synthesis of compounds in specialized organs, from resources which otherwise would be used for growth (the formation of such a reserve competes with growth); (iii) recycling which is the reutilization of compounds from some parts of the plant to be used to support subsequent growth in other parts. Resources used for regrowth were suggested to come from any of the three classes of storage mentioned above, which emphasizes the importance of studying the role of reserve storage in regrowth from the whole plant context (Sheard, 1973; Rechel, 1993).

Many factors affect accumulation of carbohydrates in storage organs of plants. These include plant factors i.e. the species of plant (Mackenzie and Wylam, 1957), ecotypes (Boller and Nösberger, 1983), the stage of growth of the plant (Nowakowski, 1969), environmental factors (Jenner, 1982) i.e. season (Mackenzie and Wylam, 1957), nutrients (Nowakowski, 1969), water and temperature (May, 1960; Weinmann, 1961), temperature and photoperiod (Boller and Nösberger, 1983; Solhaug, 1991), light (Ryle and Powell, 1976) and soil (Davidson, 1969b).

Studies on the distribution of $^{14}C$ photosynthates in white clover indicate that the apical buds and growing leaves receive their supply of carbon from the top leaves, the middle of the stem receives its carbon from centrally placed leaves, and lower stolons and branches obtain their photo assimilated carbon from older leaves (Ryle *et al.*, 1981). Danckwerts and Gordon (1989) using $^{14}C$ found in undefoliated white clover that within 24 hours most of the $^{14}C$ fixed in the fourth fully expanded leaf on the main stolon was deposited in meristems, roots, stolons and leaves, and 40 per cent was lost through respiration. Long term storage in roots and stolons accounted for 10 per cent of the assimilated carbohydrate which was later incorporated in regrowth. This development is evidently under genetic and environmental control (Wardlaw, 1990).

The levels of NSC differ in different parts of the plant. In grasses, soluble sugar (after alcohol extraction) was higher in stubble (47% of DM) than in leaf (27% of DM) and was low in the roots (less than 11% of DM), with fluctuations in concentration mostly occurring in the stubble (Vartha and Bailey, 1980). In white clover, it has been shown that cultivars (Harris *et al.*, 1983; Kang and Brink, 1995), selections within cultivars (Chapman *et al.*, 1992) and cultivars originating from different regions (Boller and Nösberger, 1983) have different carbon allocation, and different levels of reserves in storage organs.

Harris *et al.* (1983) measured WSC and starch in stolon and roots of monocultures of white clover cultivars S184 (small-leaved cultivar), S100 (medium-leaved cultivar), Menna (medium-leaved) and Olwen (medium large-leaved cultivar) as affected by cutting frequency and nitrogen application. Nitrogen were applied at 20 kg/ha (low rate) and 120 kg/ha (high rate) and the cultivars were subjected to frequently cut (five times) and infrequent cut (three times) at 5 cm height (except at the final cut when the cutting height was 2.5 cm) during 24 weeks growth period. Samples were taken during winter. No significant main effects of cutting frequency, nitrogen, and cultivar x cutting frequency and cultivar x nitrogen interactions on WSC and starch percentages were found. Stolons of S184 had higher WSC and starch percentages at the beginning of and over winter compared to S100, Menna and Olwen, resulting in better winter survival of S184 than the other cultivars (Table 2.5). In interpreting the results, the stage of growth, frequency and intensity of defoliation,

**Table 2.5.** Main effects of cultivars of white clover on dry weight of stolons and roots and their water soluble carbohydrate and starch contents in monoculture (means over three cuts). (Adapted from Harris *et al.*, 1983).

| Organ/Carbohydrates | S100 | S184 | Menna | Olwen |
|---|---|---|---|---|
| Stolon | | | | |
| DM (kg $ha^{-1}$) | 562.00 | 970.00 | 548.00 | 293.00 |
| WSC (%)** | 11.45 | 13.56 | 11.82 | 11.28 |
| WSC (kg $ha^{-1}$)* | 64.35 | 131.53 | 64.77 | 33.05 |
| Starch (%)*** | 3.05 | 6.99 | 3.79 | 4.33 |
| Root | | | | |
| DM (kg $ha^{-1}$) | 346.00 | 337.00 | 353.00 | 331.00 |
| WSC (%) | 8.09 | 8.16 | 8.19 | 8.47 |
| WSC (kg $ha^{-1}$)* | 27.99 | 27.50 | 28.91 | 28.04 |

*Concentration (%) multiplied by biomass (kg $ha^{-1}$). **boiling with water for 2 hours, ***perchloric acid-iodine precipitation methods

and also the environmental conditions should be taken into account. Hence, the results of Harris *et al.* (1983) can be contrasted to those of Kang and Brink (1995) who found in a pot experiment in a glasshouse (temperature 16-36 $^{o}C$) that stolons and roots of larger-leaved white clover Osceola contained higher concentrations of NSC (after extraction with NaOH for 90 minutes) regardless of the defoliation interval compared to medium-leaved cultivar Huia or small-leaved cultivar Aberystwyth S184.

It can be concluded that the amount of NSC (water soluble carbohydrates and starch) in storage organs depend on cultivars and environmental conditions. Larger-leaved cultivar eg. Osceola had higher NSC content in warmer temperature regimes (16-36 $^{o}$C) compared to smaller-leaved cultivars, however the reverse was true in lower temperature regimes. The extractant used in the measurements may contribute to the differences of NSC content; for example, boiling for two hours and perchloric acid-iodine precipitation methods in Harris *et al.*, 1993, and extraction with NaOH for 90 minutes in the work of Kang and Brink, 1995 to measure NSC in the cultivars.

Carbohydrate reserves increase in line with the increase in rates of growth early in development, decrease during the generative phase, and increase again during maturation. Carbohydrate reserves decrease in winter and early spring due to maintenance respiration and use in regrowth, with replenishment coinciding with the period of active shoot growth (Weinmann, 1961), and in the period soon after seed formation (Hassan and Krueger, 1980).

Wilman and Riley (1993) analysed several grassland species including *Lolium perenne* cv. Melle and *Trifolium repens* cv. Grasslands Huia for WSC and nutrient contents. The plants were grown in monoculture in 8 cm diameter pots for 12 weeks and cut 1.0 cm above ground level. They found that the leaves and stems of perennial ryegrass contained higher concentrations of WSC (after boiling with water for two hours) than white clover (Table 2.6).

Davidson (1969b) studied carbohydrate concentrations in roots of perennial ryegrass and white clover in monocultures. The percentage of total soluble carbohydrates (extracted in boiling water for two hours) was higher in white clover than in perennial ryegrass, but the reverse was true for total available carbohydrates (extracted for two hours with 0.2 N sulphuric acid). The higher total available carbohydrates might be due in part by the extraction of pectic materials by 0.2 N sulphuric acids. Because root dry weight of perennial ryegrass was larger than white clover (Davidson, 1969a), the total content of available carbohydrates was larger in perennial ryegrass than in clover.

**Table 2.6** The concentration (%) in DM of WSC in *Lolium perenne* cv. Melle and *Trifolium repens* cv. Grasslands Huia (adapted from Wilman and Riley, 1993)

| Species and parts of plants | WSC (%) |
|---|---|
| Perennial ryegrass | |
| Leaf* | 10.5 |
| Stem** | 25.7 |
| Total | 15.0 |
| White clover | |
| Leaf* | 6.1 |
| Stem@ | 11.0 |
| Total | 8.3 |

* leaf = lamina, **stem = leaf sheath, unemerged leaf, true stem and inflorescence,
@ stem = petiole and small amount of stolon

Carbon partitioning has been shown to be sensitive to environmental changes. Plants grown in low light regimes export relatively more assimilate to the growing leaves at the terminal meristem than to their roots and tillers compared with the equivalent plants in a high light regime (Ryle and Powell, 1976). Shading reduced the rates of growth, the number of tillers and the ability to replace leaf area in the event of grazing (Pierson *et al.*, 1990).

High soil nutrient levels encourage shoot growth. High nutrient levels if combined with high temperatures, and frequent defoliation reduce stolon DM and carbohydrate reserves (Chapman *et al.*, 1992). Low nutrient status often results in the accumulation of reserve carbohydrates in the stem and leaves (Wardlaw, 1990), and roots (Henry and Raper, 1991; Rufty *et al.*, 1992), which indicates that growth is retarded more than photosynthesis.

Under drought conditions, the level of water-soluble carbohydrates in the leaves of some species increases. This accumulation is sometimes regarded as osmoregulation in the leaves as response to water stress, but it results from a reduction of growth and carbohydrate utilization prior to a reduction in photosynthesis (Munns, 1988).

In summary, leaves of white clover start exporting photosynthates when the leaflets are about 10% unfolded, while in perennial ryegrass exporting begins when the leaves have reached two thirds of their mature size. The NSC are stored in the stolons and roots in white clover and the stubble and roots of perennial ryegrass. The levels of NSC stored in storage organs depend on cultivars, environmental factors and management factors. In favourable high nitrogen conditions perennial ryegrass contains higher concentration of NSC in their storage organs than white clover. When environmental conditions limit growth, accumulation of NSC in plant organs occurs. With infrequent and long interval defoliation, medium large-leaved white clover cultivars contain larger concentrations of NSC in the stolons than smaller-leaved cultivars.

### 2.3.3 The Effects of Defoliation, Nitrogen and Time of Year on Partitioning and Remobilization of Non-Structural Carbohydrates in White Clover and Perennial Ryegrass

Pasture plants are normally subjected to regular defoliation or grazing and following defoliation, the green photosynthetic organs of the shoots regrow and carbon dioxide fixation from the air is resumed. During the first 1-6 days of regrowth after clipping or grazing, reserve carbohydrates are remobilized (Prud'homme *et al.*, 1992; Danckwerts, 1993) to meet the energy requirement for growth of new photosynthetic tissue and for maintenance respiration. Hence, following defoliation the NSC pools in the storage organs decrease (Davies, 1966; Chapin *et al.*, 1990; Gallagher *et al.*, 1994). Clipping causes a greater reduction in carbohydrate concentration compared with other factors viz. low light intensity, high air temperature, and nutrient treatments (addition or starvation) (Davies, 1966; Chapin *et al.*, 1990).

Reduction in NSC reserves caused by defoliation varies according to the species of plant, its growth habits, the intensity and frequency of defoliation, the time of the year, and nutrient and water stress. Remobilization of non-structural carbohyrates from the stolon is reported to be larger when the initial content is high, and the reduction to be greater in severely defoliated plants (Baur-Höch *et al.*, 1990). In the following discussion the effects of defoliation and its interaction with other factors on partitioning and remobilization of carbohydrates are discussed.

### (a) Intensity, frequency and timing of defoliation

Intensity, frequency and timing of defoliation affect NSC levels in plants. However, cultivars of white clover have been shown to differ in their response to those treatments. Thus, Kang and Brink (1995) found that after a 55 day establishment of white clover plants grown singly in 15 cm pots at a temperature range of 16-36 $^{o}$C, defoliation intervals of 7, 14, and 28 days for 28 days had small effects on the NSC concentration in stolons and roots. The larger-leaved cultivar (Osceola) was found to accumulate more NSC than the medium-leaved cultivar (Huia) and the small-leaved-cultivar (Aberystwyth S184). Their results can be contrasted with those of Harris *et al.* (1983) who measured NSC in monocultures of white clover in the field in Aberystwyth with much lower temperatures and with five cuts prior to measurement. It was found that larger-leaved cultivar Olwen had less concentration of NSC in the stolons but slightly higher in the roots, compared to smaller-leaved cultivar S184.

In white clover, Gallagher (1993) examined the accumulation of carbohydrates in stolons of white clover as affected by defoliation. Similar levels of reducing sugars were observed in the stolons of defoliated and non-defoliated plants. Starch levels in stolons of defoliated plants declined dramatically between the first and the sixth node from the apex; from node 7 onwards starch levels were similar to those in non-defoliated stolons. Danckwerts and Gordon (1989) found that partial defoliation of the plant led to more rapid regrowth compared to total defoliation, but the latter depleted more $^{14}C$ reserves from stolons and roots. Stolon reserves were preferentially utilized compared to root reserves.

King *et al.* (1979) examined the effects of the intensity and frequency of cutting on the hot water soluble carbohydrate concentration in the tiller base of perennial ryegrass. S23 perennial ryegrass was grown for 7 weeks followed by a 6-week period of cutting treatments (i.e. a combination of weekly and 3-weekly cuts and high and low cutting height). Measurements were made during 25 days of regrowth (Figure 2.2). At the start of measurements WSC in the stubble was higher following cutting to 4 cm compared to 2 cm cutting at the same cutting frequency. It was also higher following infrequent (3 weekly) than frequent (weekly) cuttings. During the first period of 1-4 days of regrowth, the concentration of WSC decreased. Replenishment then occurred and was faster in the swards cut to 4 cm ( i.e. in 1H and 3H). They found that higher cutting height and less frequent cutting leads to higher WSC reserves available for regrowth, leading to faster regrowth and replenishment of reserves, which is also due to higher residual photosynthetic area.

Davies (1966) imposed pre-treatments to perennial ryegrass which included high and low nitrogen levels, and cutting once, twice and four times over 8 weeks on the cultivar S24 perennial ryegrass grown in pots in a monoculture. At the end of the pretreatment period it was found that percentage of WSC was more dependent on cutting frequency than on nitrogen treatments. WSC in stubble decreased immediately after cutting, and showed no consistent rise until after about a month had elapsed, a trend which was also found by others (Volenec, 1986). Defoliation reduced WSC in grass (Mackenzie and Wylam, 1957), and with repeated cutting, the progressive exhaustion of reserves led to a decline in the rate of growth until a level was reached that could be sustained by current carbon accumulation (Pilbeam *et al.*, 1986). Christiansen and Svejcar (1988) found that in heavily grazed swards of *Bothriochloa caucasia* the roots had higher total WSC compared to the leniently grazed plants, but this might be caused by higher remobilization to the shoot or higher maintenance requirements for larger root systems in leniently grazed swards.

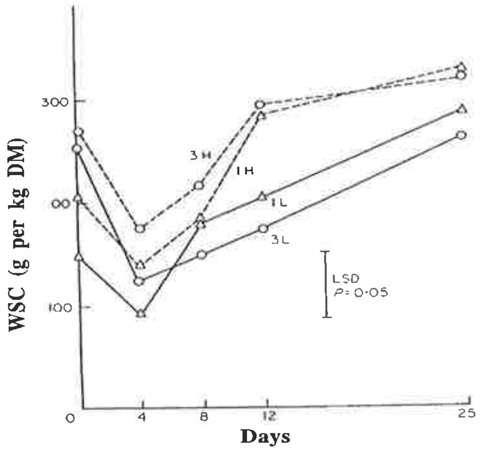



**Figure 2.2.** Effects of intensity and frequency of cutting on concentration of WSC in tiller bases during regrowth.
1L : weekly cut (6 cuts) to 2 cm prior to measurements
1H : weekly cut (6 cuts) to 4 cm prior to measurements
3L : 3-weekly cut (2 cuts) to 2 cm prior to measurements
3H : 3-weekly cut (2 cuts) to 4 cm prior to measurements. (After King *et al.*, 1979).

In summary, it can be concluded that NSC levels in the storage organs decline 1-6 days after cutting. NSC levels are affected by height and frequency of cutting. Infrequent and higher cutting height lead to higher NSC levels in the storage organ than frequent and higher cutting height. Higher cutting height and less frequent cutting also lead to higher residual leaf areas and higher current photosynthates which will lead to faster regrowth after defoliation.

### (b) Nitrogen

Defoliation and nitrogen application are two of the most important management options that affect the performance of perennial ryegrass-white clover mixtures. Chapman *et al.* (1992) studied the effects of these two factors in carbon utilization of clonal white clover. Nitrogen treatments were Nil (plant depend on nitrogen fixation for nitrogen supply), high-N (nitrogen applied at a rate to give maximum growth) and low-N (one third of rates in high-N). The first fully unfolded leaf on the main stolon (representing severe defoliation) and the first three unfolded leaves (representing lenient defoliation) were fed with $^{14}C$ and the export and translocation of carbon was measured three days later. Export of carbon in all three nitrogen treatments to young branches following severe defoliation was observed, but translocation to stolon and root tissues decreased. Total export of carbohydrates declined markedly in N-deficient plants (44% vs. 17% of $^{14}C$ assimilated for lenient vs. severe defoliation) whereas in N-sufficient plants small increases in the total export were observed (55.4% vs. 62%). In addition, in severely defoliated N-deficient plants, carbohydrate translocation to old branches ceased completely, but showed an increase in severely defoliated N-sufficient plants. This showed that plant response to a single factor may differ greatly to those of multiple factors.

Gonzalez *et al.* (1989) studied changes in stubble carbohydrate content during the regrowth of perennial ryegrass. After a 2 months period of growth, the total content of NSC in stubble represented 22% of dry matter and 60% of the soluble carbohydrate content consisted of fructans. The carbohydrate content dropped to 4.4% of DM 6 days after clipping and breakdown of polyfructans was observed up to the fourth day. Carbohydrate contents were rapidly restored during the following regrowth and after 28 days regrowth initial total soluble carbohydrate contents were reached. Nitrogen application in perennial ryegrass appeared to enhance NSC remobilization. They found in perennial ryegrass with non-limiting nitrogen supply that after defoliation 60 to 90% of soluble carbohydrates (i.e. glucose, fructose, sucrose, oligofructans and polyfructans) were remobilized during the first six days. Replenishment occurred afterwards and by day 28, levels of carbohydrate returned to levels prior to cutting (20% DM). Plants grown in a nitrogen starved medium had similar remobilization to those in N-sufficient plants, however N-starved plants maintained their fructose and glucose contents of 2% during the period of replenishment and had 2.3-fold more polyfructans than those on a non-limiting nitrogen medium.

It can be concluded that after defoliation NSC reserves were used for regrowth. However export of NSC from young regrowing leaves differed between white clover and perennial ryegrass and was affected by nitrogen nutrition and defoliation regimes. In white clover nitrogen application and defoliation increased export of NSC by young leaves but export declined markedly in nitrogen deficient plants. In perennial ryegrass nitrogen application and defoliation enhanced remobilization of NSC from storage organs, NSC level returned to that before defoliation after 28 days of regrowth in nitrogen sufficient plants, while nitrogen deficient plants had higher NSC reserves compared to nitrogen sufficient plants.

### (c) Season (Time of year)

Season (time of year) affects the partitioning and remobilization of carbohydrates in the plants. Hay *et al.* (1989) studied variation with season and node position in carbohydrate content of white clover stolons sampled from mixtures of *Lolium perenne* L. *cv.* Ellet and *Trifolium repens* L. *cv.* Grassland Kopu pastures. These mixtures were grazed infrequently (9 grazings year$^{-1}$) and severely (1-2 cm residual) by sheep. Starch content (Figure 2.3) was found to be at a minimum of 30 $\mu gmg^{-1}$ dry weight in spring (October), increasing during summer to a maximum of 146 $\mu gmg^{-1}$ in March, and gradually decreasing towards winter. Sucrose and hexose sugars were relatively constant throughout the year with a mean concentration of ca. 50 $\mu gmg^{-1}$ dry weight and 30 $\mu gmg^{-1}$ dry weight , respectively for most of the year. Node position was also found to affect the content of starch, sucrose and hexose sugars. Starch contents were highest at nodes 5 to 10 (nodes bearing true leaves numbers 10 and 11 from the apex), sucrose was lowest at the tip, progressively increased and reached asymptotically a maximum value at node number eight, while hexose content was highest at the node nearest to the tip and decreased gradually with increasing node number. From October to December the sucrose distributions were relatively constant,

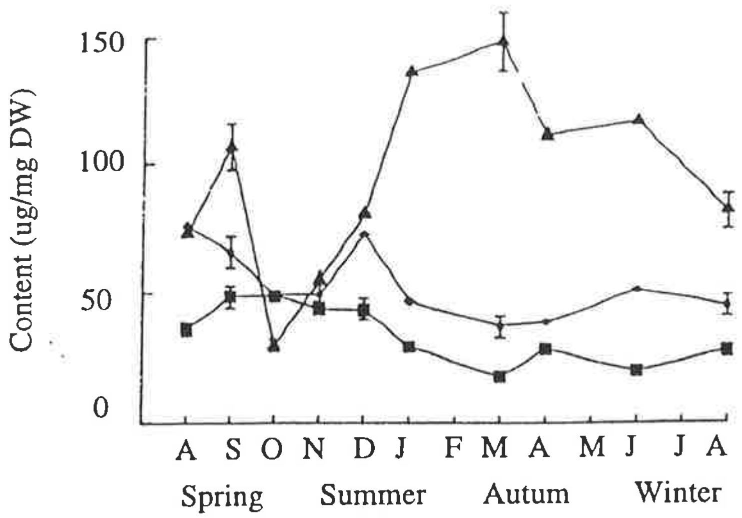



**Figure 2.3.** Seasonal variation in mean content of starch (▲), sucrose (●) and hexose sugars (■) in stolons of white clover. (S.E.s representative months are provided). (After Hay *et al.*, 1989).

but the distribution of starch and hexose sugars varied significantly. It was suggested that the nodes numbered greater than nine are chiefly involved in the transport of carbohydrates (as sugar) and have less activity in starch storage and growth (indicated by less meristematic activity and high hexose sugar content). The large variation in starch, sucrose, and hexose sugars in nodes three to eight (Figure 2.4) indicated the main branching activity occurs in that part of the plant during the spring and summer periods.

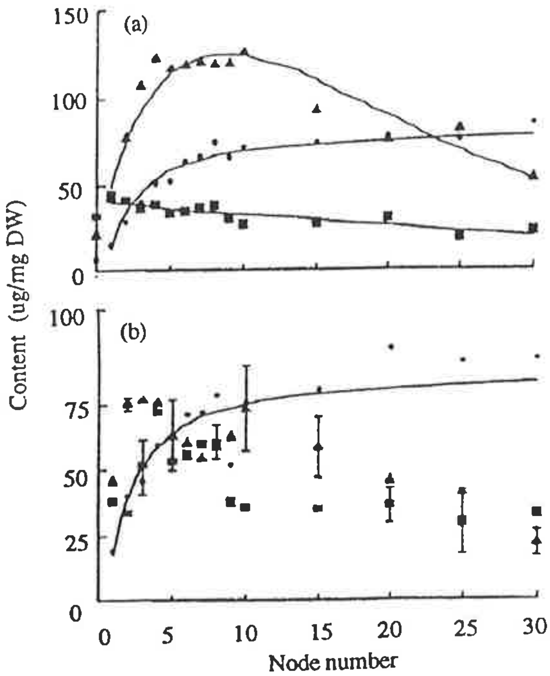



**Figure 2.4** Effect of stolon node position on content of starch (▲), sucrose (●) and hexose sugars (■). (a) Data meaned over all samplings, (b) For the October, November and December samplings. (S.E.s for starch and hexose sugars at representative nodes are shown). (After Hay *et al.*, 1989).

In summary, it can be concluded that NSC content in stolons varies with season and node position. NSC was lowest at the beginning of spring and highest in summer. NSC was lowest at the tips and progressively increased with increasing node number. Nodes number three to eight were suggested as the main branching activities, consequently samples of stolons were taken from these node positions to measure NSC in stolons in this project.

### 2.3.4 The Role of Non-Structural Carbohydrates and Other Reserves in Regrowth After Defoliation

Rapid recovery from defoliation is important if plants are to maintain their competitive position within the plant community on grazed pastures and rangelands (Richards, 1986). Radioactive carbon studies have proved, beyond doubt, that reserve carbohydrates are used for regrowth (Chung and Trlica, 1980). Starch degradation is related to the decline in the content of sucrose, glucose and fructans in young stolon parts and roots resulting from the absence of current assimilates. Before carbohydrate reserves are used sucrose is formed. In the active meristem, sucrose is transformed to glucose as a respiratory substrate. Glucose is later used for respiration which can be classed as:

(i) maintenance respiration associated with maintaining the existing biomass;

(ii) growth respiration which is associated with the synthesis of new tissues (Penning de Vries, 1975a,b).

Maintenance respiration is further divided into respiration to provide energy to:

(a) resynthesise substances that undergo renewal in metabolic processes, including enzymes, protein, RNA and membrane lipids,

(b) to maintain gradients of ions and metabolites,

(c) for processes involved in physical adaptation to changing or stressful environments. The proportion of the carbohydrate pool used in maintenance respiration is variable and increases with plant size and plant competition (Kozlowski, 1992).

While other reserves i.e. protein may also be used (Davidson and Milthorpe, 1966) to supply energy during regrowth, carbohydrates are regarded as the main source. Hence, many attempts have been made to relate the amount of NSC reserves to regrowth after defoliation. Gonzalez *et al.* (1989) and Ourry *et al.* (1988) provided evidence that the rate of regrowth of ryegrass is predominantly dependent on the size of mobile carbon and nitrogen reserves in the stubble. The amount of NSC reserves and the extent to which these reserves are utilized for regrowth and the support of physiological proceses in plants, determine their survival (Chiariello *et al.*, 1989). The efficiency of carbon allocation to meristems (from reserves and/or current photosynthates) affects tillering and subsequently, the ability of the plant to tolerate grazing (Richards and Caldwell, 1985). However, in interpreting such studies it is important to note the forms of carbohydrates that were measured. Before reserve carbohydrates in storage organs can be used for growth, starch or fructans have to be hydrolysed to sucrose for transport to growing tissues. Sucrose in turn is hydrolysed to glucose for use in respiration and growth. Hence, studies measuring WSC (which include the simple sugars glucose and sucrose) generally result in highly significant correlations with the amount of regrowth (Davies, 1966; Davidson, 1969b; King *et al.*, 1979; Hume, 1991; Fulkerson *et al.*, 1994), but where total NSC (which includes starch or fructans) was measured no correlation was identified (Davidson, 1969b; Leakey and Chancellor, 1977; Garnier and Vancaeyzeele, 1994).

Similarly Kigel (1980) found that a population of *Lolium multiflorum* Lam., which had higher level of WSC (cv. Liscate) in stubble, produced larger total leaf regrowth and higher rates of leaf elongation compared to another population with lower WSC (cv. S22). However, it is not clear whether faster initial regrowth will lead to subsequent higher competitive ability in mixtures. There is a paucity of research along this line.

It is concluded that the size of NSC reserves, current assimilates and the efficiency of utilization affect the regrowth after defoliation. In perennial ryegrass, regrowth generally has good correlation with water soluble carbohydrate content of stubble.

The importance of NSC reserve mobilization in regrowth after defoliation is difficult to establish because regrowth is also influenced by current assimilates fixed by residual leaf area or green stubble and stolons. Some workers conclude that current assimilates (hence residual leaf area) were more important than reserve carbohydrates in determining regrowth after defoliation (Ryle and Powell, 1975; Richards, 1986). Carbohydrate reserves outweigh current photosynthates as carbon source for regrowth production at least for a few days (Richards and Caldwell, 1985). After this short period, current photosynthate becomes more important than carbohydrate reserves. Current photosynthate continues preferentially allocated to the growing shoot until the demand of the shoot for photosynthate is satisfied (Culvenor *et al*, 1989b). Besides residual leaf area, stolons and cut petioles make a significant contribution to carbon uptake during the first few days of regrowth (Harris *et al.*, 1983; Davidson *et al.*, 1990), and are estimated to be 12-22% on a surface area basis as efficient as leaves in assimilating $CO_2$ (Davidson *et al.*, 1990). In continuously grazed swards of perennial ryegrass old stubble contributes less than five percent to canopy net photosynthesis (Parsons *et al.*, 1991). Chapman *et al.* (1991a,b) provided evidence that in white clover stored energy sources contribute significantly to the carbon economy of plants immediately following defoliation. They found that in the four days following defoliation 100 mg of reserve materials from the main stolon were mobilized to support growth and respiration of the whole plant, which was equivalent to 3% of the mean total weight of undefoliated plants, or the weight of three mature leaves. Large amounts of reserves were also probably mobilized from roots.

Recent studies have examined the level of NSC in relation to the number of growing points or meristematic activity in the regrowth after defoliation. It is concluded that different levels of NSC will only affect regrowth if there is no other limitation to the growth of the growing points (Busso *et al.* 1990; Pierson *et al.*, 1990). In addition, regrowth is affected by environmental conditions. In the event of environmental constraints for example drought (Busso *et al.* 1990); or shortage of nutrients (Henry and Raper, 1991; Qiu and Israel, 1994), growth is restricted earlier than photosynthesis, resulting in the accumulation of NSC in leaves and roots (Henry and Raper, 1991; Qiu and Israel, 1994). There is a burst of growth when these constraints are removed. The increase in NSC ceases upon the resupply of the nutrients.

The levels of reserves in storage organs are usually regarded as more than sufficient for the regrowth of plants (May, 1960; Weinmann, 1961) and plants use only a fraction of the stored carbohydrate for regrowth (Weinmann, 1961).

Baur-Höch *et al.* (1990) grown white clover for 37 days in a growth chamber including four weeks in different ambient $CO_2$ concentration ie. low (20 Pa $CO_2$) and high (100 Pa $CO_2$). The plants were then subjected to lenient (cutting unfolded leaves greater than 0.9 after Carlson's scale) and severe defoliation (cutting all leaves stages 0.4-0.9). It was found that NSC content in stolons was higher in plants that had been kept at 100 Pa $CO_2$ than when they had been kept at 20 Pa $CO_2$. The amount of reserves retranslocated for regrowth was found to be larger in plants with higher reserves.

There are disagreements as to the contribution of root reserves for regrowth of shoots. Some workers have suggested that plants used little of their carbohydrate root reserves (Ryle and Powell, 1975) and other non-carbohydrate components for shoot regrowth (Davies, 1965). They argued that, after defoliation, roots are under stress and root respiration alone represents a large energy burden for the plant, which amounts to 75 per cent of current net photosynthesis (Culvenor *et al.*, 1989 b). Other workers have provided proof that some reserves in the roots are used for shoot growth (Culvenor *et al.*, 1989a). They found that in subterranean clover, carbohydrates and nitrogen from roots and branches decreased during 5-9 day regrowth periods in severe defoliation treatments, while in the less severe defoliation treatments roots lost 25 per cent of their total NSC content compared to 65 per cent lost in the branches. However, roots were found to be the principal source of mobilized nitrogen.

Other workers found in *Lolium perenne* L. that, at least during the first day after defoliation, all nitrogen in the new leaves was retranslocated from organic nitrogen reserves in the roots and stubble (Ourry *et al.*, 1988), with roots mostly supplying the free amino acids (Lefevre *et al.*, 1991). Other workers observed a decrease in fresh root weight under severe defoliation (Caloin *et al.*, 1990), and Robson *et al.* (1989) suggested that reserves in roots were far less abundant compared to shoots, and in severe defoliation as the current supply of photosynthates ceased to flow, roots may exhibit a drastic reduction in their activities such as nutrient uptake, cell division and expansion, and cytokinin production.

In the legumes, nitrogen fixation represents a large energy burden to the plant, and in the roots the NSC are used for maintenance respiration to supply energy for nitrogen fixation and nutrient uptakes and transport. Defoliation has been shown to reduce nodule growth and maintenance respiration (by up to 60-70%) (Ryle *et al.*, 1986) and nitrogen fixation in white clover (Ryle *et al.*, 1985; Davidson *et al.*, 1990; Gordon *et al.*, 1990). Intensity of defoliation affects the reduction of nitrogen fixation. Lenient defoliation leads to less reduction compared to severe defoliation (Ryle *et al.*, 1989). The reserves are also used as a buffer to ensure the supply in maintaining a relatively steady fixation of $N_2$ throughout the day and night (Eckert and Raguse, 1980; Millholon and Williams, 1986).Mineral acquisition by the root system entails carbon substrate expenditure which includes maintenance respiration, respiration to support growth, loss of carbon due to root exudation and leakage, and loss incurred by symbionts. Part of this expenditure is used to support nutrient uptake processes and translocation of nutrients into the xylem (Clarkson, 1985; Kozlowski, 1992). Hence, in a competitive situation when depletion of most nutrients has occurred, large amounts of carbohydrates are probably required for respiration to supply energy for nutrient uptake.

In summary, NSC reserves from stolons and roots in white clover and stubble and roots from perennial ryegrass are used in regrowth, with reserves from stolons and stubble as the main sources. The NSC reserves are more important for regrowth production until a few days after severe defoliation, after which current photosyntahte become more important. High levels of NSC in the plants would lead to a higher ability to maintain activities and to rapid regrowth production. The number of growing points and photosynthetic activities of stolons in white clover and stubbles in perennial ryegrass also affect regrowth after defoliation. After defoliation shoot growth is favoured over root growth, but there is evidence that root reserves, i.e. nitrogen are also used in the regrowth of shoots.

Carbohydrate reserves in the roots decrease after defoliation which are used for maintenance respiration to provide energy for nitrogen fixation and other root activities.

## 2.4 The Effects of Non-structural Carbohydrate Reserves on Competitive Ability

Competitive ability for below ground growth resources of the plant is thought to be not only related to plant root features (such as root length, root hair density and degree of mycorrhyzal infection) but also to root carbohydrate levels and the flow of carbohydrates to roots (Berendse and Elberse, 1990). The residual photosynthetic area and the number of growing points available for growth are also thought to affect growth and competitive ability. However, few experiments have measured NSC in residual tissues (stubble and roots in perennial ryegrass and stolons and roots in white clover) and related these measurements to regrowth and competitive ability of perennial ryegrass and white clover in mixtures.

The amount of NSC available for regrowth following defoliation in perennial ryegrass depends on the amount of stubble present and the concentration in the stubble. Compared with lower cutting height, higher cutting height removes less stubble resulting in larger residual leaf areas and the residual biomass contains greater amount of reserves, coupled with more meristematic tissue resulting in higher rates of regrowth and thus perhaps competitive ability.

NSC levels in storage organs might affect the tolerance of white clover to high soil nitrogen levels. Kang and Brink (1995) show that larger-leaved white clover cultivars (cv. Osceola) have larger NSC concentrations than smaller-leaved cultivars (cv. S184). However, this differences are affected by environment conditions. Medium large-leaved cultivars of white clover have been shown to be better able to tolerate high soil nitrogen levels than smaller-leaved cultivars (Wilman and Asiegbu, 1982a; Eltilib and Ledgard, 1988). They have also been suggested to have higher competitive ability than smaller-leaved cultivars (Brock and Hoglund, 1974; Goodman and Collison, 1986; Eltilib and Ledgard, 1988) which might be due to their better growth and tolerance to high nitrogen level in the soil.

Richards (1986) suggested the possibility that carbohydrates previously stored in the root system are utilized in the synthesis of organic nitrogen compounds such as glutamine, asparagine, glutamate and aspartate in particular, and which are translocated to the shoot system during regrowth following defoliation. Others attribute the ability of the plant to survive grazing or defoliation to its ability to maintain adequate root growth and carbohydrate reserve levels in the roots after defoliation (Buwai and Trlica, 1977; Thom *et al.*, 1989).

In summary, the rate of regrowth after defoliation of white clover in mixtures with perennial ryegrass is thought to affect its competitive ability. The amount of regrowth is affected by NSC reserves in stolons and roots in white clover and stubble and roots in perennial ryegrass, the amount of current assimilates fixed by residual leaf area and the number of growing points. Higher cutting height will leave larger amount of stubble and reserve carbohydrates, higher amount leaf area, and greater number of growing ponts, which will increase the competitive ability of perennial ryegrass. In mixtures with perennial ryegrass, cultivars of white clover differ in their competitive ability in the presence of high soil nitrogen levels. Medium-large leaved cultivars have higher competitive ability than small-leaved cultivars in the presence of high soil nitrogen levels.

## 2.5 Conclusions and Objectives of Research

The potential of perennial ryegrass-white clover mixtures to optimize animal production has not been fully utilized due to the decline with time of white clover content from the swards. Such a decline has been attributed to lower competitive ability of white clover for above and especially below ground growth resources. Nitrogen application to boost the growth of companion grasses, and other management strategies without consideration of the requirement for growth of white clover growth, further enhance white clover demise from the swards. However, cultivars of white clover differ in their competitive ability and in mixtures with perennial ryegrass, especially in the presence of high levels of soil nitrogen. These variations have been attributed to variations in seasonal growth which is in part due to different response to temperature, morphological traits such as stolon density, stolon length, nodes number, number of leaves, size of leaves, number of growing points, physiological plasticity such as the extent of meristematic activity, speed of reconstructing new photosynthetic tissues after defoliation and maintenance of root growth to capture more growth resources. Despite extensive research along these lines comparing different cultivars, the decline of white clover from the swards persists to the present time.

Non-structural carbohydrates in plants are important to provide energy and carbon skeletons for various organic compounds for tissue construction. After defoliation or grazing the priority of growth is for reconstructing photosynthetic areas. Evidence has accumulated that during the first 4-6 days after defoliation NSC and other reserves are remobilised and used for regrowth. The speed of these processes may subsequently affect plant competitive abilities. Carbon substrate for regrowth after defoliation can also come from current photosynthates fixed by residual leaf areas, cut petioles and green stolons or stubble. If the interval between defoliation is long enough the number of new leaves and growing points formed will determine the photosynthates exported to storage tissues and roots. Supplies of current assimilates are of prime importance for the maintenance and functioning of roots, hence they will determine the success of competition for underground resources.

While NSC partitioning, accumulation and remobilization for regrowth after defoliation in perennial ryegrass and white clover have been widely studied in single plants and monocultures, their function in determining competitive abilities are relatively unknown.

The research conducted in this thesis was designed to:

(1) examine cultivar differences in accumulation of NSC in white clover and perennial ryegrass;

(2) investigate how the above differences affect subsequent growth and their competitive abilities;

(3) compare methods of studying competition to ensure that the observed effects are due to competition rather than density effects;

(4) evaluate the relative importance of the level of NSC as compared to morphological traits in affecting competitive ability of white clover in mixtures with perennial ryegrass;

(5) identify the differences among cultivars of white clover in relative contribution of reserves from stolons and roots for regrowth after defoliation; and

(6) establish whether or not differences in NSC levels will cause different tolerance of cultivars of white clover to the presence of high soil nitrogen levels in mixtures with perennial ryegrass.

# Chapter III

## Experiment I. The Effects of Defoliation on the Levels of Non-Structural Carbohydrate Reserves in Monocultures of Some Cultivars of White Clover (*Trifolium repens* L.) and Perennial Ryegrass (*Lolium perenne* L.)

### 3.1 Introduction

Evidence accumulated in the literature indicates that the availability of carbohydrates is an important feature of mechanisms of adaptation of plants to their environments (Munns, 1988; Chiariello *et al.,* 1989; Busso *et al.,* 1990). For example, carbohydrates are used in maintenance respiration to provide energy in stressful environments (Penning de Vries, 1975 a,b). In addition non-structural carbohydrate (NSC) reserves in storage organs are remobilized and used in regrowth, especially during 1-6 days after defoliation (Weinmann, 1961; Davies, 1965; Sheard, 1973; White, 1973; King *et al.*, 1979; Ourry *et al.*, 1988; Ourry *et al.*, 1989; and Hume, 1991). After defoliation NSC levels in the plants decline (Davies, 1965; King *et al.*, 1979; Ourry *et al.*, 1988; Ourry *et al.*, 1989; Hume, 1991) and then recover as photosynthesis increases. Newly formed white clover leaves start exporting carbohydrates and replenish reserves when they have reached stage 0.6 (Carlson, 1966), that is the leaves have 10% unfolded (Chapman *et al.*, 1990a), while perennial ryegrass leaves start exporting when they have reached two thirds of their mature size (Robson *et al.*, 1989).

The rapidity of reconstructing the photosynthetic tissues by means of remobilized reserves may influence the competitive ability of plants. Furthermore, the rate of replenishment of reserves during regrowth will influence the amount of reserves present when further defoliation occurs (Berendse and Elberse, 1990; Grime, 1979, Richards, 1986). In perennial ryegrass it has been found that NSC levels return to those present before defoliation 20 to 28 days after defoliation (Gonzalez *et al.*, 1989).

NSC levels in plants are also affected by cultivar, environmental conditions, frequency of defoliation and age and stage of growth of the plants. These differences may produce contrasting results. Thus, Harris *et al.* 1983) found at low Welsh temperatures in monocultures of white clover which had been cut five times, that the larger-leaved cultivar Olwen had a lower concentration of NSC in the stolons compared with smaller-leaved cultivars. In contrast, Kang and Brink (1995) found, in a glasshouse experiment at higher temperatures and only 55 days after sowing, that larger leaved cultivars had higher concentrations of NSC in the stolons and roots than smaller-leaved cultivars.

To study the influence of NSC reserves on competition between ryegrass and white clover, it would be useful to compare cultivars with different reserve NSC levels. The aim of this experiment was to measure these levels in a number of cultivars under controlled environment conditions and to choose some with different NSC reserve levels for study in competition experiments. To assist in explaining the results, information on dry matter yields and morphological traits was also collected.

## 3.2 Materials and Methods

### 3.2.1 Site and Soils

The experiment was conducted in a glasshouse in black plastic pots, 24 cm in diameter (8 L volume) with six drainage holes at the bottom. Each pot was filled with 8.5 kg of a mixture of sand and peat (4:1 by volume). To every 1.5 cubic metre of this potting mixture, fertilizers of the following composition were added: 500 g blood meal (16% N), 100 g super phosphate, 100 g potassium sulphate and 200 g calcium carbonate. The soil pH (1:10 w/v soil:water) in water was 6.0. These levels of nutrient are the same as those used for wheat experiments in pots at the Waite Institute.

During filling, the pots were tapped lightly on to the ground so that the soil surface was about 3.0 cm from the top rim of the pots. The volume of pots occupied by the soil was approximately 7.0 L.

### 3.2.2 Treatments and Design

Treatments consisted of monocultures of eight cultivars of white clover (*Trifolium repens* L.) and four cultivars of perennial ryegrass (*Lolium perenne* L.), two destructive harvests of whole plants, with three replications, resulting in a total of 72 pots arranged in a completely randomized block design. These cultivars were selected to cover a range of morphological and physiological traits (leaf size, stolon length and diameter), time of flowering, and growth habits. The characteristics of these cultivars are presented in Table 3.1. Clover seeds were obtained from Flaxley Experiment Station and from The National Clover Improvement Program at Glen Innes Experiment Station, Glen Innes, New South Wales, Australia.

These perennial ryegrass and white clover cultivars were established at a density of 16 plants pot$^{-1}$ (350 plants m$^{-2}$). In order to prevent excessively high temperatures during summer, shading cloths were placed above the glasshouse. Light intensity in this part of the glasshouse measured as photosynthetically active radiation by LICOR LI-1000 lightmeter, was ca. 650 _mol quanta m$^{-2}$ sec$^{-2}$. Temperature was controlled with an evaporative cooler and was set for the range 15-25 $^{o}$C. During the experiment, the pots were re-randomized at weekly intervals within each block to avoid microclimate effects.

**Table 3.1** Characteristics of cultivars of white clover and perennial ryegrass used

| Cultivars | Leaf-size | Time of flowering |
|---|---|---|
| A. Clover (White clover)* | | |
| 1. Tahora ( Grasslands Tahora) | small | mid-season |
| 2. Siral | medium | early |
| 3. Irrigation | medium | early |
| 4. Olwen | medium-large | early |
| 5. Huia (Grasslands Huia) | medium | early |
| 6. Pitau (Grassland Pitau) | medium | early |
| 7. Haifa | large | early |
| 8. Kopu (Grasslands Kopu) | large | mid-season |
| B. Ryegrass (Perennial ryegrass) ** | | |
| 9. Nui (Grasslands Nui) | | early |
| 10. Ellet (Grasslands Ellet) | | mid-season |
| 11. K. Valley ( Kangaroo Valley) | | Early |
| 12. Victorian* | | early |

*after Caradus (1986) **after Oram (1990)

### 3.2.3 Cultural Techniques

The clover seeds were scarified prior to sowing by rubbing with sand paper. Excess seed was sown 0.5 cm deep in six predetermined positions, evenly distributed across the soil surface. At sowing conducted on 28/10/91 an excess of commercial rhizobial inoculum mixed with water was poured on to the clover seeds. Inoculation was repeated seven days after sowing. The seedlings were thinned to one per position 21 days after sowing.

The plants were watered with 200 to 400 ml of water every two days. To prevent water stress during periods of high evaporation 25 cm diameter plastic saucers were placed at the bottom of each pot. During hot days water was applied to the saucers. Once a week these saucers were washed to get rid of residual chemicals on the saucers. In white clover any stolons which grew out of the pots were turned back inside the pots.

All the plants grew vigorously during the first growth period (7 weeks). Some cultivars came into flower. These flowers were cut off when the peduncles were less than 1.0 cm long in white clover or when the inflorescences were visible in perennial ryegrass, to prevent assimilate partitioning to flowers. After the first harvest, some plants showed signs of chlorosis especially in the younger leaves. This disappeared after adding 100 ml half strength modified Hoagland's nutrient solutions to each pot (Hammer *et al.*, 1978) (Appendix 3.1) twice weekly for two weeks.

### 3.2.4 Observations and Measurements

The first destructive harvest of 36 pots was made on 16/12/91. At the same time all plants in the remaining 36 pots were cut at 3.0 cm above the soil surface and allowed to regrow for a further four weeks before the final destructive harvest on 13/1/92.

During each of the destructive harvests, the pots were moved to a shaded room at about 8.00 am. The shoots were cut at 3.0 cm above the soil surface, and dried at 70 $^{o}C$ in a forced draft oven for weighing. The stolons and roots of white clover, and the stubble and roots of perennial ryegrass were then sampled for NSC analysis. Stolon material sampled consisted of the length from node number three, which is the node bearing unfolded leaf number three from the apex, to node number ten. Haycock (1981) showed that lamina, petiole and stolon extension at these positions is almost complete, while Gallagher *et al.* (1994) have suggested that these parts of the stolons are the main storage positions where most of the buds are active and thus ideal for studying remobilization of NSC in white clover. In contrast, younger plant parts including the apex are still growing, hence will have a variable NSC content depending on the stage of development of the young leaves. Stubble samples from perennial ryegrass (parts of the plant below 3.0 cm cutting height which consisted mostly of leaf sheaths and short basal stem) were taken at the middle of the pots.

Stubble and stolons were cut, washed free of soil and all roots were detached.

