AUTECOLOGY OF WHITE CLOVER (*Trifolium repens* L.) WITH SPECIAL REFERENCE TO THE EFFECT OF STOLON BURIAL ON BRANCH FORMATION

A thesis

in partial fulfilment of the requirements

for the degree of

Doctor of Philosophy

in Plant Science

at Masssey University

Palmerston North

New Zealand

M.J.M. HAY

1994

ABSTRACT

In moist temperate regions the persistence and productivity of white clover (*Trifolium repens* L.) in grazed pastures is dependent upon stolon growth, particularly stolon branching. However measurements of white clover in New Zealand pastures indicate that by the beginning of the growing season, generally 80% of stolon material is buried. The objective of this study was to investigate the effect of stolon burial on the branching of stolons and to utilize the findings to further the understanding of the autecology of white clover in grazed pastures.

Distinct seasonal patterns of maximal stolon burial in spring and minimal branching of stolons in spring (both on a population and plant basis) were measured in a field trial established for the monitoring of the autecology of white clover under sheep grazing. Measurements of individual axillary buds on stolons artificially buried, under a range of conditions in the field, consistently found that the major effect of burial was to reduce branching by increasing (up to threefold) the probability of mortality of axillary buds following initiation of outgrowth. Initiation of outgrowth of axillary buds on stolon tissue that had emerged from the stolon apex while on the soil surface before being buried was not, except perhaps for those buds at the two youngest nodes at the time of burial, influenced by burial.

Glasshouse experiments were performed in order to increase knowledge of factors controlling the initiation of outgrowth of axillary buds and to examine how stolon burial influenced outgrowth of axillary buds. Experiments involved measurements of the branching response of individual axillary buds on stolons subjected to differing burial/cultural treatments so that treatment responses could be separated from ontogenetic influences. Initiation of outgrowth of axillary buds was only severely lowered by burial when the node associated with a bud emerged from the stolon apex in soil and then remained in soil. This reduction in initiation of outgrowth was found to result from inhibition of bud activity rather than from loss of bud viability. As very few axillary buds in pastures are buried from emergence, it was concluded that stolon burial has little impact on the initiation of outgrowth of axillary buds in pastures. Severe deprivation of photosynthate or phosphorus within plants, induced by shading or low phosphorus supply, respectively, delayed the initiation of outgrowth of axillary buds until they were positioned six or more nodes from the stolon apex, and reduced node appearance rates which further

delayed initiation of outgrowth. However, such deprivations of resource did not change the proportion of buds that eventually initiated outgrowth. Thus together, these experiments showed that increases in apical dominance (delay in initiation of outgrowth of axillary buds) could be induced by either low levels of intraplant resource or in response to sensing of the environment. If the inhibition of axillary bud outgrowth was maintained until a bud was positioned more than eight nodes from the apex, that bud would not initiate outgrowth upon an improvement in plant growing conditions. It is suggested that there is a 'window of opportunity' for initiation of outgrowth of axillary buds limited to those nodal positions eight or less from the apex not subjected to inhibition through apical dominance.

Relationships between potentially available photosynthate within stolons and branching were further explored by measuring the starch, sucrose and hexose contents of individual internodes of stolons subjected to burial/defoliation treatments or differing seasonal growth periods and correlating these contents with branching activity of the distal axillary bud. There was no evidence to suggest that photosynthate supply was limiting branching in these pastures.

Starch content of stolons varied five-fold with season (minimal and maximal contents occurring in late spring and early autumn, respectively). This seasonal pattern in the availability of stored photosynthate within plants provided a physiological basis to underpin understanding of the very significant changes that occurred both within individual plants and populations of white clover in pastures during the early spring to mid-summer period. Such changes centre on a rapid increase in growth demand for photosynthate in early spring which decreases the starch content of stolons, whereupon stolon senescence increases markedly thereby fragmenting plants and thus reducing branching complexity and mean dry weight per plant.

The change in status of the white clover population in spring was considered to negatively influence the response of populations to cultural and environmental perturbations during late spring - early summer. It was concluded, in view of the large changes occurring within white clover populations in spring-early summer, that the greatest of the effects of stolon burial, which was an increase in the mortality of recently initiated branches during winter (a period of low growth rate), would not have major implications for the population dynamics of white clover in this environment. However if burial of a high proportion of stolon material occurred in summer it would have greater potential to significantly reduce branch establishment within populations.

ACKNOWLEDGEMENTS

I sincerely thank my chief supervisor, Associate Professor A.C.P. Chu, for his enthusiastic support, warm encouragement and valuable guidance throughout this project. I gratefully acknowledge the very valuable contribution my co-supervisor, Professor R.G. Thomas, made throughout the study. I also thank Professor J. Hodgson for his efforts as a co-supervisor over the latter stages of the project.