Stubble and stolon samples were then wrapped with blotting paper to dry. About 2.0 g of stubble and stolons (without petioles) were weighed, cut into small pieces (ca. 0.5-1.0 cm long) and dropped into test tubes (in a hot water bath) containing 20 ml 80% aqueous ethanol that was boiling. The materials were boiled for 15 minutes. This operation, from cutting to dropping into boiling 80% ethanol was completed in less than 30 minutes to reduce loss of substrates due to respiration. The tubes were then cooled and the samples submerged in aqueous 80% ethanol, individually transferred to 20 ml plastic containers (with cap), and kept in the refrigerator in the dark until analysis. Smith (1973a) showed that, compared to freeze- or oven-drying, this procedure killed the enzymes and prevented degradation and losses due to respiration and enzymatic conversion during periods of storage. Samples were also taken to determine the moisture content of stubble and stolons. All samples were dried for 48 hours at 70 $^{o}$C in a forced draught oven for dry matter (DM) determination.

To sample roots for carbohydrate determination a quadrant was cut downwards to the bottom of each pot, and the roots were detached from the stem, and washed free of soil. The roots were then dried by wrapping with blotting paper, and ca. 2.0 g of roots were cut into small pieces and dropped into tubes containing 20 ml boiling aqueous 80% ethanol. These procedures, from cutting to dropping samples into boiling ethanol were conducted in less than 30 minutes. Root samples were than treated as for stubble and stolons. Samples of roots were also taken to determine DM content. Samples were weighed to the nearest milligram. All procedures were completed on the same day for each replicate.

Laboratory analyses were conducted for ethanol soluble sugars (from now on called soluble sugars) after chlorophyll separation by chloroform (Appendix 3.2) for stolons and stubble, following Dubois *et al.* (1956). For white clover, the remaining pellets were analysed for starch, following Pucher *et al.* (1948). After treatment of the starch-iodine complex with alkali, the starch was dissolved in weak perchloric acid. In perennial ryegrass pellets fructans were analysed by the method of Dubois *et al.* (1956)

after extraction three times for 15 minutes with boiling water. Soluble sugars and fructans were quantified using sucrose as standard, while wheat starch (after overnight drying in an oven at $50^0$ C) was used as standard for starch analysis in white clover (for complete procedure see Appendix 3.2). A factor of 0.9 was used to convert sucrose equivalents to starch or fructans (Pucher *et al.*, 1948), consequently to obtain the total NSC the measured quantities of soluble sugars were added to starch or fructans after multiplying the latter with a factor of 10/9. Hence, all comparisons of NSC between white clover and perennial ryegrass were conducted on a sucrose equivalent basis. NSC contents (mg $pot^{-1}$) were obtained by multiplying total DM with concentration (mg $g^{-1}$ DM).

Stolon subsamples were also taken to measure stolon length. These subsamples were oven dried and weighed and the total stolon length was calculated from total stolon weight. Stolon diameter was measured at node number three from the apex (five samples per pot). Observations on the roots of white clovers during destructive harvests showed that abundant root nodules were present on all cultivars.

### 3.2.5 Statistical Analysis

Data were subjected to analysis of variance using Genstat 5 (Genstat 5 Committee, 1987), after appropriate transformations where necessary to ensure homogeneity of variance. Generally, a log10 transformation was sufficient. When treatment effects were significant mean separations were conducted following Duncan's Multiple Range Test (Steel and Torrie, 1960).

Analyses of variance were conducted for a factorial arrangement of cultivars and harvests, as well as for simple cultivar comparisons at each harvest. In subsequent competition studies, as plant materials remaining following defoliation are thought to be important for regrowth and competitive ability, emphasis will be placed on comparisons between cultivar means of remaining materials at individual harvests. Consequently, means at each individual harvest were presented.

Analyses of variance were also conducted for data on white clover only; however as the trends were similar, and no increase in significant effects were observed, data on all cultivar comparison were presented to show the differences between white clover and perennial ryegrass cultivars.

## 3.3 Results

### 3.3.1 Dry Matter

Table 3.2 shows the significant treatment and interaction effects from the factorial analysis and for the analysis of cultivars at each harvest for shoot, stolon or stubble and root DM, shoot:root ratio, and stolon:root ratio. The main effects of harvests were highly significant ($P<0.001$) for all plant attributes. The main cultivar effect and the cultivar x harvest interaction were not significant for shoot DM but were significant for other attributes.

**Table 3.2** Treatments and interaction effects in a factorial analysis and at each harvest for shoot DM, stolon or stubble DM, root DM, shoot:root ratio and stolon:root ratio.

| Effects | Shoot DM | Stolon or stubble DM | Root DM | Shoot:root ratio | Stolon:root ratio |
|---|---|---|---|---|---|
| Factorial Analysis | | | | | |
| Harvest number | *** | *** | *** | *** | ** |
| Cultivars | ns[@] | * | *** | *** | *** |
| Interaction | ns | * | *** | ns | * |
| Cultivars at each harvest | | | | | |
| Harvest 1 | * | *** | *** | *** | ** |
| Harvest 2 | ns | * | *** | *** | *** |

[@] ns=non-significant ($P>0.05$), *= $P<0.05$; **=$P<0.01$; ***=$P<0.001$

### (a) Shoot dry matter

Overall, DM yield was higher at harvest 2 than at harvest 1 (Figure 3.1A). Analyses at each harvest indicated that, at harvest 1, Ellet had higher shoot DM than most cultivars of white clover (Figure 3.1A) and also higher than Kangaroo Valley ($P<0.05$). All cultivars of white clover produced similar amounts of shoot DM ($P>0.05$). At harvest 2, there were no significant differences between cultivars ($P>0.05$).

### (b) Stolon or stubble dry matter

There was a significant ($P<0.05$) harvest x cultivar interaction on stolon and stubble DM (Table 3.2). DM increased markedly ($P<0.01$) from harvest 1 to harvest 2 in all cultivars used (Figure 3.1 B). At harvest 1, stolon DM yield was similar for all cultivars of white clover and lower than the stubble yields of perennial ryegrass cultivars ($P<0.05$). Nui yielded the most stubble. At harvest 2 there was no overall difference between clover and ryegrass. Olwen yielded significantly more stolon DM than Tahora, Irrigation, Huia and Pitau, while Ellet yielded more stubble than Nui and Kangaroo Valley.

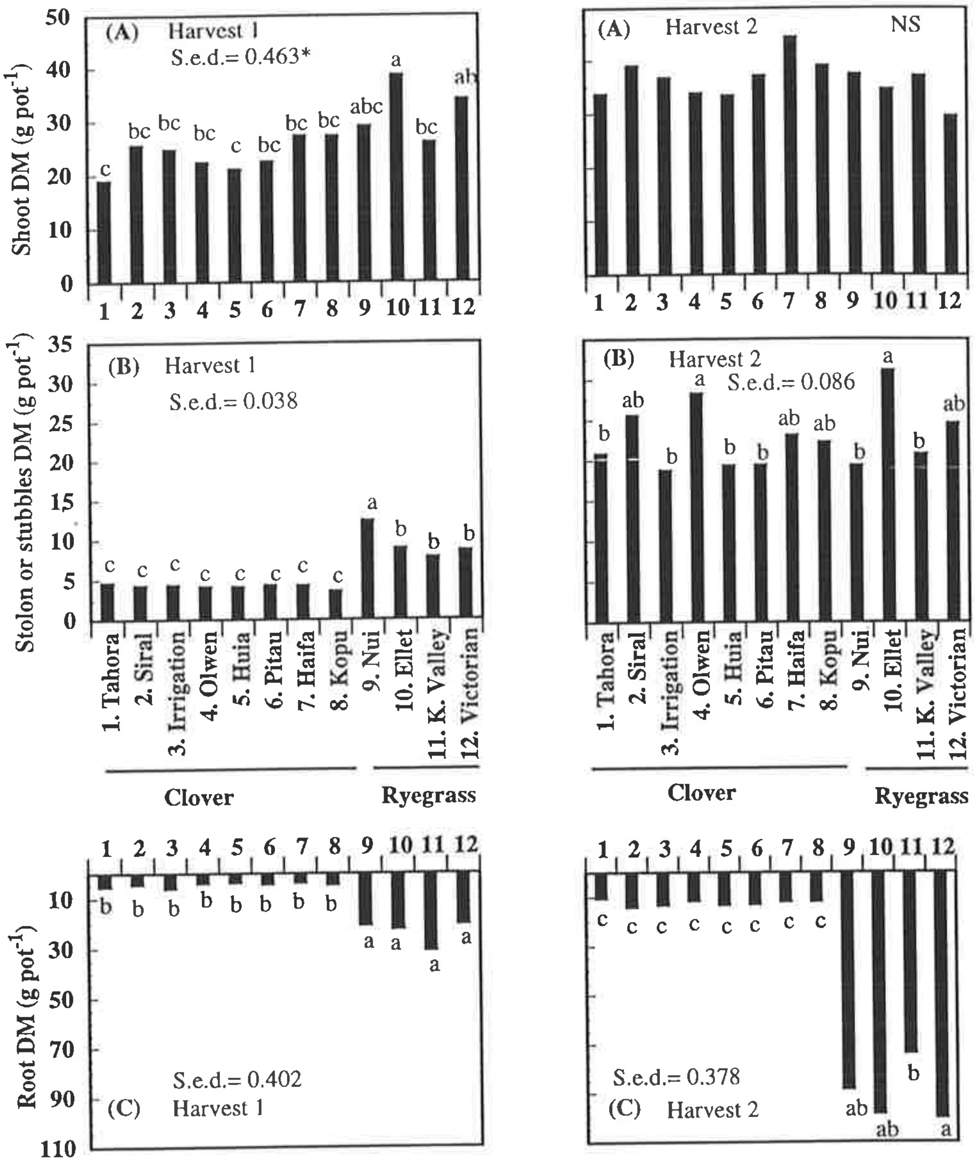



**Figure 3.1** Means of (A) shoot, (B) stolon and (C) root DM (g pot$^{-1}$) of white clover and perennial ryegrass cultivars at harvests 1 and 2. Means associated with similar letters in each diagram are not significantly different (P>0.05). NS indicates non-significant difference (P>0.05). *After log10 transformation

### (c) Root dry matter

The harvest x cultivar interaction was highly significant (P<0.01) (Table 3.2). Root DM increased significantly (P<0.05) from harvest 1 to harvest 2. Perennial ryegrass produced higher root DM (P<0.05) than white clover at both harvests, most markedly at harvest 2. Cultivars of white clover and perennial ryegrass produced similar root DM, except at harvest 2 when Kangaroo Valley had the lowest root DM of all the perennial ryegrass cultivars (P<0.05).

### (d) Shoot:root ratio and stolon:root ratio

The harvest x cultivar interaction for stolon: root or stubble:root ratio was significant (P<0.05) (Table 3.2). At each harvest, shoot:root ratios (Figure 3.2A) and stolon or stubble:root ratios (Figure 3.2B) were generally significantly higher in white clover than perennial ryegrass. There were no significant differences among cultivars except for stolon:root ratio at harvest 2, where Olwen was highest and Irrigation and Pitau lowest. Shoot:root ratio decreased from harvest 1 to harvest 2 in all cultivars of white clover and perennial ryegrass, while stolon:root ratio increased in white clover.

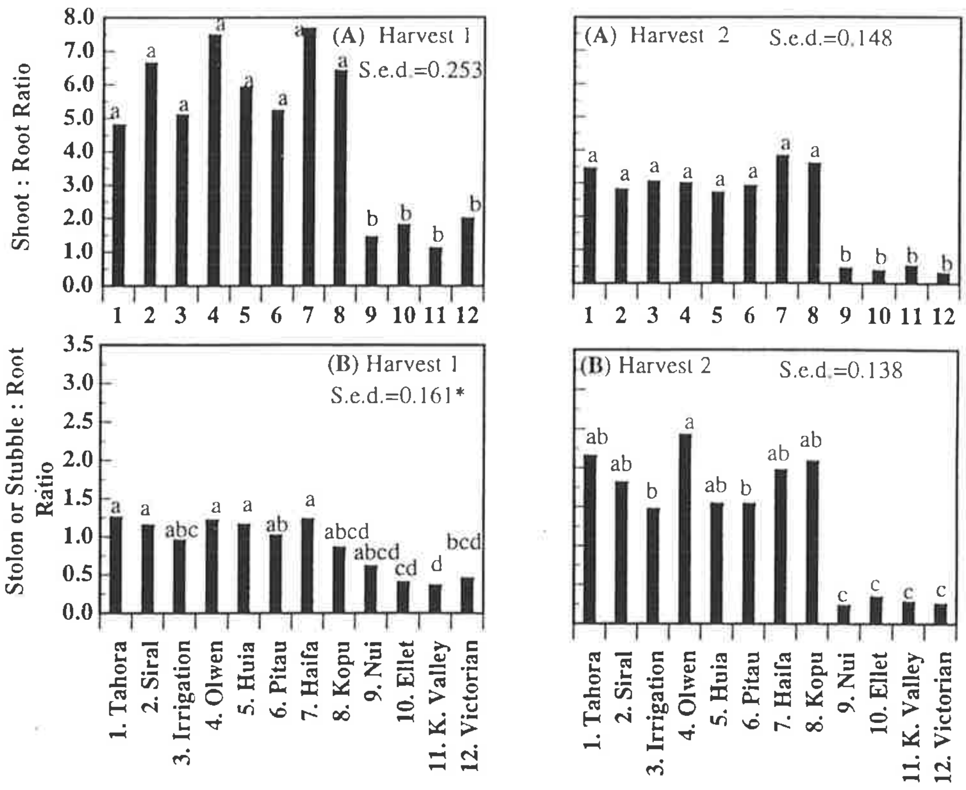



**Figure 3.2** (A) Shoot:root ratio and (B) stolon or stubble:root ratio of cultivars of white clover and perennial ryegrass at harvests 1 and 2. (Means of three replicates). Means associated with similar letters in each diagram are not significantly different ($P>0.05$). *After log10 transformation

### 3.3.2 Stolon Characteristics

Cultivars of white clover varied significantly ($P<0.01$) in stolon diameter at node number three from the apex and in stolon length (Table 3.3 ) but internode lengths were similar. Olwen (medium large-leaved) had a larger diameter than Tahora (small-leaved cultivar), but the reverse was true for stolon length. Huia had the greatest total length of stolons and Olwen, Irrigation and Kopu the lowest.

No significant cultivar x harvest interaction on stolon diameter, stolon length and internode length was found.

**Table 3.3.** Main effect of cultivars on stolon diameter (mm), stolon length (cm) and internode length (cm). (Means of six replications)

| Cultivars | Diameter (mm) | Length ($cm.pot^{-1}$) | Internode length (cm) |
|---|---|---|---|
| 1. Tahora | $2.07^{e}$ | $1989^{abc}$ | $2.77^{a}$ |
| 2. Siral | $2.65^{bcd}$ | $1527^{abcd}$ | $2.96^{a}$ |
| 3. Irrigation | $2.46^{cd}$ | $1446^{cd}$ | $2.66^{a}$ |
| 4. Olwen | $3.13^{a}$ | $911^{d}$ | $2.58^{a}$ |
| 5. Huia | $2.33^{de}$ | $2200^{a}$ | $2.81^{a}$ |
| 6. Pitau | $2.41^{cde}$ | $2165^{ab}$ | $2.99^{a}$ |
| 7. Haifa | $2.91^{ab}$ | $1476^{bcd}$ | $3.16^{a}$ |
| 8. Kopu | $2.72^{bc}$ | $1376^{cd}$ | $2.83^{a}$ |
| S.e.d. | 0.166 | 0.078* | 0.279 |

Means within the same column followed by the same superscripts are not significantly different ($P>0.05$) * After log10 transformation

### 3.3.3 Non-structural Carbohydrate Concentrations and Content in Stolons or Stubble

Treatment and interaction effects for NSC (Table 3.4A) were significant except for harvest x cultivar interaction for soluble sugars ($P>0.05$). Mean comparisons at each harvest are given for soluble sugars.

#### (a) Soluble sugars

At harvest 1, Olwen white clover (medium large-leaved cultivar) had the highest soluble sugar concentration while Huia (medium-leaved cultivar) had the lowest ($P<0.05$) (Figure 3.3A). At harvest 2, Haifa had the highest concentration ($P<0.05$), while Olwen and Huia had the lowest. Stubble of the four ryegrass cultivars had similar soluble sugar concentration and contents at both harvests. Stubble of Ellet, Kangaroo Valley and Victorian perennial ryegrass had lower soluble sugar concentrations than Haifa (large-leaved), Pitau and Irrigation (medium-leaved) white clovers, but was similar to most the other cultivars.

Content of soluble sugars per pot in stolons and stubble increased in all cultivars from harvest 1 to harvest 2 (Table 3.4A and Figure 3.4A), but the increases

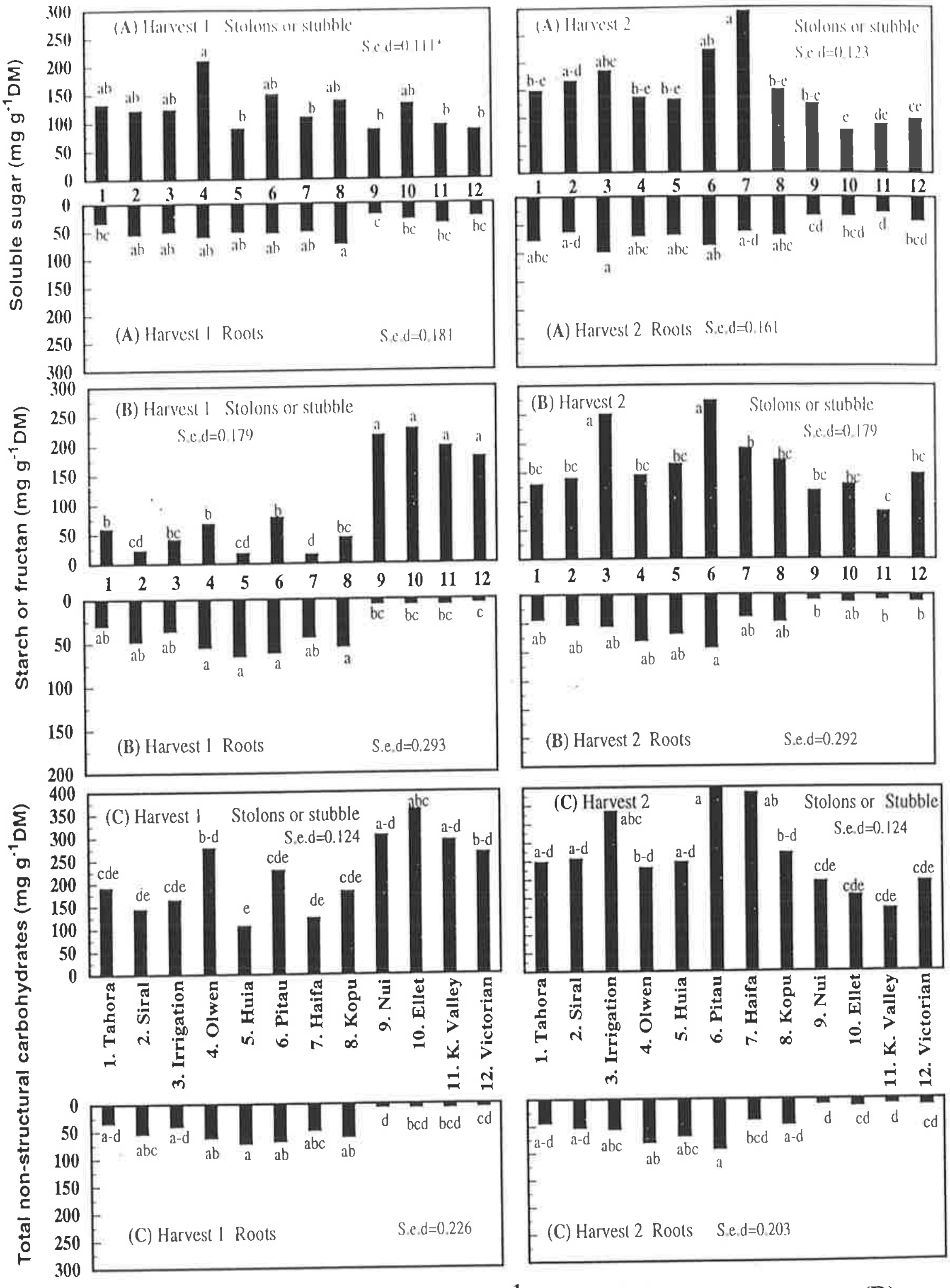



**Figure 3.3** The concentration (mg g$^{-1}$ DM) of: (A) soluble sugars, (B) starch or fructans and (C) total NSC in the stolons of white clover and stubble of perennial ryegrass cultivars and roots of each at harvest 1 and harvest 2. (Note that different scales are used). Means associated with similar letters in each diagram are not significantly different (P>0.05). *After log10 transformation. a-d indicates abcd.

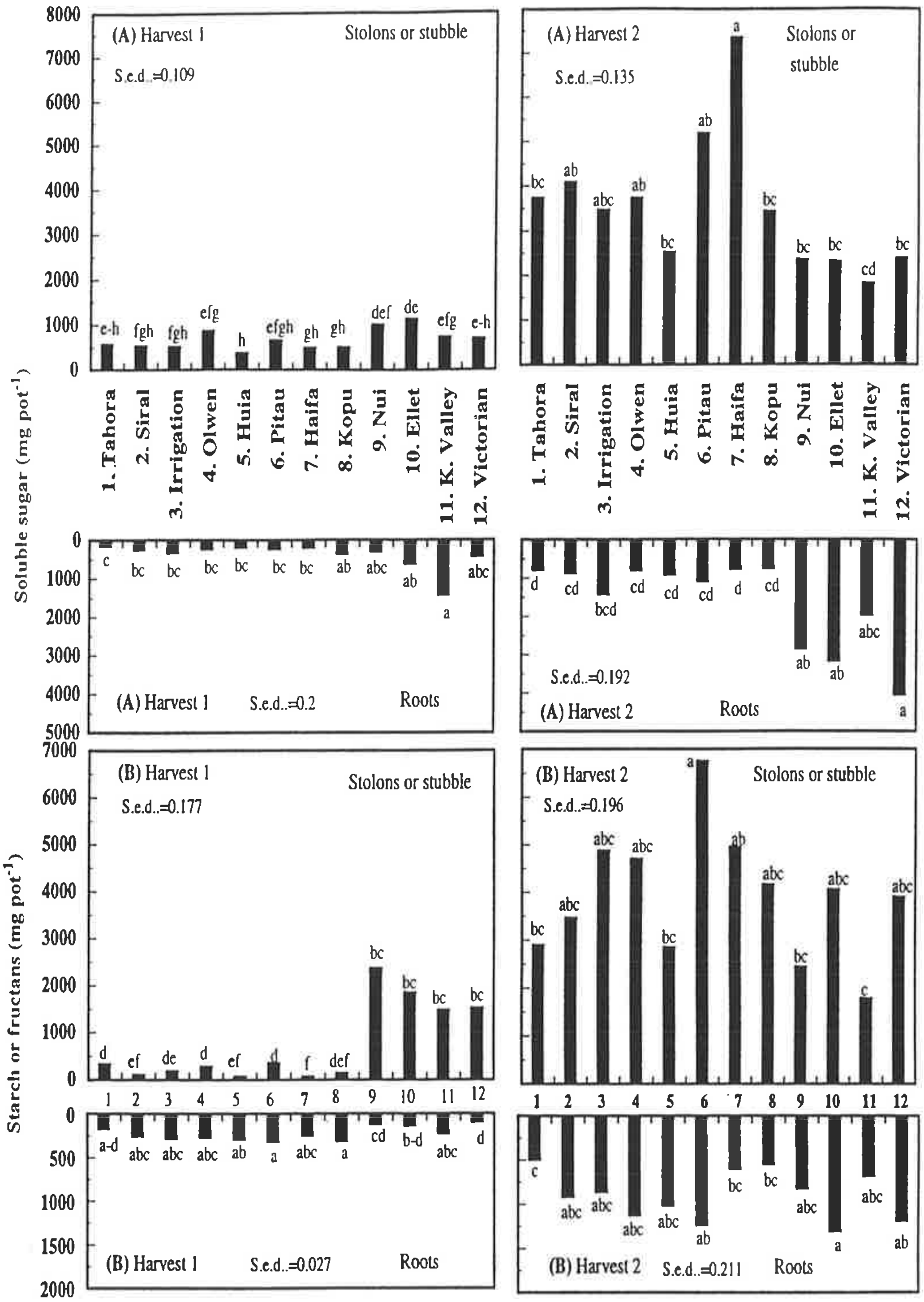



**Figure 3.4.** The content (mg pot$^{-1}$) of (A) soluble sugars and (B) starch or fructans in the stolons and roots of white clover cultivars and the stubble and roots of perennial ryegrass cultivars at harvest 1 and 2. Note that different scales are used. Means associated with similar letters in each diagram are not significantly different (P>0.05). N.S. indicates non significant (P>0.05). b-d indicates bcd.

were smaller in perennial ryegrass than in the clovers. Hence, while soluble sugar contents were slightly higher in the stubble of perennial ryegrass than in the clovers at harvest 1, they were lower at harvest 2, especially compared to Haifa white clover.

Among the white clover cultivars at harvest 1 the only two cultivars to differ significantly from each other were Olwen, with the the highest content of soluble sugars, and Huia with the lowest. At harvest 2, Haifa (large-leaved cultivar) had the highest and Huia the lowest soluble sugar content. Perennial ryegrass cultivars had similar contents of soluble sugars at both harvests.

**Table 3.4** Treatment and interaction effects in factorial analysis and at each harvest on concentration (mg g-1) and content (mg pot-1) of soluble sugars, starch or fructans and total NSC in the **(A)** stolons or stubble and **(B)** roots

| Effects | Soluble sugars | Starch or fructans | Total NSC | Soluble sugars | Starch or fructans | Total NSC |
|---|---|---|---|---|---|---|
| **(A)** Stolons or stubble | | | | | | |
| Factorial Analysis | Concentration (mg $g^{-1}$) | | | Content (mg $pot^{-1}$) | | |
| Harvest number | * | *** | ** | *** | *** | *** |
| Cultivars | * | * | * | * | *** | *** |
| Interaction | ns@ | *** | ** | *** | ** | *** |
| Cultivars at each harvest | | | | | | |
| Harvest 1 | * | *** | *** | ** | *** | *** |
| Harvest 2 | * | * | * | ** | * | * |
| **(B)** Root | | | | | | |
| Factorial Analysis | Concentration (mg $g^{-1}$) | | | Content (mg $pot^{-1}$) | | |
| Harvest number | ** | ns | * | ** | ** | ** |
| Cultivars | ** | ** | ** | ** | * | * |
| Interaction | ns@ | ns | ns | ns | ns | ns |
| Cultivars at each harvest | | | | | | |
| Harvest 1 | * | * | * | * | * | * |
| Harvest 2 | * | * | * | ** | * | * |

@ ns=non-significant, *= P<0.05; **=P<0.01; ***=P<0.001

### (b) Starch or fructans

The harvest x cultivar interaction was highly significant ($P<0.01$) for both starch concentration and starch content (Table 3.4A). Starch concentration increased ($P<0.05$) in white clover cultivars from harvest 1 to harvest 2, especially in Irrigation, Huia, Pitau, Haifa and Kopu (Figure 3.3B). At harvest 1, stubble of perennial ryegrass contained higher ($P<0.05$) fructan concentrations (on a sucrose equivalent basis) than starch levels in stolons of white clover cultivars. However, there was an increase in concentration of starch in stolons of white clover from harvest 1 to harvest 2, while levels of fructans in perennial ryegrass stubble decreased slightly. Among white clover cultivars, Olwen and Pitau had the highest and Huia, Siral and Haifa had the lowest concentrations of starch at harvest 1. At harvest 2, Pitau and Irrigation had higher concentrations than the other cultivars.

Fructan content on a sucrose equivalent basis was higher in perennial ryegrass at harvest 1 than was starch content in white clover (Figure 3.4B). Starch content increased from harvest 1 to harvest 2 in white clover, but the increases of fructant were less in perennial ryegrass than in clover. At harvest 1, Tahore, Olwen and Pitau had the highest content of starch and Siral, Huia and Haifa the lowest, but at harvest 2 Pitau had the highest and Huia the lowest starch content. In perennial ryegrass cultivars, Ellet had higher and Kangaroo Valley the lowest fructan content but the difference was not significant.

### (c) Total non-structural carbohydrates

The harvest x cultivar interaction for both total NSC concentration and content was significant ($P<0.01$) (Table 3.4A). Somewhat similar trends as those for starch were also observed for NSC, in that concentration and content were lowest in white clover cultivars at harvest 1, but while in white clover NSC levels increased from harvest 1 to harvest 2, in perennial ryegrass they decreased (Figure 3.3C and 3.5).

In white clovers, at harvest 1, Olwen had a significantly higher total NSC concentration (Figure 3.3C) and content (Figure 3.5) in stolons than Huia. At harvest 2 Olwen and Haifa did not differ and Pitau had a higher total NSC concentration than both of them. Haifa and Pitau had significantly higher total NSC contents (Figure 3.5) than Tahora, Siral and Huia. Olwen and Irrigation had significantly higher contents than Huia.

In perennial ryegrass, the total NSC concentration (Figure 3.3C) and content (Figure 3.5) were similar in all cultivars. However, at harvest 2 Ellet and Victorian appeared to have higher total NSC content and Nuia and Kangaroo Valley, but the differences were not significant.

### 3.3.4 Non-structural Carbohydrates in the Roots

The harvest x cultivar interactions for NSC in the roots were non-significant (Table 3.4B). Main effects were significant, except for the main effect of harvest number for starch or fructans ($P<0.05$). The effects of cultivar at each harvest were also significant.

#### (a) Soluble sugars

Roots of white clover cultivars had slightly higher concentrations of soluble sugars than those of perennial ryegrass at both harvests (Figure 3.3A), but the reverse was true for soluble sugar content (Figure 3.4A). Levels were similar within cultivars of white clover or perennial ryegrass.

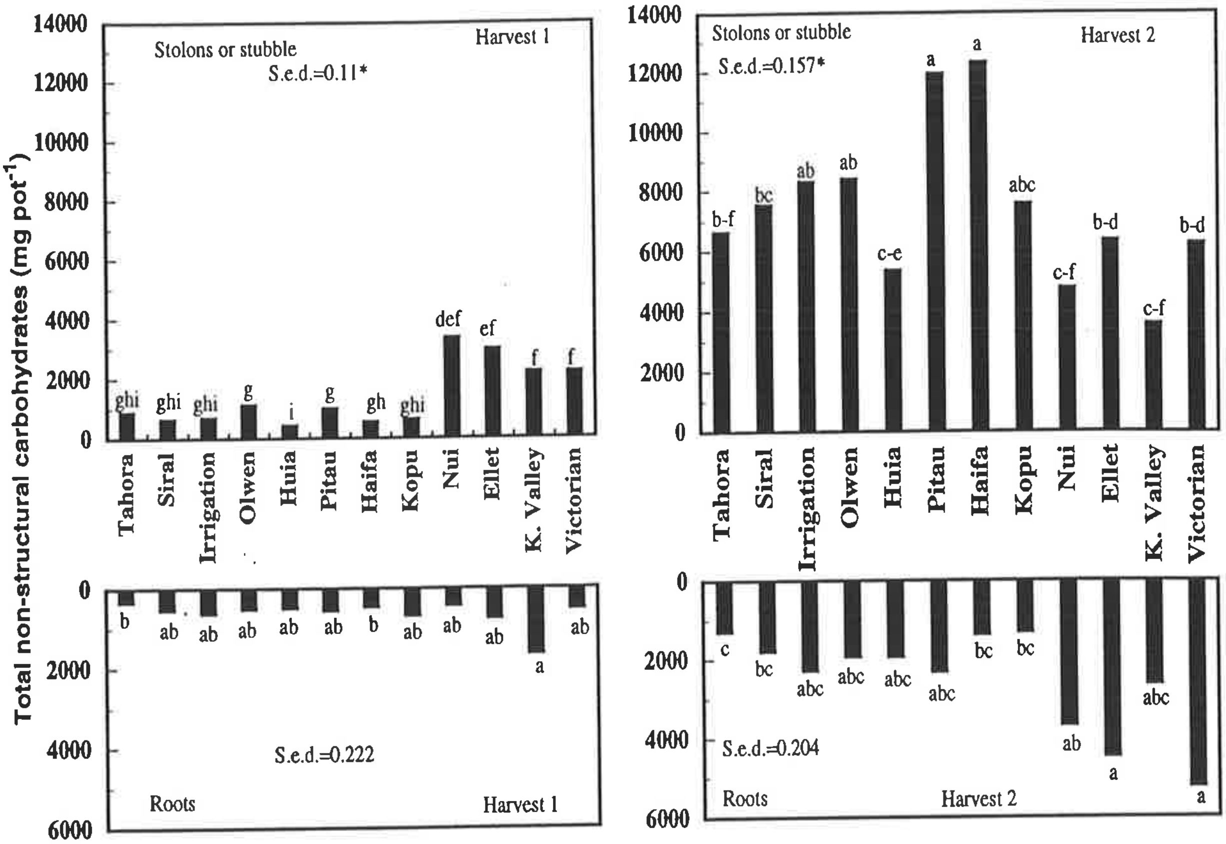



**Figure 3.5** The content (mg pot$^{-1}$) of the total NSC in stolons and roots of white clover cultivars and stubble and roots of perennial ryegrass cultivars at harvest 1 and harvest 2. Means associated with similar letters in each diagram are not significantly different (P>0.05). b-d indicates bcd. *After log10 transformation

### (b) Starch or fructans

White clover roots generally had higher starch concentrations than fructan levels in perennial ryegrass roots (Figure 3.3B). Cultivars of white clover had similar starch concentrations in their roots. The concentrations did not change significantly between harvests.

The content of starch in roots increased from harvest 1 to harvest 2 (Table 3.4B and Figure 3.4B). White clover cultivars had similar starch levels in their roots at harvest 1. At harvest 2 Pitau had significantly higher starch levels than Tahora. At harvest 1, Kangaroo Valley had a significantly higher fructan content than Victorian. At harvest 2 there were no significant differences in fructan contents between perennial ryegrass cultivars.

### (c) Total non-structural carbohydrates

Concentration and content of total NSC in the roots increased significantly from harvest 1 to harvest 2 (Table 3.4B). The total NSC concentrations were lower in perennial ryegrass roots than in white clover roots (Figure 3.3C), but the differences among white clover or ryegrass cultivars were generally not significant.

The total NSC contents in perennial ryegrass roots were higher than in white clover roots especially at harvest 2 (Figure 3.5), but within species, cultivars did not differ significantly ($P<0.05$).

## 3.4 Discussion

The high nutrient levels (especially nitrogen) during the first growth period resulted in perennial ryegrass, especially Ellet, producing higher shoot, stubble and root DM ($P<0.05$) at harvest 1 compared to white clover cultivars (Figure 3.1). At harvest 2, nitrogen in the soil in pots with perennial ryegrass monocultures might have declined, but not in pots with white clovers because of their ability to fix nitrogen, resulting in no overall differences in the shoot and stolon or stubble DM between perennial ryegrass and white clover. At harvest 2, Olwen produced higher stolon DM compared to some other cultivars (Figure 3.1B). Perennial ryegrass responded by increasing stubble DM (Figure 3.1B) and root growth more than shoot growth, typical of grass responses to nitrogen limitation in the soil (Davidson and Robson, 1985a).

Perennial ryegrass has been reported to have a larger root system than clovers (Evans, 1978) which was also observed in this experiment (Figure 3.1C). Lower shoot:root ratio (Figure 3.2A) and stolon or stubble:root ratio (Figure 3.2C) in perennial ryegrass indicate that more roots are available per unit of stubble or stolons in perennial ryegrass than in white clover. These results are in line with those of Ozanne *et al.* (1964), who found that perennial ryegrass had higher root:top ratio than white clover.

The results of this experiment agree with those of Kang and Brink (1995), who found that the larger-leaved cultivar of white clover Osceola at 55 days after sowing had greater stolon DM if defoliated at two and four week intervals compared to Huia and the smaller-leaved cultivar Aberystwyth S184. In contrast, Harris *et al.* (1983) found in Aberyswyth, Wales, that Olwen in monocultures had lower stolon DM compared to the smaller-leaved cultivar S184. However, the latter experiment was conducted at much lower temperatures and after several cuts (five) which would reduce stolon DM.

The concentrations of NSC obtained in this experiment were 90.0-200 mg $g^{-1}$ (Figure 3.3A) or 9.0-20.0% soluble sugars and 25.0-270 mg $g^{-1}$ or 2.5-27.0% starch, which are generally in line with those reported in the literature. For example, Harris *et al.* (1983) measured levels of 11.0-14.0% of water soluble carbohydrates and 3-7% for starch in the stolons and 8.0-8.5% in the roots. Similarly Hay *et al.* (1989) measured 4.0-15.0% of starch, 4.0-8.0% hexose soluble sugars, and 4.0-8.0% sucrose in stolons of Huia, while in perennial ryegrass King *et al.* (1979) reported levels of 15-30% of water soluble sugars in stubble.

NSC levels in the storage organs are affected by cultivars, nitrogen levels in the soil and defoliation regimes. For example, in this experiment Olwen white clover had higher soluble sugar concentrations in the stolons than Huia at harvest 1 (Figure 3.3A), but at harvest 2 Olwen and Huia had similar soluble sugar concentrations. This pattern also occured for starch in the stolons. However, Olwen had significantly higher stolon DM yield than Huia at harvest 2 (Figure 3.1B) leading to higher total NSC content in the stolons of Olwen at harvest 2. (Figure 3.5) This suggests a possible connection between concentration of NSC at harvest 1 and growth between harvest 1 and harvest 2.

The content of NSC in storage organs increased from harvest 1 to harvest 2 (Figures 3.3, 3.4 and 3.5) but not the concentrations of NSC (Figure 3.3). Many studies have reported that defoliation initially decreased the levels of NSC in the storage organs, but that after four weeks regrowth levels returned to that before defoliation (King *et al.*, 1979; Gonzalez *et al.*, 1989). This indicates that the frequency of defoliation as used in this experiment (four week interval) and the intensity (cut at 3.0 cm above the soil surface), coupled with favourable growth conditions, have resulted in sufficient time for regrowth and replenishment of reserves. This also indicates that complete surface cover (in monoculture) has been achieved and competition for light has occurred, hence four week defoliation interval will be maintained for competition experiments to follow.

Different accumulation of different forms of carbohydrates was observed between white clover and perennial ryegrass. At harvest 1, soluble sugar concentrations and content in the stolons except for Olwen white clover were generally similar to those in the stubble of the grasses (Figure 3.3A), but starch levels in the stolons of white clover on sucrose equivalent basis was less than fructan in stubble of perennial ryegrass, leading to higher total NSC levels in stubble than in stolons (Figure 3.5). A larger increase occurred in white clover from harvest 1 to harvest 2 than in ryegrass, resulting in a higher content of soluble sugars in white clover, especially in the larger-leaved cultivar Haifa (Figure 3.4A). This might suggest that, in perennial ryegrass by harvest 2, soluble sugars had been translocated or used to support root growth, which was more abundant in ryegrass than in clover (Figure 3.1C).

Because NSC levels in the storage organs are affected by many factors, it is difficult to find a cultivar that contains higher NSC levels in all conditions than the other cultivars. However, in the condition of this experiment some conclusions can be drawn: at harvest 1, Olwen and Pitau had higher total contents of NSC in the stolons than Huia. At harvest 2, Haifa and Pitau had the highest NSC concentration and content, however they were not significantly different to those of Olwen, Irrigation and Kopu; but significantly higher than those in Huia and Tahora. The total NSC contents in the stubble of the grasses were similar in harvest 1 and harvest 2.

The general increase from harvest 1 to harvest 2 in the levels of NSC (Figure

3.4) and in the content of NSC (Figure 3.5) in the roots, suggests defoliation had little effect on final (four weeks) accumulation of NSC in roots. Concentrations of soluble sugars, starch and total NSC were generally lower in perennial ryegrass roots than in those of white clover; however, contents were generally higher, which was caused by higher root DM (Figure 3.1C). Thus, compared to clover, perennial ryegrass partitioned more substrates to the roots, which may cause grass roots to be more efficient in acquiring below ground resources (Buwai and Trlica, 1977). The increase from harvest 1 to harvest 2 of perennial ryegrass root DM (Figure 3.1C) and total content of NSC (Figure 3.5) might be a consequence of an increase in the size of the root system with time and a decline of nitrogen in the soil. Many researchers have reported an increase of NSC levels in the roots in response to nitrogen limitation (Henry and Raper, 1991), and a burst of growth if nitrogen is applied.

The results of this experiment highlight the importance of presenting both the concentration and content of NSC. For example, soluble sugar concentrations in the roots of perennial ryegrass were lower than in clover at harvest 1 (Figure 3.3A), but if contents were considered (Figure 3.4A), the levels were higher, due to larger root DM. In white clover, at harvest 1 Olwen had higher concentrations of soluble sugar (Figure 3.3A) starch (Figure 3.3B) and total NSC (Figure 3.3C) in the stolons compared to Huia, but at harvest 2 they were similar. Olwen had higher ($P<0.05$) stolon DM than Huia at harvest 2, hence if contents per pot were considered, Olwen had higher contents of soluble sugars (Figure 3.4A), starch (Figure 3.4B) and NSC (Figure 3.5). This is in line with Richards (1986), who suggested that concentrations may be higher but the total might be less in one plant compared to the other.

The aim of this experiment was to select cultivars of white clover and perennial ryegrass with varying (low, medium and high) NSC concentration and content to be used in later experiments to find possible effects of NSC concentration and content in storage organs on competitive ability. As rates of growth (and possibly competitive ability) are also affected by current assimilates fixed by residual leaf areas, the chosen cultivars were expected to have similar morphological characters (which are expected to have similar residual leaf area). This experiment showed that these objectives were difficult to achieve. Based on the results of this experiment and morphological features, Olwen, Irrigation and Huia white clover were selected for further studies to examine the effects of NSC concentration and content in the stolons on competitive ability in mixtures with perennial ryegrass. The three cultivars have medium- to medium large-leaved size, as opposed to Pitau, Haifa and Kopu, which are large-leafed but their NSC concentration and content in the stolons vary. Larger-leafed cultivars will have shorter stolon length, lower numbers of growing points and lower residual leaf area. Thus, after defoliation, larger-leafed cultivars are likely to have lower current photosynthates compared to smaller-leafed cultivars. Hence, to ensure that as far as possible the NSC used in the regrowth will come from the reserve storage, the cultivars used in the succeeding experiments should have similar leaf size, with the difference in current photosynthates after defoliation as small as possible. Olwen and Huia had been widely used in experimental work throughout the world, and Irrigation was a popular white clover cultivar in Australia. In perennial ryegrass, cultivars Ellet and Kangaroo Valley were selected for further study.

## 3.5 Conclusions

1. During early growth perennial ryegrass, especially cv. Ellet, produced more stubble and roots than white clover. At harvest 2, Olwen and Ellet produced higher stolon or stubble DM than the other cultivars. Perennial ryegrass always produced more roots than white clover, resulting in lower shoot:root ratios or stolon or stubble:root ratios in perennial ryegrass than in clover.

2. At harvest 1 white clover cultivar Olwen had the highest soluble sugar and total NSC concentration in its stolons, Huia had the lowest, while Tahora, Siral, Pitau,

Kopu and Haifa had intermediate concentration. NSC contents were similar in all cultivars of white clover except in the stolons of Huia which contained the lowest NSC content. Stubble of all cultivars of perennial ryegrass had the same NSC concentration and content. Perennial ryegrass had higher fructans than starch concentration in clovers, resulting in a higher total NSC content in perennial ryegrass.

3. Among white clover cultivars at harvest 2, the large-leaved cultivar Haifa and the medium-leaved cultivar Pitau had the highest concentrations of NSC than other white clover cultivars which had the same NSC concentration. Haifa and Pitau also had higher NSC contents than other white clover cultivars, while Olwen, Irrigation, Tahora and Kopu were intermediate and Huia had the lowest NSC contents. Perennial ryegrass cultivars had similar levels of NSC concentrations and contents. However, there were indications that Ellet had the highest total NSC both in stubble and roots, while Kangaroo Valley had the lowest.

4. In the roots, perennial ryegrass usually had lower concentrations of soluble sugars, starch and total NSC than white clover cultivars, but contents per pot were higher.

5. In view of their similarity in leaf size and their NSC content, Olwen and Irrigation (medium to large leaf size and medium NSC content) and Huia (medium-leaf size, low NSC content) were selected for competition study.

# Chapter IV

## Experiment II. The Effects of Plant Density and Planting Design on the Competitive Ability of White Clover (*Trifolium repens* L.) in Mixtures with Perennial Ryegrass (*Lolium perenne* L.) and on the Accumulation of Non-Structural Carbohydrate Reserves

### 4.1 Introduction

The two most widely used designs for studying plant competition are the additive design and de Wit's replacement series or substitutive design (de Wit, 1960). The use of these two designs to study competitive relationships of mixtures has been compared and has been the subject of several reviews (Connolly, 1986; Austin *et al.*, 1988; Snaydon and Satorre, 1989; Taylor and Aarssen, 1989; Snaydon, 1991, 1994; Sackville Hamilton, 1994). The choice of the design in studying plant competition will depend upon the purpose of the experiment, the information available on the plants under study, and the resources of the experimenter.

The additive design is based on a constant density of one component while the density of the other is varied, resulting in different total densities across treatments. Its principal uses are in studying crop-weed competition. It is claimed to be simple, easy to interpret and closely resembling the actual conditions in the field. However, no mathematical models are available to quantify competitive relationships between components (Hall, 1978). Furthermore, the effects of interspecific competition are confounded by the effects of total plant density (Harper, 1977; Snaydon, 1991). The replacement design, which uses constant total plant density across treatments but with the relative frequencies varied (de Wit, 1960), attempts to prevent the confounding of density effects with those due to competition. It is regarded as the most elegant way to describe competition between two plant species (Harper, 1977). However, recent studies have also indicated that the relative yields (RYs) determined in grass-legume competition studies using replacement techniques are affected by total plant density and nutrient supply as well as interspecific competition (Firbanks and Watkinson, 1985; Austin *et al.*, 1988; Taylor and Aarssen, 1989). In the studies of Austin *et al.* (1988) and Taylor and Aarssen (1989), a pair of species at several densities were used. It was concluded that to study plant competition the density used should be one at which any further increase in density has no effect on yield.