I thank the late Dr R.W. Brougham and Mr J.A. Lancashire past Directors of DSIR Grasslands, for organising study leave for me and authorising the provision of the financial and physical resources to enable the study to proceed. Many DSIR Grasslands/AgResearch Grasslands staff have willingly provided assistance during the course of the study but in particular I gratefully acknowledge the following:

- Margaret Carline, for her enthusiastic and extremely competent assistance during the experimental programme, particularly in regard to her contribution to the analysis of the carbohydrate fractions of stolons
- Reneè Carson, for technical assistance both in the field and laboratory
- Jocelyn Tilbrook, for technical assistance towards the end of the project
- Yvonne Gray, Margaret Greig and Christine Van Meer for undertaking numerous tiller plug and herbage dissections
- the late Dr Siri Welwala, DSIR Applied Mathematics Division, for computing and statistical input during the early stages of the project
- Dr Gary Thomas, AgResearch Grasslands, for computing and statistical input later in the study.

Finally I thank my family for their tolerance as regards the demands this project has made on time I have had for family activities.

TABLE OF CONTENTS

Appendices

LIST OF TABLES

Chapter 3

Chapter 4

Chapter 6

Chapter 7

Appendix 5.1

LIST OF FIGURES

Chapter 3

Chapter 4

Chapter 5

LIST OF PLATES

Chapter 6

CHAPTER 1 INTRODUCTION

1.1 INTRODUCTION

Recognition of the important contribution white clover (*Trifolium repens* L.) makes within many temperate pastoral farming systems has resulted in considerable agronomic research on the species (Frame & Newbould 1986; Baker & Williams 1987). Despite this effort, detailed knowledge of the autecology of white clover in grazed swards is deficient in many areas, including, for example, detailed morphological description of the clonal fragments comprising the population of white clover in swards (Snaydon 1985; Frame & Newbould 1986). Only recently have the amounts and proportion of white clover stolon buried by soil been quantified in grazed pastures under New Zealand (Hay 1983, 1985; Hay *et al.* 1983, 1987; Hay & Chapman 1984) and UK conditions (Sackville Hamilton & Harper 1989). The range of stolon burial varies from 10 to 99%, the proportion depending chiefly upon season and rainfall. The effect of stolon burial on stolon branching, a process vitally influencing both the productivity and persistence of white clover (Beinhart 1963; Brougham *et al.* 1978; Chapman 1983), forms the basis of this study.

This study was undertaken 1985-1989, a period during which the candidate was also actively involved in parallel studies at DSIR Grasslands which contributed significant new information on the clonal growth of white clover in grazed swards (Brock *et al.* 1988; Hay *et al.* 1989a, b, 1990, 1991; Newton & Hay 1992; Newton *et al.* 1990, 1992). Although separately undertaken, considerable synergy existed between these studies and an important objective of the present study was to integrate results to provide a more detailed description of the autecology of clonally-growing white clover populations in grazed pastures. Thus the study was planned prior to, but was coincident with, a period of rapid development of conceptual knowledge of clonal growth of white clover in grazed swards.

This study therefore sought to elucidate aspects of the effect of stolon burial on the process of stolon branching and utilise this knowledge to further describe and understand the pattern of clonal growth of white clover in grazed swards in New Zealand.

1.2 OBJECTIVES

The experimental objectives were:

1. To obtain data from grazed pastures on seasonal variation in the processes of stolon burial and the density and branching characteristics of white clover populations of cultivars of contrasting morphology.
2. To identify, using simulated burial of stolon in the field and in the glasshouse, those characteristics of burial and the environmental changes induced by burial that most affect the branching processes of stolons.
3. To identify, using glasshouse studies, the developmental stage(s) of the branching process sensitive to burial.
4. To obtain data on the variation in carbohydrate content of stolons in grazed pastures in response to node ontogeny, season, defoliation and stolon burial and to relate the carbohydrate status of internodes with axillary bud outgrowth.

1.3 THESIS STRUCTURE

This thesis is presented in nine chapters. Chapter 1 is an introductory chapter and is followed by a review of literature, chapter 2, which covers clonal growth of white clover populations in pastures, correlative and environmental influences on the branching processes and stolon burial in pastures. This is followed by five experimental chapters, a discussion chapter and finally a summary chapter.

Results from a grazing trial, which monitored stolon burial and branching in white clover populations of contrasting morphology within ryegrass/white clover pastures, are presented in Chapter 3. Results of an experiment performed within the grazing trial, which applied simulated burial treatments so as to determine the characteristics of burial (date, duration, depth and proportion of stolon buried) that affect stolon branching, are reported in Chapter 4. Chapter 5 reports results from glasshouse experimentation on the response of growth and branching of white clover genotypes of contrasting morphology to variation of resources, particularly light intensity. Results from Chapter 4 suggested that the major effect of burial on branching was expressed at the youngest two nodes. A glasshouse experiment, which related burial effects on branching with node ontogeny, is reported in Chapter 6. The variation in available carbohydrates within internodes of stolons in

response to season, defoliation and burial and the association of carbohydrate fractions and branching are reported in Chapter 7.

Chapter 8 provides a synthesis of all the information of the study in relation to the clonal growth of white clover in grazed swards. A summary of major results and conclusions in Chapter 9 concludes this thesis.

CHAPTER 2 REVIEW OF LITERATURE

2.1 INTRODUCTION

This brief review of literature covers only those aspects of the agronomy of white clover (*Trifolium repens* L.) pertinent to the study and commences by examining the autecology of white clover in grazed swards. The importance of stolons and the branching of stolons for the perennation of white clover in grazed swards is emphasised. This is followed by a review of the plant (correlative) and external (environmental) factors affecting the branching processes of stolons and methods of assessing the branching of white clover in swards. Finally, information on the burial of stolon in pastures is covered so as to set the scene for this investigation of the influence of stolon burial on branching of white clover.

To allow for a more logical development of the literature review section this review will cover literature to the time of commencement of the study in 1986. Literature published after 1986 is included in the discussion of results in the appropriate chapters.

2.2 PLANT HABIT AND CLONAL GROWTH IN GRAZED SWARDS

In moist temperate environments white clover populations in established pastures have low recruitment rates (0.2 m^{-2} yr^{-1}) of genotypes from seed (Turkington *et al.* 1979; Chapman 1987) so that the major mechanism conferring persistence is vegetative propagation. As Bell (1984) defines clonal plants as "those perennials that spread and multiply by vegetative means" then, under these conditions, white clover has a clonal growth form. Although recruitment of genotypes is insignificant from a productive viewpoint (Chapman 1987) it may be essential as regards maintenance of genetic diversity of populations. For instance Watkinson (1978) calculated that recruitment of two seedlings ha^{-1} yr^{-1} was sufficient to maintain 25-30 genotypes m^{-2} in the stoloniferous clonal species *Ranunculus repens*. It should be noted however that in pastures in regions regularly subjected to severe moisture deficits, white clover may persist as a winter-annual (Blaser & Killinger 1950; Hollowell 1966; Jones 1982), and secondly that regeneration from soil seed banks can be important for the rapid colonisation of bare areas following major disturbance e.g. droughts, slips.

2.2.1 PLAGIOTROPIC HABIT

The plagiotropic habit of white clover is a feature of its clonal growth in pastures in that it confers ability for lateral spread and limits the loss of apices and axillary buds by defoliation events (Caradus 1984). Plagiotropism may be under phototropic or geotropic control or both (Newton 1986). For white clover a negative phototropic response to increasing light intensity is responsible for the horizontal growth of stolons (Thomas 1987b). In the dark negative geotropism takes precedence and stolons may grow vertically (Maige 1900; Thomas 1987b). Thus in white clover plagiotropism reflects a balance between photo- and geotropic responses operating in the following manner. The tropic responses of petioles, peduncles and stolons are all similar, only varying in degree and are negatively geotropic with the strength of the geotropic response decreasing as light intensity increases (Thomas 1987b). Hence under high light intensities stolons are adpressed to the ground and at low intensities stolons may grow upwards from the ground. Thomas (*loc. cit.*) found the bending response of stolon to be less marked when the whole plant was in darkness than when some other part of the plant was maintained in light. Hay (1983) observed that in winter/early spring many stolons in pastures after burial by wormcasting and stock treading grew vertically to the soil surface. Such stolons usually have some leaf material above ground which would confer high potential for a rapid and marked response of stolon to the darkness imposed by burial (Thomas 1987b). Application of gibberellic acid to plants growing in full light will induce the same negative geotropic growth responses as the imposition of darkness. This suggests that gibberellic acid is implicated in the control of growth responses to darkness (Fletcher & Martin 1962; Mange 1963; Thomas 1987b).

The creeping lateral movement of stolon apices through swards (Harper 1977, 1985; Burdon 1983; Turkington & Burdon 1983; Newton 1986) leads to a wandering type of growth in which genets (genotypes each derived from a unique zygote [or seed]) may spread and sample a number of different microenvironments. This type of growth is often referred to as a 'guerilla' habit (Lovett Doust 1981; Harper 1981, 1985; Newton 1986) and contrasts with species at the other end of the continuum with a 'phalanx' habit where a genet develops closely packed plant organs which exclude other genets and species from its zone of occupancy. Harper (1985) states 'white clover is a typical guerilla species and the image one forms of its populations is of mobile, migrating clones weaving among each other and within a grass-dominated sward'. However it is generally agreed (Burdon 1983;

Turkington & Burdon 1983; Chapman 1983) that although the potential for movement of apices through swards is high, susceptibility of faster growing stolons to grazing and treading damage (Chapman 1983) limits stolon elongation rates to 18 cm yr^{-1} or less. Chapman (1983) calculated that under a defoliation regime imposed by rotationally grazed cattle there was a potential for stolon elongation of 70.9 cm yr^{-1} but then measured a realised elongation of only 13.2 cm yr^{-1}. In swards more consistently defoliated by sheep under set stocking the values for potential and measured elongation were considerably less at 29.0 and 5.4 cm yr^{-1}, respectively. A high degree of intermingling of genets of white clover is indicated by the identification of three to six genets per hundred square centimetres (Cahn & Harper 1976) and 44-49 genets m^{-2} (Trathan 1983 cited by Harper 1985) within a Welsh pasture.