However, in the experiments mentioned above, plants of similar species such as grasses were used, viz. annual grasses (Taylor and Aarssen, 1989) and annual vs biennial grasses (Austin *et al.*, 1988). Furthermore, these investigators harvested the plant material only once, thus preventing the possibility of studying the effects of density after defoliation, overtime. Menchaca and Connolly (1990) examined competition between perennial ryegrass and white clover using a replacement series design with several total densities. They found that perennial ryegrass markedly affected white clover performance at the first harvest, but that this effect (measured as reduced clover yield) declined at subsequent harvests. At the later harvests, increasing density of white clover resulted in higher yields of perennial ryegrass in mixtures than in monocultures at similar density. Most recently, Sackville Hamilton (1994) estimated that values of RY would be affected by density in both additive and replacement designs. Compared to the additive design, the replacement design of de Wit was suggested to be more appropriate for calculating competition indices (Relative Replacement Rates (RRR), Relative Yields (RY), Competition Ratio (CR) and Relative Yield Total (RYT)), as the replacement design makes it possible to distinguish among competitive exclusion, coexistence, stimulation or non-competitive inhibition (Hall, 1978). Despite this, no experiment to date has compared additive and replacement designs, at several total densities and over a number of harvests, for studying competition between grasses and legumes, which have different demands for soil nutrients (e.g. nitrogen). Such an experiment was required to choose an appropriate design and density and number of harvests for subsequent experiments in this project, so that the effects of competition in terms of RY and competitive ratio (CR) would not be confounded by the effects of density used.

The objectives of the experiment reported here were:

(1) to determine the effects of plant density and harvest number on the dry matter (DM) yields of white clover and perennial ryegrass grown in pots in monocultures and mixtures;

(2) to examine the effects of density and competition design (additive and replacement series) and harvest number on the competitive ability (measured as RY and CR) of white clover in mixtures with perennial ryegrass; and

(3) to study the effects of density on the accumulation of non-structural carbohydrates (NSC) in storage organs of white clover and perennial ryegrass in monocultures.

Irrigation white clover (*Trifolium repens* cv. Irrigation) and Ellet perennial ryegrass (*Lolium perenne* cv. Ellet) were used. In Experiment I, it has been shown that Ellet was a high yielding perennial ryegrass while Irrigation (medium-leaved white clover) had yield and NSC levels between those of Huia (medium-leaved white clover) and Olwen (medium large-leaved). Both Irrigation and Ellet are widely used in perennial ryegrass-white clover mixtures in Australia.

## 4.2 Materials and Methods

### 4.2.1 Sites and Soils

The experiment was conducted in a glasshouse using 24.0 cm diameter black plastic pots. Mean daily minimum and maximum temperatures in this part of the glasshouse during the experiment were 9.8 and 25.3 $^{o}C$, respectively, with humidity ranging from 39.0 to 92.0%. The soil used was a John Innes mixture comprising 5 parts of coarse sand, 5 parts of medium loam and 4 parts of peat moss. To each half cubic metre of this soil the following nutrients were added: blood meal 600 g, potassium sulphate 300 g, super phosphate 550 g and calcium carbonate 200 g. The pH of the soil was 6.0.

### 4.2.2 Treatments and Design

Treatments consisted of six monocultures of Ellet perennial ryegrass (densities of 4, 8, 12, 16, 20, 24 plants $pot^{-1}$), six monocultures of Irrigation white clover (densities of 4, 8, 12, 16, 20, 24 plants $pot^{-1}$) and eight 50:50 mixtures (2:2, 4:4, 6:6, 8:8, 10:10, 12:12, 16:16, 20:20 plants $pot^{-1}$), resulting in a total of 20 treatments. This arrangement resulted in six mixtures representing replacement series designs and five mixtures representing additive designs. The positions of plants in the pots in the mixtures are shown in Figure 4.1. The positions of plants in monocultures were similar to those in mixtures with the same total plant densities. The treatments were arranged on glasshouse benches in a randomized complete block design with three replications. There were four harvests of shoots. The characteristics of the cultivars used are presented in Table 3.1 (Chapter III). In the analysis of variance the four harvests were treated as repeated measurements within each density. The design thus took the form of a split plot design with harvests as split plots in time.

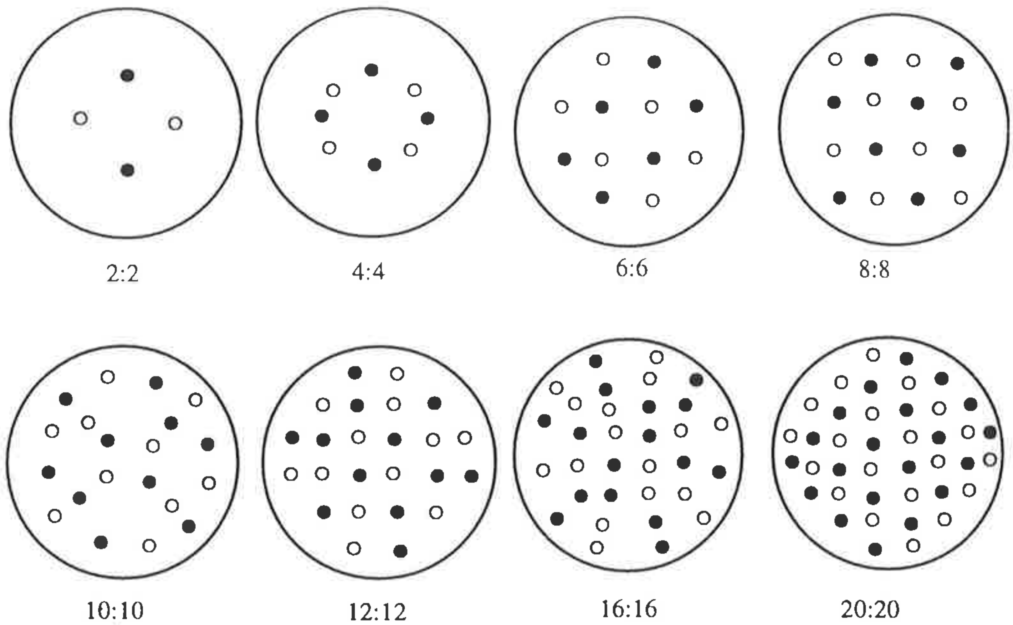



**Figure 4.1** Positions of white clover (O) and perennial ryegrass (●) across the pots in mixtures. In monocultures with the same total density, these positions were occupied by either white clover or perennial ryegrass.

### 4.2.3 Cultural Techniques

Clover seeds were obtained from Flaxley Experiment Station, from the National Clover Improvement Program at Glen Innes Experiment Station, Glen Innes, New South Wales. The seeds were sown on 5/5/92. Ten seeds of white clover and six seeds of perennial ryegrass (taking into account the germination percentage obtained prior to planting) were sown on each position. The seedlings were thinned to one plant per position at two to three weeks after sowing. A few transplantings were necessary during the first two weeks after emergence especially for white clover seedlings. Sufficient water was applied to prevent water stress. Other cultural techniques including inoculation, were similar to those described in Experiment I (Section 3.2.3). During the duration of the experiment, the pots were re-randomized at weekly intervals within each block. The distance between pots was very close, which minimized side lighting and ensured the occurrence of competition for light between grass and clover.

### 4.2.4 Observations and Measurements

After initial growth for eight weeks, the first harvest was taken on 2/7/92 by cutting the shoots 3.0 cm above the ground. The same cutting height was used for the second, third and fourth harvests which were made at five-weekly intervals on 5/8/92, 2/9/92 and 7/10/92. At each harvest, the grass shoots were separated from the legume shoots, and oven dried at 70 $^{o}$C for 48 hours. At the last harvest, stolons of white clover and stubble of perennial ryegrass were sampled for DM and non-structural carbohydrate determination. The procedures for sampling and carbohydrate analysis were presented in Section 3.2.3 and Appendix 3. RY, RYT and CR were calculated both for additive and replacement designs. Due to limitation in time and labour, carbohydrate measurements were conducted for monoculture only.

### 4.2.5. Statistical Analysis

Data were subjected to analysis of variance using Genstat 5 (Genstat 5 Committee, 1987), after appropriate transformation where necessary to stabilize the variance. Generally, a log10 or a square root transformation was sufficient. Mean separation was conducted following Duncan's Multiple Range Test (Steel and Torrie, 1960). When significant, the polynomial contrasts were calculated and correlations between density and other parameters were analysed. Analyses were done for RY, RYT (de Wit, 1960), and CR (de Wit *et al.*, 1966; Willey and Rao, 1980), for shoot DM in monocultures and mixtures and DM in storage organs (stolons or stubble). Stolon and stubble DM and NSC measured after destructive sampling at harvest 4 were analysed for each species, using a simple randomized block design with six densities and three replications.

## 4.3 Results

### 4.3.1. Shoot DM

The effects of treatments and treatment interactions on shoot DM in monocultures and mixtures are presented in Table 4.1. Interactions of density and harvest number were generally significant, except for shoot DM yield of white clover in mixtures.

**Table 4.1** The treatment main effects and interactions for shoot DM of white clover and perennial ryegrass in monocultures and mixtures

| Variables | White clover | | Perennial ryegrass | | Total |
|---|---|---|---|---|---|
| | Monoculture | Mixturc | Monoculture | Mixture | Mixture |
| Density (D) | *** | * | * | ns | *** |
| Harvest No. (H) | *** | *** | ** | *** | *** |
| D x H | *** | ns | ** | *** | * |

*, **, *** and ns indicate significant $P<0.05$, $P<0.01$ $P<0.001$ and non-significant ($P>0.05$), respectively.

#### (a) Monocultures

The density x harvest interaction for shoot DM of white clover (Figure 4.2 A) and perennial ryegrass (Figure 4.2 B) were highly significant ($P<0.01$). In white clover, shoot DM increased progressively from harvest 1 to harvest 4, but in perennial ryegrass it increased from harvest 1 to harvest 2, especially at lower densities, then decreased from harvest 2 to harvests 3 and 4.

Density had significant effects on both white clover and perennial ryegrass only at harvests 1 and 2. White clover showed an increase from lower to higher densities but there was no significant yield increase at densities above 16 plants pot$^{-1}$. In perennial ryegrass, there was a marked yield increase from lower to higher densities at harvest 1. However, at harvest 2, there was a significant decrease at the highest density.

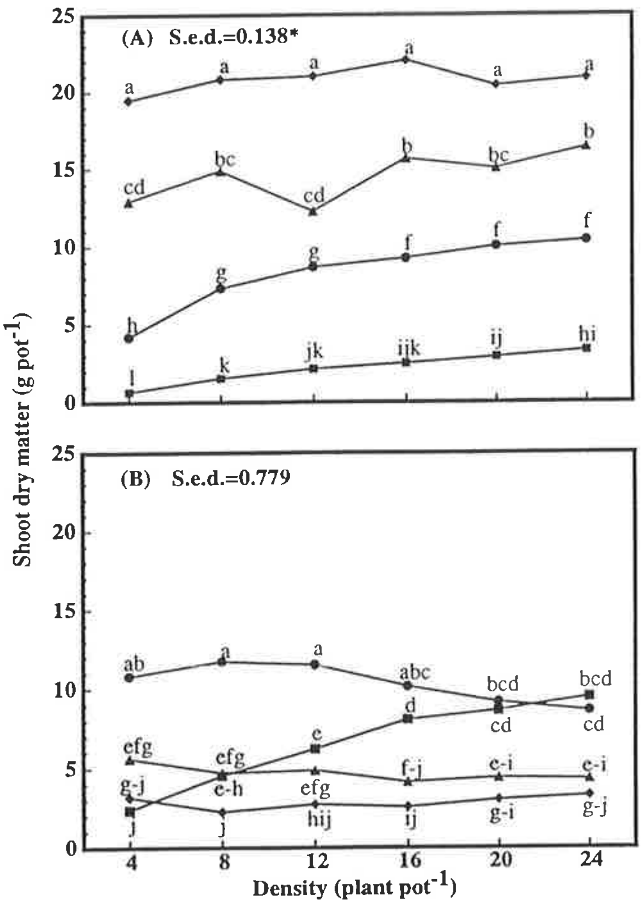



**Figure 4.2** The effects of density x harvest interactions on shoot DM (g pot$^{-1}$) of (A) white clover and (B) perennial ryegrass at H1 (■), H2 (●), H3 (▲), and H4 (◆) in monocultures. * After square root transformation. Data points within each graph associated with similar letters are not significantly different (P>0.05). e-i indicates efghi

Curves were fitted to the yield-density relationships for each species at each harvest. The quadratic relationships were found to give a better fit than the logistic and Mitscherlich relationships (Appendix 4.1).

### (b) Mixtures

There were significant main effects of density (P<0.05) and harvest (P<0.01) on shoot DM yield of white clover in mixtures (Table 4.1). Mixed pots with densities in mixtures above 2 plants pot$^{-1}$ had similar yields of clover (Figure 4.3), and yield increased at later harvests (Table 4.2).

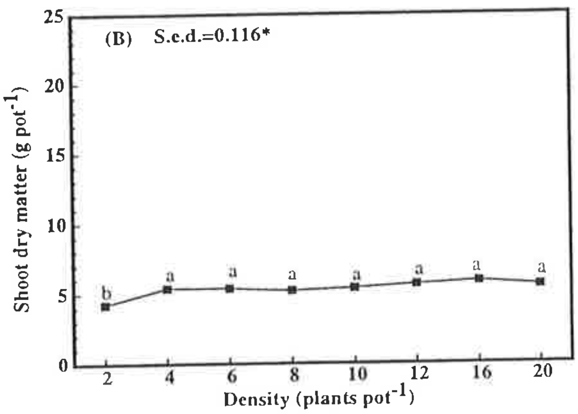



**Figure 4.3** The main effect of density on shoot DM yield of white clover in mixtures. Data points in each graph associated with the same letter are not significantly different (P>0.05).

The density x harvest interaction for shoot DM of white clover in mixtures (Figure 4.4A) was non-significant (P>0.05); however, it is presented to show levels of individual harvest yields and for comparison with shoot DM of perennial ryegrass in mixtures (Figure 4.4B) where the interaction was significant (P<0.05). Shoot DM yields of white clover and perennial ryegrass in mixtures showed similar trends to those in monocultures (Figure 4.2), especially in perennial ryegrass where yields in mixtures were slightly lower than in monocultures. In the case of white clover, yields were considerably lower in mixtures than monocultures and there was no significant effect of density above 4 plants pot$^{-1}$.

**Table 4.2** The main effect of harvest number on shoot DM of white clover in mixtures

| Harvest No. | Shoot DM (g pot$^{-1}$) |
|---|---|
| H1 | 1.32$^{d}$ |
| H2 | 2.67$^{c}$ |
| H3 | 5.72$^{b}$ |
| H4 | 11.62$^{a}$ |

Means associated with the same letter are not significantly different (P>0.05)

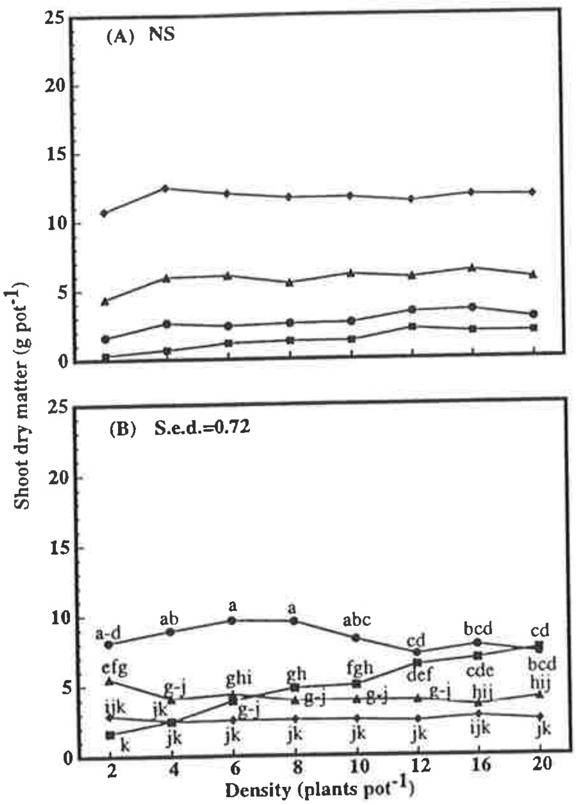



**Figure 4.4** The effects of density x harvest interactions on shoot DM (g $pot^{-1}$) of (A) white clover and (B) perennial ryegrass at H1 (■), H2 (●), H3 (▲), and H4 (◆) in mixtures. * After square root transformation. Data points in each graph with letters in common are not significantly different (P>0.05). g-j indicates ghij

### (c) Total DM yield in mixtures

The density x harvest interaction for total shoot DM yield in mixtures (Table 4.1) was significant (P<0.05). An increase in density increased the total shoot DM yield in mixtures (Figure 4.5) only at harvest 1, but afterwards there were no effects of density on yields.

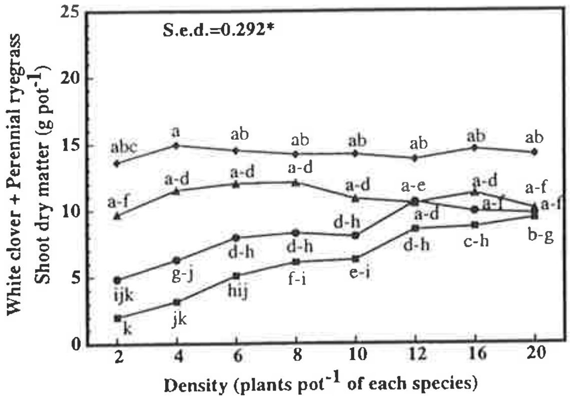



**Figure 4.5** The effects of density x harvest interactions on total shoot DM (g pot$^{-1}$) of mixtures at H1 (■), H2 (●), H3 (▲), and H4 (◆). * After square root transformation. Data points in each graph with letters in common are not significantly different (P>0.05). d-g indicates defg

## (d) Comparison of yields of species and of monocultures and mixtures

Shoot DM in monocultures and in mixtures in replacement diagrams (Figure 4.6) showed that at harvest 1, total yield of the mixtures were between the monoculture yields of ryegrass and clover, with ryegrass yields highest. Total yields in mixtures were higher than monoculture yields of both species only at harvest 2 when yields of the two monocultures were similar and the yields of one of the species in mixtures was almost as high as in monoculture. At harvests 3 and 4 yields of white clover monocultures were much higher than perennial ryegrass monocultures. Yields of the mixtures were then intermediate between yield of two monocultures. Perennial ryegrass yields were low but similar in mixtures and in monocultures; whereas yields of white clover were much lower in mixtures than in monocultures. In mixtures, perennial ryegrass generally yielded more than white clover up to harvest 2, but the reverse was true at subsequent harvests.

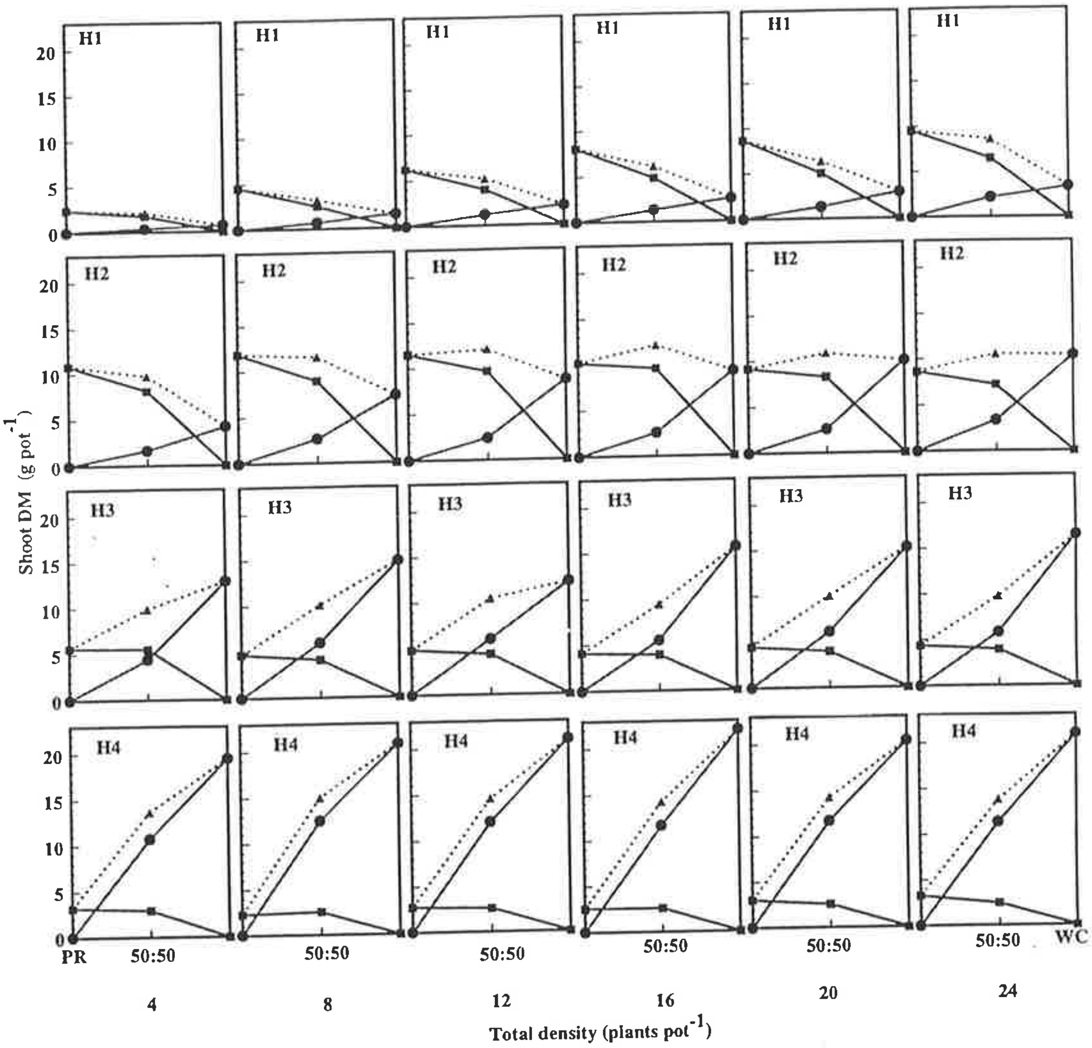



**Figure 4.6** Shoot DM (gpot$^{-1}$), in replacement design, of monocultures of perennial ryegrass (PR) and white clover (WC) and in 50:50 mixtures, at harvest 1 (H1) to harvest 4 (H4), at the total density of 4, 8, 12, 16, 20, and 24 plants pot$^{-1}$. (■ PR, ● WC and ▲ total DM).

The de Wit diagrams also show that perennial ryegrass was usually more competitive during the first two harvests, with suppression (reduction from the expected yield) of white clover prominent at harvest 2. White clover was more competitive during the last two harvests. de Wit's diagrams indicate that perennial ryegrass yield was hardly reduced at all by white clover.

The diagram sequence shows that, although marked changes in competitive relationships occurred from harvest to harvest, there was little if any effect of density within harvests.

### 4.3.2 RY, RYT and CR in Replacement and Additive Designs

Harvest had highly significant effects (P<0.001) on all variables, in both replacement and additive designs (Table 4.3), but the effects of density were significant (P<0.05) only for white clover RY in the additive design. The density x harvest number interaction was significant only for RY of white clover in the replacement design (P>0.05).

**Table 4.3** The effects of treatments and interaction on RY, RYT, and CR of shoot of white clover (wc) and perennial ryegrass (pr) in (A) replacement and (B) additive designs

| Variables | (A) Replacement design | | | |
|---|---|---|---|---|
| | RYwc | RYpr | RYT | CR (RYwc/RYpr) |
| Density (D) | ns | ns | ns | ns |
| Harvest No. (H) | *** | *** | * | *** |
| D x H | * | ns | ns | ns |
| | (B) Additive design | | | |
| Density (D) | * | ns | ns | ns |
| Harvest No. (H) | *** | * | *** | *** |
| D x H | ns | ns | ns | ns |

*, *** and ns indicate significant P<0.05, P<0.001 and non-significant (P>0.05), respectively.

(a) **Relative yield**

**RY of white clover in replacement design**. In white clover, the density x harvest interaction was significant (P<0.05) (Table 4.4A). However, there are very few significant differences and no clear trend for RY to vary with total plant density.

**RY of white clover in additive design**. The density x harvest interaction for RY of white clover was non-significant (P>0.05); however, for comparative purposes, this interaction is presented in Table 4.4B. RYs of white clover in the additive design (Table 4.4B) appeared clearly higher than those of the replacement design only at harvest 1 at all densities and at harvest 2 at the lowest density (i.e. 4 plants pot$^{-1}$). (See also Table 4.7).

The main effects of density and harvest on RY of white clover were significant

($P<0.05$) (Table 4.3). Over all harvests, RY of white clover was highest at a density of 4 plants $pot^{-1}$ (Table 4.6) with no significant differences among the other densities. Over all densities, RY of white clover was highest at harvest 1, similar at harvests 2 and 3 and increased again at harvest 4 (Table 4.7).

**Table 4.4** The effects of density x harvest number (H) interaction on RY of shoot DM of white clover in (A) replacement and (B) additive design

| Monoculture density (plants $pot^{-1}$) | (A) Replacement design | | | |
|---|---|---|---|---|
| | H1 | H2 | H3 | H4 |
| 4 | $0.51^{abcd*}$ | $0.39^{def}$ | $0.34^{ef}$ | $0.56^{ab}$ |
| 8 | $0.45^{bcde}$ | $0.36^{cdef}$ | $0.40^{cdef}$ | $0.60^{a}$ |
| 12 | $0.56^{ab}$ | $0.28^{f}$ | $0.53^{abc}$ | $0.57^{ab}$ |
| 16 | $0.51^{abcd}$ | $0.28^{f}$ | $0.35^{ef}$ | $0.53^{abc}$ |
| 20 | $0.52^{abcd}$ | $0.26^{f}$ | $0.40^{cdef}$ | $0.57^{ab}$ |
| 24 | $0.64^{a}$ | $0.32^{ef}$ | $0.35^{ef}$ | $0.54^{ab}$ |
| S.e.d | 0.081** | | | |
| (plants pot- | (B) Additive design | | | |
| 4 | 1.02 | 0.63 | 0.47 | 0.64 |
| 8 | 0.83 | 0.35 | 0.37 | 0.56 |
| 12 | 1.00 | 0.39 | 0.45 | 0.54 |
| 16 | 0.76 | 0.38 | 0.40 | 0.53 |
| 20 | 0.66 | 0.28 | 0.38 | 0.59 |

*Means in each column in each design followed by the same superscripts are not significantly different ($P>0.05$). **After square root transformation

**Table 4.5** The effects of density x harvest number (H) interactions* on RY of shoot DM of perennial ryegrass in (A) replacement and (B) additive design

| Monoculture density (plants pot$^{-1}$) | (A) Replacement design | | | |
|---|---|---|---|---|
| | H1 | H2 | H3 | H4 |
| 4 | 0.70 | 0.75 | 0.99 | 1.00 |
| 8 | 0.54 | 0.75 | 0.88 | 1.09 |
| 12 | 0.63 | 0.83 | 0.93 | 0.95 |
| 16 | 0.60 | 0.96 | 0.94 | 1.02 |
| 20 | 0.58 | 0.90 | 0.89 | 0.86 |
| 24 | 0.67 | 0.83 | 0.96 | 0.77 |
| (plants pot- | (B) Additive design | | | |
| 4 | 1.06 | 0.83 | 0.74 | 0.78 |
| 8 | 1.05 | 0.80 | 0.83 | 1.13 |
| 12 | 1.03 | 0.62 | 0.8 | 0.91 |
| 16 | 0.86 | 0.79 | $0.87^{a}$ | 1.13 |
| 20 | 0.87 | 0.79 | 0.95 | 0.83 |

* The interactions are not significantly different (P>0.05).

**Table 4.6** The main effects of density on RY of white clover in additive design

| Monoculture density (plants pot$^{-1}$) | RY |
|---|---|
| 4 | $0.69^{a*}$ |
| 8 | $0.53^{b}$ |
| 12 | $0.59^{ab}$ |
| 16 | $0.52^{b}$ |
| 20 | $0.48^{b}$ |
| S.e.d | 0.047 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05)

**Table 4.7** The main effect of harvest on RY of white clover in replacement and additive designs

| Harvest No. | RY of white clover additive design | RY of white clover replacement design |
|---|---|---|
| 1 | $0.53^{a}$ | $0.85^{a}$ |
| 2 | $0.31^{a}$ | $0.41^{c}$ |
| 3 | $0.40^{a}$ | $0.41^{c}$ |
| 4 | $0.56^{a}$ | $0.57^{b}$ |
| S.e.d | 0.024 | 0.041 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05)

**RY of perennial ryegrass in replacement design**. The main effect of harvest on RY of perennial ryegrass was highly significant (P<0.01) but the density x harvest interaction for RY (Table 4.5) was non-significant (P>0.05).

**Table 4.8** The main effect of harvest on RY of perennial ryegrass in replacement and additive designs

| Harvest No. | RY of ryegrass replacement design | RY of ryegrass additive design |
|---|---|---|
| 1 | 0.62[b]* | 0.97[a]* |
| 2 | 0.84[a] | 0.77[b] |
| 3 | 0.93[a] | 0.84[ab] |
| 4 | 0.95[a] | 0.96[a] |
| S.e.d | 0.062 | 0.069 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05)

RY of perennial ryegrass was lowest at harvest 1 (Table 4.8), and was similar for harvests 2 to 4.

**RY of perennial ryegrass in additive design**. The main effect of harvest on RY of perennial ryegrass was also significant (P<0.05) (Table 4.3). RYs of perennial ryegrass were highest at harvests 1 and 4 (Table 4.8). Only at harvest 1 did there appear to be a clear difference between additive and replacement designs ie. RY was higher in the additive design.

**(b) Competitive ratio of white clover with respect to perennial ryegrass**

In the replacement series, the main effect of harvest on CR was highly significant (P<0.001) (Table 4.9), but the overall density effect and density x harvest interaction were not significant (Table 4.3). The results for the additive design were very similar to those of replacement design (Table 4.9). The changes in CR from harvest to harvest (a decrease followed by an increase) and the lack of significant density effects are also illustrated by Figure 4.6

**Table 4.9** The main effects of harvest on CR of white clover with respect to perennial ryegrass in (A) replacement and (B) additive design

| Harvest No. | (A) Replacement design | (B) Additive design |
|---|---|---|
| 1 | 0.85[a*] | 0.89[a] |
| 2 | 0.38[c] | 0.54[c] |
| 3 | 0.47[c] | 0.54[c] |
| 4 | 0.63[b] | 0.64[b] |
| S.e.d | 0.08** | 0.069 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05). ** After square root transformation

### (c) Relative yield total

In each design, there was a significant harvest effect (Table 4.3). Time trends are very similar for the two designs (Table 4.10), although the additive design gave a much higher overall value at harvest 1 probably because of the higher overall density than in replacement series. The fact that RYT values exceeded 1 indicates that, overall the two species were not mutually exclusive, a result of N-fixation in the legume. However, the values at harvests 1 and 2 may not have been significantly greater than 1, indicating that they were mutually exclusive at that time, when N-fixation had been operating for only a short time.

**Table 4.10** The main effect of harvest on RYT in replacement and additive designs

| Harvest No. | Replacement | Additive |
|---|---|---|
| | RYT | |
| 1 | 1.14$_b$ | 1.83$_a$ |
| 2 | 1.15$_b$ | 1.17$_c$ |
| 3 | 1.33$_a$ | 1.25$_c$ |
| 4 | 1.37$_a$ | 1.53$_b$ |
| S.e.d | 0.08 | 0.078 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05)

### 4.3.3 Stolon and Stubble DM in Monocultures and Mixtures

Density had no significant effects (P>0.05) on stolon and stubble DM in monocultures (Table 4.11A) or in mixtures at the final harvest (Table 4.11B). Mean yields of stolons in the mixtures were less than half yields in white clover monocultures. In contrast yields of ryegrass stubble in the mixtures were only a little less than yields in the monocultures.

**Table 4.11** The main effects of density on DM (g pot$^{-1}$) of white clover stolons and perennial ryegrass stubble at final harvest in (A) monocultures and (B) in mixtures

| (A) Monoculture density (plants pot$^{-1}$) | Stolon DM (g pot$^{-1}$) | Stubble DM (g pot$^{-1}$) |
|---|---|---|
| 4 | 14.78$^{a*}$ | 6.07$^{a}$ |
| 8 | 14.81$^{a}$ | 5.31$^{a}$ |
| 12 | 15.28$^{a}$ | 6.03$^{a}$ |
| 16 | 15.04$^{a}$ | 5.61$^{a}$ |
| 20 | 13.42$^{a}$ | 5.85$^{a}$ |
| 24 | 14.02$^{a}$ | 6.12$^{a}$ |
| Mean | 14.56 | 5.83 |
| S.e.d | 1.126 | 0.357 |

| (B) Density in mixtures of each species (plants pot$^{-1}$) | Stolon DM (g pot$^{-1}$) | Stubble DM (g pot$^{-1}$) |
|---|---|---|
| 2 | 4.99$^{a}$ | 4.19$^{a}$ |
| 4 | 5.65$^{a}$ | 4.35$^{a}$ |
| 6 | 5.78$^{a}$ | 4.31$^{a}$ |
| 8 | 5.73$^{a}$ | 4.55$^{a}$ |
| 10 | 6.23$^{a}$ | 4.48$^{a}$ |
| 12 | 4.40$^{a}$ | 3.74$^{a}$ |
| 16 | 5.16$^{a}$ | 4.52$^{a}$ |
| 20 | 5.22$^{a}$ | 4.52$^{a}$ |
| Mean | 5.40 | 4.33 |
| S.e.d | 0.697 | 0.424 |

*Means within the same column in the same table followed by the same superscripts are not significantly different (P>0.05)

### 4.3.4 Non-structural Carbohydrates in Stolons and Stubble in Monocultures

The main effects of density on concentration and content of soluble sugars, starch and total NSC in the stolons of white clover were non-significant (P>0.05) (Table 4.12 and 4.13), except for a higher starch concentration and content in the stolons at a density of 12 plants pot$^{-1}$.

**Table 4.12** The effects of density (plants pot$^{-1}$) on NSC pools of white clover stolons

| Monoculture Density | Concentration (mg g$^{-1}$) | | | Content (mg pot$^{-1}$) | | |
|---|---|---|---|---|---|---|
| (plants pot$^{-1}$) | Sugar | Starch | Total NSC | Sugar | Starch | Total NSC |
| 4 | 136$^{a*}$ | 27.0$^{b}$ | 153$^{a}$ | 2010$^{a}$ | 399$^{d}$ | 2409$^{a}$ |
| 8 | 186$^{a}$ | 29.2$^{b}$ | 191$^{a}$ | 2755$^{a}$ | 432$^{abc}$ | 3187$^{a}$ |
| 12 | 195$^{a}$ | 44.7$^{a}$ | 239$^{a}$ | 2980$^{a}$ | 683$^{a}$ | 3663$^{a}$ |
| 16 | 177$^{a}$ | 29.6$^{b}$ | 207$^{a}$ | 2726$^{a}$ | 445$^{ab}$ | 3171$^{a}$ |
| 20 | 168$^{a}$ | 22.1$^{bc}$ | 190$^{a}$ | 2255$^{a}$ | 297$^{bcd}$ | 2552$^{a}$ |
| 24 | 152$^{a}$ | 15.7$^{c}$ | 161$^{a}$ | 2131$^{a}$ | 220$^{cd}$ | 2351$^{a}$ |
| S.e.d | 35.8 | 0.075** | 48.9 | 421 | 2.62** | 587.8 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05). ** After log10 transformation

However, there were significant quadratic relationships between density (X) and NSC (Y) (Table 4.13) with $r^2$ ranging from 0.56 - 0.83. These curves are in agreement with apparent peaks in values at 12 - 14 plants pot$^{-1}$.

**Table 4.13** The relationships between plant density (X) and the concentration (mg g$^{-1}$) and content (mg pot$^{-1}$) of NSC (Y) in the stolons of white clover, and the density at which NSC peaked as predicted by the curves

| NSC | Relationships | | $r^2$ | NSC peaked at density |
|---|---|---|---|---|
| Sugars | (mg g$^{-1}$) | $Y=101.2+12.62X - 0.45X^2$ | 0.66* | 14.0 |
| | (mg pot$^{-1}$) | $Y=2039.8+129.5X - 5.44X^2$ | 0.83* | 11.9 |
| Starch | (mg g$^{-1}$) | $Y=14.6+3.60X - 0.1529X^2$ | 0.56* | 11.8 |
| | (mg pot$^{-1}$) | $Y=-208.8+56.08X - 2.40X^2$ | 0.71* | 11.7 |
| Total NSC | (mg g$^{-1}$) | $Y=105.5+16.71X - 0.608X^2$ | 0.73* | 13.5 |
| | (mg pot$^{-1}$) | $Y=1646.7+262.8X - 10.1X^2$ | 0.78* | 13.0 |

* and ** indicate significant P<0.05 and P<0.01, respectively.

The effects of density on NSC in stubble of perennial ryegrass were non-significant (P>0.05) (Table 4.14). The relationships between density and NSC were quadratic (Table 4.15) but mostly non-significant and with low $r^2$ values.

**Table 4.14** The effects of density on NSC pools in perennial ryegrass stubble in monocultures

| Density | Concentration ($mg\ g^{-1}$) | | | Content ($mg\ pot^{-1}$) | | |
|---|---|---|---|---|---|---|
| (plants $pot^{-1}$) | Sugar | Starch | Total NSC | Sugar | Starch | Total NSC |
| 4 | $91.9^{a*}$ | $143.8^{a}$ | $236^{a}$ | $562^{a}$ | $880^{a}$ | $1442^{a}$ |
| 8 | $99.5^{a}$ | $148.7^{a}$ | $248^{a}$ | $530^{a}$ | $788^{a}$ | $1318^{a}$ |
| 12 | $78.4^{a}$ | $121.4^{a}$ | $200^{a}$ | $457^{a}$ | $735^{a}$ | $1210^{a}$ |
| 16 | $97.8^{a}$ | $144.5^{a}$ | $242^{a}$ | $537^{a}$ | $803^{a}$ | $1341^{a}$ |
| 20 | $85.3^{a}$ | $107.7^{a}$ | $193^{a}$ | $499^{a}$ | $635^{a}$ | $1134^{a}$ |
| 24 | $80.2^{a}$ | $107.1^{a}$ | $187^{a}$ | $486^{a}$ | $666^{a}$ | $1152^{a}$ |
| S.e.d | 18.36 | 29.93 | 37.4 | 111.5 | 188.5 | 239.7 |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05).

**Table 4.15** The relationships between plant density (X) and the concentration ($mg\ g^{-1}$) and content ($mg\ pot^{-1}$) of NSC (Y) in stubble of perennial ryegrass

| NSC | | Relationships | $r^2$ |
|---|---|---|---|
| Sugars | ($mg\ g^{-1}$) | $Y=92.2+0.33\ X - 0.032\ X^2$ | 0.05 ns |
| | ($mg\ pot^{-1}$) | $Y=59.3-10.2\ X + 0.262\ X^2$ | 0.12 ns |
| Starch | ($mg\ g^{-1}$) | $Y=146.3+0.02\ X - 0.073\ X^2$ | 0.42 ns |
| | ($mg\ pot^{-1}$) | $Y=-92.3-15.3\ X - 0.173\ X^2$ | 0.57* |
| Total NSC | ($mg\ g^{-1}$) | $Y=238.8 + 0.31\ X - 0.105\ X^2$ | 0.25 ns |
| | ($mg\ pot^{-1}$) | $Y=1506- 23.2\ X - 0.35\ X^2$ | 0.50* |

* and ns indicate significant P<0.05 and non-significant (P>0.05), respectively.

## 4.4 Discussion

The confinement of the density response of each species in monoculture, to the first and second harvests and to the lower densities illustrates a common result of growing plants from seed.

Young plants need a relatively high density to make full use of resources of nutrients, water and light. However, as they become larger, the density required for the full use of resources becomes less, and there is no response to higher densities.

Growing the plants in mixtures did not change the density response pattern, although the response was less pronounced especially in white clover. Thus, because the yield of white clover was greatly reduced by association with perennial ryegrass, the density effects were also much smaller at later harvests than at harvest 1.

The reduced effects of density in the mixture after harvest 1 show themselves as an almost constant level of total yield of the mixture regardless of density at any one harvest. This is in contrast to the increase in yields of white clover with each successive harvest (as the plants become larger) and the decreasing yield of perennial ryegrass with time (as soil nitrogen is depleted). The consequence of these changes is that after harvest 1, the combined yield of clover and grass in the mixtures did not change markedly with time. In view of the above results, it is therefore not surprising that the indices of competition did not respond significantly to density changes, although in contrast, there were significant differences between harvests, which were caused by changes in relative yields of each species.

Nevertheless there were indications that, in order to minimize density effects from the first harvest onwards, an optimum density could be chosen. A density of 16 plants $pot^{-1}$ for monocultures and 8 of each species in mixtures fits these criteria, and will be used in the next experiment, although the exact number of 16 plants $pot^{-1}$ did not appear critical. Higher numbers than 16 plants $pot^{-1}$ creates practical difficulties in precise sowing and transplanting if required for uniformity of the population throughout the pot.

This experiment also helps provide understanding of the dynamics of competitive grass-legume relationships, which will be referred to in association with later experiments. The replacement series diagrams (Figure 4.6) illustrate these dynamics and are supported by the data for RY and CR. It is clear that white clover and ryegrass are not suppressed at the first harvest, apparently because competition between the two species had hardly begun. At the second harvest white clover yields were clearly depressed below expected values while perennial ryegrass yields were only a little lower than monoculture yields. Thus ryegrass was at a competitive advantage, as shown by the low values (less than 1) of CR (Table 4.9). The subsequent increases in CR from harvest 2 and 3 to harvest 4 i.e. an increase in the competitive ability of white clover were achieved by increases in RY of white clover (Table 4.4) concurrent with maintenance of high values in perennial ryegrass (Table 4.5). This period (Harvests 2-4) would appear to be a suitable one over which to study the relationship between the levels of NSC and CR. This was done in subsequent experiments.

The significant curvilinear relationship between white clover NSC levels (Y) and density (X) with apparent maxima of NSC levels at densities of around 12-14 plants $pot^{-1}$ (Table 4.13) and the smaller effect of density response in the levels of NSC in perennial ryegrass monocultures supports the conclusions drawn from DM yield data that total plant density will not be a factor in determining the results of subsequent experiments to study the relation of NSC levels and competition above the density of 12-14 plant $pot^{-1}$.

Neither replacement nor additive design led to significant differences in density effects ($P>0.05$) on RY and CR provided that the total monoculture density of 16 plants $pot^{-1}$ was used.

White clover and perennial ryegrass have some degree of complementarity of resources (especially nitrogen) which might lead to coexistence (Evans *et al.*, 1985; Turkington, 1989; Turkington and Harper, 1979). This resulted in RYT generally greater than unity (Table 4.3, 4.10) which might also cause no significant effects of density on their CR.

Both stolon and stubble DM yields per pot at the end of the experiment were independent of density, which is consistent with shoot yield data. NSC content in stolons peaked at a density of about 12 plant $pot^{-1}$ (Table 4.12) which coincided with highest shoot DM yield (Figure 4.3 and Figure 4.5). This may indicate that complete surface cover had been achieved at this density. Hence, competition for light was also occuring at this density . The significant curvilinear relationships between density and NSC suggests that the balance between the NSC storage and utilization is density dependent. The reduction in NSC content at the higher densities is probably due to respiration of shaded leaves which are not photosynthesizing. In ryegrass stubbles, non-structural carbohydrate concentration and content were similar ($P>0.05$) across densities (Table 4.15) which might also indicate that perennial ryegrass was limited by nitrogen in the soil. Nitrogen deficiency will limit leaf growth and reduce photosynthesis rates, resulting in similar NSC levels across densities.

These results suggested that to examine morphological traits and carbohydrate storage as the possible mechanisms affecting competitive ability of white clover, studies should be directed to these periods (Harvest 2-4). Either replacement design or additive design is appropriate, since relative yields and competitive ratio were independent of plant density above 12 plant $pot^{-1}$ in both designs (Table 4.13). However, as the competitive ratio (which will be used throughout this thesis) as measured in the replacement design was independent of (initial) density (disproving the notion that in replacement design competition effects are confounded with density effects), replacement design is preferable (due to the availability of mathematical formula). This is in accordance with Sackville Hamilton (1994) who pointed out that replacement design was more appropriate in studying coexistence, competitive exclusion and competitive ability of plants.

## 4.5 Conclusions

1. Density had significant effects on shoot DM and RY of white clover and perennial ryegrass only during initial growth (harvest 1). During the following regrowth, no effects of (initial) densities on shoot growth, RY, RYT and CR were evident.

2. Suppression (reduction from the expected yield) of white clover was evident at harvest 2. Suppression diminished at subsequent harvests as shown by RY values. Athough pėrennial ryegrass growth was limited by soil nitrogen, it was never suppressed by white clover. Its RY values were also close to 1 at harvest 3 and 4.

3. The use of thc replacement experiment and the additive experiment gave almost the same results in terms of RYs and CRs. The only difference was during early growth when mixtures in the additive design led to slightly higher shoot DM yields and RY's compared to the replacement design. To exclude the effects of density from that of competition, and based on DM yield data, both replacement and additive designs were suitable for studying competitive abilities of components in mixtures provided the measurements were focused at harvest 2 at a total density above 8 plants $pot^{-1}$.

4. The densities used, at the frequency and intensity of defoliation applied, did not cause significant differences in pools of NSC. In stubble, NSC pools were similar across all densities used. Significant culvilinear relationships of density (X) and NSC pools (Y) in stolons of white clover indicated that density of 12-14 plant $pot^{-1}$ would give maximum NSC pools, above these densities the NSC pools declined, which might indicate intense competition.

5. Hence to study NSC as a possible determinant of competitive ability, competitive relationships must be studied over at least two harvests at densities above 12 plants $pot^{-1}$·in monocultures. Replacement design is to be preferred because of the value of the diagrams to illustrate RY and CR results, and the ease of comparison of results with published data.

# Chapter V

## Experiment III. The Effects of Morphological Traits and Non-Structural Carbohydrate Reserves on Competitive Ability of White Clover (*Trifolium repens* L.) with Respect to Perennial Ryegrass (*Lolium perenne* L)

### 5.1 Introduction

In white clover-perennial ryegrass swards, maintaining white clover content of up to 50% on the dry weight basis has been suggested should be the objective of management strategies (Mason, 1993), if a high animal productivity is to be achieved. However, this objective seems to be difficult to achieve due to the decline of white clover content in mixtures over time. A recent survey of farm situations in Britain, New Zealand and Australia showed that long term white clover-perennial ryegrass mixtures rarely had as much as 20 per cent clover, and some contained as low as two per cent (Frame and Newbould, 1986).