Herein lies the essence of the population biology of white clover in grazed swards. The population is organised at two levels in that it comprises a number of genets with each genet comprising any number of spatially and physiologically independent modular units (clonal fragments) (Snaydon 1985). Henceforth, an independent clonal fragment will be referred to as a plant. Thus response of a white clover population to any perturbation reflects the net effect of changes at both the genotype or genetic level and at the clonal fragment or plant level of organisation. While this study focuses on processes at the plant level it should be appreciated that genetic variation in response can also induce changes in the genetic structure of populations e.g. Chapman (1983), by measuring the proportion of cyanogenic individuals in populations, demonstrated that the genetic structure of populations differed in response to system of grazing and species of grazing stock.

2.2.2 GROWTH OF CLONAL FRAGMENTS (PLANTS)

The basic plant unit of white clover in grazed pastures usually has a main stolon growing forward at the apex and decaying at the base (Erith 1924; Kershaw 1959; Beinhart *et al.* 1963; Hollowell 1966; Harvey 1970; Chapman 1983) with a number of branches each of which also grows forward at the apex (Chapman 1983). This plant unit (clonal fragment) is one part of a genet derived from successful establishment of a seedling which subsequently fragmented, upon death of the seminal root system (Sanderson 1966), into several discrete, genetically-identical plants. The plant or clonal fragment just described produces further genetically identical plants through the processes of branching and decay of old basal stolon tissue. A branch is released to form a separate plant upon

the death of the node on the parent stolon from which it originated (see Fig. 1, Chapman 1983). These processes are continual and are the basis of persistence and perennation of white clover in swards.

The balance between the rates of growth at apices, death of basal stolon and production and survival of apices are the important determinants of both the size of individual plants and ultimately the net gain or loss of stolon biomass in a population. Generally in clonal plants, the rates of death of old tissue and production of new tissue are in balance and both increase or decrease in response to seasonal growth conditions to maintain mean plant size (Cook 1985). However, for white clover, evidence from stolon density measurements in swards in Manawatu, New Zealand, indicates that the rate of death exceeds the rate of formation of stolon in late spring-early summer (Hay 1983, 1985; Hay *et al.* 1983). At this time total stolon DW m^{-2} decreases by 30-50%, a loss accounted for by a 70% decrease in the buried stolon pool where the older basal stolon resides. This is also accompanied by a significant increase in the length per unit weight of stolon, further evidence of morphological changes in stolon systems occurring at this time. The increase in the death rate of basal stolon tissue relative to rate of formation of new stolon tissue should result in a reduction in mean plant dry weight and in the complexity of the branching systems of stolons in spring.

Conversely, as there appears to be a cyclical seasonal pattern (Hay 1985; Hay *et al.* 1987) over summer, plants must recover mean plant size and complexity of branching by forming new stolon tissue at a rate greater than that of the death of older basal tissue. The dynamic nature of the growth of individual plants and intermingling of plants under field conditions make identification of individuals very difficult and consequently few studies have attempted to investigate factors influencing the morphology of individual plants under field conditions. However, Pascoe (1973) mapped the surface portions of the stolon systems of plants and found grazing events to have very variable effects on both survival and fragmentation of the stolon system. In the only other reported study, Kershaw (1959) measured the length of the stolon systems of plants in populations from six Welsh pastures and found variation both among and within sites. Frame & Newbould (1986) emphasise the requirement for increased knowledge of the growth of individual plants within swards.

Erith (1924) recognised that growth of a plant from seed from April to July (northern hemisphere) reflected three attributes; node appearance rate, position (number

of nodes from apex) at which the first branch formed and proportion of nodes that branch. For clonally growing plants it is the combination of variation over time in these three factors and in the rate of death of basal nodes that determines the number of metamers (nodes) and modules (stolons) comprising an individual plant. A metamer is defined as the repeating structured unit differentiated from an apical meristem (Barlow 1989) and for white clover comprises of a node, internode, axillary bud, subtending leaf and two root primordia. A module (stolon) is defined as the string of all metamers derived from a single apical meristem.

While information on the growth at apices delineating the appearance of new nodes is substantive both under controlled conditions (including Mitchell 1956; Mitchell & Lucanus 1962; Beinhart 1963; King *et al.* 1978; Boller & Nosberger 1983; Hoglund & Williams 1984) and field conditions (including Brougham 1962; Curll & Wilkins 1982; Davies & Evans 1982; Wilman & Asiegbu 1982a,b; Chapman 1983; Korte & Parsons 1984; Hollington & Wilman 1985), that pertaining to the death rate of nodes at the bases of stolons is scarce. The basal node of the main or primary stolon of plants often supports a well developed root system (Wilman & Asiegbu 1982b; Chapman 1983; Hay 1983; Sackville Hamilton 1987b) and the life expectancy of such nodes is 100 days greater than that of non-rooted nodes (Sackville Hamilton 1987b). This arises because the death of nodes occurs in cohorts in contrast to their birth, which occurs one at a time at regular intervals. When the basal rooted node dies, all unrooted nodes distal to it before the next node with a living root die at the same time (Sackville Hamilton 1987b). The variable rate of progression of node death at the stolon base, proceeding acropetally in stages, and pausing at nodes with well developed adventitious roots, introduces temporal variability to the size of individual plants in that both the number and size of branches released upon death of a cohort of parent stolon nodes is highly variable. The death processes of basal nodes are responsive to seasonal changes (Hay 1983, 1985) and, given the predictable rate of recruitment of nodes at the apex, small variations in the rate of death of basal nodes are likely to have profound effects on both the size of individual plants and mean plant size of populations via a direct effect on loss of primary stolon and, perhaps more importantly, an indirect effect on the fragmentation of plants. Given the potential that change in the balance between birth and death rates of nodes has to alter mean plant size, it would appear that physiology of senescence and death at the base of stolon is an area requiring further investigation.

2.2.3 MORPHOLOGY

Morphology of white clover in grazed pastures contrasts starkly with that of glasshouse grown plants. White clover in the field commonly has only two to four leaves on well established stolons (Korte & Parsons 1984; Chapman 1986; Davies & Jones 1986), roots and branches at 17-33% and 27-43% of nodes, respectively (Chapman 1983) and 55-60% of total dry weight (DW) in stolon tissue (Cowling 1961; Hay *et al.* 1986) whereas glasshouse plants can have 13 leaves on well established stolons (Hoshino 1974), roots and branches at all mature nodes and only 21% of total DW in stolon tissue (Wilkinson & Gross 1967). These differences may precipitate both quantitative and qualitative changes in performances of genotypes in these environments and highlight the hazards in extrapolating results from the glasshouse to the field (Caradus 1984). In addition in grazed swards there is a considerable loss of apices associated with grazings (Pascoe 1973). Chapman (1983) measured a loss of 6% of main stolon apices per week during summer, which included losses associated with disease and pest attack and whole plant death as well as those associated with grazing. This continual loss of apices in swards requires a rate of branching of stolons that is sufficient to replace lost apices if the population is to persist. Indeed it has long been recognised that growth and survival of white clover are strongly dependent on stolon development and replacement by branching (Knight 1953; Beinhart 1963; Beinhart *et al.* 1963; Schillinger & Leffel 1964; Hollowell 1966; Chapman 1983; Frame & Newbould 1986).

2.2.4 STOLON FUNCTION

The high investment of plant dry weight in stolon tissue reflects the significance of stolons to the functioning of white clover in grazed swards. Nodes on stolons harbour the primordia capable of producing new apices and nodal roots, so any management causing stolon loss will have adverse effects on the population. Stolon internodes serve to space leaves, axillary buds and nodal roots so as to minimise overlap of resource depletion zones (Harper 1985). Stolon tissue is considered a storage organ for carbohydrate, stored in the form of starch (Bulter & Bailey 1973), in that stored starch is remobilised and utilised during winter (Wood & Sprague 1952; Ruelke & Smith 1956; Vez 1961; Murphy 1982; Harris *et al.* 1983) and after defoliation events (Tesar & Ahlgren 1950; Stewart & Bear 1951; Moran *et al.* 1953; Vez 1961; Murphy 1982). Evidence suggests a storage function of stolon as regards phosphorus (Hay *et al.* 1985, 1986) and possibly for other

nutrients but not nitrogen (Hay *et al.* 1985). Photosynthesis by stolons can contribute > 5% to total plant photosynthesis in the field (Harris *et al.* 1983; Korte & Parsons 1984). Our recent studies have demonstrated stolons in pastures to have a nutrient absorptive capacity which for phosphorus accounts for *c.* 5% of phosphorus uptake (Hay & Dunlop 1982; Hay *et al.* 1982, 1986; Dunlop & Hay 1985). Often substantial amounts of older basal stolon, without leaves, branches or roots, functions as a two way pipe transporting water and nutrients from roots to leaves (Ueno & Williams 1967; Hoshino 1974) and transporting photosynthate from leaves to roots (Ryle *et al.* 1981). Older stolon tissue tends to persist and function in this fashion when it links apical growth zones to an active taproot at the basal node (Sackville Hamilton 1987b).

The substantive allocation of plant resource to stolon, the previously detailed significance of stolon branching to growth and persistence in grazed swards and the low percentage of axillary buds that successfully form a branch (Chapman 1983), indicate that greater understanding of the basis of persistence and productivity will only accrue from quantifying the activity of axillary buds in populations. It is their biology which ultimately determines the population dynamics within clonally growing white clover. As there is one axillary bud at each node and considerable phenotypic (Harris & Brougham 1968; Hill 1977; King *et al.* 1978; Briseno de la Hoz & Wilman 1981; Hay & Baxter 1984) and genetic (Erith 1924; Knight 1953; Caradus 1986) variation in internode length, estimates of branching per stolon or stolon length or per unit area have limited value for study of population dynamics as measures are confounded by effects of numbers of buds and bud ontogeny. Thus attention must focus on activity of individual axillary buds and accommodate effects of bud ontogeny.

2.3 STOLON BRANCHING IN WHITE CLOVER

Axillary bud primordia are initiated within the apical bud of a stolon in the axil of leaf primordia positioned two or three from the apical dome (Thomas 1987b). Development is uninhibited for 5 plastochrons before ceasing at the stage leaflets of the subtending leaf unfold. Growth at this stage is repressed by the presence of the apical bud and further development may be initiated after *c.* two plastochrons in favourable conditions. This review covers the development of the axillary bud from the stage where the bud is formed but dormant and subtended by an unfolding leaf. The process of outgrowth of an axillary bud involves three phases which are; initiation of axillary bud outgrowth (observed

as appearance of the first leaf beyond the stipule, 0.1 Carlson (1966) scale), survival and establishment of the developing bud (0.2-1.0 Carlson scale) and survival and development of the young branch (production of up to seven leaves on the branch). Each of these stages have differing physiological and environmental requirements. These stages of vegetative reproduction may be considered analogous to the later stages of sexual reproduction involving germination of the seed, survival and establishment of the seedling and survival and development of the young plant.