In mixtures with grasses, white clover growth and competitive ability depend on nitrogen levels in the soil. High nitrogen levels promote grass growth leading to the competitive suppression of white clover. Low nitrogen levels in the soils reduce grass growth and release white clover from competitive suppression. However, cultivars of white clover have been shown to differ in their ability to compete with perennial ryegrass in the presence of high nitrogen levels in the soil, and medium-to large-leaved cultivars have been shown to be more tolerant (Wilman and Asiegbu, 1982a,b; Goodman and Collison, 1986). The biological bases of differential responses are not clear.

Experiments have been conducted to identify plant traits that contribute to the superior competitive ability of plants in mixtures. One such approach was to construct regression analyses between plant traits ( e.g. height, biomass) and competitive ability when comparing cultivars (Breese and Hill, 1973; Willey and Rao, 1980; Mather *et al.*, 1982; Gaudet and Keddy, 1988). Willey and Rao (1980), based on de Wit's replacement technique, calculated competitive ratios (CR) which are the ratios of RYs of the two components, and correlated these ratios with plant traits. They found significant linear regressions of CRs and plant traits such as height and days required to reach maturity. While Willey and Rao (1980) intended CR to be used for studying competition in crop intercropping, it is also relevant in pasture studies (de Wit *et al.*, 1966).

Gaudet and Keddy (1988) found a strong multiple linear relationship between plant traits (biomass and height) and competitive ability (Section 2.1.3b) defined as relative ability of test species to reduce the growth of a common phytometer (indicator species). The above ground biomass of the test plants was inversely related to the biomass of the phytometer, while other traits (leaf area, shoot:root ratio) were not related.

Studies on white clover-perennial ryegrass associations have proposed morphological traits that possibly relate to their performances in mixtures. These, for white clover, include leaf size, stolon branching, and number of growing points (Caradus *et al.*, 1991; Caradus and Mackay, 1991). Many workers have reported that white clover had higher specific leaf area (SLA) compared to perennial ryegrass (Poorter and Remkes, 1990; Parsons *et al.*, 1991 and Barthram and Grant, 1994) which would mean a greater return of photosynthates per unit of carbon invested in new leaf areas (Barthram and Grant, 1994). It has also been suggested that this might support the contention of strong correlation between SLA and success of white clover in mixtures. However, the importance of SLA would depend on the degree of light interception by the leaves.

Non-structural carbohydrate (NSC) reserves in plants are used in regrowth and for maintenance respiration in the absence of current assimilates after defoliation (Baur-Höch *et al.*, 1990), and may influence competitive abilities of the plants (Grime, 1979; Braakhekke, 1980; Berendse and Elberse, 1990). Most studies to provide evidence of the remobilization of NSCs in regrowth after defoliation have been conducted in monocultures (Davies, 1966; Davidson, 1969b; King *et al.*, 1979; Fulkerson *et al.*, 1994). In such studies, levels of NSCs were varied by pre-treatments involving shading, variation in frequency and intensity of defoliation, exposing the plants to air with different $CO_2$ concentration, or combinations of those approaches. The results of those experiments confirmed the notion that NSCs are important, at least during the initial few days, for regrowth after defoliation in plants in monocultures (King *et al.*, 1979).

It has also been suggested that in plants exposed to stress (moisture stress, nutrient deficiencies, low light intensities due to cloud cover, cold temperatures) and disturbances (continuous or hard grazing, defoliation) the reserves play an important role and may affect their competitive abilities (Grime, 1979). For instance, nitrogen deficiency has been shown to cause an accumulation of NSC in plants, and this is mobilized if deficiency is corrected (Henry and Raper, 1991; Qiu and Israel, 1994). Despite this no experiments to date specifically examine the effects of NSCs on competitive relations in white clover-perennial ryegrass mixtures.

The experiment reported here was designed to:

(1) examine whether or not cultivars of white clover which grow more vigorously in monocultures during vegetative growth will have a higher competitive ability in mixtures with perennial ryegrass;

(2) examine if cultivars of white clover with higher NSC in monoculture grow more vigorously than cultivars with lower NSC;

(3) investigate whether or not cultivars of white clover that accumulate higher levels of NSCs in mixture before defoliation will have a higher competitive ability during regrowth following defoliation; and

(4) examine the relative importance of morphological traits measured at the same time as NSC compared to NSC reserves in conferring higher competitive ability on white clover in mixtures.

The results of experiment I were used to select cultivars possessing different NSC concentration and content. The objective 4 above will be assessed by regression analyses relating plant traits and levels of NSCs to CRs and comparing the correlation coefficients to identify traits that contributed most to competitive abilities (Willey and Rao, 1980).

## 5.2 Materials and Methods

### 5.2.1 Sites and Soil

Pots and soil used were the same as used in experiment II (Section 4.2.1). During the first half of the experiment, the pots were kept on the benches outside in the open (Plate 1) because of inadequate space in the glasshouse, but during the second half, when half of the plants had been destructively harvested, the remaining pots were kept on benches in a glasshouse.

### 5.2.2 Treatments and Design

Three cultivars of white clover and two cultivars of perennial ryegrass were grown as monocultures and as the 50:50 mixtures, with four replications. These plant combinations were subjected to two destructive harvests to make a total of 88 pots, arranged in a randomized complete block design. The cultivars were chosen to provide a range of levels of NSC in storage organs as found in Experiment I and shown in Table 5.1.

**Plate 5.1.** Overview of experiment III when the pots were put outside on the benches

### 5.2.3 Cultural Techniques

Cultural techniques were as described in experiment II (Chapter IV). In experiment II (Chapter IV) it was found RYs and CR were not affected by density above about 8 plants pot$^{-1}$. Hence, to study NSCs as a possible mechanism affecting competitive ability, the total density used in this experiment was 16 plants pot$^{-1}$.

**Table 5.1.** Characteristics of cultivars of perennial ryegrass and white clover used in the experiment, in terms of NSC content (mg pot$^{-1}$) and leaf-size

| Cultivars | NSC content* and leaf size |
|---|---|
| Perennial ryegrass | |
| 1. Ellet | The same NSC content as Kangaroo Valley |
| 2. Kangaroo Valley | The same NSC content as Ellet |
| White clover | |
| 3. Huia | Low NSC content, medium leaf size |
| 4. Irrigation | Medium NSC content, medium leaf size |
| 5. Olwen | Medium NSC content, medium-large leaf size |
| *based on results of experiment I (Chapter III). | |

The experiment was sown on 30/9/92. The first, non-destructive harvest (H1) was made on 25/11/92. Regrowth was allowed for four weeks before the first destructive harvest (H2) on 23/12/92 of half of the pots. The second non-destructive harvest was taken (H3) from the remaining plants after a four week regrowth on 20/1/93. The final (second) destructive harvest (H4) was conducted after a further four weeks regrowth on 17/2/93. All harvests were made by cutting at 3.0 cm above the soil surface. At destructive harvests stolons and stubble were separated from the roots and the samples were washed free of soil. All harvests, samplings, extractions and analyses of NSCs were conducted in the manner described in Sections 3.2.3 and 4.2.3. The time between sampling and dropping stolon or stubble samples into boiling 80 per cent ethanol was kept below 30 minutes to prevent loss of substrates due to respiration.

### 5.2.4 Observations and Measurements

During the experiment the following attributes were measured on plants in mixtures:

#### (a) White clover

(1) *height* was measured, before each harvest, as petiole length of fully expanded leaves number three from apex (five measurements per pot). These leaves were chosen because observations showed that they were usually elevated above the canopy;

(2) *leaf appearance rates* were measured during the seven day period of regrowth after harvest 2, where the number of leaves at stage 0.7 on Carlson's scales (Carlson, 1966) were recorded on three marked stolons (see Appendix 3.1);

(3) *area of 10 fully expanded leaves* (leaves number three) were measured at each harvest by a Paton electronic planimeter. This sampling position was chosen because the leaves were fully unfolded and so would have been exporting photosynthates (Chapman *et al.*, 1990a; Woledge, 1988). The laminae of these leaves were separated from the petiole, oven dried at 70 $^{0}$C for 48 hours, weighed and specific leaf areas calculated;

(4) *shoot* DM harvested and treated as described in Section 4.2;

(5) *stolon* DM, measured at destructive harvests 2 and 4, and stolon length, diameter and NSC concentration and content were determined as in Sections 3.2 and 4.2. Stolon lengths were measured on five randomly selected stolons per pot, and total length for the whole pot was obtained by calculation. Stolon diameters were estimated from five measurements per pot, at node number three using calipers. All samples were oven dried in a forced-draught oven at 70 $^{0}$C for 48 hours before weighing.

Limitations in bench space in the glasshouse as well as labour made it not possible to have destructive harvest in all harvests and to measure residual leaf areas in this experiment.

**(b) Perennial ryegrass**

(1) *shoot* DM was measured at each harvest by cutting at 3.0 cm above the soil surface.

(2) *stubble* ( plant materials below 3.0 cm minus roots) DM was measured at destructive harvests 2 and 4, and NSC concentration and content were determined as in Section 3.2 and 4.2.

Results of experiment II (Chapter III) showed that yield reductions of white clover by associated perennial ryegrass were prominent at harvest 2 and harvest 3. Hence, the destructive harvest was conducted at harvest 2 when storage organs were sampled for NSC analyses. RY, RYT and CR of shoot DM were calculated. Willey and Rao (1980) used the correlation between CR and plant characteristics to identify plant traits that related to competitive ability of plants in mixtures. These approaches will be followed here.

### 5.2.5 Statistical Analysis

Data were subjected to analysis of variance using Genstat 5 (Genstat 5 Committee, 1987) after transformation where necessary to ensure the homogeneity of variances. Mean separations were conducted following Duncan's Multiple Range Test (Steel and Torrie, 1960).

DM yields of shoots in monocultures and mixtures were analysed as split-plots experiments involving three white clover, and two perennial ryegrass cultivars as main plots and the four harvests as repeated measurements. Stolon and stubble DM yields in mixtures were analysed in factorial arrangements with only two harvests. In an attempt to identify plant traits in white clover that relate to cultivar differences in competitive ability, regression analyses were conducted for the relationships between those traits which were measured at or before harvest 3 and the CR which was also calculated at harvest 3. The same analyses were conducted for the relationship between NSCs and CRs. The NSC concentration and content in the stolons sampled at harvest 2 (destructive harvest) were related to CRs determined at harvest 3. The same was done for stolon biomass, stolon length and diameter. Height of white clover, leaf appearance rates, leaf areas, specific leaf areas and stolon diameter were measured before harvest 3 and related to CRs at harvest 3. In the regression analyses treatment means averaged over replications were used to prevent confounding of the experimental error and residual sum of squares (Gomez and Gomez, 1984). Initially, a polynomial regression was fitted to the data. However $r^2$ values were generally lower than for a linear regression; hence the results of linear regressions are presented, as done by Willey and Rao, (1980).

## 5.3 Results

### 5.3.1 Shoot Dry Matter Yield

Shoot DM yields of white clover in monocultures increased from harvest 1 to 3 and decreased from harvest 3 to 4 (Table 5.2 and Figure 5.3). Olwen had higher shoot DM yields than Huia at harvests 3 and 4 ($P<0.05$). Irrigation usually had intermediate yields.

Perennial ryegrass growth was vigorous up to harvest 1, with DM yields in monocultures and mixtures exceeding those of white clover (Table 5.2 and Figure 5.3). However shoot DM yield decreased in the subsequent harvests.

In mixtures, the white clovers increased progressively their contribution of DM from harvest 1 to harvest 4, resulting in the highest yields at harvests 3 and 4 (Table 5.2

and Figure 5.3). The differences among white clover cultivars were evident at harvest 3, with Huia producing significantly higher yields (P<0.05). However while yields were similar from harvests 3 to 4 in both Huia and Irrigation, in Olwen yield increased in the same interval (P<0.05). In perennial ryegrass shoot DM was highest (P<0.05) during harvest 1 and decreased at the following harvest. Perennial ryegrass DM yields in mixtures exceeded those of white clover at harvests 1 and 2, but at the following harvests the reverse was true.

**Table 5.2** The effects of cultivar x harvest interactions on shoot DM yields of white clover and perennial ryegrass in monocultures and mixtures

| | H1 | H2 | H3 | H4 |
|---|---|---|---|---|
| Cultivars | White clover monocultures (g pot$^{-1}$) | | | |
| Huia | 6.85$^{h*}$ | 15.87$^{f}$ | 29.90$^{b}$ | 21.00$^{e}$ |
| Irrigation | 7.40$^{h}$ | 11.98$^{g}$ | 31.95$^{ab}$ | 23.92$^{d}$ |
| Olwen | 7.76$^{h}$ | 13.40$^{fg}$ | 34.53$^{a}$ | 26.89$^{c}$ |
| S.e.d. | 1.40 | | | |
| Cultivars | White clover in mixtures (g pot$^{-1}$) | | | |
| Huia | 2.09$^{e}$ | 3.86$^{de}$ | 23.61$^{a}$ | 21.10$^{abc}$ |
| Irrigation | 2.55$^{e}$ | 5.37$^{d}$ | 20.05$^{c}$ | 20.53$^{bc}$ |
| Olwen | 2.34$^{e}$ | 4.05$^{de}$ | 19.50$^{c}$ | 23.12$^{ab}$ |
| S.e.d. | 1.26 | | | |
| Associated | Perennial ryegrass in mixtures (g pot$^{-1}$) | | | |
| Huia | 7.80$^{ab}$ | 6.74$^{cd}$ | 4.15$^{e}$ | 2.80$^{f}$ |
| Irrigation | 7.15$^{bc}$ | 6.31$^{d}$ | 4.40$^{e}$ | 3.26$^{f}$ |
| Olwen | 8.16$^{a}$ | 6.31$^{d}$ | 4.09$^{e}$ | 2.51$^{f}$ |
| S.e.d. | 0.38 | | | |
| Cultivars | Perennial ryegrass monocultures (g pot$^{-1}$) | | | |
| Ellet | 10.76$^{a}$ | 6.28$^{c}$ | 6.14$^{c}$ | 4.12$^{d}$ |
| K.Valley | 11.40$^{a}$ | 7.81$^{b}$ | 5.43$^{c}$ | 3.73$^{d}$ |
| S.e.d. | 0.53 | | | |

* Means associated with similar letters among cultivars and between harvests are non-significantly different (P>0.05)

The total shoot DM yields in mixtures (white clover + perennial ryegrass) increased (P<0.001) from harvest 1 to harvest 3 and levelled off from harvest 3 to harvest 4 (Figure 5.1). Cultivar and cultivar x harvest effects were non-significant (P<0.05).

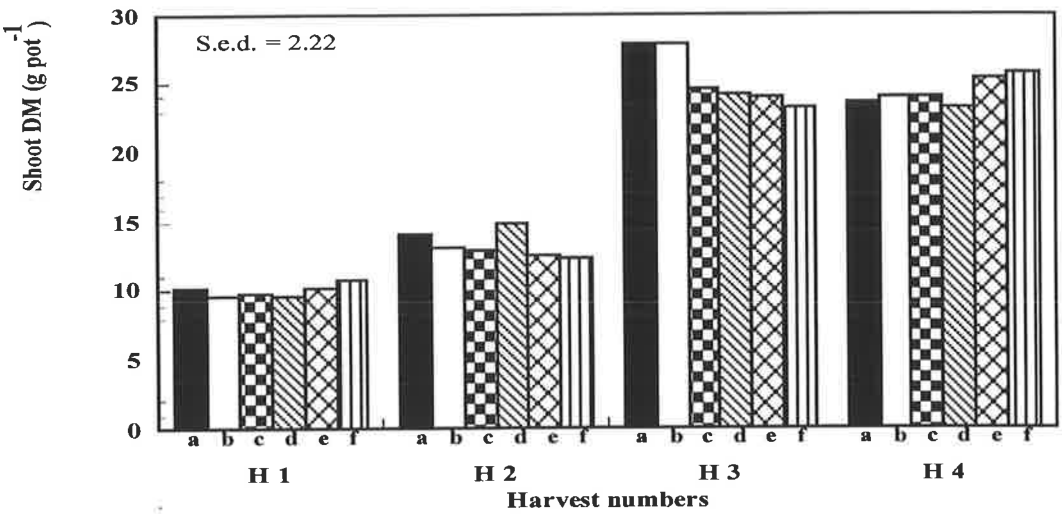



**Figure 5.1** The effects of cultivars on total shoot DM yield (g pot$^{-1}$) in mixtures (white clover + perennial ryegrass) at H1 (harvest 1) to H4 (means of four replications. (a) Huia-Ellet, (b) Irrigation-Ellet, (c) Olwen-Ellet, (d) Huia-K. Valley, (e) Irrigation-K. Valley, (f) Olwen-K. Valley.

### 5.3.2 Proportions of White Clover in Mixtures

The white clover cultivar x perennial ryegrass cultivar x harvest interaction (Table 5.3) was significant ($P<0.05$). The mixture was grass dominant at harvests 1 and 2, and strongly legume dominant thereafter. At individual harvests the proportion of white clover was similar regardless of the cultivar of perennial ryegrass, except at harvest 1 where Irrigation had higher values than Huia in association with Kangaroo Valley and at harvest 2 when Huia in the mixture with K. Valley had the lowest proportion.

**Table 5.3** The effects of the white clover x perennial ryegrass cultivar x harvest interaction on the proportion (%) of white clover in mixtures (means of four replicates)

| White clover cultivars | Associated perennial ryegrass cultivars | White clover proportion (%) | | | |
|---|---|---|---|---|---|
| | | H 1 | H2 | H3 | H4 |
| Huia | Ellet | 24.8$^{ghi*}$ | 42.3$^{ef}$ | 82.5$^{bcd}$ | 87.0$^{abc}$ |
| | K.Valley | 17.5$^{i}$ | 30.0$^{g}$ | 86.5$^{abcd}$ | 89.3$^{abc}$ |
| Irrigation | Ellet | 25.0$^{ghi}$ | 47.0$^{e}$ | 80.5$^{d}$ | 85.0$^{abcd}$ |
| | K. Valley | 28.0$^{gh}$ | 46.3$^{e}$ | 83.5$^{abcd}$ | 89.3$^{abc}$ |
| Olwen | Ellet | 21.5$^{hi}$ | 36.5$^{f}$ | 82.8$^{bcd}$ | 90.5$^{a}$ |
| | K. Valley | 23.0$^{ghi}$ | 41.8$^{ef}$ | 82.3$^{cd}$ | 90.3$^{ab}$ |
| S.e.d. | | 0.03 | | | |

* Means associated with the same letters are not significantly different (P>0.05)

### 5.3.3 Relative Yields

There were highly significant main effects of white clover cultivar (P<0.01) and harvest (P<0.001), and the white clover x harvest interaction was significant (P<0.001). RYs of the white clover cultivars were similar at harvest 1. They increased with time thereafter, but at different rates. At harvest 2 Irrigation had the highest RY but at harvests 3 and 4 Huia had the highest RY (Table 5.4).

**Table 5.4** The effects of white clover cultivar x harvest interaction on the RY of shoot DM of white clover

| White clover cultivars | RY | | | |
|---|---|---|---|---|
| | H1 | H2 | H3 | H4 |
| Huia | 0.32$^{e*}$ | 0.25$^{e}$ | 0.80$^{b}$ | 1.00$^{a}$ |
| Irrigation | 0.35$^{e}$ | 0.46$^{d}$ | 0.60$^{c}$ | 0.85$^{b}$ |
| Olwen | 0.31$^{e}$ | 0.29$^{e}$ | 0.57$^{c}$ | 0.86$^{b}$ |
| S.e.d. | 0.05 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05).

For perennial ryegrass, the main effect of harvest was highly significant (P<0.001). RYs of perennial ryegrass shoot DM increased from harvest 1 (0.70) to harvest 2 (0.93), but decreased again and became similar at harvests 3 (0.74) and 4

(0.73) (Appendix 5.2A). Other effects of treatments were non-significant (P>0.05). The effect of cultivar of perennial ryegrass x harvest number interaction on the RY of perennial ryegrass was non-significant (Appendix 5.2B).

Comparing RY of white clover (Table 5.4) to RY of perennial ryegrass (Appendix 5.2B) it was clear that perennial ryegrass had higher RY at least at harvests 1 and 2 suggesting that white clover was suppressed in mixtures.

### 5.3.4 Competitive Ratio

The effects of harvest and white clover x harvest interaction on CR ($RY_{wc}/RY_{pr}$) were significant (P<0.001). There was no significant change in CR from harvest 1 to harvest 2 (Figure 5.2). Thereafter, however, CR increased markedly for all white clover cultivars but at different rates. Thus while Irrigation initially had the highest CR value, it finally had the lowest. Huia and Olwen had similar values at harvests 1,2 and 4 but Huia was higher at harvest 3.

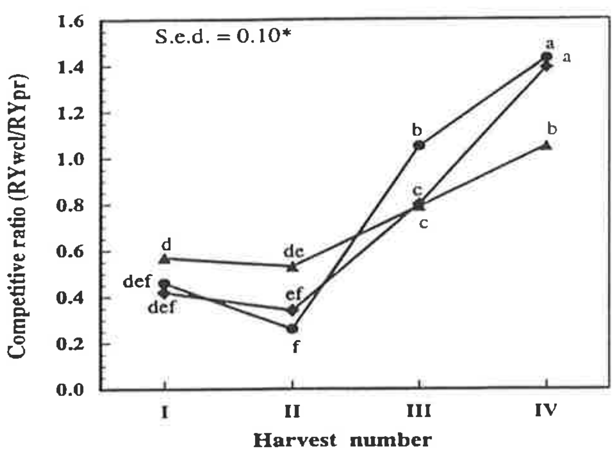



**Figure 5.2** The effects of the white clover cultivar x harvest interaction on CR of white clover with respect to perennial ryegrass (● Huia, ▲ Irrigation, ◆ Olwen. Values associated with similar letters, are not significantly different (P>0.05).

The competitive relationships between cultivars of perennial ryegrass and white clover are presented in the de Wit diagram (Figure 5.3). The diagrams show for example that at harvest 2, Huia and Olwen suffered more from competition than Irrigation (see also Table 5.4) but that Huia was the most competitive cultivar at harvests 3 and 4. At harvests 3 and 4, white clover yielded more than ryegrass both in monocultures and mixtures, but the perennial ryegrass was not suppressed below expected yields (yields in monocultures at the same density as in mixture).

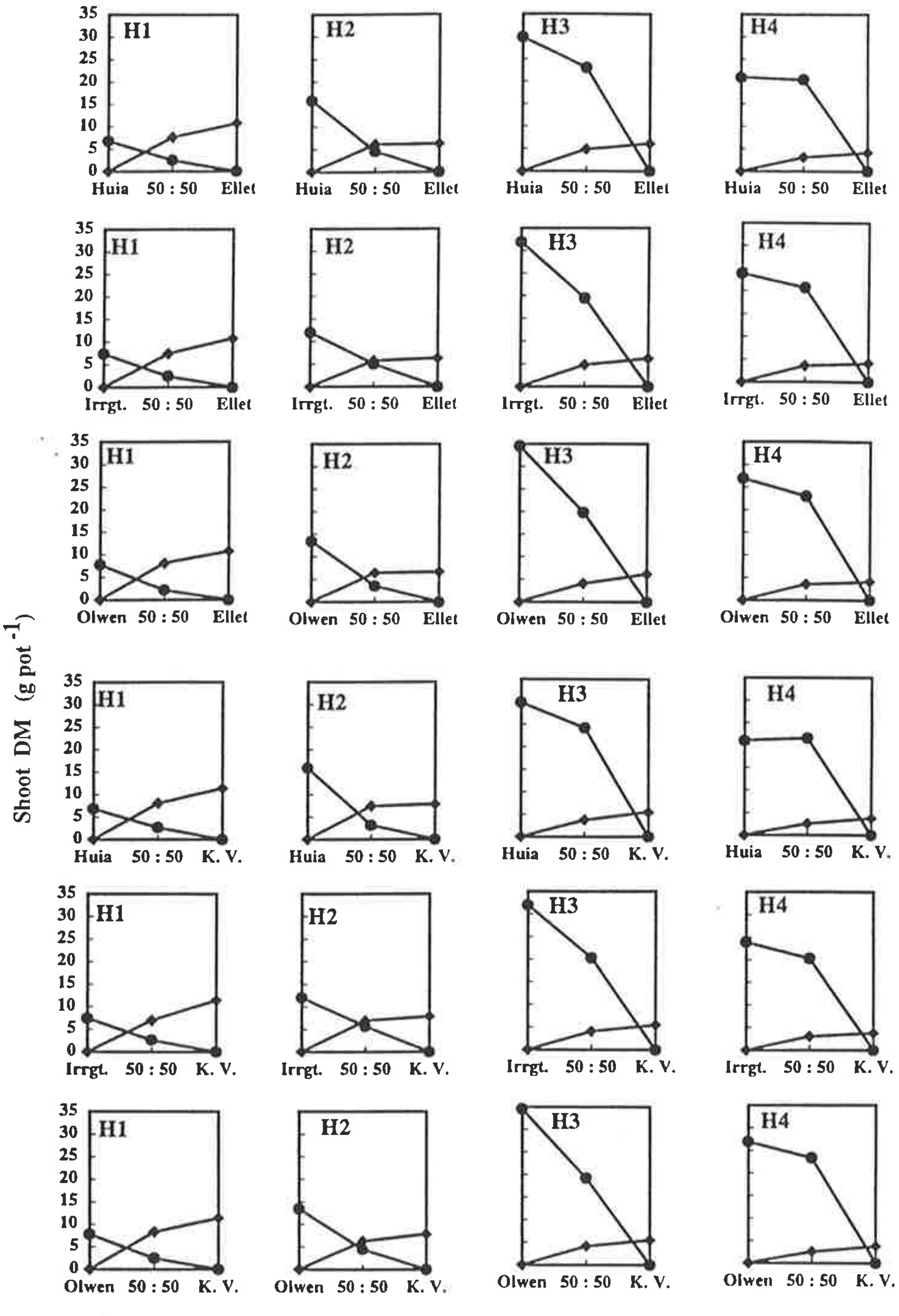



**Figure 5.3** Shoot DM (g $pot^{-1}$) of Huia, Irrigation, and Olwen white clover, Ellet and Kangaroo Valley perennial ryegrass in monocultures and 50:50 mixtures at H1, H2, H3, and H4 (● white clover; ▲ perennial ryegrass.

### 5.3.5 Relative Yield Total

The relative yield totals (RYT) were equal to 1 at harvest 1 (Figure 5.4). Thereafter, RYT increased to values around 1.4-1.6. At harvests 3 and 4 Olwen had the lowest RYT.

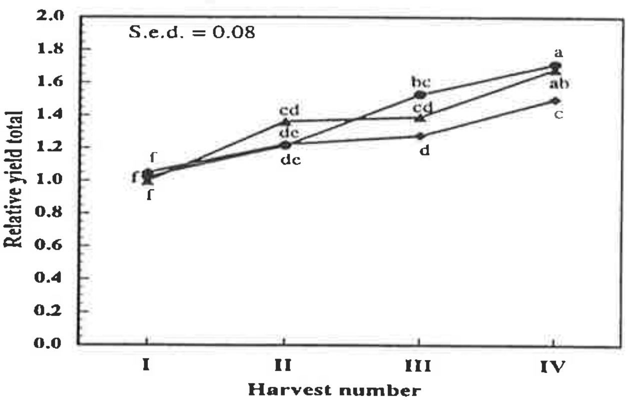



**Figure 5.4** The effect of white clover cultivar and harvest number on RYT (● Huia, ▲ Irrigation, ◆ Olwen. Values associated with similar letters are not significantly different (P>0.05).

### 5.3.6 Morphological Characteristics of White Clover in Mixtures.

#### (a) Height (petiole length)

Olwen was usually higher than Huia and Irrigation, and these differences increased at the later harvests (Figure 5.5).

#### (b) Stolon length and diameter

The cultivar x harvest interactions for stolon length and stolon diameter (Table 5.5) were highly significant ($P<0.001$). Stolon length increased 4 to 5-fold from harvest 2 to harvest 4. It was lowest in Olwen at both harvests and highest in Huia at harvest 4 (Table 5.5). Stolon diameter did not vary significantly between harvests. Olwen had the thickest stolon and Huia the thinnest.

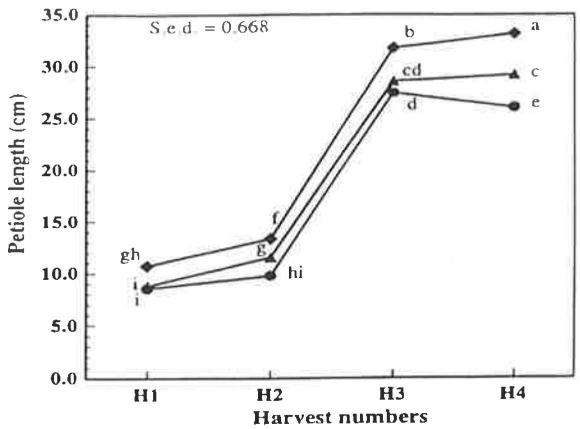



**Figure 5.5** The effects of interaction of white clover cultivars and harvests on height (petiole length) of white clover (J Huia, H Irrigation, F Olwen. Values associated with similar letters, are not significantly different (P>0.05)

**Table 5.5** The effects of interaction of white clover cultivars x harvest on stolon length (cm $pot^{-1}$) and stolon diameter (mm) of white clovers cultivars

| Cultivars | Stolon length (cm $pot^{-1}$) | | Stolon diameter (mm) | |
|---|---|---|---|---|
| | Harvest 2 | Harvest 4 | Harvest 2 | Harvest 4 |
| Huia | $455.87^{d*}$ | $2346.81^{a}$ | $1.58^{d*}$ | $1.66^{cd}$ |
| Irrigation | $486.41^{d}$ | $1795.55^{b}$ | $1.80^{bc}$ | $1.87^{b}$ |
| Olwen | $234.13^{e}$ | $1255.35^{c}$ | $2.34^{a}$ | $2.36^{a}$ |
| S.e.d. | 125.17 | | 0.078 | |

*Means associated with the same superscripts are not significantly different (P>0.05).

### (c) Leaf area and specific leaf area of 10 fully unfolded leaves at harvest

Olwen had the highest (P<0.001) leaf area especially at harvests 3 and 4, while Huia had the lowest leaf area and Irrigation was intermediate (Table 5.6). Huia had higher (P<.0.01) specific leaf area than both Irrigation and Olwen (Table 5.7). The effects of other treatments or interactions on specific leaf areas were non-significant (P>0.05).

**Table 5.6** The effects of white clover cultivar x harvest interaction on leaf area ($cm^2$) measured on ten fully unfolded leaves of white clover (means of four replicates)

| White clover cultivars | Leaf area ($cm^2$) | | | |
|---|---|---|---|---|
| | H1 | H2 | H3 | H4 |
| Huia | $61.1^{gh*}$ | $55.7^{h}$ | $109.7^{d}$ | $85.7^{e}$ |
| Irrigation | $70.0^{fg}$ | $70.0^{fg}$ | $129.4^{c}$ | $101.3^{d}$ |
| Olwen | $78.9^{ef}$ | $79.4^{ef}$ | $194.7^{a}$ | $157.7^{b}$ |
| S.e.d | 0.35** | | | |

*Means associated with the same superscripts are not significantly different (P>0.05).
**After square root transformation

### (d) Leaf appearance rates

Leaf appearance rates at day 5 were highest (P<0.05) for Huia and lowest for Olwen. Irrigation had similar leaf appearance rate with Olwen (Table 5.7).

**Table 5.7** Main effects of white clover cultivars on their specific leaf area ($cm^2\ g^{-1}$) measured at harvest 3 and leaf appearance rates (leaves/3 stolon apices/week) measured after harvest 2

| White clover cultivars | Specific leaf area ($cm^2\ g^{-1}$) | Leaf appearance rates (leaves/3 stolon apices/week) |
|---|---|---|
| Huia | $390^{a*}$ | $5.53^{a*}$ |
| Irrigation | $345^{b}$ | $5.29^{b}$ |
| Olwen | $369^{b}$ | $5.04^{b}$ |
| S.e.d. | 0.014** | 0.014** |

*Means associated with the same superscripts are not significantly different (P>0.05).
**After log10 transformation

## 5.3.7 DM of Stolons and Stubble in Mixtures

Except for the effects of harvest, no effect of treatment was significant on stolon and stubble DM (P>0.05) at harvests 2 and 4 (destructive harvests) (Figure 5.6, Appendix 5.3.1).

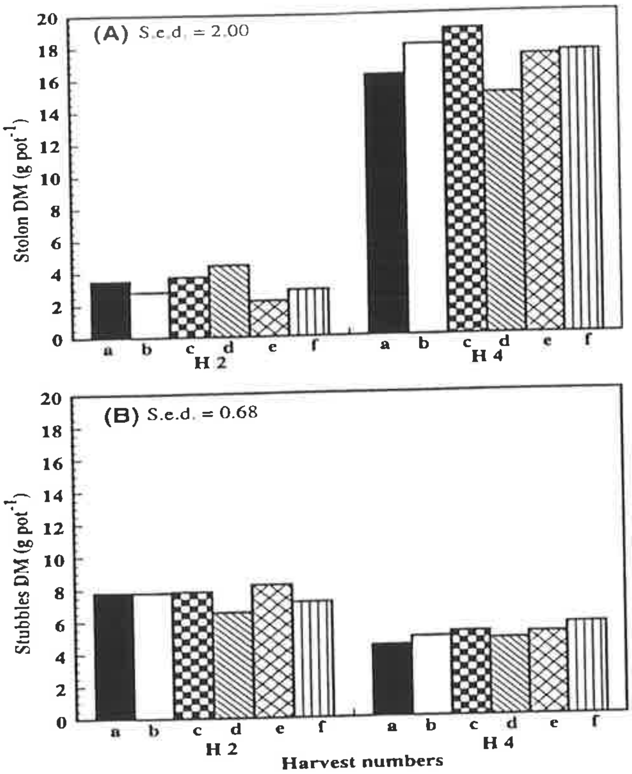



**Figure 5.6** The effects of pasture cultivars on (A) total stolon DM yield (g pot$^{-1}$) and on (B) total stubble DM yield (g pot$^{-1}$) in mixtures at H2 (harvest 2) and H4 (means of four replicates). (a) Huia-Ellet, (b) Irrigation-Ellet, (c) Olwen-Ellet, (d) Huia-K. Valley, (e) Irrigation-K. Valley, (f) Olwen-K. Valley.

### 5.3.8 Non-structural Carbohydrates (NSC) in the Stolons and Stubble in mixtures

Treatments with significant effects on concentration and content of NSCs in stolons and stubble are presented in Appendix 5.3.2. Differences between harvests were the most frequently significant, but there were some significant cultivar effects. Except for sugar content the effects of interaction of cultivars and harvest were non-significant ($P>0.05$). Means of NSC concentration and NSC content, for stolons and stubble, for all monocultures and mixtures of harvests 2 and 4 are shown in Appendices 5.13 and 5.14.

#### (a) Soluble sugars

White clover cultivars differed significantly ($P<0.01$) in concentration of sugar in their stolons (Table 5.8A), Olwen had the highest concentration, and Huia the

In stubble, perennial ryegrass in mixtures with Olwen had the lowest soluble sugars (Table 5.8B), while in mixtures with Huia and Irrigation, perennial ryegrass stubble had the highest soluble sugars concentration.

**Table 5.8** (A) The main effects of white clover cultivars on the concentration of soluble sugars in stolons of white clover in mixtures and (B) the main effects of associated white clover cultivar on stubble of perennial ryegrass in mixtures

| Cultivars | (A) Stolons | (B) Stubble |
|---|---|---|
| | Sugars (mg $g^{-1}$) | |
| Huia | $148.48^{b*}$ | $68.98^{a}$ |
| Irrigation | $171.13^{ab}$ | $67.45^{a}$ |
| Olwen | $201.70^{a}$ | $58.70^{b}$ |
| S.e.d | $0.038^{**}$ | $0.037^{**}$ |

*Means within the same column associated with the same superscripts are not significantly different (P>0.05). ** After log10 transformation

The white clover cultivar x harvest interaction for soluble sugar content (mg $pot^{-1}$) in the stolons was significant (P<0.05) (Table 5.9).

No significant effects of treatments on the content of sugars in stubble were evident except for the effects of harvest (Appendix 5.3.2). Sugar content was about four times as high at harvest 1 as at harvest 2 (Appendix 5.4).

**Table 5.9** The effects of white clover cultivar x harvest interaction on soluble sugar content in stolons of white clover in mixtures

| Cultivars | Sugars (mg $pot^{-1}$) | |
|---|---|---|
| | Harvest 2 | Harvest 4 |
| Huia | $336.10^{c*}$ | $2115.21^{a}$ |
| Irrigation | $587.39^{b}$ | $1996.66^{a}$ |
| Olwen | $404.93^{c}$ | $2697.34^{a}$ |
| S.e.d | $0.084^{**}$ | |

*Means associated with the same superscripts are not significantly different (P>0.05). ** After log10 transformation

### (b) Starch or fructans

Starch concentrations were lower at harvest 4 compared to harvest 2 in stolons of white clover (Appendix 5.5), but starch content was much higher at Harvest 4 (Appendix 5.6). No effects of other treatments or interactions were evident ($P>0.05$). Fructan concentrations were lower ($P<0.05$) in stubble of Kangaroo Valley compared with Ellet (Table 5.10). Fructan concentration decreased in stubble at later compared to early harvests (Appendix 5.7), while fructan content increased (Appendix 5.7). Ellet had a higher content of fructan compared to Kangaroo Valley (Table 5.10).

**Table 5.10** The main effects of perennial ryegrass cultivars on fructan concentration and content in stubble in mixtures

| Cultivars | Fructan concentration (mg $g^{-1}$) | Fructan content (mg $pot^{-1}$) |
|---|---|---|
| Ellet | 126.93[a]* | 1578.04[a] |
| Kangaroo Valley | 101.18[b] | 1395.19[b] |
| S.e.d | 0.041** | 0.045** |

*Means within the same column associated with the same superscripts are not significantly different ($P>0.05$). ** After log10 transformation

### (c) Total non-structural carbohydrates

The total NSC concentration (mg $g^{-1}$) and content (mg $pot^{-1}$) at harvests 2 and 4 in monocultures and mixtures are presented in Figure 5.7 and Figure 5.8, respectively and also in Appendices 5.13 and 5.14. NSC concentrations were always higher in white clover, both in monocultures and mixtures. Huia had the lowest total NSC concentration in the monocultures and in most mixtures. In terms of the NSC concentration, the de Wit diagrams indicate that perennial ryegrass was never disadvantaged by white clover, except for Kangaroo Valley in mixtures with Olwen at harvest 4. At harvest 2 and harvest 4 the total NSC contents of white clover cultivars were lower in mixtures than in monocultures (Figure 5.8). At harvest 4, white clover had higher total NSC content than perennial ryegrass both in monocultures and in mixtures. The main effects of harvest number on total NSC concentrations and contents of white clover and ryegrass in mixtures were significant ($P<0.001$). Concentrations were lower at harvest 4 than at harvest 2. (Appendices 5.9 and 5.10). NSC content was much higher at harvest 4 than at harvest 2 in white clover (Appendix 5.11) but much lower for perennial ryegrass (Appendix 5.12). These effects are also apparent in Figures 5.7 and 5.8.

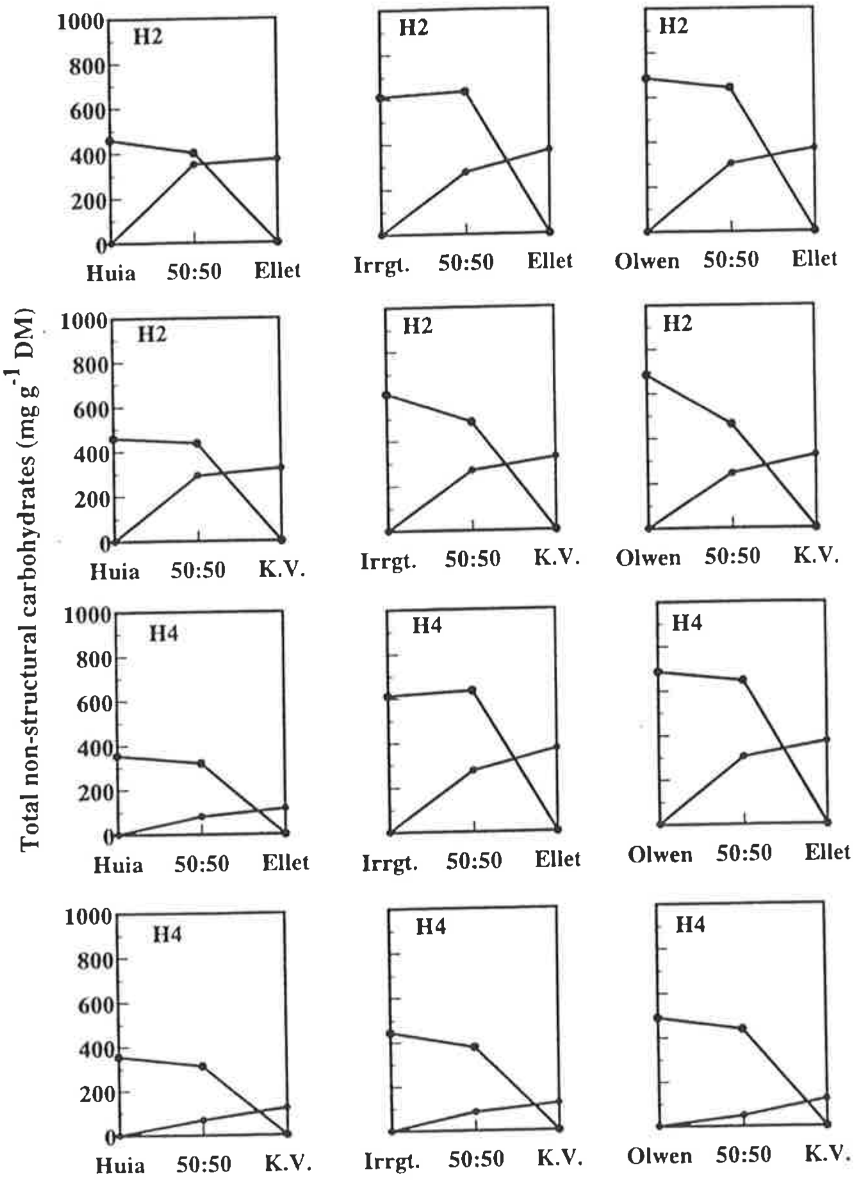



**Figure 5.7.** Total NSC concentration in the stolons of white clover and stubble of perennial ryegrass cultivars in monocultures and mixtures at harvest 2 and harvest 4. (● white clover, ■ perennial ryegrass)

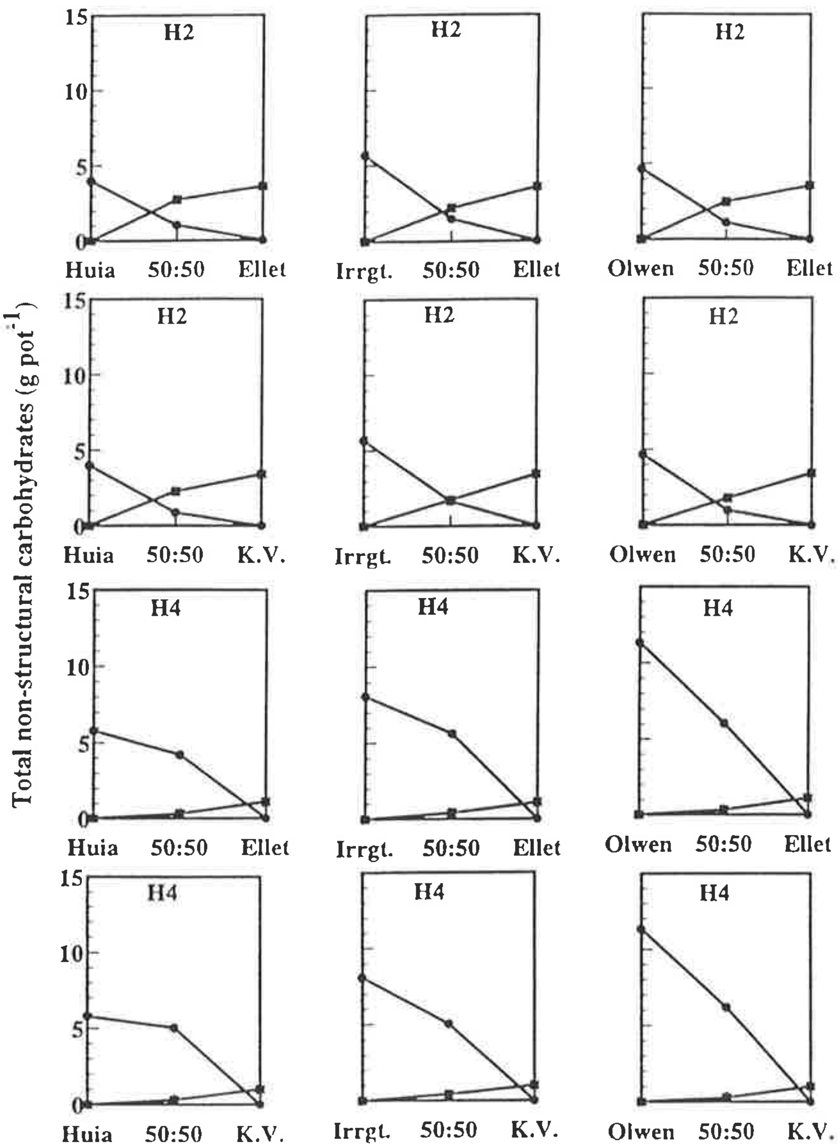



**Figure 5.8.** Total NSC contents in the stolons of white clover and stubble of perennial ryegrass cultivars in monocultures and mixtures at harvest 2 and harvest 4. (● white clover, ■ perennial ryegrass)

There were significant main effects of white clover cultivars ($P<0.05$) for total NSC concentration (Table 5.11) in the stolons. Olwen had the highest NSC concentration, Huia the lowest and Irrigation intermediate. In stubble, there were significant main effects ($P<0.001$), associated white clover cultivars (Table 5.12) and perennial ryegrass cultivars ($P<0.05$) (Table 5.13). Perennial ryegrass in mixtures with Olwen had the lowest NSC concentration in their stubble compared with other mixtures (Table 5.12). Stubble of Ellet had a higher total NSC concentration than Kangaroo Valley (Table 5.13).