Dormancy in vegetative buds may arise from correlative factors i.e. factors originating within the plant such as apical dominance, or environmental factors i.e. the conditions surrounding each bud site (Romberger 1963). Given that axillary buds of white clover are in a dormant state as nodes become differentiated from the apical bud (Thomas 1987b), understanding of the processes of branching is enhanced by separately considering correlative and environmental factors affecting outgrowth.

2.3.1 CORRELATIVE INFLUENCES

2.3.1.1 GENOTYPE

Genotypic variation for branching frequency (Erith 1924; Knight 1953; Beinhart *et al.* 1963; Williams 1983; Thomas 1981; Hoglund & Williams 1984), exemplified by the often quoted 'viney' and 'non viney' clones of Beinhart *et al.* (1963), indicates that a genetic component contributes to correlative influences on branching.

2.3.1.2 APICAL DOMINANCE

Apical dominance is the control exerted by apical portions of the shoot over the outgrowth of lateral buds (Phillips 1975). In white clover this is seen as the inhibition of outgrowth of axillary buds near the apical bud so that branching occurs at older nodes on the stolon (Thomas 1987b). The number of nodes basipetal to the apical bud at which development of axillary buds is inhibited is an expression of the current extent of apical dominance exerted by an apex. Expression of apical dominance varies with genotype (Erith 1924), shading (Harvey 1979; Newton 1986), nutrient supply (Harvey 1979; Thomas 1987b) and season (Thomas 1987b). Any limitation in the supply of resource (light, nutrients and possibly water; but see Thomas (1984)) increases the extent of apical

dominance (Harvey 1979; Newton 1986; Thomas 1987b) which suggests that control of apical dominance may be aligned with mechanisms governing intra-plant changes in resource allocation described by source-sink strength relationships. However the internal control of apical dominance in white clover has been little studied (Thomas 1987b) and knowledge in general of internal control of apical dominance is not equivocal (Phillips 1975; Trewavas 1981; Hillman 1984, 1986). Although studied in little detail, variation in the extent of apical dominance is a mechanism by which white clover can rapidly respond to a heterogenous environment and is an attribute which makes an important contribution to the high levels of phenotypic plasticity displayed by white clover (Harris & Brougham 1968; Hill 1977; Brougham *et al.* 1978; Horikawa 1986a, b).

2.3.1.3 NODAL POSITION OF THE AXILLARY BUD

A consequence of apical dominance is that the potential of an axillary bud to initiate outgrowth varies with the position of the node bearing it; i.e. outgrowth is rarely initiated while buds occupy the two node positions immediately basipetal to the apical bud (Erith 1924; Harvey 1979; Sackville Hamilton 1987a; Thomas 1987b). In addition, in the field, initiation of outgrowth of axillary buds at nodes positioned > 8 from the apex is uncommon (Chapman 1983). The net result is that effective initiation of outgrowth of axillary buds seems to occur only while buds are positioned at nodes beyond the influence of apical dominance but less than nine from the apex.

2.3.1.4 ROOT PRESENCE

Evidence regarding the correlative influence of the presence of a root at a node on the initiation of outgrowth of the associated axillary bud is contradictory. Solangaarachchi (1985) concluded that initiation of branch outgrowth was not influenced by presence or absence of a root at the node whereas Thomas (1987b) found initiation of axillary bud outgrowth only at nodes where nodal root growth had been previously stimulated by provision of moisture to the node. However the experimental plant material used by Thomas (*loc. cit*) had been grown in pots in a nutrient-poor mix, with stolons prevented from contacting any moist substratum, a pretreatment which resulted in inhibition of axillary bud outgrowth at nodes six or more basal to the youngest leaf with unfolded leaflets. There is a clear need for further work to clarify the correlative influences of nodal root presence on initiation of axillary bud outgrowth.

The survival, development and productivity of a branch have consistently been found to be enhanced by the presence of a root at the node of origin of the branch (Knight 1953; Trautner & Gibson 1966; Chapman 1983; Solangaarachchi 1985) which suggests a close physiological relationship between the root and the branch presumably concerning the supply of water and nutrients (Chow 1966; Chapman 1983) to the branch and photosynthates from branch to root.

2.3.1.5 LEAF PRESENCE

Thomas (1987b) found apical dominance was removed upon excision of the three oldest leaf primordia within the apical bud and suggested the repressive influences on bud growth are associated with young leaf primordia rather than the apical meristem itself. The presence of a subtending leaf and/or petiole also has an inhibitory influence on the development of the axillary bud at the associated node (Carlson 1966b; Harvey 1979; Newton 1986) despite the fact that vascular connections between the leaf at one node are with the axillary bud and root at the next youngest node (Thomas 1987a). The same inhibitory mechanism, originating within leaf tissue, could explain both these effects. On stolons shaded for three months prior to experimentation, leaf presence had a stimulatory effect on development of the axillary bud (Davies & Evans 1983). An explanation may be that assimilates within these stolons were insufficient to support outgrowth of buds in the absence of a supply of current photosynthate from the subtending leaf. All the same, this result indicates that the correlative effect of leaf presence is only one factor operating in the balance which determines outgrowth of axillary buds.