**Table 5.11.** Main effect of white clover cultivar in mixture on the total NSC concentration in the stolons

| White clover cultivars | Total NSC (mg $g^{-1}$) |
|---|---|
| Huia | $391.28^{b}$ |
| Irrigation | $487.54^{ab}$ |
| Olwen | $527.07^{a}$ |
| S.e.d | 0.117 |

*Means followed by the same superscripts are not significantly different ($P>0.05$).
**After log10 transformation

**Table 5.12**. Main effect of associated white clover cultivar on the total NSC concentration in the stubble of perennial ryegrass

| White clover cultivars | Total NSC (mg $g^{-1}$) |
|---|---|
| Huia | $196.85^{a}$ |
| Irrigation | $175.78^{a}$ |
| Olwen | $164.22^{b}$ |
| S.e.d | 0.037 |

*Means followed by the same superscripts are not significantly different ($P>0.05$).
**After log10 transformation

**Table 5.13.** Main effect of perennial ryegrass cultivar on the total NSC concentration in their stubble in mixtures

| Perennial ryegrass cultivars | Total NSC (mg $g^{-1}$) |
|---|---|
| Ellet | 191.26[a] |
| Kangaroo Valley | 166.64[b] |
| S.e.d | 0.031 |

*Means followed by the same superscripts are not significantly different (P>0.05).
**After log10 transformation

### 5.3.9 Relationships of Plant Traits and Non-structural Carbohydrates (X) of White Clover in Mixture to Competitive Ratio (Y)

The regression analysis between SLA and CR is highly significant (P<0.01), with $r^2$=0.86 (Table 5.14). Other traits had non-significant (P>0.05) regression with CR.

**Table 5.14** The relationships for white clover between CR at harvest 3 (dependent variable) and plant traits at harvest 3 and NSC pools at harvest 2 (independent variables)

| Plant traits and NSC pools | Level of significance | $r^2$ | Equation |
|---|---|---|---|
| Height (petiole length) | ns@ | 0.29 | Y = 2.115-0.043 X |
| Shoot biomass (g $pot^{-1}$) | ns | 0.51 | Y = 1.418 - 0.122 X |
| Leaf area ($cm^2$) | ns | 0.24 | Y = 1.442 - 0.047 X |
| Specific leaf area ($cm^2$ $g^{-1}$) | ** | 0.86 | Y = -1.724+ 0.0068 X |
| Leaf appearance rate | ns | 0.10 | Y = 0.169 + 0.132 X |
| Stolon biomass (g $plant^{-1}$) | ns | 0.09 | Y = 1.078 - 0.061 X |
| Stolon length ( cm $pot^{-1}$) | ns | 0.03 | Y = 0.852+ 0.0006 X |
| Stolon diameter (mm) | ns | 0.04 | Y = 1.341 - 0.272 X |
| Soluble sugars | | | |
| Concentration (mg $g^{-1}$) | ns | 0.42 | Y = 1.493 - 0.0035 X |
| Content (mg $pot^{-1}$) | ns | 0.45 | Y = 1.22 - 0.0007X |
| Starch | | | |
| Concentration (mg $g^{-1}$) | ns | 0.05 | Y = 1.018 - 0.0004X |
| Content (mg $pot^{-1}$) | ns | 0.18 | Y = 1.127 - 0.0003X |
| Total NSC | | | |
| Concentration (mg $g^{-1}$) | ns | 0.15 | Y = 1.174 - 0.0004X |
| Content (mg $pot^{-1}$) | ns | 0.30 | Y = 1.209 - 0.0003X |

@ns=non-significant (P> 0.05), ** =significant at P<0.01

### 5.3.10 Relationships of RY of Shoot Dry Matter (X) and Non-structural Carbohydrates (Y)

The shapes of the curves in the de Wit diagram of shoot DM at harvest 1 (Figure 5.1) and NSC content in the stolons at harvest 2 (Figure 5.8) were very similar, suggesting relationships (Table 5.15). Similar trend occurred between shoot DM at harvest 3 and NSC content in the stolons at harvest 4. Regression analyses showed a significant ($P<0.05$) positive relationship between RY shoot DM (X) at harvests 1 and 3 and RY of NSC content in the stolons (Y) at the following harvests, i.e. 2 and 4 respectively (Table 5.15). Other relationships were non significant.

**Table 5.15** The relationships between RY of shoot DM and NSC content in the stolons at various harvests

| Relationships | Level of significance | $r^2$ | Equation |
|---|---|---|---|
| RY shoot DM at harvest 1 (X) and RY of NSC at harvest 2 (Y) | *@ | 0.74 | Y = 0.051 - 0.618 X |
| RY shoot DM at harvest 3 (X) and RY of NSC at harvest 4 (Y) | * | 0.73 | Y = -0.014 - 1.034 X |
| RY NSC content at harvest 2 (X) and RY of shoot DM at harvest 3 (Y) | ns | 0.29 | Y = 0.19 + 1.75 X |
| RY shoot DM at harvest 2 (X) and RY of NSC at harvest 2 (Y) | ns | 0.12 | Y = 0.24 + 0.11 X |
| RY shoot DM at harvest 4 (X) and RY of NSC at harvest 4 (Y) | ns | 0.52 | Y = -0.25 + 0.90 X |

@* =significant at $P<0.05$, ns = non-significant ($P>0.05$)

## 5.4 Discussion

In experiment II (Chapter IV) it has been shown that RY and CR were independent of density above the total density of about 8 plants $pot^{-1}$, hence the same should apply for competitive relationships in this experiment, with the total density of 16 plants $pot^{-1}$. The results of this experiment are also consistent with those of experiment II in that at harvest 1, mixtures of white clover and perennial ryegrass were close to mutually exclusive with RYT close to 1 (Figure 5.4) indicating that both species competed for similar resources (de Wit, 1960) presumably because the N-fixing system of white clover had not been fully developed (de Wit *et al.*, 1966).

Nitrogen availability in the soil is the most important factor affecting the yield of grass in white clover-perennial ryegrass mixtures (Rhodes and Ngah 1983, Harris, 1987) as might also be the case in this experiment. Hence, up to H 1 when soil N was still high, DM yields of perennial ryegrass were higher than white clover both in monocultures and mixtures (Table 5.2), resulting in a low proportion of white clover in mixtures (Table 5.3). RY values of white clover were usually less than 0.5 (Table 5.4) and RYT values were close 1 (Figure 5.4).

As the experiment continued, perennial ryegrass depleted soil N and its shoot DM yields declined while white clover, being largely independent of soil N, increased its growth both in monocultures and mixtures (Table 5.2) especially at H3 and H4, resulting in higher proportions of white clover (Table 5.3), higher RYs (Table 5.4) and values of RYT which were higher than unity, indicating coexistence in that perennial ryegrass and white clover did not compete for similar resources (de Wit, 1960; de Wit *et al.*, 1966; Hall, 1974). Despite this, the de Wit diagrams (Figure 5.3) and RY values (Appendix 5.2A) showed that perennial ryegrass was not suppressed by the presence of white clover, as DM yield of perennial ryegrass cultivars in mixtures were generally higher than the expected yields. The results of this experiment agree with those of Bakhuis and Kleter (1965), Harris and Thomas (1973), Harris (1978) and Menchaca and Connolly (1990) and confirm that the effects of perennial ryegrass on white clover were strong initially but these effects declined over time, in spite of high RY values in ryegrass.

It is noteworthy that at H2 shoot DM yields of white clover in monocultures were higher compared to perennial ryegrass (Table 5.2); however in mixtures white clover DM yields were less. This indicates a suppression of white clover (reduction of yield below the expected yield) especially at harvest 2, as is evident in Figure 5.3. This trend occurred despite the increase in proportions of white clover in the swards from 17-28% at harvest 1 to 30-46% at harvest 2 which further increased to 85-90% at harvest 4 (Table 5.3). This shows that ranking of species yields in monocultures is not a good indicator of the ranking of these species in mixtures. Furthermore the relative ranking changes with time due to changes in both soil nitrogen levels and competitive ability. This is certainly supported by Table 5.2. The results of this experiment also agree with those of Weiner (1980) who found that in monocultures, *Trifolium incarnatum* yields were higher than that of *Lolium multiflorum*, but in mixtures their yields were less.

Cultivar differences in the response of white clover to the presence of perennial ryegrass are also important. Thus, Huia which had a lower DM yield than Olwen in monocultures at H3 and H4, had a higher yield in mixtures at H3 (Table 5.2). This occurred in spite of similar total yields of mixtures at each harvest (Figure 5.1), and total yield over all harvests (Appendix 5.1). This different varietal response resulted in higher RY (Table 5.4) and RYT (Figure 5.4) of Huia at H3 and H4 compared to Olwen, which suggests that Huia would be more competitive than Olwen with respect to perennial ryegrass. The differences in white clover cultivar responses to the presence of perennial ryegrass result in different time trends in CR (Figure 5.2), thus different ranking of cultivars.

The reasons for the higher RY (Table 5.4) and CR (Figure 5.4) of Huia compared to Olwen at H3 might be due to longer stolons (Table 5.5) (Archer and Robinson, 1989) and thus higher stolon density (Caradus *et al.*, 1991). This implies a higher residual leaf area and higher density of growing points. It might also be caused by thinner stolons resulting in larger surface areas of Huia as stolons have been shown to be capable of photosynthesis (Harris *et al.*, 1983; Davidson *et al.*, 1990).

The almost total lack of significant relationship between plant traits and CR indicates that no single trait, with the possible exception of SLA has a direct relationship with competitive ability. However, since the traits were measured only in mixtures at only one harvest, the result does not rule out the possibility of more complex relationships which may vary overtime. Because the relationship between CR and SLA was highly significant, with a high correlation, it should be considered as a possible indicator of competitive ability. No information is available on cultivar variation in SLA in white clover in mixtures with perennial ryegrass. Higher SLA could be of competitive advantage in competition for light and for capturing more light per unit of leaf DM. However, it must be remembered that cause and effect can not be identified simply from significant difference in SLA i.e. it is not known whether variations in SLA are a cause or an effect of variations in competitive ability.

There are reports in the literature indicating that in mixtures with perennial ryegrass, Olwen produces higher yields, absorbs more available nitrogen from the soil and fixes more nitrogen than the smaller-leaved cultivar S184 (Brock, 1971; Brock and Hoglund, 1974; Goodman and Collison, 1986). The associated grass also took up more nitrogen when grown with S184 than with Olwen. However, when soil available nitrogen declined a time lag occurred for Olwen which was necessary to develop its N-fixation system fully (Brock, 1971; Brock and Hoglund, 1974). This factor might also contribute to a lower CR ($P<0.05$) of Olwen at H3, but then a similar value to that of Huia at H4 (Figure 5.3).

While the stolon and stubble DM yields in mixtures did not differ significantly among cultivars used (Figure 5.6), the NSC concentration did. Consistent with the results of experiment I (Chapter III) Huia in mixture had a lower concentration of soluble sugars (Table 5.8) and starch (Figure 5.9) and total NSC concentration than

Olwen (Table 5.11). However, the higher NSC levels in Olwen were not translated into significantly higher growth of Olwen in mixture with perennial ryegrass (Table 5.2) and did not cause higher CR of Olwen compared with Huia (Figure 5.2). The higher NSC levels in Olwen than in Huia might be caused by larger requirements for maintenance and growth of the longer stolons and faster leaf appearance rates of Huia.

The de Wit's diagram for total NSC concentration (Figure 5.7) indicated that perennial ryegrass cultivars were not suppressed by white clover. However in terms of total NSC contents (Figure 5.8), the diagram showed similar trends as those for shoot DM, viz. white clover was depressed at H2 and gained at H4. King *et al.* (1979) and Gonzalez *et al.* (1989) found in defoliated perennial ryegrass that NSC returned to initial levels four weeks after defoliation, thus the interval used in this experiment (four weeks) might enable white clover to replenish its reserves. However the relationships found between RY of DM at a particular harvest and RY (content of stolon NSC) at the next harvest indicates that competition reduces the replenishment of carbohydrate reserves in the same way as it has reduced DM production at the previous harvest. This is in agreement with the suggestion of Davidson and Robson (1986) that the shoot regrowth soon after defoliation might affect the outcomes of competition rather more than the biomass yield measured at the end of the growth periods. In addition plants use only a fraction of reserves for their regrowth after defoliation (Weinmann, 1961), continued growth and competitive ability being determined by residual leaf areas, number of growing points, and leaf area development (Briske, 1986; Caldwell, 1986; Busso *et al.*, 1990; Piersons *et al.*, 1990). This may explain the apparent lack of effect of NSC reserves on CR values in the present experiment. It may be possible that NSC levels affect competitive ability in the absence of residual leaf area or under shade.

## 5.5 Conclusions

From the results of this experiment the following conclusions were drawn:

1. Olwen (*Trifolium repens* cv. Olwen) produced higher shoot dry matter yields in monocultures compared to Irrigation and Grasslands Huia at harvests 3 and 4, but higher yield in monocultures did not always result in to higher yield in mixtures.

2. CR of Irrigation was highest at the first two harvests and became lowest at the following two harvests. The reverse was true for Huia, while Olwen had a similar trend to Huia except at harvest 3 when CR of Olwen was lower than Huia.

3. Higher yield in monocultures did not result in higher competitive ability, measured as CR, in mixtures with perennial ryegrass. This occurred in spite of increasing proportions of white clover in the mixtures over time.

4. Olwen had higher NSC's in mixtures with perennial ryegrass than Huia but this was not translated into higher CR compared to Huia.

5. Competitive ability, measured as CR of white clover with respect to perennial ryegrass, was significantly related to specific leaf areas. Other traits had no relationship to competitive ability in the conditions of this experiment.

6. The growth of perennial ryegrass decreased from harvest to harvest presumably because of decreasing soil nitrogen availability. However, RYs remained high, presumably due to a transfer of nitrogen from the legume to the grass.

# Chapter VI

# Experiment IV. The Effects of Cultivars, Defoliation, and Nitrogen Application on Competitive Ability of White Clover with Respect to Perennial Ryegrass. The Relative Importance of Morphological Traits and Non-Structural Carbohydrate Reserves

## 6.1 Introduction

Cultivar, defoliation and nitrogen application are three of the most important factors under the control of management, affecting the performance of white clover-perennial ryegrass mixtures (Rhode, 1981; Rhodes and Ngah, 1983).

Chapman *et al.* (1990b) provided evidence that the immediate response to defoliation in white clover was an increase in specific leaf area ($cm^2$ $g^{-1}$), while assimilation rates (net photosynthesis x area), were similar to the non-defoliated plants. Cultivars of white clover are known to vary in their yield response to competition from perennial ryegrass (Widdup and Turner, 1983; Frame, 1990; Caradus and Chapman, 1991). They also vary in their tolerance of the presence of high soil nitrogen, with larger-leaved cultivars e.g. Olwen being more tolerant than smaller-leaved cultivars (Wilman and Asiegbu, 1986b; Caradus, 1986; Goodman and Collison, 1986). The reasons for this nitrogen tolerance are not known.

Martin and Field (1984) found that nitrogen application of 200 $kgha^{-1}$ with defoliation at 4-weekly intervals increased the competitive ability of perennial ryegrass relative to white clover, mainly due to an increase in shading by the grass leaf. Frame and Boyd (1987a,b) found that nitrogen applications to white clover-ryegrass mixtures resulted in a lower proportion of white clover. In contrast, in the absence of nitrogen application, defoliation benefits white clover over ryegrass. However, different species and cultivars differ in their response to nitrogen and defoliation. For example, small-leaved white clover cultivars in mixtures with grasses have been shown to have a higher yield than larger-leaved cultivars and maintain their proportion in the swards under frequent and severe defoliation (Frame and Boyd, 1987). The mechanisms of these differences are not well understood. Studies have considered morphological traits i.e. number of growing points, node length, number of branches and root number (Caradus *et al.* 1991). Non-structural carbohydrates (NSC) while being acknowledged to be important in plant regrowth, have never been implicated in the competitive ability of pasture plants.

Nitrogen applications have been found to enhance NSC remobilization in perennial ryegrass after defoliation (Gonzalez *et al.*, 1989), with 60-90% of soluble carbohydrates being remobilized during the first six days of regrowth. Soluble carbohydrates return to initial levels or higher after about 4 weeks (King *et al.*, 1979).

Results of experiment I (Chapter III) have indicated that in monocultures, white clover cv. Huia had a lower concentration and content of NSC in stolons than Olwen, which was confirmed in experiment III (Chapter V). However, in mixtures, while Huia had less NSC concentration and content, its competitive ability as measured by the competitive ratio ($RY_{wc}/RY_{pr}$) was higher than that of Olwen at harvest 3. The decline of nitrogen availability in the soil at this time might have played a role in this response, because Olwen might absorb more nitrogen and be more competitive at higher levels of soil nitrogen than at lower levels (Goodman and Collison, 1986). Thus, the role of NSC in determining competitive ability of white clover, in association with perennial ryegrass may depend on the level of nitrogen. As there is no information on this in the literature, these aspects are investigated in the following experiment.

The aims of the experiment reported here are to determine:

(1) how defoliation and nitrogen application affect the levels of NSCs in stolons of white clover cultivars in mixtures with perennial ryegrass;

(2) whether or not the above responses would affect the competitive ability of white clover in the following regrowth; and

(3) the relative importance of morphological traits and NSC as affected by defoliation and nitrogen application on the competitive abilities of white clover cultivars with respect to perennial ryegrass.

## 6.2 Materials and Methods

### 6.2.1 Sites and Soils

The sites, pots and soil used were the same as used in experiment III (See Chapter V).

### 6.2.2 Treatments and Design

Pretreatments were applied to all pots as three cuts prior to the application of nitrogen and defoliation treatments. Cutting after the initial eight weeks of growth (Cut 1) and two more cuts (Cut 2 and Cut 3) each after four weeks of regrowth, were applied to all pots, to allow the plants to achieve complete surface cover and reduce soil nitrogen as in Experiment III (Chapter V).

Treatments consisted of: two monocultures of white clover cultivars (Olwen and Huia), one monoculture of perennial ryegrass (Ellet) and their 50:50 mixtures; two levels of defoliation intensity viz. (i) lenient (L): cut after four weeks, 3.0 cm above the soil surface and (ii) severe (S): cut weekly (four cuts), all unfolded leaves (stage 0.6 Carlson's scale or higher) being harvested (at 3.0 cm above the soil surface if the petioles were longer than 3.0 cm or the leaf blades with ca. 0.5 cm petiole); two rates of nitrogen application viz. (i) none (N0) and (ii) 75 kg $ha^{-1}$ (N1). There were two destructive harvests each with two replicates to make a total of 80 pots arranged in a completely randomized block design. The time table for planting, cutting, and destructive harvests are presented in Figure 6.1.

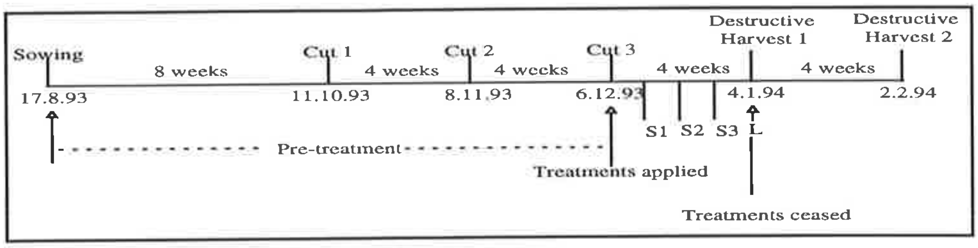



**Figure 6.1** Time table for sowing, cutting, application of treatments and destructive harvests. (S=severe cutting, L=lenient cutting). S4 was conducted on 4.1.94.

Those treatments were designed to vary the levels of NSC in stolons of white clover and stubble of perennial ryegrass at destructive harvest 1 (DH1). The effects of this variation on competitive ability measured as competitive ratio (CR) were further examined at destructive harvest 2 (DH2).

### 6.2.3 Cultural Techniques

Cultural techniques were similar to those described in experiment II (Chapter IV). The total density used was 16 plants pot$^{-1}$. Excess seeds were sown, and seedlings were thinned to get the correct number and uniform size during the period of three weeks after sowing. Specific rhizobial inoculum was applied as described in the previous experiment.

Nitrogen was applied as $NH_4NO_3$ in split applications, 50 kg ha$^{-1}$ N immediately after Cut 3 and a further 25 kg ha$^{-1}$ N two weeks later, both in 200 ml solution. After pretreatments, half of the pots were subjected to lenient cutting and the other half to severe cutting. Destructive harvest 1 was conducted four weeks after the last pretreatment cut (Cut 3) to half of the pots, the remaining pots were allowed to regrow for a further four weeks before the second destructive harvest.

During the initial two week pretreatment period the plants were affected by earth aphids. Omethoate was applied at 0.15 L $ha^{-1}$. Applications were repeated two weeks afterwards with higher rates as the insects showed some degree of resistance to the chemicals. Some white clovers were affected by the spray and showed leaf necrosis, however, these symptoms disappeared after two weeks and before the first cut the plants showed excellent, luxuriant growth.

In pots receiving nitrogen treatments, the plants grew vigorously, potentially shading the adjacent pots. Hence, immediately after treatment applications, each pot was surrounded by a cylinder of black mosquito netting that could be slid up and down to adjust to the height of the plants and thus prevent spreading of the foliage (Plate 6.1).

White clover was cut to a height of 3.0 cm above the soil surface, except in pots receiving the severe defoliation (S1, S2, S3 and S4) treatment in which the blades of all unfolded leaves with petiole length less than 3.0 cm, were cut together with ca. 0.5 cm petiole. All perennial ryegrass was cut to a height of 3.0 cm above the soil surface. Thus, variations in the concentration and content of NSC in response to defoliation and nitrogen application were obtained in the stolons of white clover and the stubble of perennial ryegrass at destructive harvest 1. How these variations would affect the competitive ability of white clover was studied during regrowth from destructive harvest 1 to destructive harvest 2.

### 6.2.4 Observations and Measurements

Pretreatment periods (C1, C2 and C3) were the same as H1, H2 and H3 in experiment III (Chapter V) for monocultures and mixtures involving Huia, Olwen and Ellet, during which the growth of perennial ryegrass declined at the latter harvest which was thought to be due to declining nitrogen availability in the soil. At C1, C2 and C3 only shoot DM was recorded.

**Plate 6.1.** Overview of experiment IV

At DH1 and DH2, the following were recorded: for white clover shoot DM, petiole length of the fully expanded leaves number 3-5 (five measurements per pot); leaf area of ten fully expanded leaves number 3-5 and their specific leaf area; stolon length and diameter. All measurements and sampling (dry matter, morphological traits, NSC) were as described in Experiment III (Chapter V). At each harvest, white clover and perennial ryegrass were separated and oven-dried at 70 $^0$C for 48 hours, as described previously. Shoot DM in the four severe defoliation treatment harvests was pooled for comparison with the single harvest of the lenient (L) defoliation treatment. Stolons and stubble in mixtures were sampled at DH1 and DH2 for NSC extraction as described in Chapter III (Experiment I). Stolon length and diameter were recorded as in Experiment IV (Chapter V). The RY and CR were also calculated as in Experiment IV.

### 6.2.5 Statistical Analysis

Shoot DM, RY and CR of shoots during pretreatments were analysed as factorial experiments with cutting as repeated measurements.

Shoot and stolon (or stubble) DM in monocultures and mixtures at DH1 and DH2 were subjected to analysis of variance as a factorial experiment using Genstat 5 (Genstat 5 Committee, 1987), after transformation where necessary to ensure homogeneity of variance. Significant mean separations were conducted following Duncan's Multiple Range Test (Steel and Torrie, 1960). The relative yield (RY) of shoot DM and CR were also subjected to the analysis of variance. In effect, analysis of variance involved four factors each at two levels, namely cultivars (Olwen and Huia), harvests (destructive harvests 1 and 2), nitrogen applications (none and 75 kg ha$^{-1}$) and defoliations (lenient and severe).

Regression analyses between plant traits and NSC pool (independent variables) and CR (dependent variable) were conducted using NSC pools in stolons extracted at DH1 and plant traits in the regrowth measured before DH2, while CRs were measured using shoot DM at DH2.

## 6.3 Results

### 6.3.1 Shoot Dry Matter

#### (a) Shoot dry matter, RY and CR at pre-treatment cutting (C1, C2 and C3)

There were no effects ($P>0.05$) of white clover cultivar on clover shoot DM in monocultures or mixtures (Appendix 6.1). Similarly, there were no effects of cultivars on RY of white clover (Appendix 6.1). White clover cultivar also had no effect ($P>0.05$) on shoot DM of associated perennial ryegrass in mixtures, and the same was true for RY of perennial ryegrass (Appendix 6.2). Shoot DM of white clover increased ($P<0.01$) from C1 to C3 (Appendix 6.1), while that of perennial ryegrass decreased (Appendix 6.2).

RY of white clover decreased ($P<0.05$) from C1 to C2 then increased again from C2 to C3 (Appendix 6.1) while those of ryegrass increased ($P<0.05$) from C1 to C2 then decreased again from C2 to C3 (Appendix 6.2).

There was no significant effect of white clover cultivar on their CR with respect to ryegrass, although Olwen tended to have the higher values. (Appendix 6.1). CR of white clover with respect to ryegrass decreased ($P<0.05$) from C1 to C2 then increased again from C2 to C3, but no values exceeded 0.51.

#### (b) Shoot dry matter at destructive harvests

There were highly significant depressing effects of severe defoliation ($P<0.001$) on all parameters recorded. At DH2, white clover had a higher biomass than at DH1, resulting in significant main effects of harvest, while the reverse was true for perennial ryegrass.

**Monocultures**. The nitrogen x defoliation x harvest interactions on shoot DM of white clover (Table 6.1) and perennial ryegrass (Table 6.2) in monocultures were significant (P<0.05). With the lenient defoliation regime, and nitrogen application shoot DM yield of white clover was higher (P<0.05) at DH1 than at DH2; no significant effects were observed in the absence of nitrogen fertilizer. Severe defoliation decreased (P<0.05) shoot DM of white clover compared to the lenient defoliation regime especially at DH1. Following severe defoliation, shoot DM increased from DH1 to DH2 and was higher (P<0.05) with addition of nitrogen than without.

**Table 6.1** The nitrogen x defoliation x harvest interaction on shoot DM (g pot$^{-1}$) of white clover in monocultures at destructive harvests 1 and 2

| Destructive Harvest | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Monoculture shoot DM (g pot$^{-1}$) | | | |
| DH1 | 15.25$^{ab}$* | 16.26$^{a}$ | 1.36$^{d}$ | 1.62$^{d}$ |
| DH2 | 14.36$^{ab}$ | 12.20$^{bc}$ | 10.16$^{c}$ | 13.62$^{ab}$ |
| S.e.d. | 1.385 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

Nitrogen application markedly increased (P<0.05) shoot DM of perennial ryegrass monoculture except under severe defoliation at DH1. Severe defoliation markedly reduced shoot DM of ryegrass at DH1 (P<0.05) but had little effect at DH2 (Table 6.2).

**Table 6.2** The nitrogen x defoliation x harvest interaction on shoot DM (g pot$^{-1}$) of perennial ryegrass in monocultures at destructive harvest 1 and 2

| Destructive Harvest | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Monoculture shoot DM (g pot$^{-1}$) | | | |
| DH1 | 2.39$^{bc}$* | 14.31$^{a}$ | 0.44$^{f}$ | 0.91$^{ef}$ |
| DH2 | 1.50$^{de}$ | 2.80$^{b}$ | 1.09$^{ef}$ | 2.06$^{cd}$ |
| S.e.d. | 0.329 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Mixtures**. Nitrogen application significantly (P<0.05) reduced shoot DM of white clover (Figure 6.2A) (Plate 6.2). The harvest x defoliation interaction was significant (P<0.001). Severe defoliation markedly reduced shoot DM at DH1 (Figure 6.2B), but the effect was no longer significant at DH2.

In perennial ryegrass, the harvest x defoliation x nitrogen interaction was highly significant (P<0.001) (Figure 6.3). Nitrogen application increased shoot DM of perennial ryegrass at DH1 especially with lenient defoliation (Plate 3). By DH2, the residual nitrogen response was small, with no remaining effect of defoliation treatment. Without nitrogen application, DM yields were similar regardless of the intensity of defoliation and harvest.

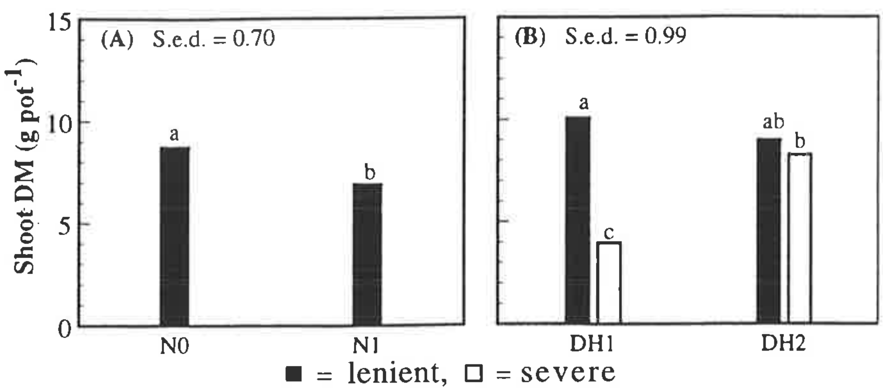



**Figure 6.2** The effects of (A) nitrogen application and (B) interaction of harvest x defoliation on shoot dry matter (DM) of white clover in mixtures. Means associated with similar letters are not significantly different (P>0.05).

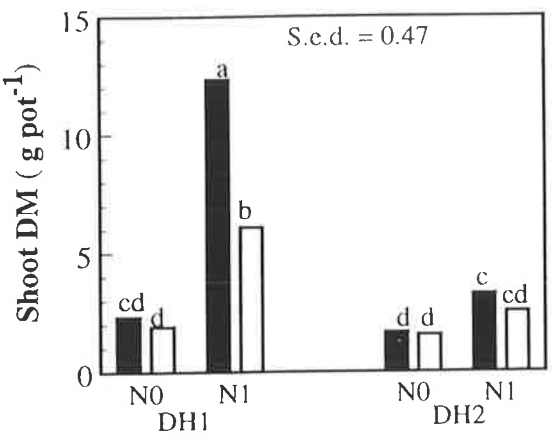



**Figure 6.3** The interaction of harvest x defoliation x nitrogen on shoot DM of perennial ryegrass in mixtures (■ = lenient, □ = severe). Means associated with similar letters are not significantly different (P>0.05).

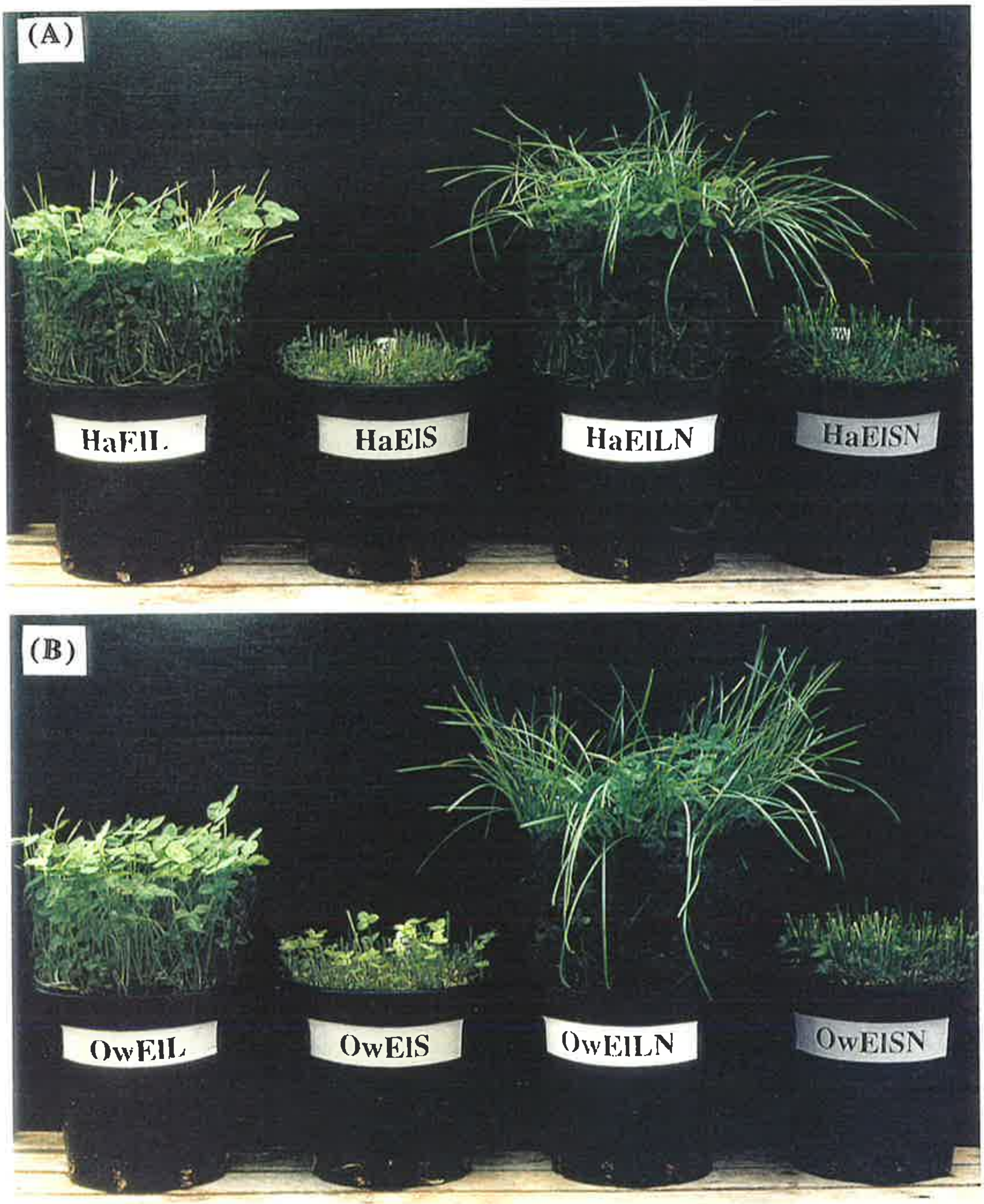



**Plate 6.2.** The effects of white clover cultivars, defoliation regime and nitrogen application on the growth of white clover-perennial ryegrass mixtures, before destructive harvest 1. (A) Mixture of Huia and Ellet, (B) mixtures of Olwen and Ellet. (Ha=Huia, Ow=Olwen, El=Ellet, S=severe defoliation, L=lenient defoliation, N=with nitrogen application

**Total shoot DM in mixtures**(white clover+perennial ryegrass). The harvest x defoliation and harvest x nitrogen application interactions were significant (P<0.001). Significant (P<0.05) decrease in yield through severe defoliation (Figure 6.4A) and increase due to nitrogen application (Figure 6.4B) occurred at harvest 1 only.

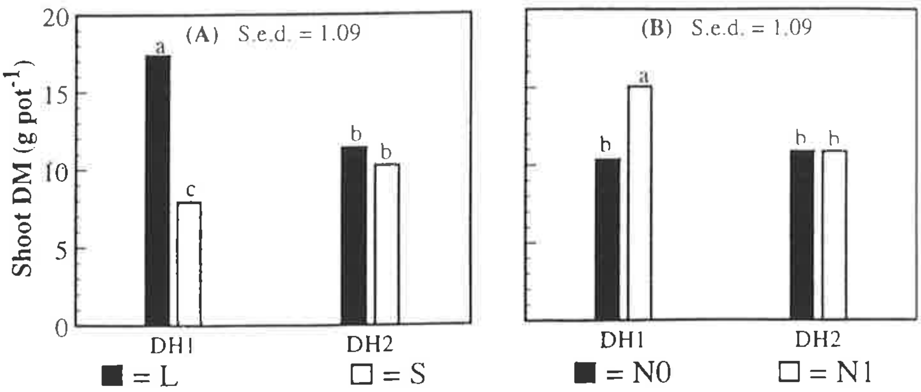



**Figure 6.4** The interaction of (A) harvest x defoliation, and (B) harvest x nitrogen application on total shoot DM (white clover+perennial ryegrass) in mixtures.

**Proportion of white clover in mixtures.** The harvest x nitrogen application interaction was significant (P<0.001). Nitrogen application reduced (P<0.05) the proportion of white clover at both harvests (Figure 6.5), but white clover proportion increased from harvest 1 to harvest 2.

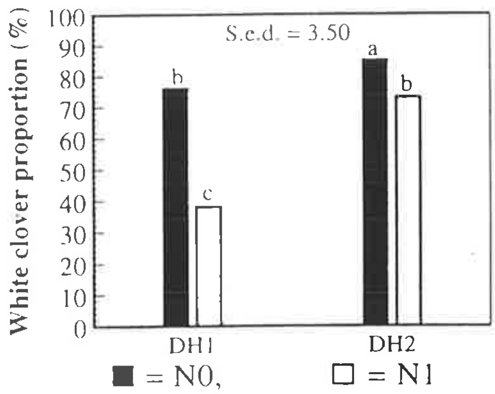



**Figure 6.5** The interaction of harvest x nitrogen application on white clover proportion in mixtures.

### 6.3.2 Dry Matter of White Clover Stolons in Mixtures at Destructive Harvests

The main effects of harvests, defoliation and nitrogen on stolon DM (Figure 6.6) were highly significant (P<0.001). Stolon DM was lowest at DH1 (Figure 6.6A), with severe defoliation (Fig. 6.6B) and with nitrogen application (Figure 6.6C).

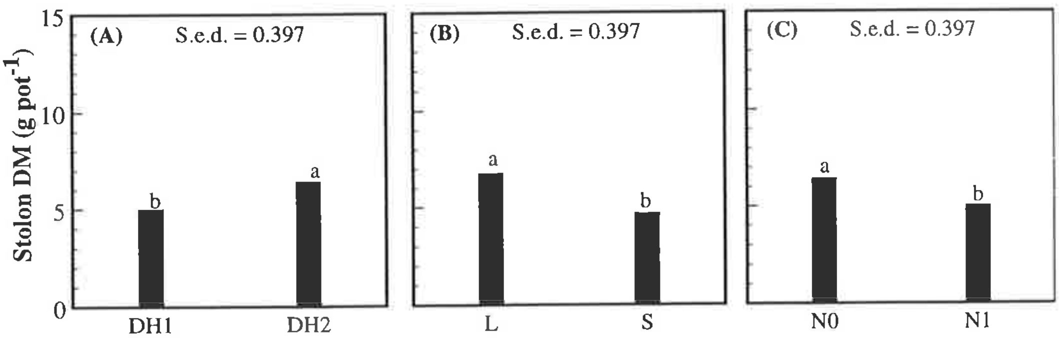



**Figure 6.6** The effects of harvests (A), defoliation regimes (B) and nitrogen application (c) on stolon DM of white clover in mixtures. Means associated with similar letters are not significantly different (P>0.05).

### 6.3.3 Dry Matter of Ryegrass Stubble in Mixtures

There was a significant main effect of harvests (P<0.001) and the defoliation x nitrogen interaction was significant (P<0.05). Without added nitrogen, lenient defoliation resulted in the lowest stubble DM (Figure 6.7B) but with added nitrogen lenient defoliation resulted in the highest stubble DM.

### 6.3.4 Relative Yield of White Clover

The harvest x cultivar interaction on RY of white clover shoots was significant ($P<0.05$) (Figure 6.8A). Other treatments and interactions were non-significant ($P>0.05$).

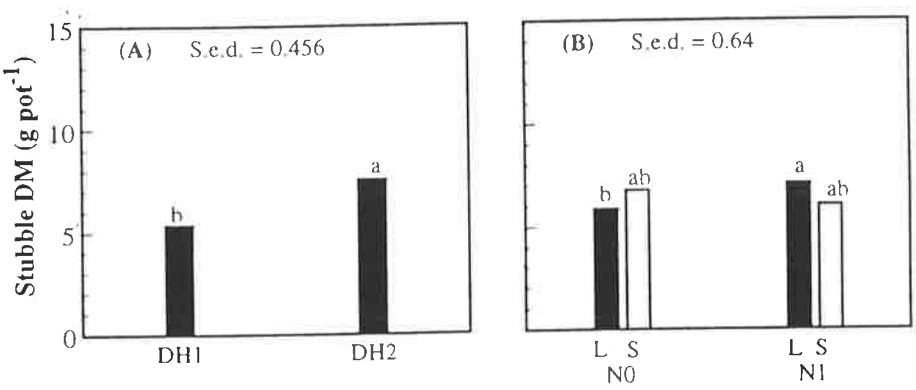



**Figure 6.7** The effects of **(A)** destructive harvest and **(B)** defoliation regimes x nitrogen application on stubble DM of perennial ryegrass. Means associated with similar letters in each graph are not significantly different ($P>0.05$).

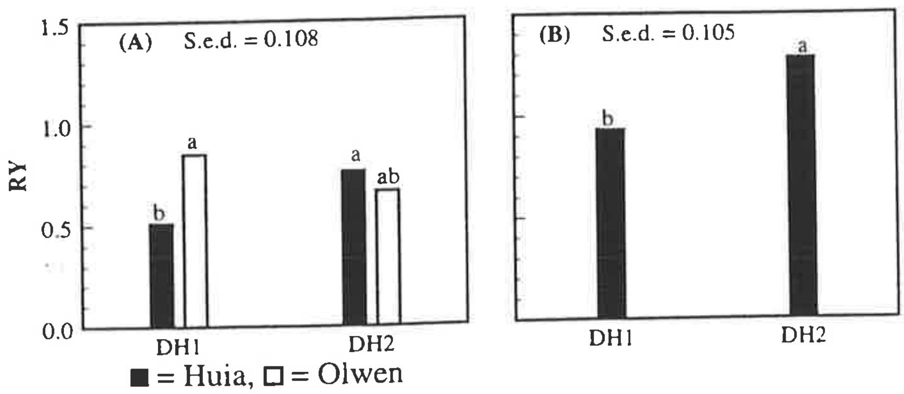



**Figure 6.8** (A) The cultivar x destructive harvest interaction on RY of white clover and (B) the effects of destructive harvest on RY of perennial ryegrass. Means associated with similar letters in each graph are not significantly different ($P<0.05$).

At DH1, Olwen had a higher RY than Huia, but at DH2 their RYs were the same. Thus RY increased from harvest 1 to harvest 2 for Huia but not Olwen.

### 6.3.5 Relative Yield of Perennial Ryegrass

There was a significant increase ($P<0.001$) from DH 1 to DH 2 of RY of perennial ryegrass (Figure 6.8B).

### 6.3.6 Relative Yield Total (RYT)

Relative yield totals were consistently higher than unity indicating the two species were not mutually exclusive. Except for the main effect of harvest no other treatment effects were significant ($P>0.05$). Relative yield total was highest at DH2 (Table 6.3).

**Table 6.3** The main effects of destructive harvest on RY total

| Destructive harvest | RYT |
|---|---|
| DH1 | 1.50$^{b}$ |
| DH2 | 1.99$^{a}$ |
| S.e.d. | 0.15 |

*Means associated with the same superscripts are not significantly different ($P>0.05$)

### 6.3.7 Competitive Ratio of White Clover ($RY_{wc}/RY_{pr}$)

In Section 6.3.1 (a) it was shown that there was no significant effect of cultivar of white clover on their CRs at pre-treatment cuttings; however there were significant effects of treatments on CR of white clover with respect to perennial ryegrass at destructive harvests.

There were significant main effects of defoliation ($P<0.05$), and nitrogen ($P<0.05$), and the harvest x cultivar x nitrogen interaction was significant ($P<0.05$). Severe defoliation decreased CR compared to lenient defoliation (Figure 6.9A). At DH1 without nitrogen application, both Huia and Olwen had similar CR values (Figure 6.9B), but with nitrogen application Olwen had a higher CR than Huia. At harvest 2, without nitrogen application Olwen had a higher CR, but with nitrogen application Olwen had a lower (P<0.05) CR than Huia.

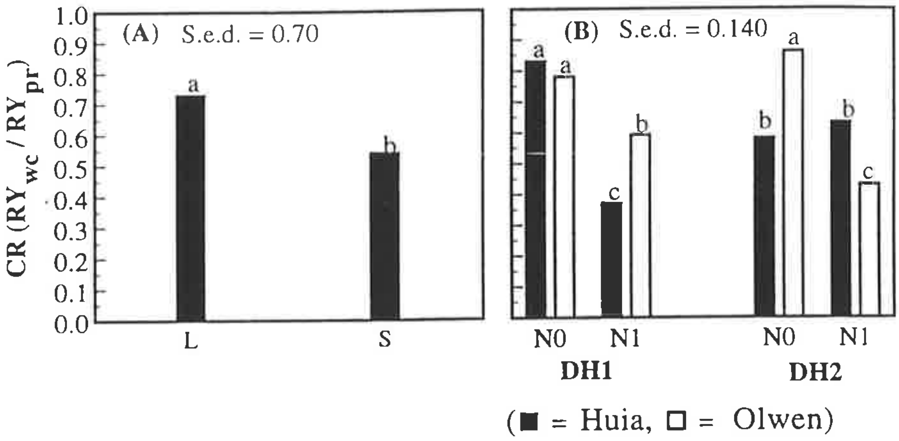



**Figure 6.9** Main effects of (A) defoliation regime and (B) harvest x cultivar x nitrogen interaction on CR of white clover with respect to perennial ryegrass. Means associated with similar letters in each graph are not significantly different (P<0.05).

### 6.3.8 Morphology of White Clover in Mixtures

#### (a) Petiole length

There were highly significant main effects of harvest (P<0.01) and cultivars (P<0.01) and defoliation x harvest interaction on petiole length. At DH2 white clover had longer petioles than at DH1 (data not presented), while Olwen had longer petioles than Huia (Table 6.4).

**Table 6.4.** The main effects of white clover cultivar on petiole length (cm) of white clover

| White clover cultivars | Petiole length (cm) |
|---|---|
| Huia | $14.81^{b}$ |
| Olwen | $17.66^{a}$ |
| S.e.d. | 0.81 |

*Means associated with the same superscripts are not significantly different (P>0.05)

At DH1 lenient defoliation resulted in greater petiole length than severe defoliation, but at DH2 no such differences were observed (Table 6.5). Petiole length was similar for both defoliation treatments at DH2.

**Table 6.5** The effects of defoliation regimes on petiole length (cm) at destructive harvest 1 and 2

| Destructive harvest | Petiole length (cm) | |
|---|---|---|
| | Lenient | Severe |
| DH1 | 22.50[a]* | 6.75[c] |
| DH2 | 16.83[b] | 18.85[b] |
| S.e.d. | 1.16 | |

*Means associated with the same superscripts are not significantly different (P>0.05)

### (b) Stolon length

All main effects of treatments on stolon length (cm $pot^{-1}$)were significant (P<0.05), as were defoliation x harvest, nitrogen x harvest, cultivar x nitrogen and cultivar x defoliation x nitrogen interactions (P<0.05).

Huia had greater stolon length than Olwen (P<0.05) regardless of nitrogen application and defoliation regime (Table 6.6). Lenient defoliation with applied nitrogen reduced (P<0.05) stolon length in Huia but in Olwen this effect was not significant. With severe defoliation there were no significant effects of nitrogen application on stolon length.

**Table 6.6** The white clover cultivar x defoliation regime x nitrogen interaction for stolon length (cm $pot^{-1}$) (means of four replicates)

| White clover cultivars | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Stolon length (cm) | | | |
| Huia | 1936.0[a]* | 1379.8[bcd] | 1436.7[bc] | 1735.8[ab] |
| Olwen | 973.6[cde] | 717.2[e] | 902.1[de] | 525.1[e] |
| S.e.d. | 217.9 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

### (c) Stolon diameter

There were significant main effects of harvest ($P<0.05$) and cultivar ($P<0.001$) on stolon diameter of white clover. Stolon diameter was less at DH2 than at DH1 (Table 6.7), and Olwen had thicker stolons than Huia ($P<0.05$) (Table 6.8).

**Table 6.7** Stolon diameter (mm) of white clover at destructive harvest 1 and 2

| Destructive harvest | Stolon diameter (mm) |
|---|---|
| DH1 | $1.66^{a*}$ |
| DH2 | $1.52^{b}$ |
| S.e.d. | 0.07 |

*Means associated with the same superscripts are not significantly different ($P>0.05$)

**Table 6.8** The effects of white clover cultivar on stolon diameter (mm)

| White clover cultivar | Stolon diameter (mm) |
|---|---|
| Huia | $1.24^{a*}$ |
| Olwen | $1.94^{b}$ |
| S.e.d. | 0.07 |

*Means associated with the same superscripts are not significantly different ($P>0.05$)

### (d) Area of 10 fully expanded leaves

Main effects of harvest, cultivar and defoliation, and the harvest x defoliation interaction were highly significant ($P<0.001$). Olwen had a much higher leaf area than Huia (Table 6.9).

**Table 6.9** The effects of white clover cultivar on their leaf area ($cm^2$/10 leaf blades)

| White clover cultivar | Leaf area ($cm^2$/10 leaf blades) |
|---|---|
| Huia | $27.66^{a*}$ |
| Olwen | $46.46^{b}$ |
| S.e.d. | 2.99 |

*Means associated with the same superscripts are not significantly different ($P>0.05$)

Severe defoliation reduced leaf area at DH1 but not at DH2 (Table 6.10). Lenient defoliation resulted in similar leaf area at DH1 and DH2, but severe defoliation resulted in less leaf area at DH1 compared to DH2.

**Table 6.10** The effects of defoliation regimes on leaf area ($cm^2$/10 leaf blades) at destructive harvest 1 and 2 . Means of eight replicates

| Destructive harvest | Defoliation regimes | |
|---|---|---|
| | Lenient | Severe |
| DH1 | 48.64$^{a*}$ | 16.37$^{c}$ |
| DH2 | 44.15$^{ab}$ | 39.06$^{b}$ |
| S.e.d. | 4.23 | |

*Means associated with the same superscripts are not significantly different (P>0.05)

### (e) Specific leaf area (SLA)

The cultivar x nitrogen x harvest and defoliation x nitrogen x harvest interactions were significant ($P<0.05$). Huia had the highest ($P<0.05$) SLA at DH1 in the absence of nitrogen application, (Table 6.11). All other treatment means were lower and similar.

**Table 6.11** The white clover cultivar x nitrogen x destructive harvest interaction on the SLA ($cm^2\ g^{-1}$) of white clover (means of four replicates)

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Specific leaf area ($cm^2\ g^{-1}$) | | | |
| DH1 | 519.8$^{a*}$ | 396.3$^{b}$ | 385.8$^{b}$ | 413.5$^{b}$ |
| DH2 | 400.8$^{b}$ | 397.1$^{b}$ | 403.6$^{b}$ | 348.1$^{b}$ |
| S.e.d. | 44.4 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

Again at harvest 1 in the absence of nitrogen, SLA was highest following severe defoliation (Table 6.12).

**Table 6.12** The defoliation regimes x nitrogen x destructive harvest interaction for the SLA ($cm^2\ g^{-1}$) of white clover (means of four replicates)

| Destructive harvests | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Specific leaf area ($cm^2\ g^{-1}$/10 leaf blades) | | | |
| DH1 | $412.1^{ab*}$ | $387.5^{b}$ | $493.5^{a}$ | $422.3^{ab}$ |
| DH2 | $400.0^{ab}$ | $371.5^{b}$ | $404.3^{ab}$ | $373.7^{b}$ |
| S.e.d. | 44.4 | | | |

*Means associated wth the same superscripts are not significantly different (P>0.05)

### 6.3.9 Non-structural Carbohydrates in Stolons of White Clover in Mixtures

The effects of treatment and treatment combinations on NSC in stolons are presented in Table 6.13A. Defoliation was the most important factor affecting the concentration and content of NSC in stolons with very highly significant ($P<0.001$) effects except for the sugar content as mg $pot^{-1}$.

#### (a) Sugar concentration and content

**Sugar concentration.** The cultivar x nitrogen x harvest interaction was significant ($P<0.05$) (Figure 6.10A). At DH1, Olwen had a higher sugar concentration without nitrogen than with it. The reverse occurred at DH2. Huia had similar sugar concentrations regardless of nitrogen level or harvest number. Huia had a lower level of sugar than Olwen at DH1 without nitrogen.

**Table 6.13** The significance of effects of treatments and interactions on concentration and content of sugars, starch and total NSC in (A) stolons of white clover and (B) stubble of perennial ryegrass

| (A) Main effects and interactions | Sugars | | Starch | | Total NSC | |
|---|---|---|---|---|---|---|
| | mg $g^{-1}$ | mg $pot^{-1}$ | mg $g^{-1}$ | mg $pot^{-1}$ | mg $g^{-1}$ | mg $pot^{-1}$ |
| Harvest (Hv) | ns | ns | ns | ns | ns | ns |
| Cultivar (Cv) | ns | ns | ns | ns | ns | ns |
| Defoliation (Defl) | *** | * | *** | *** | *** | *** |
| Nitrogen (N) | ns | ns | ns | ns | ns | ns |
| Cv x Hv | ns | ns | * | * | ns | ns |
| Hv x Defl | ** | ns | ns | * | ** | ns |
| Cv x Defl | ns | ns | ns | * | ns | ns |
| Hv x N | * | ns | ns | ns | * | * |
| Cv x N | ns | ns | ns | ns | ns | ns |
| Defl x N | ns | ns | ns | ns | ns | ns |
| Cv x N x Hv | * | ns | * | * | * | ns |
| Hv x Defl x N | * | ns | ns | ns | * | ns |
| CvxHv x Defl x N | ns | ns | ns | ns | ns | ns |

| (B) Main effects and interactions | Sugars | | Fructan | | Total NSC | |
|---|---|---|---|---|---|---|
| | mg $g^{-1}$ | mg $pot^{-1}$ | mg $g^{-1}$ | mg $pot^{-1}$ | mg $g^{-1}$ | mg $pot^{-1}$ |
| Harvest (Hv) | * | ns | *** | ns | *** | ns |
| Cultivar (Cv) | *** | *** | *** | *** | *** | *** |
| Defoliation (Defl) | ns | ns | ns | ns | ns | ns |
| Nitrogen (N) | *** | *** | *** | *** | *** | *** |
| Cv x Hv | ns | * | *** | *** | *** | ** |
| Hv x Defl | ns | ns | ns | ns | ns | ns |
| Cv x Defl | ns | ns | ns | ns | ns | ns |
| Hv x N | * | ** | *** | *** | *** | *** |
| Cv x N | *** | *** | *** | *** | *** | *** |
| Defl x N | ns | ns | ns | ns | ns | ns |
| Cv x N x Hv | * | ns | *** | *** | *** | *** |
| Hv x Defl x N | ns | ns | ns | ns | * | ns |
| CvxHv x Defl x N | ns | ns | ns | ns | ns | ns |

*, **,*** = significant at 5%, 1 and 0.1%, respectively; ns = P>0.05

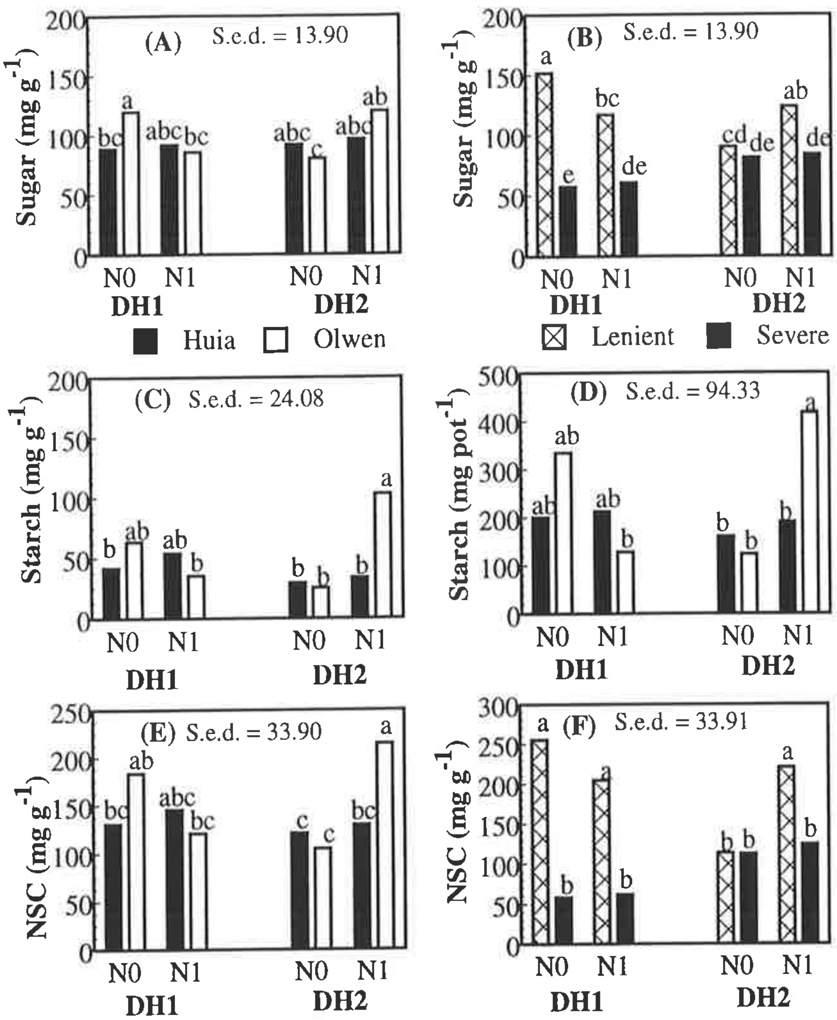



**Figure 6.10** The effects of treatment interactions on (A) and (B) sugar concentration; (C) starch concentration and (D) starch content and (E) total NSC concentration and (F) total NSC content in stolons of white clover in mixtures. Notice that different scales are used in the graphs. Means associated with the same letters are not significantly different (P<0.05).

The harvest x defoliation x nitrogen interaction was significant (P<0.0.05) Severe defoliation with nitrogen application reduced (P<0.05) sugar concentration in white clover at both harvests (Figure 6.10B) compared to lenient defoliation, but in the absence of nitrogen application, severe defoliation only reduced soluble sugars at DH1. Under severe defoliation stolons contained similar (P>0.05) sugar concentrations regardless of nitrogen application or harvest.

**Sugar content.** Sugar content was only affected significantly by defoliation treatment (Table 6.14). Severe defoliation caused a significant (P<0.05) reduction in sugar content in stolons.

**Table 6.14** Main effects of defoliation on sugar content (mg $pot^{-1}$) in stolons of white clover

| Defoliation | Sugar content (mg $pot^{-1}$) |
|---|---|
| Lenient | $633.78^{a*}$ |
| Severe | $222.08^{b}$ |
| S.e.d. | 59.93 |

*Means associated with the same superscripts are not significantly different (P>0.05)

### (b) Starch concentration and content

**Starch concentration.** The cultivar x nitrogen x harvest interaction was significant (P<0.05) (Figure 6.10C). Huia and Olwen had similar starch concentrations regardless of treatment or harvest except at DH2 when Olwen with N application had the highest (P<0.05) starch concentration in the stolons.

**Starch content.** The same trends as for starch concentration were observed for starch content (Figure 6.10D).

### (c) Total Non-structural Carbohydrate Concentration and Content

**Total NSC concentration.** The cultivar x nitrogen x harvest and defoliation x nitrogen x harvest interactions were significant (P<0.05). At DH1, Huia and Olwen had similar total NSC concentrations regardless of nitrogen application (Figure 6.10E), but at DH2 Olwen receiving nitrogen had the highest (P<0.05) total NSC concentration.

Severe defoliation with nitrogen application reduced (P<0.05) total NSC concentration in white clover at both harvests (Figure 6.10F) compared to lenient defoliation, but in the absence of nitrogen application, severe defoliation only reduced total NSC concentration at DH1. Under severe defoliation stolons contained the same (P>0.05) levels of total NSC concentration regardless of nitrogen application or harvest.

**Total NSC content.** The main effect of defoliation (P<0.001) and the nitrogen x harvest interactions were significant (P<0.05). Severe defoliation decreased markedly (P<0.01) the total NSC content in stolons compared to lenient defoliation (Table 6.15).

Nitrogen application resulted in the lowest (P<0.05) total NSC content at DH1, but the greatest at DH2 (Table 6.16).

**Table 6.15** Main effects of defoliation on total NSC content (mg pot$^{-1}$) in stolons of white clover

| Defoliation | Total NSC content (mg pot$^{-1}$) |
|---|---|
| Lenient | 1013.04$^{a}$* |
| Severe | 283.75$^{b}$ |
| S.e.d. | 108.33 |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Table 6.16** The effects of nitrogen x harvest interaction on NSC content (mg pot$^{-1}$) in stolons

| Destructive harvests | Nitrogen application | |
|---|---|---|
| | N0 | N1 |
| DH1 | 748.04$^{ab}$* | 452.82$^{c}$ |
| DH2 | 610.62$^{b}$ | 782.09$^{a}$ |
| S.e.d. | 153.21 | |

*Means associated with the same superscripts are not significantly different (P>0.05)

## 6.3.10 The Non-structural Carbohydrates in Stubble of Perennial Ryegrass in Mixtures

Effects of treatments and treatment combinations on the NSC components in the stubble of perennial ryegrass are presented in Table 6.13B. All main effects of cultivar of associated white clover and of nitrogen were significant, and the interactions involving cultivar x nitrogen x harvest were significant except for the sugar content. There was no effect of defoliation.

### (a) Sugar concentration and content.

**Sugar concentration**. Stubble of ryegrass in mixtures with Olwen, in the absence (but not in the presence) of nitrogen application had higher (P<0.05) sugar concentrations at both harvests (Figure 6.11A) than with Huia ($P>0.05$).

**Sugar content.** Stubble of ryegrass in mixtures with Olwen had higher ($P<0.05$) sugar content at both destructive harvests (Figure 6.11B) than in mixtures with Huia. In the absence of nitrogen application stubble of ryegrass in mixtures with Olwen had higher sugar content than in mixtures with Huia (Figure 6.11C).

### (b) Fructan concentration and content

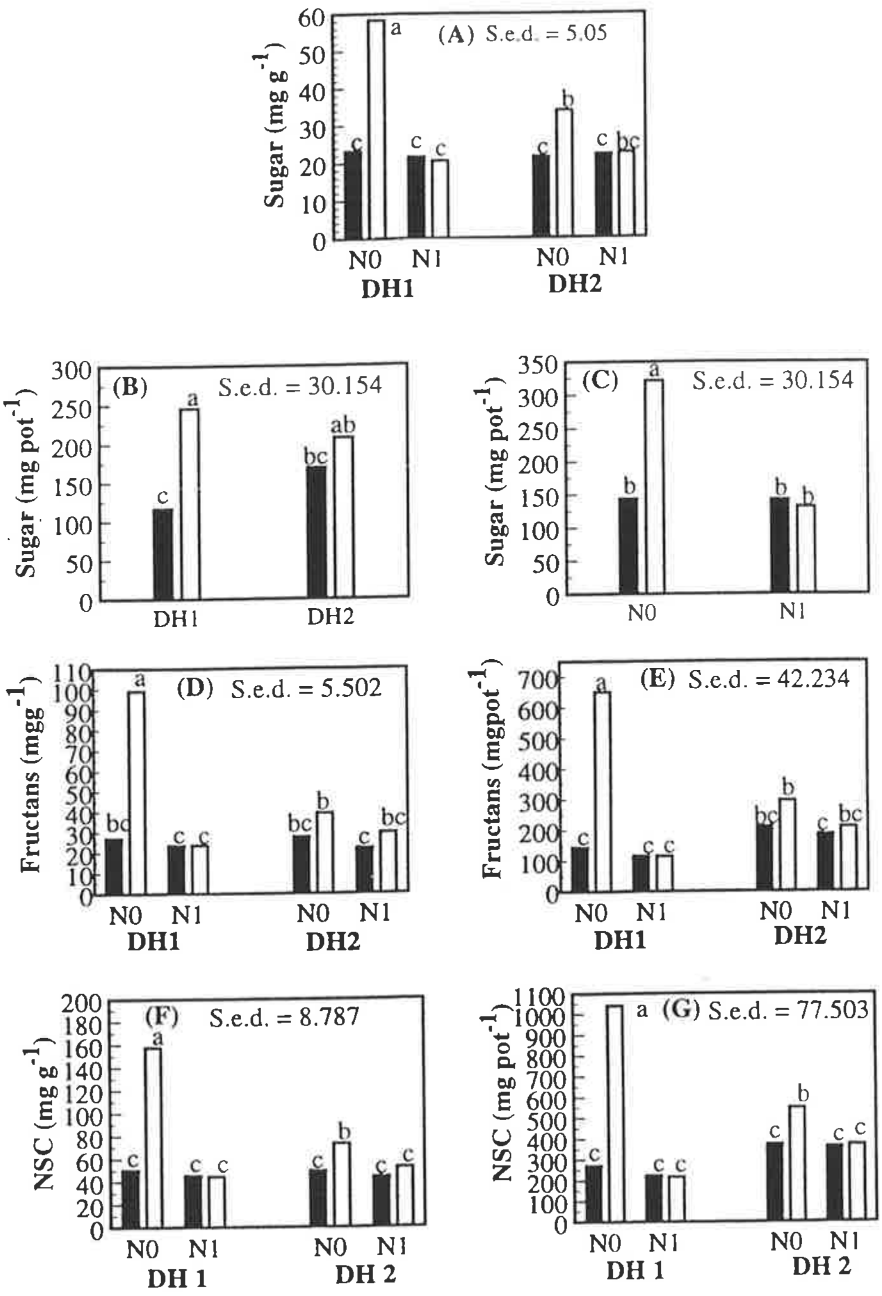
**Fructan concentration.** Fructan concentration was higher ($P<0.05$) in stubble of perennial ryegrass in association with Olwen than with Huia, in the absence of nitrogen application (Figure 6.11D) at harvest 1, but not at harvest 2 ($P>0.05$).

**Fructan content.** Fructan content was higher ($P<0.05$) in stubble of perennial ryegrass in association with Olwen than with Huia in the absence of nitrogen application (Figure 6.11E), at harvest 1 ($P>0.05$).

### (c) Total non-structural carbohydrate concentration and content

**Total NSC concentration.** Total NSC concentration was highest ($P<0.05$) in stubble of perennial ryegrass in association with Olwen than with Huia in the absence of nitrogen application (Figure 6.11F), at both harvests ($P>0.05$).

**Total NSC content.** Total NSC content (Figure 6.11G) was highest ($P<0.05$) in stubble of perennial ryegrass in association with Olwen than with Huia in the absence of nitrogen application, at both harvests ($P>0.05$).




(Associated white clover ■ = Huia, □ = Olwen)

**Figure 6.11** The effects of treatment interactions on (A) sugar concentration, (B) and (C) sugar content (D) fructan concentration, (E) fructan content, (F) NSC concentration, (G) NSC content in ryegrass stubble. Means associated with similar letters are not significantly different (P>0.05). Notice that different scales are used in the graphs.

### 6.3.11 Relationships between Competitive Ratio (Y axis) with Plant Traits and Non-structural Carbohydrates (X axis) in Stolons

Regression analyses (Section 6.2.5) were conducted between CRs (Y) of shoot

DM of white clover (measured at DH2) and plant traits (measured at DH2) and NSCs (X) in stolons of white clover measured at DH1 (Table 6.17). Competitive ratio had significant correlation with shoot biomass ($P<0.05$) , and height.

**Table 6.17** The relationships between CR of white clover (Y) measured at DH2 to plant traits measured at DH2 and NSC level (X) measured at DH1

| Plant traits and NSC pools | Level of significant | $r^2$ | Equation |
|---|---|---|---|
| Height (petiole length) | * | 0.52 | Y = 0.111+0.0325 X |
| Shoot biomass (g $pot^{-1}$) | * | 0.65 | Y = 0.132 + 0.0647 X |
| Leaf area | ns | 0.31 | Y = 0.377 + 0.007 X |
| Specific leaf area ($cm^2$ $g^{-1}$) | ns | 0.18 | Y = 0.053 + 0.0014 X |
| Stolon biomass (g $plant^{-1}$) | ns | 0.29 | Y = 0.168 + 0.079 X |
| Stolon length ( cm $pot^{-1}$) | ns | 0.02 | Y = 0.168 + 0.079 X |
| Stolon diameter (mm) | ns | 0.09 | Y = 0.418 + 0.138 X |
| Sugars | | | |
| Concentration (mg $g^{-1}$) | ns | 0.21 | Y = 0.349 + 0.003 X |
| Content (mg $pot^{-1}$) | ns | 0.39 | Y = 0.428 + 0.049X |
| Starch | | | |
| Concentration (mg $g^{-1}$) | ns | 0.26 | Y = 0.537 + 0.0021 X |
| Content (mg $pot^{-1}$) | ns | 0.39 | Y = 0.519 + 0.0006 X |
| Total NSC | | | |
| Concentration (mg $g^{-1}$) | ns | 0.07 | Y = 0.442 + 0.0014 X |
| Content (mg $pot^{-1}$) | ns | 0.02 | Y = 0.063 - 0.002 X |

* = significant at 5%, ns = non-significant ($P>0.05$)

### 6.3.12 Relationships of RY of Shoot Dry Matter (X) of White Clover and Non-structural Carbohydrates (Y)

The relationship between RY of NSC content of white clover stolon at DH1 (Y) and RY of shoot DM at Cut 3 (X) was significant for Olwen but not Huia (Table 6.18). The regression between RY of NSC content at DH2 (Y) and shoot DM at DH1 (X) was non-significant ($P>0.05$). For these analyses treatment means of the data involved were used.

**Table 6.18** The relationships between RY of white clover shoot DM at C3 (X) and RY of NSC content in the stolons at DH1 (Y) and between RY of white clover shoot at DH1 (X) and RY of NSC in the stolons at DH2 (Y) for Huia and Olwen

| Relationships | Level of significance | $r^2$ | Equations |
|---|---|---|---|
| RY of shoot DM at Cut 3 | | | |
| and RY of NSC at DH1 | | | |
| Huia | ns | 0.02 | Y = 0.356 + 0.103 X |
| Olwen | * | 0.95 | Y = -0.352 + 1.726 X |
| RY shoot DM at DH1 and | | 0.02 | |
| RY of NSC at DH2 | | | |
| Huia | ns | 0.17 | Y = 0.380 + 0.118 X |
| Olwen | ns | 0.06 | Y = 0.22 + 0.045 X |

@* =significant at $P<0.05$; ns=non-significant,

## 6.4 Discussion

The decline of shoot DM of perennial ryegrass from C1 to C3 (Appendix 6.2), and the increase of DM of white clover (Appendix 6.1), confirmed the inference in experiment III (Chapter V) that nitrogen availability declined during this period.

The observations that nitrogen application disadvantaged white clover (Martin and Field, 1984; Frame and Boyd, 1987b; Harris, 1987) were also confirmed in this experiment as indicated by white clover which received nitrogen application after C3, yielding lower shoot DM in mixtures (Figure 6.2A) but not in monocultures (Table 6.1) and therefore a lower CR (Figure 6.9). This is a confirmation of the previous observation (Chapter IV) of the difference in response of white clover in the presence compared with the absence of perennial ryegrass. Nitrogen application also reduced the proportion of white clover in the mixtures (Figure 6.5) and reduced stolon DM (Figure 6.6C) regardless of the cultivar and defoliation regime. In contrast nitrogen application increased shoot DM of perennial ryegrass in both monocultures (Table 6.2) and in mixtures at DH1 (Figure 6.3) resulting in concomitant decrease of CR of white clover (Figure 6.11). This is in line with the finding of Martin and Field (1984) that nitrogen application increased competitive ability of perennial ryegrass. However, the rates of nitrogen used in this experiment still imposed a limit to grass growth at DH2, resulting in a decrease of shoot DM of perennial ryegrass during the period from DH1 to DH2 and only a small increase ($P<0.05$) with nitrogen application at lenient defoliation (Figure 6.3). This result suggests that most of the nitrogen applied (75 kg $ha^{-1}$ N in split applications) was used up during the periods prior to DH1. Some workers applied nitrogen as high as 250-360 kg $ha^{-1}$ (Martin and Field, 1984; Frame and Boyd, 1987a), but a moderate rate was used in this experiment to encourage grass growth without severe effects on white clover.

The effects of nitrogen on carbohydrate reserves were similar in the grass and legume in some respects. Thus significant nitrogen effects occurred only in Olwen or in grass associated with Olwen. Furthermore, at harvest 1, the response to nitrogen was negative for both grass and legume. However, at harvest 2, the response to nitrogen was positive in Olwen, though still negative in Ellet. These responses probably had different causes in the grass and legume. The negative response to nitrogen in the grass may have been due to a priority for shoot growth rather than carbohydrate storage when nitrogen is applied. However, in Olwen, the negative response to nitrogen may have been a consequence of higher competition from ryegrass with nitrogen application reducing both the DM and carbohydrate concentration in stolons. This is supported by the positive correlation found, for Olwen only, between RY for DM at the last pretreatment cut and the RY for NSC at DH 1 (Table 6.18).

The higher stolon NSC concentration at harvest 2 with nitrogen application does not seem to be consistent with the above explanations or with the higher CR in the absence of nitrogen application at harvest 2. It appears that changes in the levels of nitrogen fixation in the two nitrogen treatments as the soil nitrogen is depleted may affect relative carbohydrate levels. This complex situation is difficult to predict. Hence there was no correlation between RY for DM at DH1 and NSC at DH2 (Table 6.18).

The lack of NSC response to nitrogen in Huia and in grass associated with Huia is difficult to explain but the differences between the two clover cultivars illustrate why there is no overall correlation between carbohydrate reserves and CR at the same harvest (Table 6.17 and 6.18). It is possible that Olwen and Huia differ in the nitrogen contribution to ryegrass. If so, it would complicate the relationships between competition and carbohydrate levels.

The contrasting effects of severe defoliation on ryegrass and white clover ensured that the DM yield and carbohydrate levels of white clover in mixtures and the CR were consistently much lower with severe defoliation than with lenient. Thus in this case there seems to be a clear relationship between competitive ability and concurrent levels of carbohydrates, at least at harvest 1.

The strong recovery of white clover shoot DM from severe defoliation in both monoculture (Table 6.1) and mixture (Figure 6.2B) at harvest 2 was not accompanied by such strong recovery of carbohydrates, probably because the priority for NSC utilization after defoliation was for shoot growth rather than reserve replenishment, at least in the short period of recovery between harvest 1 and harvest 2 (Appendix 6.3). At the same time the responses of CR of Huia and Olwen to nitrogen application differed at harvest 2 (Figure 6.9). Thus, the relationship between CR and NSC levels was a changing and complex one, influenced by changing soil nitrogen levels and recovery from defoliation as well as by cultivar differences. Such complexities may also have contributed to the lack of significant correlation between CR and other plant traits at the same harvest (Table 6.17).

The evidence from this experiment indicates (as in Experiment 3) that competition affects not only the RY of white clover but also the RY of NSC at the following harvest. However, this relationship may be altered or negated by the changes imposed on white clover, by the declining influence with time of nitrogen application and severe defoliation. There appears to be no evidence that carbohydrate levels at one harvest affect CR at the next. Rather, white clover recovered quickly from the brief setbacks imposed by nitrogen and severe defoliation, indicating that other factors such as root carbohydrate reserves and nitrogen fixation may be important.

Some workers have observed that in the event of unfavourable environmental conditions growth will be affected earlier than photosynthesis, resulting in the build up of NSC in plant organs (Henry and Raper, 1991; Qiu and Israel, 1994). Hence, soil nitrogen shortages in the absence of N application during the periods from DH1 to DH2, could have resulted in the accumulation of NSC in the stubble of Ellet (Figure 6.11). There would be a burst of growth if the shortage was alleviated, as is normally observed if N is applied to N-starved grass. In this case, an increase in N availability results in better regrowth of perennial ryegrass than white clover.

In the absence of nitrogen application, the generally higher carbohydrates in the stubble of perennial ryegrass in association with Olwen than with Huia (Figure 6.11) indicated that (i) the growth of perennial ryegrass was restricted by low nitrogen status of the soil, (ii) Olwen was more competitive for nitrogen toward perennial ryegrass compared with Huia, this being also suported by higher CR of Olwen compared with Huia at DH2 (Figure 6.9 B).

In the presence of nitrogen application, carbohydrates in stubble of perennial ryegrass were similar for the two cultivars of white clover (Figure 6.11) i.e. perennial ryegrass was not affected differentially by the two cultivars of associated white clover. However, CR of Olwen was higher ($P<0.05$) than Huia at DH1 (Figure 6.9 B) but became lower at DH2. As soil nitrogen had declined at DH2, this indicated that Olwen was favoured more than Huia by higher soil N in competition with perennial ryegrass. It would be interesting to see the response of these two cultivars of white clover in terms of carbohydrate content in competition with perennial ryegrass, if nitrogen application was increased to 200-300 kg N $ha^{-1}$ as used by other workers, instead of 75 kg $ha^{-1}$ that was used in this experiment.

The white clovers were independent of soil nitrogen, and their shoots as well as their stolons grew and may eventually have released nitrogen to the soil. This would have contributed to the coexistence or equilibrium generally observed in white clover - perennial ryegrass mixtures (de Wit *et al.*, 1966;Harris and Thomas, 1973; Hall, 1974a,b). Perennial ryegrass might benefit from nitrogen transference from the legumes as was indicated by the increase of RY from DH1 to DH2 (Figure 6.8B).

Goodman and Collison (1986) reported that in mixtures Olwen was more competitive and fixed more nitrogen than a smaller-leaved cultivar, S24, in the presence of high soil nitrogen. This result was supported at DH1, with nitrogen application, by higher RY (Figure 6.8A) and higher CR ($RY_{wc}/RY_{pr}$) (Figure 6.9B) of Olwen than Huia. In contrast, in the absence of nitrogen application, CR of Olwen was similar to that of Huia at DH1. Furthermore, the application of nitrogen and lenient defoliation, which could be expected to increase the competitive ability of ryegrass, significantly decreased stolon length of Huia but not that of Olwen (Table 6.6).

At DH2, with the decline of nitrogen in the soil, the CR of white clover with respect to perennial ryegrass in N1 increased in Huia ($P<0.05$), but decreased in Olwen (Figure 6.9B). This might indicate a faster transformation of sugars to starch in Olwen or that faster regrowth or maintenance resulted in a lower sugar concentration at lenient defoliation or in the presence of nitrogen in the soil. Olwen should have higher rates of photosynthesis than Huia due to larger leaf area (Table 6.9), while the SLA was similar for both cultivars (Table 6.11). This may also mean that during the period of regrowth (four weeks) from DH1 to DH2 Huia favoured shoot growth at the expense of stolons or roots, while Olwen partitioned more photosynthates to stolons or roots. This was shown in the higher starch content (Figure 6.10D) and higher total NSC concentration in stolons of Olwen compared to Huia at DH2 with nitrogen application (Figure 6.10E). However, the lower yields of shoot DM of perennial ryegrass at DH2 than at DH1 suggesting soil nitrogen depletion, may mean that lower soil nitrogen reduced uptake by Olwen. The higher total NSC in stolons of Olwen than Huia at DH2 would enable Olwen to compete for soil nitrogen as reported by Goodman and Collison

(1986). This result might also mean that Olwen may require higher soil nitrogen to achieve high DM production as also found by Frame and Boyd (1987a) in that larger-leaved cultivars Olwen and Linda were more productive at higher nitrogen rates of 240 - 360 kg $ha^{-1}$ than smaller or medium-leaved cultivars such as Huia.

The higher CR of Huia than Olwen at DH2 at high nitrogen might be due to (i) greater number of growing points resulting in higher shoot growth in the presence of nitrogen, (ii) higher current assimilates fixed by Huia through its longer (Table 6.6), thinner stolons (Table 6.7) than Olwen, and differential allocation of current photosynthates for the growth of shoots, stolon formation and storage filling (Steinlein *et al.*, 1993). Nitrogen might also have been depleted by perennial ryegrass. This is clearly shown in higher starch in stolons of Olwen than Huia (Figure 6.10D). Due to limitation in bench space in the glasshouse and limitation in labour the residual leaf areas could not be measured. Hence, differential residual leaf areas between Olwen and Huia could not be discussed. However Huia had longer (Table 6.6), thinner stolons (Table 6.7) and greater number of growing points than Olwen (see Chapter VII) suggesting that Huia had larger residual leaf areas than Olwen leading to the higher CR of Huia than Olwen at DH2 at high nitrogen.

The higher specific leaf area (SLA) in Huia than Olwen in the absence of nitrogen application (Table 6.11) agrees with the results in experiment III. Even though in the present experiment the higher SLA of white clover with severe defoliation than with lenient defoliation (Figure 6.12) was non-significant ($P>0.05$) the increases in SLA due to defoliation agrees with the finding of Chapman *et al.* (1990b) that the immediate response of white clover to defoliation is the increase of SLA to maximize light absorption per unit carbon cost.

White clover in mixture produced significantly higher shoot DM in the absence of N application (Figure 6.2A), and severe defoliation generally decreased NSC in stolons (Figure 6.10F) (except in the absence of N application at DH2), which might indicate a trade off for the growth of shoot at the expense of the stolon as found by

Tesar and Ahlgren (1950).

The correlation of CR with shoot biomass and height of white clover but not with other traits (Table 6.17), might indicate that in the conditions of this experiment shoot competition was more important than root competition, which was caused by the decline of nitrogen level in the soil resulting in low shoot DM yield of perennial ryegrass at DH2 (Figure 6.3). These results also indicated that shoot biomass and height were more important than NSC concentration and content in affecting competitive ability of white clover.

## 6.5 Conclusions

On the basis of the results of the experiment the following can be concluded :

1. In mixtures, nitrogen application and severe defoliation reduced shoot DM of white clover, and the proportion of shoot DM of white clover to total and stolon DM. In perennial ryegrass nitrogen application increased shoot DM at harvest 1 but this effect disappeared at harvest 2.

2. Relative yield of Olwen white clover was higher than Huia at destructive harvest 1 (DH1) (when soil nitrogen level was still high) but no difference was observed at destructive harvest 2 (DH2).

3. In white clover severe defoliation caused a decrease in NSC levels in the stolons.

4. In perennial ryegrass shoot regrowth was not related to NSC levels in the stubble.

5. Nitrogen application (75 kg N $ha^{-1}$) resulted in similar sugar and NSC levels at DH1 in stolons of Olwen and Huia, but in the absence of nitrogen application stolons of Olwen contained higher ($P<0.05$) sugar levels than Huia. As there were no

differences in growth (DM) of stolons of the two cultivars at low nitrogen, the difference in sugar level can not be due to N effects on growth. Olwen response in term of NSC in the stolon is not explained by a differential effect of N on the growth of Olwen and Huia. It could be that in the absence of nitrogen application, more sugar was used by Huia than by Olwen for respiration to provide energy and substrates for the increase inlength of stolons (Table 6.6) and for the greater growth of SLA (Table 6.11). In addition, the similar NSC in Olwen and Huia at DH1 with applied nitrogen might indicate that more NSC was used in Olwen for root growth and activity (see Chapter VII).

6. In the absence of nitrogen application, stubble of perennial ryegrass at DH1 had higher sugar concentration, total NSC concentration and NSC content in mixtures with Olwen than with Huia. As there was no difference in regrowth of ryegrass at harvest 2 in association with the two cultivars of white clover, NSC content at DH2 did not influence subsequent growth of ryegrass.

7. At DH1, white clover cv. Olwen in mixtures with Ellet was more competitive (had higher competitive ratio) than Huia in the presence but not in the absence of applied nitrogen. At DH2 after applied nitrogen was depleted, the reverse occurred.

8. Competitive ratio was more strongly related to above ground biomass and petiole length of white clover than NSC concentration or content.

# Chapter VII

# Experiment V. Effects of Non-structural Carbohydrate Reserves and Nutrient Supply on The Regrowth of White Clover and Perennial Ryegrass in Mixtures

## 7.1 Introduction

In the absence of current assimilates after defoliation, white clover and perennial ryegrass remobilize their reserves to provide energy for maintenance respiration and regrowth (Baur-Höch *et al.*, 1990). Thus, plants with higher non-structural carbohydrate (NSC) reserves should have more regrowth after defoliation. However, the speed of recovery from defoliation would also depend on the residual leaf area, the number of the growing points and favourable environmental conditions for regrowth. Hence, recovery would depend on the levels of NSC reserves and current assimilates.

Rechel (1993) suggested that in order to study the amount of reserves available for regrowth, the whole plants (stubble and roots) should be examined. One way to do this is to measure the etiolated regrowth of plants as an indicator of available reserves (Davies, 1965; Kigel, 1980; Richards and Caldwell, 1985; Rechel, 1993). In such experiments, the plants are usually put in the dark in controlled environments, or are covered by inverted plastic pots painted white to prevent a rise in temperature around the plants. The regrowth is then cut regularly until it ceases. Using this technique, Richards and Caldwell (1985), for example, were able to show that the more grazing tolerant *Agropyron desertorium* consistently produced more growth in the absence of photosynthesis than *A. spicatum*. This indicated the value of NSC reserves for grazing tolerance. Thus, the technique detects differences in regrowth due to differences in NSC reserves, which may be important from the point of view of competition.

In the previous experiments it was established that Olwen white clover had greater pools of NSC in its stolons compared to Huia (Chapter III). However, while both cultivars had similar competitive ratio initially, as growth continued and mineral and nitrogen availability declined, competitive ratio of Olwen declined and Huia had a higher competitive ratio (Chapter V). While this seemed to be related to the lower competitive ability of Olwen under low nitrogen conditions, the influence of the number of growing points and level of carbohydrate reserves on competitive ability also needs to be understood.

In previous experiments, only NSCs in the stolons were considered. This was because extraction of NSC has to be done within 30 minutes of harvesting the plant material, to avoid loss of NSC by respiration. Washing, separation and subsampling of roots from mixtures grown in soil would take much more than 30 minutes. Nevertheless, there is evidence of the remobilization of root reserves for regrowth after defoliation (Culvenor *et al.*, 1989b; Davidson *et al.*, 1990). Hence, in the present experiment sand culture was used to facilitate separation of the roots. Provision was also made to examine the relative importance of remobilization of NSC from stolons and from roots.

Cultivars of white clover have been shown to differ in their tolerance to high levels of nitrogen in the soils (Brock and Hoglund, 1974; Wilman and Asiegbu, 1982a,b). Previous experiments confirmed that Olwen required higher nitrogen from the soil than Huia for its growth. However, how the levels of nitrogen and other nutrients in the soil would affect the regrowth of Olwen and Huia in mixtures, is not known. After defoliation, shoot regrowth is favoured at the expense of root growth. In the absence of a supply of current assimilates to the roots, root activities decline, resulting in only a small contribution of mineral nitrogen to the growth of the shoot (Marriott and Haystead, 1990). However, higher levels of mineral nitrogen and other nutrients in the soil might reduce energy requirement for root activities. Hence, this would lead to higher regrowth.

The experiment reported here was designed to examine the hypotheses :

(1) in mixtures, a white clover cultivar with larger reserves of NSC (Olwen) will have more etiolated regrowth after defoliation than a cultivar with lower levels of NSC (Huia);

(2) cultivar with larger reserves of NSC (Olwen) will also have a better regrowth in the presence of sufficient soil nutrients than a cultivar with lower levels of NSC (Huia);

(3) a cultivar of white clover with long and thin stolons and higher node numbers (Huia) will have more growing points per unit area (and thus more leaves) than a cultivar with thick and short stolons and lower node number (Olwen); and

(4) the remobilization of NSCs from the storage organs is proportional to the quantities of NSCs available as reserves.

The results of this experiment is expected to explain the reasons for higher competitive ratio of Huia compared to Olwen as observed in Experiment III (Chapter V).

## 7.2 Materials and Methods

The experiment was conducted in pots in a glasshouse similar to previous experiments, during the January-March periods of 1994. Temperatures were controlled by evaporative cooling and were set for the range 11-25 $^{O}$C.

### 7.2.1 Treatments and Design

Treatments comprised the factorial combination of two cultivars of white clover with perennial ryegrass (2 mixtures x 3 nutrient levels x 2 light levels for regrowth) replicated three times in a randomized complete block design. In addition, an extra six pots (three pots for each mixture) were destructively harvested at the commencement of treatment applications to measure the level of NSC 7 weeks after establishment (at the time of harvesting).

White clover *cv.* Olwen and *cv.* Huia were each mixed with perennial ryegrass *cv.* Ellet (50:50 mixture) and were grown for seven weeks prior to application of three nutrient treatments which consisted of three levels i.e. Nil - deionized water; nitrogen (N) only; and N plus other nutrients. The concentrations of N only and N plus other nutrients were similar to those in 1/2 strength Hoagland's solution. There were two light treatments i.e. etiolated (E) - covered by inverted plastic pots to prevent photosynthesis, and light (F)- exposed to full light.

### 7.2.2 Cultural Techniques

Plastic pots (18 cm diameter, 20 cm height) were filled with 5.5 kg coarse sand which had been sieved with sieve number 10 (2.8 mm). Total plant density used was 12 plants pot$^{-1}$ (adjusted to give similar density to those in previous experiments).

Preliminary trials conducted to find optimum nutrient solution application showed that 400 ml of half strength Hoagland's solution twice weekly gave maximum dry matter yield after six weeks regrowth. Hence, this rate was used subsequently during the 7 week establishment period for all pots (Hammer *et al.* 1978) (Appendix 3.3).

Plastic saucers were placed underneath the pots to catch and retain excess nutrient solution. Nutrients were added twice weekly. Similar volumes of deionized water were applied to pots with Nil nutrient treatment. Deionized water was applied to all pots as necessary to prevent water stress. Once a week the pots were flushed with tap water and the plastic saucers were emptied and washed to prevent the accumulation of nutrients in the saucers (Hocking and Meyer, 1991). During the establishment period, mosquito wire cylinders were placed around the pots and raised as the plants grew taller, to prevent shading between adjacent pots. After the establishment period, the plants were cut at 3.0 cm above ground level, and the pots were flushed with deionized water to leach out the residual nutrients in the sand (Marriott and Haystead, 1990), followed by the application of appropriate nutrient treatments. Water collected after flushing the pots with 5.0 L deionized water indicated only a negligible amount of nutrients as measured by ICP (compared to deionized water). Hence all pots were flushed with 5.0 L of deionized water.

Etiolated regrowth was obtained by covering each pot with an inverted plastic pot painted white to minimize the rise in temperature beneath it (Plate 7.1).

### 7.2.3 Measurements

After seven weeks establishment, the extra six pots were destructively harvested, shoots and roots and stolons of white clover were separated from those of grasses. Roots were separated from stolons and stubble and washed free of sand. The stolons, stubble and roots were sampled for NSC analyses.

The regrowth was cut at intervals of 2-3 days for two weeks and shoots of clover and grass were separated. Yields of the harvests were pooled for statistical analysis. It has been shown that white clover leaves start to export photosynthates at stage 0.6 of Carlson's classification (Appendix 3.1) i.e. when the leaves are about 10 percent unfolded (Chapman *et al.*, 1990a). Hence, to prevent export of assimilates to stolons by plants in the light, regrowth of all leaves older than this stage in pots with the light treatment was harvested on days 2, 4, 7, 10 and 14 and the number of harvested leaves counted. At the same times, all other plants (dark treatment) were also harvested. Grass and clover leaves in the etiolated treatment were cut at 3.0 cm above the soil surface. White clover petioles in the light treatment were generally shorter than 3.0 cm, and the leaves were cut with ca. 1.0 cm of petiole attached. Preliminary trials also showed that in plants covered with inverted white plastic pots regrowth ceased after two weeks. Hence, the experiments were terminated after two weeks of regrowth. At the end of the experiment, plants were destructively harvested. Stolon length, stolon diameter and stolon dry matter of white clover and stubble dry matter of perennial ryegrass were measured. The stolons and stubble were sampled and analysed for NSCs in the manner previously described (Section 3.3.1). Roots were also washed free of sand and the white clover and perennial ryegrass roots separated. Sampling of the roots for NSC determinations were only conducted for one replicate. All of the procedures for sampling of stolons and stubble and roots for analysis for each pot were conducted in less than half an hour to minimize loss of substrates due to the activity of respiratory enzymes. The same procedures were applied to the six pots destructively harvested at the commencement of treatment application.


**Plate 7.1.** Overview of experiment V

### 7.2.4. Statistical Analysis

Data were subjected to analysis of variance using Genstat 5 (Genstat 5 Committee, 1987), after transformation where necessary to ensure homogeneity of variance. Mean separation was conducted using Duncan's Multiple Range Test (Steel and Torrie, 1960).

## 7.3 Results

### 7.3.1 Total Dry Matter and NSC after Establishment

Dry matter and their NSC content seven weeks after planting (before respective treatments were applied) are presented in Figure 7.1. Soluble sugars, starch or fructans and total NSC are presented in Appendix 7.1. Huia and Olwen had similar shoot (Figure 7.1A), stolon (Figure 7.1B) and root DM (Figure 7.1D). However, Huia had a higher ($P<0.05$) content of NSC in the stolons compared to Olwen (Figure 7.1C). The reverse was true ($P<0.05$) for total NSC in the roots with Olwen roots having three times the root NSC content of Huia, in spite of similar root DM (Figure 7.1E). Ellet had similar stubble DM regardless of the associated white clover cultivar (Figure 7.1A), but higher root DM in mixtures with Huia than with Olwen (Figure 7.1D).