2.3.1.6 PRESENCE OF A FLOWER

The initiation of a flower primordium within the apical bud is precocious in that it occurs in the axil of the first leaf primordium rather than at the third leaf primordium where most vegetative axillary buds are initiated (Thomas 1962, 1987c). Conditions conducive to flowering also favour the early initiation of vegetative axillary bud primordia relative to the apical dome (Thomas 1962). Sackville Hamilton (1982) reported an increase in the proportion of vegetative axillary buds at nodes adjacent to a node with a flower that initiated outgrowth. On the other hand, many reviews of white clover growth (Burdon 1983; Turkington & Burdon 1983; Frame & Newbould 1986; Thomas 1987b, c) do not note the association of increased axillary bud outgrowth at nodes adjacent to a

flower. This discrepancy may arise because bud survival is variable following initiation of outgrowth, being dependent upon the local microenvironment (Chapman 1983; Sackville Hamilton 1982), and most studies are insufficiently detailed to monitor both the initiation of axillary bud outgrowth as well as branch establishment.

The presence of a flower has a correlative influence on internode development as the length of the internode basipetal to the first initiated flower is increased and often the acropetal internode is shorter than internodes associated with vegetative axillary buds (Booysen & Laude 1964; Thomas 1980; Thomas 1987c).

2.3.2 ENVIRONMENTAL FACTORS

2.3.2.1 TEMPERATURE

Different stages of the development of an axillary bud into a lateral branch stolon have different temperature optima in much the same way as various plant organs respond differently to temperature (Mitchell 1956; Mitchell & Lucanus 1962; Williams & Hoglund 1978; Hart 1987). For instance, the initiation of axillary bud formation in the apical bud is more precocious (i.e. axillary buds form at nodes nearer the apical dome) at low temperatures (10°C) (Thomas 1962), whereas initiation of outgrowth of axillary buds at nodes emerged from the apical bud is precocious at higher temperatures (20°C). However, the proportion of buds that initiate outgrowth at emerged nodes decreases as temperature increases from 10°C (Beinhart 1963; Hoglund & Williams 1984). As the rate of node/leaf appearance increases with increasing temperature up to 30°C (Mitchell 1956; Beinhart 1963) at already formed apices (Mitchell 1956; Mitchell & Lucanus 1962; Beinhart 1963; Machler & Nosberger 1977; Boller & Nosberger 1983; Hoglund & Williams 1984; Hart 1987) the rate of development of newly formed branches increases with temperature. Thus as temperature increases, the rate of production of new apices reflects the balance between an increase in rate of production of new nodes (axillary buds), a decrease in the proportion of buds that develop and a decrease in nodal position relative to the apex at which outgrowth occurs. These processes compensate to some extent and thus provide a rather broad temperature range (17°-23°C) for optimal shoot production (Beinhart 1963; Hart 1987).

2.3.2.2 LIGHT

There are four aspects of the light regime that influence plant growth; photoperiod, intensity, spectral quality and total irradiation. Unfortunately the independent action of each of these variables largely remains undefined for white clover. The strong interactions between temperature and light regime on white clover growth have long been recognised (Blackman 1934; Mitchell 1956; Brougham 1962; Beinhart 1963; Brougham *et al.* 1978; Hart 1987) and this along with the often correlated seasonal variation in all these parameters has meant that detailed understanding of the effects of the light regime on aspects of white clover growth is incomplete.

Many studies confound the effects of photoperiod (daylength) and total incident irradiation e.g. Mitchell & Lucanus 1962; Nosberger *et al.* 1983; Boller & Nosberger 1983). However Thomas (1987c) used low light intensity supplementary lighting and break lighting during the dark period to demonstrate that leaves sense photoperiod and transmit a signal both acropetally and basipetally that induces initiation of reproductive development within the apices of stolons. As flowering increases the initiation of outgrowth of vegetative buds at adjacent emerged nodes (Sackville Hamilton 1982) photoperiod can indirectly affect probability of branching. In experiments where the effects of photoperiod were confounded with changes in total irradiation it was reported that at lower temperatures ($\leq$ 10°C) a decrease in photoperiod did not affect the rate of node appearance (Mitchell & Lucanus 1962; Boller & Nosberger 1983) whereas at higher temperatures (18° and 30°C) a shorter photoperiod decreased the rate of node appearance (Mitchell & Lucanus 1962). These experiments also found the proportion of nodes branching to increase or decrease as photoperiod decreased from 16 h to 12 h (Boller & Nosberger 1983) or from 16 h to 8 h (Mitchell & Lucanus 1962), respectively.