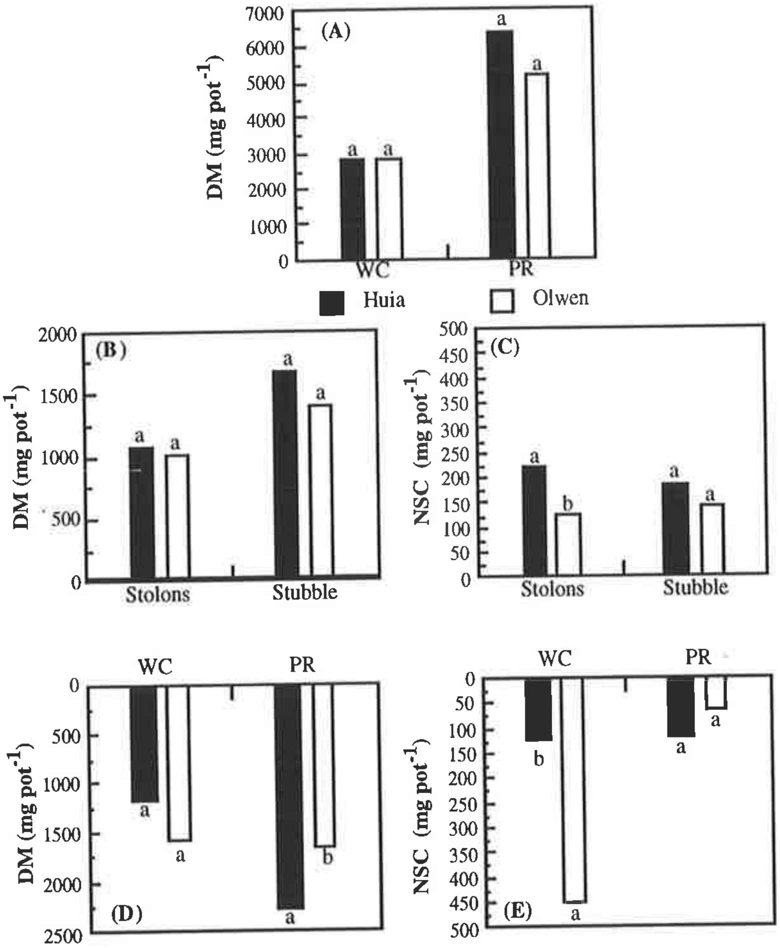



**Figure 7.1** Mean DM yield (mg pot$^{-1}$) of (A) shoot, (B) stolons and stubble of perennial ryegrass with different associated white clover cultivars; (C) total non-stuctural carbohydrates, and (D) roots of white clover and perennial ryegrass with different associated white clover cultivars and (E) total NSC in their roots seven weeks after planting. Means of each variable in each graph associated with the same letters are not significantly different (P>0.05). WC=white clover, PR=perennial ryegrass

Perennial ryegrass stubble and roots had similar total NSC regardless of the associated white clover cultivar (Figure 7.1B,C).

### 7.3.2 Total Dry Matter from Shoot Regrowth

Plants receiving light treatments were healthy and green in colour, while etiolated regrowth was whitish indicating that covering the plant with inverted pots was able to prevent completely the formation of chlorophyll and photosynthesis. There was a very highly significant (P<0.001) main effect of light on all parameters recorded, with plants in the sun outyielding plants in the etiolated treatments. The effects of nutrient treatments were non-significant (P<0.05). Hence, in the following sections only the effects of cultivars and light and their interactions are presented.

There were highly significant (P<0.01) effects of white clover cultivar and light treatment on total shoot DM regrowth. Olwen had a significantly (P<0.05) higher dry matter in regrowth than Huia (Figure 7.2A) and plants in the light produced higher shoot DM in regrowth than plants in the dark (Figure 7.2B).

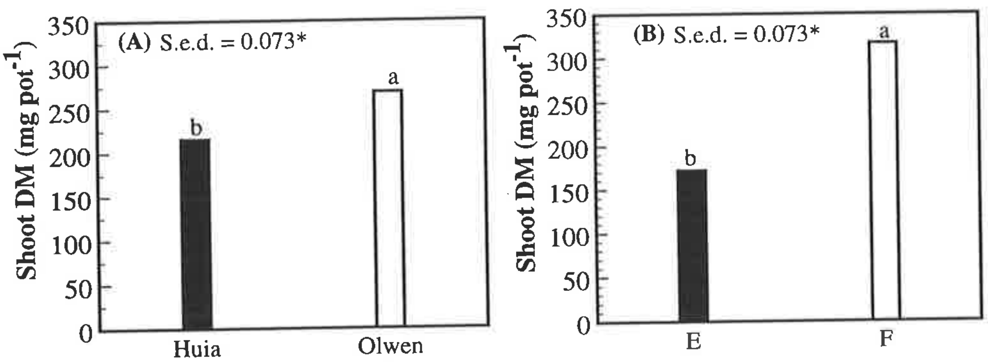



**Figure 7.2** The main effect of (A) white clover cultivars and (B) light treatment on the amount of regrowth. Means associated with the same letters are not significantly different (P>0.05). * After log10 transformation

The effects of white clover cultivar x light x nutrient interaction on their shoot regrowth are presented in Figure 7.3A. While this interaction was not significant (P>0.05), it is useful in showing the levels of yield of the cultivars in the individual treatments.

The cultivar x light x nutrient interaction for shoot DM of perennial ryegrass was significant (P<0.05). Only in the N-only treatment in the light (Figure 7.3B) were there significant (P<0.05) differences between Ellet associated with Olwen and Huia: in that treatment, Ellet produced significantly (P<0.5) more DM in association with Huia than with Olwen.

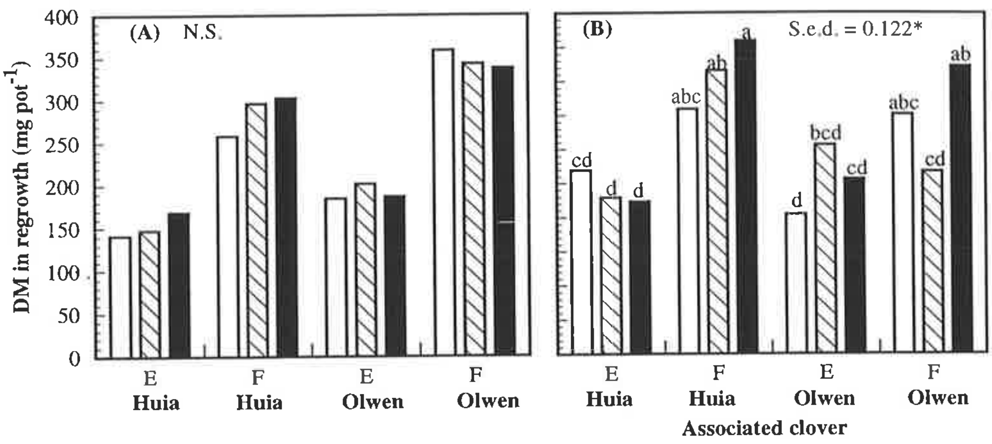



**Figure 7.3** The effects of (A) cultivar x light x nutrient interaction on DM in regrowth of shoots of white clover, and (B) associated clover x light x nutrient interaction on shoot DM in regrowth of Ellet (□ = nil, = ⧅ nitrogen, ■ = complete; E=etiolated, F=full light). Means associated with similar letters are not significantly different (P>0.05). * After log10 tranformation.

### 7.3.3 Dry Matter of Plant Components and Morphology of Stolons at Final Harvest (after 2 weeks regrowth)

#### (a) Stolon and stubble dry matter

Stolon and stubble DM yields were significantly (P<0.05) higher in the full light treatment than without light (Figure 7.4 A,B). There were no other significant main effects or interactions, i.e. no treatment differences for either of stolon or stubble.

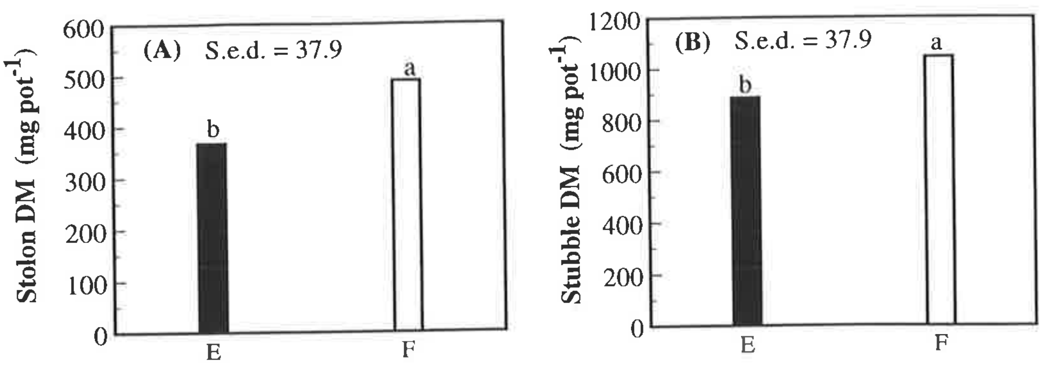



**Figure 7.4** The effects of light on (A) the stolon of white clover and (B) the stubbles of perennial ryegrass. (E=etiolated, F=full light). Means associated with the same letters are not significantly different (P>0.05). Notice that different scales are used in the graphs.

### (b) Stolon morphology

Huia had significantly (P<0.05) longer and thinner stolons, with a higher node number compared to Olwen (Table 7.1).

**Table 7.1** The effects of white clover cultivars on stolon length (cm $pot^{-1}$), diameter (mm) and node numbers (nodes $pot^{-1}$)

| White clover cultivars | Stolon | | |
|---|---|---|---|
| | Length (cm $pot^{-1}$) | Diameter (mm) | Node number (nodes $pot^{-1}$) |
| Huia | $119.1^{a}$* | $1.53^{b}$ | $64.1^{a}$ |
| Olwen | $57.3^{b}$ | $2.12^{a}$ | $32.4^{b}$ |
| S.e.d. | 11.0 | 0.10 | 0.1 |

*Means associated with the same letters in the same column are not significantly different (P>0.05)

### (c) Root dry matter

Olwen had significantly (P<0.001) higher root yields than Huia (Figure 7.5A). There was a significant (P<0.05) light x nutrient interaction in perennial ryegrass (Figure 7.5 B). Ellet root yield in the full light treatment was significantly higher (P<0.05) with Hoagland solution than with N alone.

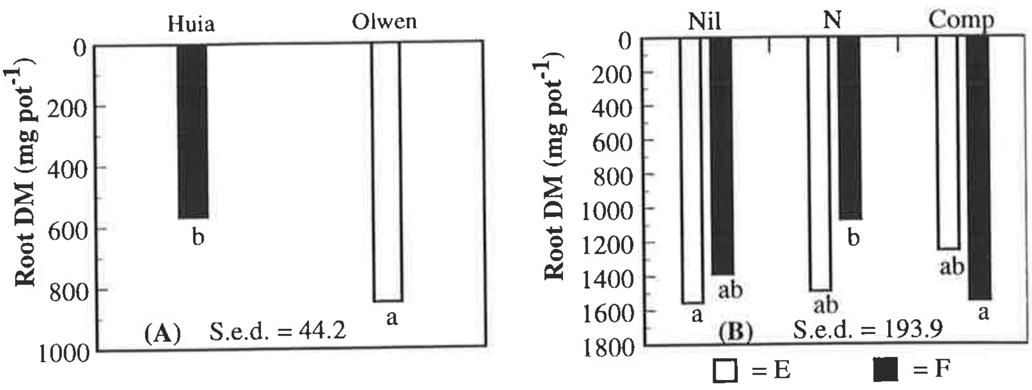



**Figure 7.5** The effects (A) of white clover cultivar on the root yield of white clover and (B) of light and nutrient levels on root yield of perennial ryegrass. Means associated with the same letters are not significantly different (P>0.05)

### (d) Total biomass

White clover had a higher (P<0.05) biomass (shoot regrowth + final stolons/stubble and root) in the light compared to that in the dark (Figure 7.6A), and Olwen had higher (P<0.05) biomass than Huia (Figure 7.6B). Ellet had similar biomass (P>0.05) regardless of light (Figure 7.6C) and cultivar of associated white clover (Figure 7.6D).

### (e) Total leaf number of white clover produced during regrowth

The positive effect of light on total leaf number per pot produced during the two weeks of regrowth was highly significant (P<0.001) (Figure 7.7A) with Huia producing significantly (P<0.05) more leaves than Olwen (Figure 7.7B).

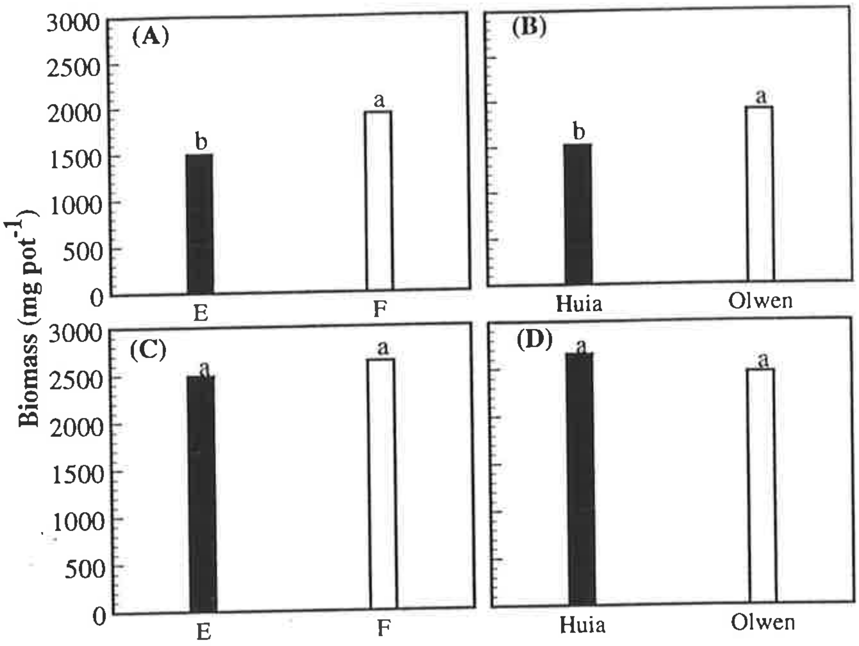



**Figure 7.6** The effects of (A) light and (B) white clover cultivar on the biomass of white clover and (C) light and (D) associated white clover cultivar on the biomass the of perennial ryegrass. (E=etiolated, F=full light). Means associated with the same letters are not significantly different (P>0.05). Notice that different scales are used in the graphs.

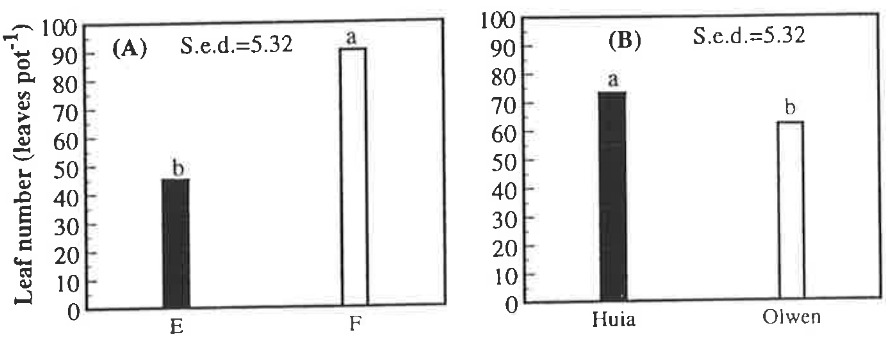



**Figure 7.7** The effects of (A) light and (B) white clover cultivars on white clover leaf production during regrowth. (E=etiolated, F=full light). Means associated with the same letters are not significantly different (P>0.05)

### 7.3.4 NSC Remaining in White Clover Stolons at the Final Harvest

#### (a) Sugars

The cultivar x light x nutrient interaction was significant ($P<0.05$). Olwen receiving nitrogen and complete nutrients in the dark had the highest remaining sugar concentration compared to other treatments (Figure 7.8). Sugar content was lowest in the stolon of Huia in the dark (Figure 7.9) compared to in the light or compared to Olwen.

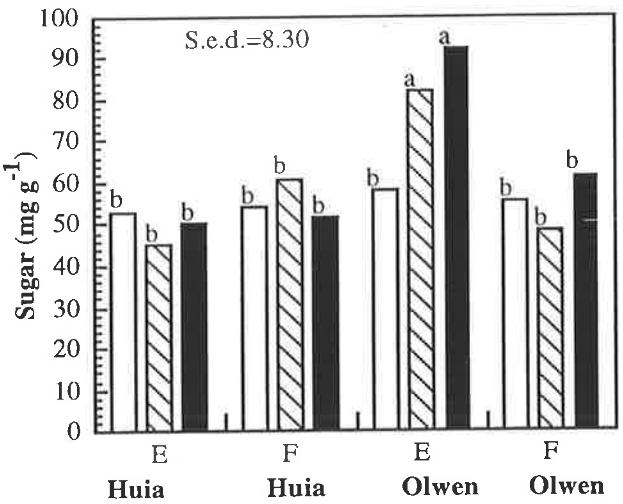



**Figure 7.8** The effects of (**a**) cultivars x light x nutrient interaction on sugar concentration in stolons of white clover at the final harvest. ( □ = nil, ▧ = nitrogen, ■ = complete; E=etiolated, F=full light). Means associated with the same letters are not significantly different (P>0.05)

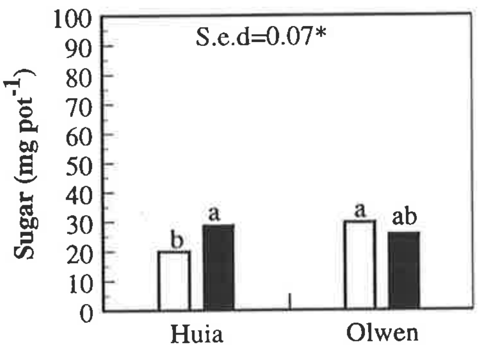



**Figure 7.9** The effects of cultivars x light interaction on sugar content in stolon of white clover at the final harvest. (□ = etiolated, ■ = light). Means associated with the same letters are not significantly different (P>0.05)

**(b) Starch**

The main effect of cultivar on starch concentration and content were significant (P<0.05), while the effects of other treatments or treatment combinations were non-significant. Olwen had higher starch concentration (Figure 7.10A) and content (Figure 7.10B) than Huia.

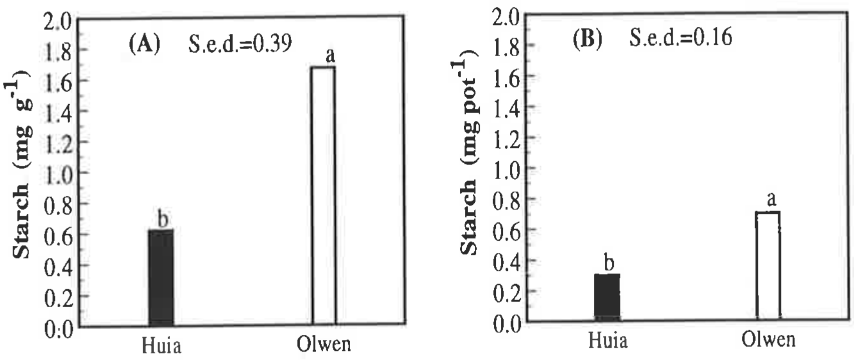



**Figure 7.10** The effects of cultivar of white clover on (A) starch concentration and (B) starch content in the stolon of white clover at the final harvest. Means associated with the same letters are not significantly different (P>0.05)

### (c) Total NSC

The cultivar x light x nutrient interaction on the total NSC concentration in the stolon was highly significant (P<0.05). The total NSC concentration was highest in the stolons of Olwen receiving nitrogen and complete nutrient application in the dark (Figure 7.11), while Huia had similar total NSC regardless of treatment, which were similar to Olwen in the light or with receiving water only (nil treatment).

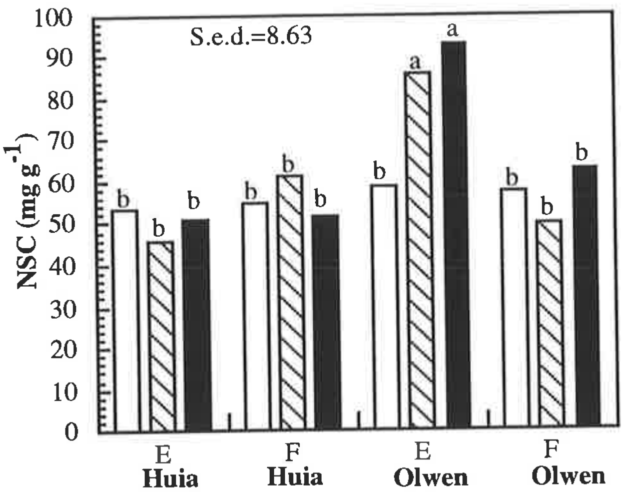



**Figure 7.11** The effects of cultivar x light x nutrient interaction on total concentration in stolons of white clover at the final harvest. (□ = nil, = ⧅ nitrogen, ■ = complete). (E=etiolated, F=full light). Means associated with the same letters are not significantly different (P>0.05)

The total NSC content in the stolon of Huia was lower in the dark treatment than the light. Huia in the light had similar total NSC content to Olwen (Figure 7.12). The percentage of total NSC content remaining was higher in Olwen than in Huia (Appendix 7.2).

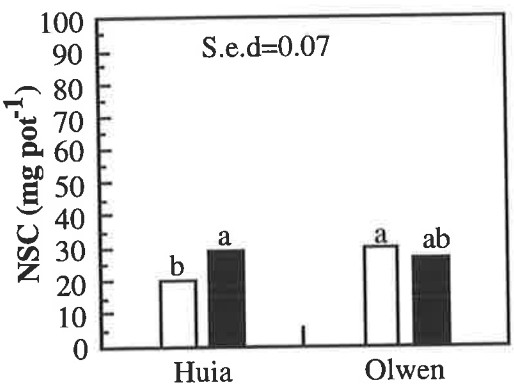



**Figure 7.12** The effects of cultivar x light interaction on the total NSC content in stolons of white clover at the final harvest. (□ = etiolated, ■ = light). Means associated with the same letters are not significantly different (P>0.05)

### 7.3.5 Non-structural Carbohydrates in the Roots

Because of the time limitation, the NSC in the roots were analysed for one replicate only. The effects of cultivars of white clover in etiolated and full sun on the total NSC is presented in Figure 7.13. The NSCs in the root were lower in the etiolated treatment than with full lighting, especially for starch content. Olwen had higher residual NSC than Huia.

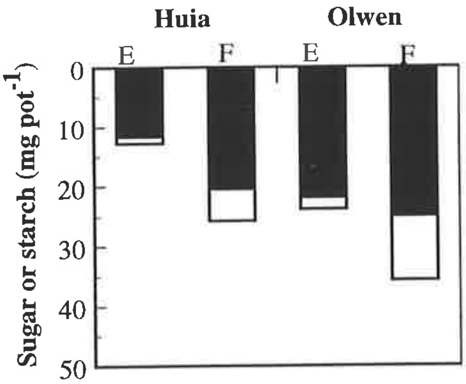



**Figure 7.13.** The effects of cultivar and light on sugar (■) and starch (□) content in root of white clover

The percentage of the total NSC remaining in the roots was also lower in Olwen than in Huia (Appendix 7.2).

### 7.3.6 Total Non-structural Carbohydrates Remobilised

The total NSCs remobilised during two weeks of regrowth were calculated by substracting the NSCs at the end of the experiment from NSCs at the the time of treatment application (after seven weeks initial growth). There was a highly significant ($P<0.001$) main effect of cultivar of white clover on the non-structural carbohydrate remobilised from the stolon, with Huia remobilising twice as much NSCs per pot as Olwen (Figure 7.14A). In the roots, Olwen remobilised more non-structural carbohydrates than Huia (Figure 7.14B and Table 7.2). The proportion of available NSC content remobilized from stolons were higher ($P<0.05$) in Huia than in Olwen (Table 7.2).

NSC content remobilized from stolons were higher ($P<0.05$) in Huia than in Olwen (Table 7.2).

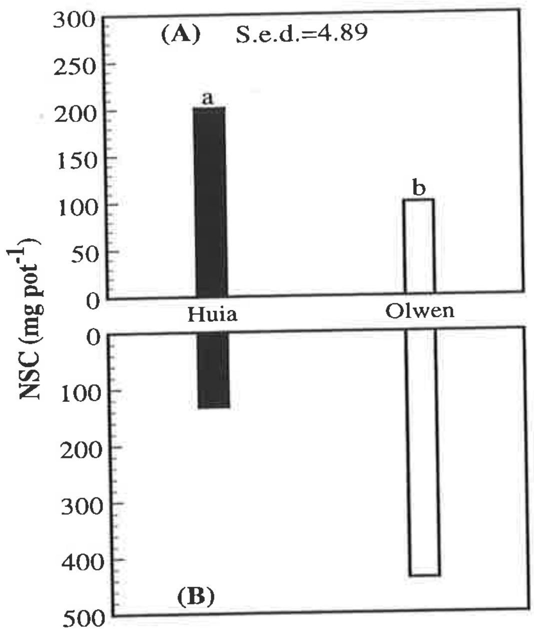



**Figure 7.14** The main effect of cultivar on the total loss of NSCs from the (A) stolon (means of three replications), and (B) root (one replication)

**Table 7.2** The effects of white clover cultivars on proportion of loss of available NSC content (%) from stolons and roots.

| White clover cultivars | Stolons (%) | Roots** (%) |
|---|---|---|
| Huia | 89.02a* | 85.1 |
| Olwen | 77.30b | 93.5 |
| S.e.d. | 48.68 | |

*Means associated with the same letters in the same column are not significantly different ($P>0.05$). ** one replicate only

### 7.3.7 The Total Non-structural Carbohydrates Remaining in the Ryegrass Stubble

Only the main effect of light, had a significant effect on sugar, fructan and total NSC content in stubble of perennial ryegrass.

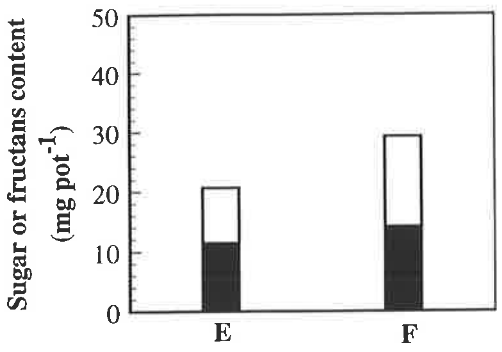



**Figure 7.15** Main effects of light on sugar (■) and fructans (□) content (mg $pot^{-1}$) in the stubble of Ellet

The effects of treatments and the interaction on NSC remaining in the roots of perennial ryegrass were non-significant (P>0.05). However, perennial ryegrass in mixtures with Olwen had higher percentage of total NSC remaining in the roots (Appendix 7.2).

## 7.4 Discussion

In the present experiment, plants were grown in sand and the nutrients applied during the establishment period had been shown to give maximum growth. In such conditions competition for light might be more important than competition for nutrients. The better yield of roots of Ellet in mixture with Huia than with Olwen at the end of the establishment period (seven weeks) (Figure 7.1D) suggested that in mixtures with Ellet, Olwen was more competitive than Huia. However, shoot DM yield of Huia and Olwen in mixture with Ellet were similar, consistent to those of experiment 3 (Chapter 5) except at harvest 3 when Huia had higher shoot DM yield (see Table 5.2). Huia and Olwen showed different accumulation of NSC in mixtures with perennial ryegrass, i.e. Huia accumulated higher (P<0.05) levels of NSC in stolons (Figure 7.1C), while Olwen had higher (P<0.05) NSC in the roots (Figure 7.1E). In totals (stolons and roots) Olwen had the highest NSC levels, resulting in higher (P<0.01) total DM of shoot regrowth of Olwen (Figure 7.2A) during the two weeks regrowth period.

Nitrogen treatments affected shoot DM of perennial ryegrass in the regrowth. The lower total DM in the regrowth of Ellet in mixtures with Olwen than with Huia and in the presence of nitrogen in the light (Figure 7.3B) support the findings reported in the literature (Goodman and Colison, 1986) that Olwen was more competitive than Huia in the presence of nitrogen. In addition, at the end of the experiment in the light as well as in the dark, higher NSC in the roots of Olwen compared to Huia (Figure 7.13) may indicate a greater ability to maintain root growth, to provide energy for active absorption of nutrients (Penning de Vries, 1975a,b), and to provide substrate for biological nitrogen fixation (Ryle *et al.*, 1989). Olwen remobilized more NSC from its roots than Huia (Figure 7.14B) and a higher percentage of its available NSC (Appendix 7.2), which may affect future growth and competitive ability, especially if defoliation is severe and conducted at short intervals. The hypothesis that remobilization of NSC from storage organs is proportional to the quantities of NSC available as reserves is supported by the results for both Huia and Olwen which started with different levels in both stolons and roots. Interestingly, the higher initial levels of NSC in Olwen were due to greater amounts of NSC in the roots rather than in the stolons. Not only were more NSCs mobilized in Olwen than in Huia, but Olwen also had higher levels of NSC in the roots than did Huia at the end of the experiment.

The defoliation treatment in this experiment (every 2-3 days) resulted in most of the remaining NSC being in the form of soluble sugars (Figure 7.8), while the remaining starch in white clover (Figure 7.10) and fructans in perennial ryegrass (Figure 7.15) were very low.

The results of the present experiment showed that nitrogen and other nutrients in the growth medium caused the levels of sugar (Figure 7.8) and NSC (Figure 7.11) in stolons of Olwen at the end of experiment to remain higher in the dark treatment than in the light. As N had no significant effects on regrowth (Figure 7.3A), the effects of N on sugars can not be through effects on growth. Instead the effects of N on NSC may be more directly on either the production of sugars (or transport to the stolons) or on the consumption of sugars. It is possible that more sugar may have been transported from the roots of Olwen to the stolon in the presence of N.

The lower root DM in perennial ryegrass in the presence of nitrogen compared to Nil nutrients and the addition of complete nutrients (Figure 7.5B) suggested that perennial ryegrass favoured shoot growth more than root growth at high nitrogen which also indicated the adverse effects of the combination of defoliation and nitrogen application on root growth.

The results of this experiment also pointed to the differences in NSC remobilization for regrowth after defoliation between Olwen and Huia. Huia remobilized more ($P<0.05$) NSC from the stolons (Figure 7.14A) but less from the roots (Figure 7.14B) compared to Olwen. As pointed out earlier, the greater amounts remobilised by Olwen compared to Huia, were derived from the roots. However stolons of Huia contained similar NSC content in the light to Olwen (Figure 7.12). Coupled with a higher number of leaves (Figure 7.7B), these results suggest that Huia in the light will have higher regrowth rates than Olwen as observed in harvest 3 in mixture with perennial ryegrass (Table 5.2).

Huia might produce more current photosynthates (Chapman *et al.*, 1991a,b) compared to Olwen, because of a higher ($P<0.05$) leaf number (Figure 7.7 B) and longer, thinner stolons with a higher ($P<0.05$) number of nodes (Table 7.1) as also observed by Caradus *et al.*, (1991), resulting in a higher surface area. These traits might be responsible for the higher competitive ratio of Huia than Olwen as observed at harvest 3 in Experiment 3.

## 7.5 Conclusions

From the results of this experiment it can be concluded that :

1. In mixtures with Ellet, Olwen was more competitive compared to Huia resulting in higher shoot DM in regrowth of Olwen than Huia and lower shoot DM in regrowth of Ellet in mixtures with Olwen than with Huia, at high nitrogen levels.

2. As nutrient treatments had no effects on the DM in the shoot regrowth of Olwen and Huia the white clover had accumulated sufficient nutrients to support regrowth.

3. Huia had a higher number of leaves, longer stolons with a higher number of nodes which which could explain the higher competitive ability as observed in Experiment 3 at harvest 3.

4. In the regrowth, Huia remobilized more NSC from the stolons compared to Olwen, the reverse is true from the roots. As most of the soluble NSC in roots and stolons is remobilised during regrowth after defoliation, the differences between cultivars in the remobilization of NSC was a reflection of the initial distribution of carbohydrates, that is more NSCs were remobilised from roots of Olwen than roots of Huia because of the higher initial levels in the roots of Olwen than Huia. The reverse is also true for NSCs remobilised from stolon.

5. The accumulation of sugar in the stolons of Olwen which is due to the transport from the roots, in the dark in the presence of nitrogen, and higher levels of NSCs remaining in the roots of Olwen might cause the tolerance of Olwen to high levels of soil nitrogen reported in the literature, because more sugars would be available for maintenance respiration, regrowth and utilization of the nitrogen.

# Chapter VIII

## General Discussion and Conclusions

In white clover-perennial ryegrass mixtures proportions of 30-50 percent of white clover on a dry weight basis are required to achieve high nitrogen contributions to the system by white clover and high animal outputs from the land (Martin, 1960; Harris and Thomas, 1973; Curll, 1982). However, white clover proportions in the swards generally decline with time to below 20 percent, with the consequences of lower N-inputs and lower animal production. The declines have been attributed to the higher competitive ability of perennial ryegrass, especially in the presence of high soil nitrogen levels (Curll, 1982; Frame and Newbould, 1986; Frame, 1987).

The objectives of the studies reported in this thesis are to investigate (i) cultivar differences in accumulation of non structural carbohydrate (NSC) during vegetative growth and regrowth after defoliation in white clover and perennial ryegrass; (ii) how the above differences affect their competitive abilities and subsequent regrowth; (iii) the relative importance of the level of NSC as compared to morphological characteristics in competition between perennial ryegrass and white clover; (iv) the differences among cultivars of white clover in relative contribution of reserves from stolons and roots in regrowth after defoliation, and (v) whether cultivars and differences in management strategies (i.e. defoliation, mineral nutrition application) will affect the NSC levels and the outcome of competition.

These topics will be discussed in this chapter. Initially however, the experimental approach and some general matter concerning competitive relationships arising from the experiments will be discussed.

Five glasshouse experiments comprising monocultures and 50:50 mixtures of cultivars of white cover and perennial ryegrass were conducted during the period from 1991 to 1994. The de Wit replacement design was used to quantify the competitive relationships of the association. The competitive ratios (CRs) were calculated and used to compare the competitive ability of cultivars. Regression analyses were constructed between CR (Y) and NSC concentration and content and plant traits (X) to compare the correlations and possible importance of plant traits in determining competitive ability. The traits used are those frequently reported in the literature from competition experiments.

## 8.1 Experimental Approach in Studying Competition and Competitive Ability in White Clover-Perennial Ryegrass Mixtures

The investigation to compare the effects of plant density on yield and competitive ability showed that yield and RY values increase with increasing density at early harvests. This has prompted other workers to suggest the dependence of competitive ability on density. However, due to the stoloniferous habit of clover and tiller fprmation in ryegrass, after the plants are fully established, a complete soil surface cover is achieved, the density effects disappear. Even from the beginning, there is no effect of density on the CR. Therefore it appears that competitive effects can be studied at any density but that to avoid density effects on yield and RY, a total density of around 8-16 plants per pot of 24 cm diameter, should be used and the experiment should be continued for more than two harvests. Higher densities are impractical because of difficulties in maintaining homogeneity of size and distribution in the pots.

The most commonly used design for pasture competition studies is the replacement design of de Wit. However, many other investigators have used an additive design which gives a higher number of plants in the mixtures than in the monocultures.

Experiment 2 (Chapter 4) showed that the higher number of plants in the mixture initially increased RY in this design compared with the replacement design especially at low density. However, this effect disappeared at later harvests. Furthermore, there was no difference between designs in the CR values.

This experiment therefore, shows that both replacement and additive designs can be used to study competition by means of CR. In the following experiments, the de Wit replacement designs were used because these designs are widely used, thus the results could be related to the information available in the literature.

The de Wit replacement diagrams indicated that yield reduction (deviation from the expected yields) of white clover occurred after harvest 1 (Figures 4.6 and 5.1). This is consistent with the observations of depressions of white clover yield and proportions in field situations. Accordingly, to examine the proposition that NSC levels in the stolons may affect the subsequent competitive abilities, measurements of NSC levels were conducted at harvests 2 and 4 (Chapter V).

## 8.2 Competitive Relationships in White Clover-Perennial Ryegrass Mixtures

The results of the present experiments were in agreement with those reported in the literature in showing that perennial ryegrass initially is more competitive (Chapters IV and V) than white clover which is due to high nitrogen in the soil, but in the subsequent growth, as nitrogen in the soil is depleted white clover becomes more competitive (Bakhuis and Kleter, 1965; Harris and Thomas, 1973; Davidson *et al.*, 1986; Davidson and Robson, 1990; Menchaca and Connolly, 1990). In the present experiments, the improved competitive ability of white clover with time was expressed as higher proportions of white clover (Table 5.2) and its RY (Table 5.3) and higher CR (Figure 5.4). Consequently, after harvest 2 (Experiments 2 and 3) the proportions of white clover were in excess of the 30 percent, suggested by Harris and Thomas (1973) as a condition to achieve equilibrium. The RYTs were generally greater than unity, indicating complementarity (coexistence) in resource acquisition.

The responses of white clover cultivars in mixtures with perennial ryegrass differed from their responses as monocultures (Chapters V and VI). Higher shoot DM yields of clover in monocultures were not related to higher yields in mixtures which was in agreement with Weiner (1980). The ultimate aims of planting white clover-perennial ryegrass mixtures are to obtain a high total shoot DM production (white clover + perennial ryegrass) containing a high proportion of white clover. These aims were achieved in the present experiments over a series of harvests. Furthermore, the total shoot DM production obtained in mixtures was the same regardless of cultivar of white clover (Chapters V and VI).

The durations of the experiments reported in this thesis were too short to allow sufficient nitrogen contribution of white clover to the system to show large impact on the growth and competitive ability of perennial ryegrass. This was clearly shown in the consistent decline of shoot DM yields of perennial ryegrass with time while that of white clover increased (Chapters IV, V and VI). However, high RY's of perennial ryegrass are an indication of at least a small amount of nitrogen transfer. To provide experimentally, the higher soil nitrogen conditions found in older white clover-perennial ryegrass swards, without too severe suppression of the white clover, moderate rates of nitrogen (75 kg $ha^{-1}$ N) in two split applications were applied (Chapter VI). This gave information on competitive effects and NSC changes. In this situation, the high nitrogen and its effects on growth of perennial ryegrass lasted for only one harvest. Therefore, this experiment confirmed what has been found by many others e.g. Martin and Field (1984); Frame and Newbould (1987b) that nitrogen application increases the competitive ability of perennial ryegrass and generally disadvantages white clover. Thus, in Experiment 4, nitrogen application reduced temporarily ($P<0.05$) not only shoot DM of white clover (Figure 6.2A) but also stolon DM (Figure 6.4C). The extent of the reduction was similar regardless of cultivars and defoliation regimes.

## 8.3 The Effects of Cultivars, Defoliation and Nitrogen on NSC Levels in Stolons and Stubble

Although in Experiment 1 significant differences were detected between cultivars of white clover in NSC levels, it was found that the differences were not consistent from one harvest to the next and were also influenced by nitrogen and defoliation treatments. In contrast, there were rarely any cultivar differences in NSC in perennial ryegrass.

Some investigators have claimed that NSC levels in stolons and roots of white clover are correlated with leaf size. For instance, Kang and Brink (1995) found that stolons and roots of the larger-leaved cultivar Osceola contained a higher concentration of NSC, compared to the medium-leaved cultivar Huia and the small-leaved cultivar Aberystwyth S184. In the present experiments, in monocultures (Chapters III and IV) and in mixtures (Chapters IV and V) Olwen, a medium large-leaved cultivar contained higher ($P<0.05$) concentrations of NSC in the stolons compared to the medium-leaved cultivar Huia. However, this trend was also modified by nutrient supply and defoliation. Thus when the mixtures were supplied with nutrient levels that gave maximum yields, the medium large-leaved cultivar Olwen accumulated a lower level of total NSC content in the stolons than Huia (Chapter VII). However, during the etiolated regrowth following high nutrient levels Olwen produced higher shoot DM yields than Huia. Thus, the higher growth of Olwen compared to Huia can not be attributed to the NSC levels of its stolons, but could be attributed to the higher NSC in its roots. NSC in roots therefore may be of greater important in determining shoot growth than NSC levels in the stolons. At low soil nitrogen levels limiting growth of perennial ryegrass, accumulation of NSC in the stubble occurred. On a sucrose equivalent basis, perennial ryegrass had higher fructan levels in the stubble than starch in stolons of white clover (Chapter III). The significance of this finding for the adaptation of white clover and perennial ryegrass to their environment is yet to be elucidated.

Severe defoliation combined with nitrogen application caused a reduction in NSC content in white clover (Chapter VI). Nitrogen limitation generally causes the accumulation of NSC in the storage organs (Henry and Raper, 1991; Qiu and Israel, 1993). This occurred in perennial ryegrass in the present experiments. It is noteworthy that Olwen white clover also accumulated more NSC at the low soil nitrogen level than at higher. This trend did not occur in Huia. This indicates that Olwen may require higher soil nitrogen levels for maximum growth and utilization of NSC. This in agreement with the findings of Goodman and Collison (1986) who found that Olwen absorbed more soil nitrogen and fixed more nitrogen than a smaller leaved-cultivar, in high nitrogen conditions compared with low nitrogen. This may also be related to similar findings of Eltilib and Ledgard (1988) comparing larger leaved-cultivars (high nitrogen preference) with smaller leaved-cultivars (low nitrogen preference). This difference in response to nitrogen is often referred to as a difference in tolerance to nitrogen.

There were also other important differences between Olwen and Huia in the distribution of NSC, which became apparent in mixtures at high levels of total plant nutrients. Thus in Experiment 5 (Chapter VII), shoot DM of Olwen and Huia were similar, yet Huia initially partitioned more photosynthates to the stolons (Figure 7.1C) and less to the roots. This is the opposite to what occurred in monocultures in Experiment 1 (Chapter III), suggesting a different response in the white clover cultivars to competition from perennial ryegrass. The end result at the high nutrient level used was that Olwen was more competitive than Huia and this was associated with relatively greater partitioning of NSC to the roots than stolons. The subsequent greater amount of regrowth from Olwen than Huia therefore indicates a greater mobilization of NSC from the roots in Olwen. This again stresses the importance of defining cultivar differences, which has received little attention in research on the effect of NSC on competition.

## 8.4 Variations in Cultivars of White Clover in their Competitive Ability with Perennial Ryegrass

The results of the present experiments confirmed the previous findings reported in the literature that cultivars of white clover differed in competitive ability in mixtures with perennial ryegrass (Chapters V and VI). Measurements on CR and on the NSC levels in the stolons and stubble of perennial ryegrass suggested that Olwen was more competitive than Huia in mixtures with perennial ryegrass (Chapters V, VI and VII). The data showed that this was not consistent over time, being affected by soil nitrogen levels and the stage of growth of the plants. Thus, when soil nitrogen levels were high, Olwen was more competitive than Huia (Chapters VI and VII). As soil nitrogen become depleted (Chapter VI), the competitive ability of Olwen declined and that of Huia increased. Similarly, the early flowering cultivar Irrigation promoted shoot growth at the onset of flowering, resulting in higher competitive ability than both Huia and Olwen during the beginning of the experiment (Chapter V).

The relative competitive abilities of the white clover cultivars also changes with time, as shown in Experiment 3 (Chapter V) (Figure 5.3). Huia had the lowest CR at harvest 2, but the highest at harvest 3. By harvest 4, the CR's of Olwen and Huia were similar and much higher than the CR of Irrigation. This suggest a capacity for Olwen to recover from initial lower CR values at low soil nitrogen levels.

## 8.5 The Relative Importance of Morphological Traits and NSC in Determining Competitive Ability

The hypothesis that concentration or content of NSC at one harvest was correlated with subsequent competitive ability (CR) has not been substantiated in the present series of experiments. Rather, the converse has been shown i.e. that the effect of competition on white clover measured as RY for DM at one harvest is reflected in the RY for NSC at the next.

One explanation of the low correlation between NSC with subsequent CR may be that NSC levels have been more than adequate to support regrowth and competitive ability of white clover. The one treatment where NSC levels may have been sub-optimal was the severe defoliation in Experiment 4 (Chapter VI).

The use of plant traits (other than NSC) to explain differences in competitive ability, though widely measured in competition studies is also largely unsupported by the present studies. However, the high correlation between SLA and CR and the moderate correlation between height and CR may be worthy of further investigation. However, these correlations were significant and reasonably high in only one of two experiments.

The high relationship between CR and SLA in Experiment 3 (Chapter V) agrees with the observations of Barthram and Grant (1994). They correlated SLA with the proportion of white clover in mixed swards. Such a correlation might indicate that competition for light was dominant in this experiment. The correlation with height may indicate the same.

Because of the correlations shown above between SLA and height and CR, it seems worthwhile continuing to investigate these correlations. However, experiments should be designed to determine cause and effect and to distinguish between the effects of particular environmental and management factors. The lack of correlation between NSC and subsequent CR does not necessarily mean that NSC reserves havenorole in competition, but this will only be determined by experiments which provide more precise understanding of cause and effect.

## 8.6 Conclusions

While the results of these experiments have yet to be verified in field conditions some conclusions and possible implications can be drawn :

1. The NSC concentration and content in stolons were affected by cultivars, defoliation, soil nitrogen levels and stage of growth. Hence, Olwen a medium large-leaved white clover cultivar in monocultures initially had a higher soluble sugar concentration than Huia a medium-leaved white clover cultivar but was similar to Huia at later harvests. In mixtures, Olwen had a higher soluble sugar concentration than Huia in the absence of nitrogen application.

2. Olwen was more competitive than Huia with respect to perennial ryegrass and more tolerant in the presence of high soil nitrogen levels in the conditions of this experiment. However, this trend was not consistent being affected by soil nutrients and stage of growth.

3. When soil nitrogen levels were low accumulation of soluble sugars in the stolons of Olwen occurred but not in Huia. This could result in greater growth in Olwen than Huia when nitrogen was resupplied. However, Olwen remobilised more total NSC than Huia from its roots, which in the longer term would have resulted in lower roots activities leading to the decline of Olwen in the swards.

4. Huia can be more competitive than Olwen with respect to perennial ryegrass, part of this might be due to the decline of soil nitrogen and higher leaf numbers relatively of Huia compared to Olwen.

5. Non-structural carbohydrate levels in the storage organs were less important

in determining competitive ability than morphological traits. However, the relative important of morphological traits that affecting competitive ability varied in different environment.

6. In areas with high levels of fertility or if nitrogen fertilizers are applied Olwen should be used in mixtures with perennial ryegrass. To ensure a suitable proportion of white clover in the swards, planting Olwen and Huia at the same time in mixtures with perennial ryegrass might be beneficial.

7. Further research is required to examine the impact of nitrogen application and defoliation on the remobilization of NSC from the roots and root activities of different cultivars of white clover in mixtures with perennial ryegrass. The effects of environmental stress (water stress, nutrient stress, cold stress, defoliation) on NSC levels in the stolons and the subsequent effects on yield and competitive ability when the stress is relieved merit further studies.

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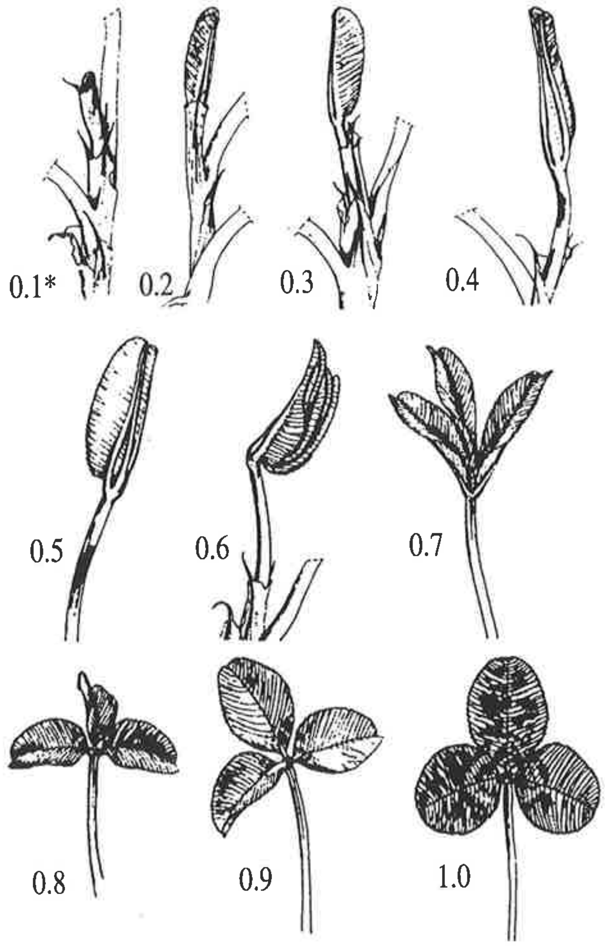
# Appendices




**Appendix 2.1** Ten stages of morphologic development of ladino clover leaves (After Carlson, 1966)

| | |
|---|---|
| 0.0* | Leaf bud is not visible in axil of preceding leaf |
| 0.1 | Approximately 25% of the folded leaf is visible as it emerges from its membranous stipule |
| 0.2 | Folded leaf is completely visible but the petiole remains enclosed in its membranous stipule |
| 0.3 | Petiole is visible and all leaflets are tightly folded |
| 0.4 | A slight separation of individual leaflets is apparent at the midvein |
| 0.5 | Individual leaflets are folded but are starting to separate from each other. (Occasionaly, 1 leaflet may be entirely separated from the other 2) |
| 0.6 | Leaflets are clearly separated from each other, and each leaflet is approximately 10% unfolded. |
| 0.7 | Leaflets are approximately 30% unfolded. |
| 0.8 | Leaflets are approximately 60% unfolded. |
| 0.9 | Leaflets are approximately 90% unfolded. |
| 1.0 | Leaflets are 95% unfolded or slightly cupped |

**Appendix 3.1** Nutrient solution used. The following solution concentrates were prepared in distilled or demineralized water and made up to a final solution of 10 litres using 50 ml of A, B and C and 5 ml of D. The pH of the final solution was adjusted to 6.0 with 0.1 N HCl or 0.1 N KOH. All the stock solutions were stored in a cool, darkened area (adapted from Hammer *et al.*, 1978).

| | |
|---|---|
| **Concentrate A** | |
| Salt | g $L^{-1}$ |
| $KNO_3$ | 50.55 |
| $KH_2PO_4$ | 13.61 |
| $MgSO_4.7H_2O$ | 49.30 |
| **Concentrate B** | |
| $Ca(NO_3)_2.4H_2O$ | 118.08 |
| **Concentrate C** | |
| $FeSO_4.7H_2O$ | 2.49 |
| NaEDTA* | 2.61 |
| **Concentrate D** | |
| $H_3BO_3$ | 2.850 |
| $MnSO_4.H_2O$ | 1.538 |
| $ZnSO_4.7H_2O$ | 0.219 |
| $CuSO_4.5H_2O$ | 0.078 |
| $H_2MoO_4$ | 0.020 |

* The iron and sequestrene were dissolve in 80% of the final volume of water, then the base was added . The solution was aerated vigourously overnight and made up to volume. Solution was stored at $5^{o}$ C and if precipitation occurred, it was discarded and remade.

## Appendix 3.2 Non-Structural Carbohydrate Analysis

**A) Extraction**

For tissue containing chlorophyll it is necessary to perform all step.
For tissue containing no pigments (e.g. roots) step d is not necessary.

**Solvent for extraction :** 80 % aquaeus ethanol

**Extraction**

Weigh 2.0 g fresh tissue. Drop tissue (small pieces c. 1.0 cm) fresh into 20 ml 80% ethanol which is boiling. Boil for 15 minutes cool and store in the refrigerator in the dark.

a. Grind tissue to a fine pulp, using mortar and pestle.

b. Decant slurry into 50 ml centrifuge tube and wash mortar and pestle with a further 5 ml of 80% ethanol, into the same 50 ml centrifuge tube.

c. Centrifuge for 10 minutes at 2, 000 r.p.m. This separates a pellet from the supernatant.

d. Decant the supernatant into 50 ml centrifuge tube and add 10.7 ml distilled water and 3.3 ml chloroform and shake to separate out the chlorophylls into the subnatant (bottom) chloroform layer and centrifuge - as in (**c**). Decant the supernatant into a 25 ml volumetric flask and discard the chloroform layer.

e. To the pellet (containing starch and other ethanol soluble carbohydrates) add 5 ml 80% ethanol and resuspend. Heat the slurry to boiling in a water-bath, remove and allow to cool before centrifuging at 2,000 r.p.m for 5 minutes. Decant the supernatant into the 25 ml volumtric mentioned in step (d). Make up to volume with 80% ethanol.

**B) Ethanol Soluble Sugars Analysis**

**Reagents required:**

1. 500 ml concentrated sulphuric acid in an automatic dispenser (5 ml volumes)
2. 500 ml 5% aqueous phenol solution
3. Standard sucrose solution 10 mg in 100 ml (keep frozen)

**Procedures:**

1. Select a set of 150 x 18 mm glass test-tubes clean and free from dust or cellulose (e.g. paper) fibres
2. Take 0.1 ml of 80% ethanol extract and add 0.9 ml of water. Add 1 ml of 5% aqueous phenol. (Readable O.D. range 0.1 - 0.8. If O.D. is above the readable range, take a smaller quantity of extract. If below, you will have to concentrate

the 80% ethanol extract by heating) to evaporate the solvent at ca. 60 °C.

3. Pump a jet of concentrated sulphuric acid (5 ml) into the centre of the sample.
4. Measure the optical density at 490 nm when the tubes have cooled
5. Include a standard curve of sucrose (5 to 70 ug per tube)

**C) Starch Determination of Clovers**

1. Transfer the pellet from stage **(e)** to a 15 ml glass centrifuge tube by rinsing with 80% ethanol. Centrifuge (for 10 minutes in 2,000 rpm) Add the supernatant to the volumetric flask in **(d).** Dry the pellet in the oven at ca. 50° C. Include in each run 3 tubes containing about 5 mg of starch (weight known accurately) as standards. Follow the starch schedule, as follows :
2. Add a little clean acid-washed sand to each tube.
3. Add 2.7 ml water to each tube.
4. Cover the tubes with a glass marble and heat on a boiling water bath for 15 minutes.
5. Put a glass rod in each tube and crush and grind the pellet finely (same with standard starch). Allow to cool to room temperature.
6. Add 2 ml 72% Perchloric acid and stir immediately, noting the time. Start one tube at a time at approx. 2 minute intervals.
7. Grind each tube in turn. After a total of 15 to 20 minutes from adding the Perchloric acid, add 2 ml water and stir.
8. When all tubes have been diluted, withdraw the rods and centrifuge for a few minutes (turn off when the centrifuge reaches 3,000 rpm) (5 mins).
9. Decant the supernatant into a 10 ml volumetric flask.
10. Add another 1.25 ml of water to the residue (pellet), stir and add 0.95 ml of 72% perchloric acid.
11. Stir and grind a further 10 to 15 minutess. Dilute with 0.95 ml of water and centrifuge as above.
12. Add the supernatant to the 10 ml flask and make to volume with water, mix.
13. Transfer 5 ml of perchloric acid extract to a clean dry 15 ml centrifuge tube.
14. Add 2.5 ml 20% NaCl and 1 ml of $I_2$/KI reagent. Mix. Allow to stand for at least 20 minutes. <u>This step can be left overnight.</u>
15. Centrifuge as above.
16. Decant supernatant very carefully to avoid loss of precipitate.
17. Suspend the pellet (residue) in 2.5 ml of alcoholic NaCl-tap the tube to suspend (gets rid of soluble substances in perchloric acid extract, e.g. sugar).
18. Centrifuge as above; decant carefully.
19. Add 2.0 ml alcoholic NaOH. Gently tap the tube and leave until the blue colour has gone. <u>Leave overnight if necessary.</u>

20. Centrifuge, decant and wash with 5 ml alcoholic NaCl centrifuge and decant.
21. Add 2.5 ml water and a glass rod, stir and then add 2.5 ml 0.1 M Perchloric acid.
22. Heat with stirring in boiling water bath for 3 minutes.
23. Decant into 10 ml volumetric flask.
24. Rinse the tube and rod in 2.5 ml of 0.05 M Perchloric acid. Heat 3 minutes. Add the contents to the 10 ml volumetric flask and make to volume with water. Filter through glass fibre paper or centrifuge.
25. Dilute 0.2 to 10 ml for 5 mg starch, as necessary so that the solution containins between 20 and 70 ug starch per ml, and use <u>immediately</u> for phenol determinations
26. Standard curve of wheat starch (10-100 µg per tube). Prepare standard wheat starch solution by dissolving 100 mg of wheat starch in hot 0.05 M perchloric acid and stir until disolved, make to 100 ml.

**D) Fructosan Determination for Perennial Ryegrass**

1. Dry the pellet from stage **(e)** oven at ca. $50^{o}$ C
2. Suspend the pellet from stage **(e)** in 5 ml of water, boil for 15 minutes and centrifuge.
3. Decant the supernatant into volumetric flask.
4. Re-extract the pellet twice more with boiling water.
5. Pool the supernatants. Make to volume. Centrifuge.
6. Phenol test for the measurement of fructans.

**Reagent**

1. <u>PERCHLORIC ACID</u>
AR 72% w/w (1.67 - 1.72)

2. <u>I2-KI</u>
7.5 g Iodine and 7.5 g KI are ground with 150 ml water; dilute to 250 ml, filter by suction

3. <u>ALCOHOLIC NaOH</u>
350 ml absolute ethanol, and 50 ml water and 50 ml of 5N NaOH are diluted to 500 ml with water

3. <u>ALCOHOLIC NaCl</u>
350 ml absolute ethanol, and 80 ml water are mixed with 50 ml aquaeous sodium chlorude (20%) and then diluted to 500 ml with water

4. <u>PERCHLOROC ACID</u>
<u>0.1 MOLAR</u> 13.95 g (or 8.35 ml) 72% AR perchloric acid make up to 1 L

6. <u>20% NaCl</u> 20 g of NaCl in 100 ml of water

**Appendix 4.1** Relationships between shoot DM yield (Y) with density in monocultures (X) of white clover and perennial ryegrass at different harvest

| Harvest | Equation | $r^2$ |
|---|---|---|
| | White clover | |
| H1 | $Y = 0.783 + 0.089X - 0.0017X^2$ | 0.99*** |
| H2 | $Y = 1.671 + 0.155X - 0.0037X^2$ | 0.97** |
| H3 | ns | ns |
| H4 | ns | ns |
| | Perennial ryegrass | |
| H1 | $Y = 0.267 + 0.703X - 0.012X^2$ | 0.99*** |
| H2 | $Y = 10.615 + 0.181X - 0.12X^2$ | 0.89* |
| H3 | ns | ns |
| H4 | ns | ns |

*, **, *** and ns indicate significant P<0.05, P<0.01, P<0.001 and non-significant (P>0.05), respectively

**Appendix 5.1** The effects of cultivar of white clover and perennial ryegrass interaction on total shoot DM yields (over four harvests)

| Cultivars of white clover | Perennial ryegrass (g pot$^{-1}$) | |
|---|---|---|
| | Ellet | K. Valley |
| Huia | 72.30$^{b*}$ | 74.64$^{b}$ |
| Irrigation | 69.30$^{a}$ | 69.53$^{b}$ |
| Olwen | 69.65$^{a}$ | 69.49$^{a}$ |
| S.e.d | 2.895 | |

*Means within the same column followed by the same superscripts are not significantly different (P>0.05)

**Appendix 5.2** (A) The main effect of harvest number on the RY of shoot DM of perennial ryegrass

| Harvest number | RY |
|---|---|
| H1 | 0.70$^{b*}$ |
| H2 | 0.93$^{a}$ |
| H3 | 0.74$^{b}$ |
| H4 | 0.73$^{b}$ |
| S.e.d. | 0.04 |

*Means followed by the same superscripts are not significantly different (P>0.05).

**Appendix 5.2** (B) The effects of cultivar of perennial ryegrass x harvest number interaction on the RY of shoot DM of perennial ryegrass

| Cultivars of perennial ryegrass | RY | | | |
|---|---|---|---|---|
| | H1 | H2 | H3 | H4 |
| Ellet | 0.72$^{a}$ | 0.98$^{a}$ | 0.74$^{a}$ | 0.74$^{a}$ |
| Kangaroo Vally | 0.68$^{a}$ | 0.88$^{a}$ | 0.73$^{a}$ | 0.72$^{a}$ |

*Means followed by the same superscripts are not significantly different (P>0.05).

**Appendix 5.3.1** Means of (A) stolon DM of white clover (g $pot^{-1}$) in monocultures and mixtures with perennial ryegrass at harvest 2 (H2) and harvest 4 (H4), and (B) stubble DM of perennial ryegrass in mixture with white clover at H2 and H4.

| 6. Cultivars measured | Associated cultivars | H2 | H4 |
|---|---|---|---|
| | | Monoculture (g $pot^{-1}$) | |
| Huia | | 8.67 | 16.35 |
| Irrigation | | 9.29 | 18.22 |
| Olwen | | 6.79 | 23.07 |
| | | Mixtures (g $pot^{-1}$) | |
| Huia | Ellet | 2.60 | 13.22 |
| Irrigation | Ellet | 2.37 | 18.00 |
| Olwen | Ellet | 1.72 | 13.90 |
| Huia | K. Valley | 1.97 | 16.11 |
| Irrigation | K. Valley | 3.43 | 13.33 |
| Olwen | K. Valley | 2.11 | 14.17 |

| 7. Cultivars measured | Associated cultivars | H2 | H4 |
|---|---|---|---|
| | | Monoculture (g $pot^{-1}$) | |
| Ellet | | 9.64 | 9.58 |
| K. Valley | | 10.57 | 8.15 |
| | | Mixtures (g $pot^{-1}$) | |
| Ellet | Huia | 7.75 | 3.97 |
| Ellet | Irrigation | 7.94 | 4.87 |
| Ellet | Olwen | 8.2 | 4.77 |
| K. Valley | Huia | 7.72 | 4.54 |
| K. Valley | Irrigation | 6.43 | 4.82 |
| K. Valley | Olwen | 7.34 | 5.47 |

**Appendix 5.3.2** Main effects and treatment interactions with significant effects on NSC concentration (mg g$^{-1}$) and content (mg pot$^{-1}$)

| Main effects and interactions | Stolons | Stubble |
|---|---|---|
| | Sugar concentration (mg g$^{-1}$) | |
| White clover cultivars | **(a) | * |
| Harvest | ns | *** |
| | Sugar content (mg pot$^{-1}$) | |
| Harvest | *** | *** |
| White clover x harvest | * | ns |
| | Starch concentration (mg g$^{-1}$) | |
| Harvest | ** | *** |
| Perennial ryegrass cultivars | ns | * |
| | Starch content (mg pot$^{-1}$) | |
| Harvest | *** | *** |
| Perennial ryegrass cultivars | ns | * |
| | NSC concentration (mg g$^{-1}$) | |
| Harvest | ** | *** |
| White clover cultivars | * | * |
| Perennial ryegrass cultivars | | * |
| | NSC content (mg pot$^{-1}$) | |
| Harvest | *** | *** |
| Perennial ryegrass cultivars | ns | * |

(a) *, **, *** indicated significant P<0.05, P<0.01; P<0.001, respectively. ns=non-significant different (P>0.05)

**Appendix 5.4** The main effect of harvest number on the sugar content (mg pot$^{-1}$) perennial ryegrass stubble

| Harvest number | Sugar content (mg pot$^{-1}$) |
|---|---|
| H2 | 718.00$^{a*}$ |
| H4 | 169.21$^{b}$ |
| S.e.d. | 0.04** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.5** The main effect of harvest number on the starch concentration (mg g$^{-1}$) of white clover stolons

| Harvest number | Starch concentration (mg g$^{-1}$) |
|---|---|
| H2 | 358.21$^{a*}$ |
| H4 | 231.60$^{b}$ |
| S.e.d. | 0.07** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.6** The main effect of harvest on the starch content (mg pot$^{-1}$) of white clover stolons

| Harvest | Starch content (mg pot$^{-1}$) |
|---|---|
| H2 | 830.30$^{b*}$ |
| H4 | 3488.37$^{a}$ |
| S.e.d. | 0.09** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.7** The main effect of harvest number on the fructan concentration (mg g$^{-1}$) perennial ryegrass stubble

| Harvest number | Fructan concentration (mg g$^{-1}$) |
|---|---|
| H2 | 193.92$^{a*}$ |
| H4 | 33.89$^{b}$ |
| S.e.d. | 0.04** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.8** The main effect of harvest number on the fructan content (mg pot$^{-1}$) perennial ryegrass stubble

| Harvest | Fructan content (mg pot$^{-1}$) |
|---|---|
| H2 | 1149.67$^{b*}$ |
| H4 | 1823.56$^{a}$ |
| S.e.d. | 0.05** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.9** The main effect of harvest on the NSC concentration (mg g$^{-1}$) of white clover stolons

| Harvest | NSC concentration (mg g$^{-1}$) |
|---|---|
| H2 | 509.3$^{a*}$ |
| H4 | 368.5$^{b}$ |
| S.e.d. | 0.05** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.10** The main effect of harvest number on the NSC concentration (mg $g^{-1}$) perennial ryegrass stubble

| Harvest number | NSC concentration (mg $g^{-1}$) |
|---|---|
| H2 | 287.80$^{a*}$ |
| H4 | 70.09$^{b}$ |
| S.e.d. | 0.03** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.11** The main effect of harvest number on the NSC content (mg $pot^{-1}$) of white clover stolons

| Harvest number | NSC content (mg $pot^{-1}$) |
|---|---|
| H2 | 1189.8$^{b*}$ |
| H4 | 5412.5$^{a}$ |
| S.e.d. | 0.07** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.12** The main effect of harvest number on the NSC content (mg $pot^{-1}$) perennial ryegrass stubble

| Harvest number | NSC content (mg $pot^{-1}$) |
|---|---|
| H2 | 2187.04$^{a*}$ |
| H4 | 327.96$^{b}$ |
| S.e.d. | 0.04** |

*Means followed by the same superscripts are not significantly different (P>0.05). ** Log 10 transformation

**Appendix 5.13** Means of NSC concentration (mg pot$^{-1}$) in (A) stolons of white clover in monocultures and mixtures with perennial ryegrass at harvest 2 (H2) and harvest 4 (H4), and (B) stubble DM of perennial ryegrass in mixture with white clover at H2 and H4.

| (A) Cultivars measured | Associated cultivars | H2 | H4 |
|---|---|---|---|
| | | Monoculture (g pot$^{-1}$) | |
| Huia | | 460 | 355 |
| Irrigation | | 612 | 444 |
| Olwen | | 687 | 491 |
| | | Mixtures (g pot$^{-1}$) | |
| Huia | Ellet | 400 | 319 |
| Irrigation | Ellet | 632 | 333 |
| Olwen | Ellet | 644 | 434 |
| Huia | K. Valley | 436 | 312 |
| Irrigation | K. Valley | 481 | 376 |
| Olwen | K. Valley | 463 | 437 |

| (B) Cultivars measured | Associated cultivars | H2 | H4 |
|---|---|---|---|
| | | Monoculture (g pot$^{-1}$) | |
| Ellet | | 379 | 113 |
| K. Valley | | 323 | 121 |
| | | Mixtures (g pot$^{-1}$) | |
| Ellet | Huia | 349 | 79 |
| Ellet | Irrigation | 275 | 78 |
| Ellet | Olwen | 302 | 65 |
| K. Valley | Huia | 292 | 67 |
| K. Valley | Irrigation | 266 | 83 |
| K. Valley | Olwen | 243 | 47 |

**Appendix 5.14** Means of NSC content (mg $pot^{-1}$) in (A) stolons of white clover in monocultures and mixtures with perennial ryegrass at harvest 2 (H2) and harvest 4 (H4), and (B) stubble DM of perennial ryegrass in mixture with white clover at H2 and H4.

| (A) Cultivars measured | Associated cultivars | H2 | H4 |
|---|---|---|---|
| | | Monoculture (g $pot^{-1}$) | |
| Huia | | 3987 | 5804 |
| Irrigation | | 5686 | 8089 |
| Olwen | | 4659 | 11326 |
| | | Mixtures (g $pot^{-1}$) | |
| Huia | Ellet | 1041 | 4216 |
| Irrigation | Ellet | 1499 | 5995 |
| Olwen | Ellet | 1110 | 6032 |
| Huia | K. Valley | 861 | 5027 |
| Irrigation | K. Valley | 1652 | 5011 |
| Olwen | K. Valley | 976 | 6194 |

| (B) Cultivars measured | Associated cultivars | H2 | H4 |
|---|---|---|---|
| | | Monoculture (g $pot^{-1}$) | |
| Ellet | | 3566 | 1083 |
| K. Valley | | 3415 | 986 |
| | | Mixtures (g $pot^{-1}$) | |
| Ellet | Huia | 2708 | 314 |
| Ellet | Irrigation | 2183 | 380 |
| Ellet | Olwen | 2477 | 310 |
| K. Valley | Huia | 2257 | 304 |
| K. Valley | Irrigation | 1710 | 400 |
| K. Valley | Olwen | 1785 | 257 |

**Appendix 6.1** The effect of cultivar on shoot DM (g $pot^{-1}$) of white clover in monocultures and mixtures at C1, C2, and C3.

| White clover cultivar | Monoculture shoot DM (g $pot^{-1}$) | | |
|---|---|---|---|
| | C1 | C2 | C3 |
| Huia | 2.37 | 14.13 | 21.99 |
| Olwen | 1.66 | 12.09 | 21.84 |
| Means | 2.01$^{c*}$ | 13.11$^{b}$ | 21.92$^{a}$ |
| | Mixture shoot DM (g $pot^{-1}$) | | |
| Huia | 0.72 | 2.83 | 9.67 |
| Olwen | 0.65 | 2.74 | 8.85 |
| Means | 0.69$^{c*}$ | 2.79$^{b}$ | 9.26$^{a}$ |
| | RY | | |
| Huia | 0.30 | 0.22 | 0.42 |
| Olwen | 0.39 | 0.26 | 0.42 |
| Means | 0.45$^{a*}$ | 0.24$^{b}$ | 0.42$^{a}$ |

*Means within the same row associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.2** (A) Shoot DM of perennial ryegrass in monocultures, and (B) The effect of associated white clover cultivar on shoot DM (g $pot^{-1}$) and RY of Ellet perennial ryegrass in mixtures at C1, C2, and C3.

| | (A) Monoculture shoot DM (g $pot^{-1}$) | | |
|---|---|---|---|
| | C1 | C2 | C3 |
| Perennial Ryegrass | 12.86$^{a*}$ | 9.49$^{b}$ | 5.27$^{c}$ |
| Associated white clover cultivar | (B) Mixture shoot DM (g $pot^{-1}$) | | |
| | C1 | C2 | C3 |
| Huia | 10.49 | 9.22 | 4.57 |
| Olwen | 9.91 | 9.04 | 4.27 |
| Means | 10.20$^{a*}$ | 9.13$^{b}$ | 4.42$^{c}$ |
| | RY | | |
| Huia | 0.83 | 0.99 | 0.89 |
| Olwen | 0.78 | 0.96 | 0.82 |
| Means | 0.80$^{b*}$ | 0.98$^{a}$ | 0.85$^{b}$ |

*Means within the same row associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.3** The nitrogen x defoliation x harvest interaction* on NSC content (mg pot$^{-1}$) in stolons of white clover in mixtures at DH 1 and DH 2

| Destructive Harvest | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Monoculture shoot DM (g pot$^{-1}$) | | | |
| DH1 | 1301 | 762 | 194 | 143 |
| DH2 | 807 | 1181 | 414 | 382 |

*This interaction is non-significant (P>0.05)

**Appendix 6.3.1** The effects of destructive harvest number, cultivar of white clover, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg ha$^{-1}$ (N1) on dry matter and NSC in stolons of white clover in mixtures with ryegrass.

| Destructive harvest number | Cultivar | Defoliation | Nitrogen | DM (gpot$^{-1}$) | NSC (mgg$^{-1}$) | NSC (mgpot$^{-1}$) |
|---|---|---|---|---|---|---|
| DH1 | Huia | L | N0 | 4.72 | 206 | 973 |
| | | | N1 | 3.87 | 237 | 917 |
| | | S | N0 | 2.87 | 55 | 158 |
| | | | N1 | 3.20 | 54 | 173 |
| | Olwen | L | N0 | 5.34 | 305 | 1629 |
| | | | N1 | 3.53 | 172 | 608 |
| | | S | N0 | 3.68 | 63 | 232 |
| | | | N1 | 1.63 | 70 | 114 |
| DH2 | Huia | L | N0 | 7.77 | 131 | 1018 |
| | | | N1 | 6.75 | 158 | 1067 |
| | | S | N0 | 3.37 | 111 | 374 |
| | | | N1 | 4.51 | 101 | 456 |
| | Olwen | L | N0 | 6.21 | 96 | 596 |
| | | | N1 | 5.56 | 233 | 1296 |
| | | S | N0 | 3,98 | 114 | 454 |
| | | | N1 | 2.10 | 147 | 309 |

**Appendix 6.3.2** The effects of destructive harvest number, cultivar of white clover, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg $ha^{-1}$ (N1) on dry matter and NSC in stubble of ryegrass in mixtures.

| Destructive harvest number | Cultivar | Defoliation | Nitrogen | DM ($gpot^{-1}$) | NSC ($mgg^{-1}$) | NSC ($mgpot^{-1}$) |
|---|---|---|---|---|---|---|
| DH1 | Huia | L | N0 | 5.44 | 54 | 294 |
| | | | N1 | 6.48 | 167 | 1082 |
| | | S | N0 | 5.03 | 39 | 196 |
| | | | N1 | 4.84 | 45 | 218 |
| | Olwen | L | N0 | 5.26 | 47 | 247 |
| | | | N1 | 6.71 | 149 | 1000 |
| | | S | N0 | 4.87 | 52 | 253 |
| | | | N1 | 2.41 | 45 | 217 |
| DH2 | Huia | L | N0 | 7.42 | 53 | 393 |
| | | | N1 | 3.43 | 80 | 574 |
| | | S | N0 | 9.10 | 57 | 464 |
| | | | N1 | 7.55 | 49 | 370 |
| | Olwen | L | N0 | 7.80 | 46 | 356 |
| | | | N1 | 7.84 | 67 | 525 |
| | | S | N0 | 7.05 | 37 | 261 |
| | | | N1 | 6.71 | 56 | 376 |

**Appendix 6.3.3** The effects of destructive harvest number, cultivar of white clover, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg $ha^{-1}$ (N1) on dry matter and NSC in stolons of white clover in monocultures.

| Destructive harvest number | Cultivar | Defoliation | Nitrogen | DM ($gpot^{-1}$) | NSC ($mgg^{-1}$) | NSC ($mgpot^{-1}$) |
|---|---|---|---|---|---|---|
| DH1 | Huia | L | N0 | 10.03 | 250 | 2507 |
| | | | N1 | 9.02 | 171 | 1542 |
| | | S | N0 | 6.36 | 55 | 350 |
| | | | N1 | 6.74 | 66 | 445 |
| | Olwen | L | N0 | 11.00 | 363 | 3982 |
| | | | N1 | 10.62 | 226 | 2401 |
| | | S | N0 | 4.88 | 64 | 312 |
| | | | N1 | 5.82 | 66 | 384 |
| DH2 | Huia | L | N0 | 13.34 | 186 | 2480 |
| | | | N1 | 14.13 | 147 | 2077 |
| | | S | N0 | 8.12 | 102 | 828 |
| | | | N1 | 8.04 | 156 | 1254 |
| | Olwen | L | N0 | 12.54 | 210 | 2633 |
| | | | N1 | 14.29 | 370 | 5287 |
| | | S | N0 | 7.02 | 208 | 1460 |
| | | | N1 | 9.98 | 205 | 2048 |

**Appendix 6.3.4** The effects of destructive harvest number, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg $ha^{-1}$ (N1) on dry matter and NSC in stubble of ryegrass in monocultures.

| Destructive harvest number | Defoliation | Nitrogen | DM ($gpot^{-1}$) | NSC ($mgg^{-1}$) | NSC ($mgpot^{-1}$) |
|---|---|---|---|---|---|
| DH1 | L | N0 | 7.91 | 184 | 1455 |
| | | N1 | 8.85 | 177 | 1566 |
| | S | N0 | 6.80 | 56 | 381 |
| | | N1 | 5.56 | 52 | 289 |
| DH2 | L | N0 | 8.87 | 87 | 772 |
| | | N1 | 12.57 | 106 | 1332 |
| | S | N0 | 7.85 | 54 | 424 |
| | | N1 | 9.45 | 69 | 652 |

**Appendix 6.3.5** The effects of destructive harvest number, cultivar of white clover, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg $ha^{-1}$ (N1) on dry matter and NSC in stubble of ryegrass in mixtures.

| Destructive harvest number | Cultivar | Defoliation | Nitrogen | DM ($gpot^{-1}$) | NSC ($mgg^{-1}$) | NSC ($mgpot^{-1}$) |
|---|---|---|---|---|---|---|
| DH1 | Huia | L | N0 | 5.44 | 54 | 294 |
| | | | N1 | 6.48 | 167 | 1082 |
| | | S | N0 | 5.03 | 39 | 196 |
| | | | N1 | 4.84 | 45 | 218 |
| | Olwen | L | N0 | 5.26 | 47 | 247 |
| | | | N1 | 6.71 | 149 | 1000 |
| | | S | N0 | 4.87 | 52 | 253 |
| | | | N1 | 2.41 | 45 | 217 |
| DH2 | Huia | L | N0 | 7.42 | 53 | 393 |
| | | | N1 | 3.43 | 80 | 574 |
| | | S | N0 | 9.10 | 57 | 464 |
| | | | N1 | 7.55 | 49 | 370 |
| | Olwen | L | N0 | 7.80 | 46 | 356 |
| | | | N1 | 7.84 | 67 | 525 |
| | | S | N0 | 7.05 | 37 | 261 |
| | | | N1 | 6.71 | 56 | 376 |

**Appendix 6.3.6** The effects of destructive harvest number, cultivar of white clover, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg ha$^{-1}$ (N1) on dry matter and NSC in stolons of white clover in monocultures.

| Destructive harvest number | Cultivar | Defoliation | Nitrogen | DM (gpot$^{-1}$) | NSC (mgg$^{-1}$) | NSC (mgpot$^{-1}$) |
|---|---|---|---|---|---|---|
| DH1 | Huia | L | N0 | 10.03 | 250 | 2507 |
| | | | N1 | 9.02 | 171 | 1542 |
| | | S | N0 | 6.36 | 55 | 350 |
| | | | N1 | 6.74 | 66 | 445 |
| | Olwen | L | N0 | 11.00 | 363 | 3982 |
| | | | N1 | 10.62 | 226 | 2401 |
| | | S | N0 | 4.88 | 64 | 312 |
| | | | N1 | 5.82 | 66 | 384 |
| DH2 | Huia | L | N0 | 13.34 | 186 | 2480 |
| | | | N1 | 14.13 | 147 | 2077 |
| | | S | N0 | 8.12 | 102 | 828 |
| | | | N1 | 8.04 | 156 | 1254 |
| | Olwen | L | N0 | 12.54 | 210 | 2633 |
| | | | N1 | 14.29 | 370 | 5287 |
| | | S | N0 | 7.02 | 208 | 1460 |
| | | | N1 | 9.98 | 205 | 2048 |

**Appendix 6.3.7** The effects of destructive harvest number, lenient (L) and severe (S) defoliation, and rates of nitrogen application of none (N0) and 75 kg $ha^{-1}$ (N1) on dry matter and NSC in stubble of ryegrass in monocultures.

| Destructive harvest number | Defoliation | Nitrogen | DM ($gpot^{-1}$) | NSC ($mgg^{-1}$) | NSC ($mgpot^{-1}$) |
|---|---|---|---|---|---|
| DH1 | L | N0 | 7.91 | 184 | 1455 |
| | | N1 | 8.85 | 177 | 1566 |
| | S | N0 | 6.80 | 56 | 381 |
| | | N1 | 5.56 | 52 | 289 |
| DH2 | L | N0 | 8.87 | 87 | 772 |
| | | N1 | 12.57 | 106 | 1332 |
| | S | N0 | 7.85 | 54 | 424 |
| | | N1 | 9.45 | 69 | 652 |

**Appendix 6.4** The effect of cultivar on competitive ratio of white clover with respect to perennial ryegreass at C1, C2, and C3.

| | (C) CR | | |
|---|---|---|---|
| | C1 | C2 | C3 |
| Huia | 0.36[ab*] | 0.22[b*] | 0.47[a] |
| Olwen | 0.50[a*] | 0.27[b*] | 0.51[a] |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.5** The effect of white clover cultivar x nitrogen x destructive harvest interaction on sugar concentration (mg $g^{-1}$) in stolons of white clover (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Sugar concentration (mg $g^{-1}$) | | | |
| DH1 | 88.71[bc*] | 91.74[abc] | 119.89[a] | 85.75[bc] |
| DH2 | 91.81[abc] | 96.14[abc] | 79.92[c] | 111.64[ab] |
| S.e.d. | 13.90 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.6** The effect of nitrogen x defoliation x harvest interaction on sugar concentration (mg $g^{-1}$) in stolons of white clover at destructive harvests 1 and 2 (means of four replicates).

| Destructive harvest | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Sugar concentration (mg $g^{-1}$) | | | |
| DH1 | 151.57[a*] | 117.01[bc] | 57.03[e] | 60.48[de] |
| DH2 | 90.46[cd] | 123.85[ab] | 81.27[de] | 83.93[de] |
| S.e.d. | 13.90 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.7** The effect of white clover cultivar x nitrogen x destructive harvest interaction on starch concentration (mg $g^{-1}$) in stolons of white clover (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Starch concentration (mg $g^{-1}$) | | | |
| DH1 | 41.54[b*] | 53.74[ab] | 63.51[ab] | 35.07[b] |
| DH2 | 28.90[b] | 33.28[b] | 24.89[b] | 103.06[a] |
| S.e.d. | 24.08 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.8** The effect of white clover cultivar x nitrogen x destructive harvest interaction on starch content (mg $pot^{-1}$) in stolons of white clover (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Starch concentration (mg $pot^{-1}$) | | | |
| DH1 | 200.16[ab*] | 212.33[ab] | 334.03[ab] | 127.65[b] |
| DH2 | 159.92[b] | 190.12[b] | 123.22[b] | 416.32[a] |
| S.e.d. | 94.33 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.9** The effect of nitrogen x defoliation x harvest interaction on starch content (mg $pot^{-1}$) of white clover stolons at destructive harvests 1 and 2 (means of four replicates).

| Destructive harvest | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Starch concentration (mg $pot^{-1}$) | | | |
| DH1 | 529.73$^{a*}$ | 336.70$^{ab}$ | 4.46$^{c}$ | 3.28$^{c}$ |
| DH2 | 170.25$^{bc}$ | 480.37$^{a}$ | 112.89$^{c}$ | 126.07$^{c}$ |
| S.e.d. | 94.33 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.10** The effect of white clover cultivar x nitrogen x destructive harvest interaction on NSC concentration (mg $g^{-1}$) in stolons of white clover (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | NSC concentration (mg $g^{-1}$) | | | |
| DH1 | 130.25$^{*}$ | 145.48$^{abc}$ | 183.39$^{ab}$ | 120.82$^{bc}$ |
| DH2 | 120.72$^{bc}$ | 129.43$^{bc}$ | 104.80$^{c}$ | 214.70$^{a}$ |
| S.e.d. | 33.901 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.11** The effect of nitrogen x defoliation x harvest interaction on NSC concentration (mg $g^{-1}$) in stolon of white clover at destructive harvests 1 and 2 (means of four replicates).

| Destructive harvest | Lenient | | Severe | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | NSC concentration (mg $g^{-1}$) | | | |
| DH1 | 255.35$^{a*}$ | 204.41$^{a}$ | 58.29$^{b}$ | 61.89$^{b}$ |
| DH2 | 113.42$^{b}$ | 220.39$^{a}$ | 112.09$^{b}$ | 123.74$^{b}$ |
| S.e.d. | 33.90 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.12** The effect of associated white clover cultivar x nitrogen x destructive harvest interaction on sugar concentration (mg $g^{-1}$) in stubble of perennial ryegrass (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Sugar concentration (mg $g^{-1}$) | | | |
| DH1 | 23.15$^{c*}$ | 21.56$^{c}$ | 58.29$^{a}$ | 20.56$^{c}$ |
| DH2 | 21.46$^{c}$ | 22.15$^{c}$ | 33.90$^{b}$ | 22.69$^{bc}$ |
| S.e.d. | 33.901 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.13** The effects of associated white clover cultivar x harvest interaction on sugar content (mg pot$^{-1}$) in stubble of perennial ryegrass.

| Destructive harvests | Huia | Olwen |
|---|---|---|
| | Sugar content (mg pot$^{-1}$) | |
| DH1 | 116.95$^{c*}$ | 246.04$^{a}$ |
| DH2 | 169.39$^{bc}$ | 207.72$^{ab}$ |
| S.e.d. | 30.154 | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.14** The effects of associated white clover cultivar x nitrogen interaction on sugar content (mg pot$^{-1}$) in stubble of perennial ryegrass.

| Destructive harvests | N0 | N1 |
|---|---|---|
| | Sugar content (mg pot$^{-1}$) | |
| Huia | 143.99$^{b*}$ | 142.34$^{b}$ |
| Olwen | 321.72$^{a}$ | 132.04$^{b}$ |
| S.e.d. | 30.154 | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.15** The effect of associated white clover cultivar x nitrogen x destructive harvest interaction on fructan concentration(mg g$^{-1}$) in stubble of perennial ryegrass (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Fructan concentration (mg g$^{-1}$) | | | |
| DH1 | 27.16$^{b*}$ | 23.54$^{c}$ | 99.21$^{a}$ | 23.71$^{c}$ |
| DH2 | 27.63$^{bc}$ | 22.06$^{c}$ | 39.23$^{b}$ | 29.57$^{bc}$ |
| S.e.d. | 5.50 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.16** The effect of associated white clover cultivar x nitrogen x destructive harvest interaction on fructan content (mg g$^{-1}$) in stubble of perennial ryegrass (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | Fructan concentration (mg pot$^{-1}$) | | | |
| DH1 | 144.50$^{c*}$ | 116.38$^{c}$ | 650.75$^{a}$ | 115.21$^{c}$ |
| DH2 | 211.80$^{bc}$ | 185.75$^{c}$ | 295.68$^{b}$ | 210.15$^{bc}$ |
| S.e.d. | 42.23 | | | |

*Means associated with the same superscripts arex not significantly different (P>0.05)

**Appendix 6.17** The effect of associated white clover cultivar x nitrogen x destructive harvest interaction on NSC concentration (mg $g^{-1}$) in stubble of perennial ryegrass (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | NSC concentration (mg $g^{-1}$) | | | |
| DH1 | 50.30$^{c*}$ | 45.09$^{c}$ | 157.50$^{a}$ | 44.27$^{c}$ |
| DH2 | 49.09$^{c}$ | 44.21$^{c}$ | 73.13$^{b}$ | 52.27$^{c}$ |
| S.e.d. | 8.79 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.18** The effect of associated white clover cultivar x nitrogen x destructive harvest interaction on NSC content (mg $pot^{-1}$) in stubble of perennial ryegrass (means of four replicates).

| Destructive harvest | Huia | | Olwen | |
|---|---|---|---|---|
| | N0 | N1 | N0 | N1 |
| | NSC concentration (mg $pot^{-1}$) | | | |
| DH1 | 270.45$^{c*}$ | 224.35$^{c}$ | 1041.10$^{a}$ | 217.15$^{c}$ |
| DH2 | 373.90$^{bc}$ | 362.45$^{c}$ | 548.97$^{b}$ | 372.30$^{bc}$ |
| S.e.d. | 77.50 | | | |

*Means associated with the same superscripts are not significantly different (P>0.05)

**Appendix 6.19** The effect of cultivar, defoliation and nitrogen treatment on (A) DM of stolons of white clover and stubble of perennial ryegrass in monoculture (g $pot^{-1}$).

| (A) Cultivars measured | Defoliation treatment | Nitrogen treatment | Destructive Harvest 1 | Destructive Harvest 2 |
|---|---|---|---|---|
| | | | Stolon DM (g $pot^{-1}$) | |
| Huia | Lenient | N0 | 9.99 | 13.36 |
| | | N75 | 8.95 | 14.09 |
| | Severe | N0 | 6.39 | 8.14 |
| | | N75 | 6.89 | 10.02 |
| Olwen | Lenient | N0 | 10.70 | 12.44 |
| | | N75 | 10.55 | 14.36 |
| | Severe | N0 | 4.89 | 7.2 |
| | | N75 | 5.88 | 9.76 |
| | | | Stubble DM (g $pot^{-1}$) | |
| Ellet | Lenient | N0 | 7.93 | 8.71 |
| | | N75 | 8.85 | 12.39 |
| | Severe | N0 | 6.80 | 7.94 |
| | | N75 | 5.68 | 9.37 |

**Appendix 7.1** Non-structural carbohydrates in the stolons and roots of white clover and stubble and roots of perennial ryegrass in mixtures seven weeks after planting (means of three replicates)

| Cultivar measured | | Huia | Olwen | Ellet | Ellet |
|---|---|---|---|---|---|
| Associated cultivars | | Ellet | Ellet | Huia | Olwen |
| Stolons or Stubble | NSC | Stolons | Stolons | Stubble | Stubble |
| | Sol. Sugar (mgg$^{-1}$) (mg pot$^{-1}$) | 198.5<br>127.4 | 169.6<br>68.2 | 77.3<br>132.2 | 68.1<br>92.8 |
| | Fructan or Starch (mgg$^{-1}$) (mg pot$^{-1}$) | 155.7<br>98.1 | 141.8<br>59.5 | 31.7<br>53.9 | 33.7<br>47.0 |
| | NSC (mgg$^{-1}$) (mg pot$^{-1}$) | 354.2<br>225.6 | 311.4<br>127.7 | 109.0<br>186.1 | 101.8<br>139.8 |
| Roots | NSC | White clover | White clover | Perennial ryegrass | Perennial ryegrass |
| | Sol. Sugar (mgg$^{-1}$) (mg pot$^{-1}$) | 59.3<br>68.4 | 108.0<br>173.0 | 34.9<br>81.8 | 28.1<br>45.9 |
| | Fructan or Starch (mgg$^{-1}$) (mg pot$^{-1}$) | 51.5<br>57.9 | 177.4<br>278.7 | 14.2<br>33.1 | 12.7<br>20.8 |
| Total | NSC (mgg$^{-1}$) (mg pot$^{-1}$) | 110.9<br>126.3 | 285.4<br>451.7 | 49.1<br>114.9 | 40.7<br>66.7 |
| | NSC(mg pot$^{-1}$) | 351.9 | 579.4 | 301.0 | 206.5 |

**Appendix 7.2** Means (averaged over nutrient treatments) of total NSC content at initial (seven week growth) and remaining at final harvest and the percentage of NSC; (A) in the stolons of white clover and stubble of perennial ryegrass cultivars, (B) roots

| (A) Cultivars measured | Associated cultivars | Initial NSC (mg pot$^{-1}$) | Light treatment | NSC remaining (mg pot$^{-1}$) | NSC remaining Percent* |
|---|---|---|---|---|---|
| | | | Stolons | | |
| Huia | Ellet | 225.60 | E | 20.00 | 8.86 |
| | | | F | 28.97 | 12.84 |
| Olwen | Ellet | 127.70 | E | 30.30 | 23.72 |
| | | | F | 26.30 | 20.60 |
| | | | Stubble | | |
| Ellet | Huia | 18.61 | E | 19.53 | 10.49 |
| | | | F | 31.83 | 16.82 |
| Ellet | Olwen | 139.8 | E | 21.77 | 15.57 |
| | | | F | 26.60 | 19.03 |

| (B) Cultivars measured | Associated cultivars | Initial NSC (mg pot$^{-1}$) | Light treatment | Final NSC (mg pot$^{-1}$) | Final NSC Percent* |
|---|---|---|---|---|---|
| | | | Root of white clover | | |
| Huia | Ellet | 126.30 | E | 15.10 | 11.95 |
| | | | F | 25.17 | 19.92 |
| Olwen | Ellet | 451.70 | E | 22.78 | 5.04 |
| | | | F | 35.68 | 7.90 |
| | | | Root of perennial ryegrass | | |
| Ellet | Huia | 114.90 | E | 22.07 | 19.20 |
| | | | F | 36.11 | 31.42 |
| Ellet | Olwen | 66.70 | E | 27.19 | 41.82 |
| | | | F | 28.99 | 43.46 |

*(remaining/initial) x 100%