The light intensity required for light saturation varies with temperature and optima for specific growth processes (i.e. axillary bud outgrowth, node appearance rate) vary (Beinhart 1962, 1963, 1964; Boller & Nosberger 1983). However a detailed study of the influence of light intensity on the growth processes of white clover, such as has been made for *Lolium perenne* L. (Hunt & Halligan 1981; Hunt & Thomas 1985), is required. It is known that as light intensity decreases, the rates of node appearance (Beinhart 1963; Jelmini & Nosberger 1978; Arnott & Ryle 1982) and proportion of nodes branching (Beinhart 1963; Sanderson 1966) progressively decrease while the number of nodes from

the apex to first node with a branch increases (Sanderson 1966; Harvey 1979; Davies & Evans 1983; Newton 1986). However a contrary result was obtained in mid-summer at Clemson, South Carolina when shading to 33% of sunlight favoured the proportion of nodes branching in spaced plants grown in the field (Trautner & Gibson 1966).

Light transmission and reflection from white clover leaves, in common with that of other species (Holmes & Smith 1977; Holmes 1981) has a low red (R) : far-red (FR) ratio and a deficiency of blue light (Scott *et al.* 1968) relative to unfiltered light. In general, decreasing the R:FR ratio of light incident on shoots results in increased internode lengths and inhibition of lateral bud growth, although species differ in the extent of their response (Grime 1966). Phytochrome is involved in the sensing and initiation of morphogenetic responses to changes in R:FR ratio of incident light (Smith 1975). Available evidence suggests the response of white clover to alterations in light quality is similar to that exhibited in other species; a decrease in the R:FR ratio of incident light from six to four increased petiole and stolon length while increases in intensity of the blue wavelengths altered morphology towards compactness with plants having short petioles and stolons (Warrington & Mitchell 1976). As far back as 1962, Brougham suggested that the R:FR ratio of incident light in different zones within the sward canopy was a factor influencing differential development of organs of white clover (Brougham 1962). The creeping horizontal growth of stolons at the base of sward canopies, the ability to vary petiole length in response to the light environment (Brougham 1962; Brougham *et al.* 1978), the variation occurring with changes in plant density in the proportion of nodes that develop branches (Erith 1924; Sanderson 1966) and the high degree of phenotypic plasticity exhibited by the species (Hill 1977; Brougham *et al.* 1978; Horikawa 1986a, b) all suggest white clover is a species capable of sensitive response to alterations in quality of incident irradiation. However a clear demonstration of the effects of changing R:FR ratio of incident irradiation, over and above the effects of light intensity, at the axillary bud sites causing a change in the outgrowth of buds, such as has been reported for ryegrass species and other grass species (Deregibus *et al.* 1983, 1985; Casal *et al.* 1985), remains to be reported (Hart 1987).

Where the intensity of irradiation is at or below light saturation the total quantity of light supplied may have an effect, independent of that of photoperiod, on the growth processes influencing axillary bud development. In general, understanding of the independent effects of photoperiod, intensity, spectral quality and quantity of

photosynthetically active incident irradiation on the processes influencing branching in white clover is incomplete and there is a need for detailed studies of the interaction of these parameters with temperature.

2.3.2.3 NUTRIENT (PHOSPHORUS) SUPPLY

Although some general principles cover the effects of deficiency in supply of most nutrients, there are differences in plant response to deficiency in supply of individual nutrients as nutrients vary with respect to specificity of the plant processes they influence. For this reason, this section will consider in particular the effect of phosphorus (P) supply, as P is the major nutritional input into pastoral enterprises in New Zealand (Levy 1970) and variation in P supply is an experimental treatment within one of the experiments reported in Chapter 5.

In general, the extent of stolon or rhizome branching in many clonal species is positively related to the availability of nutrients (Wareing 1964; McIntyre 1965; Ginzo & Lovell 1973; Slade & Hutchings 1987). For white clover it is not known whether this arises solely from influences of P supply on apical dominance or if changes in the proportion of nodes basal to the first branching node that branch also contribute to this result. Whereas some clonal species such as *Glechoma hederacea* respectively increase or decrease internode length in nutrient poor or rich mediums, leading to a resulting concentration of the clone in resource rich areas (Slade & Hutchings 1987), in white clover, limitation in P supply induces the converse response (Sackville Hamilton 1980). Thus as limitation in P supply decreases both internode length and branching frequency it severely reduces both the spread and productivity of white clover in pastures (Sears *et al.* 1955; Levy 1970; Frame & Newbould 1986). In contrast to this response, limited soil nitrogen supply induces increases in internode length and branching frequency (Hoglund & Williams 1984). This response may have evolved as a result of natural selection favouring the facilitation of rapid colonisation of low soil nitrogen sites where the nitrogen fixation process confers white clover an advantage over competing species with no nitrogen fixation capacity. The interaction of soil mineral nitrogen supply and rate of nitrogen fixation on the supply of nitrogen to white clover is complex (Hoglund & Brock 1973) and further investigation of the response of nodulated clonal fragments to variation in mineral nitrogen supply is required if the mechanisms underpinning the suppression of white clover in mixed swards with nitrogenous fertiliser use are to be fully understood.

Deprivation in P supply increases the extent of apical dominance (Phillips 1975) and this is evident in white clover as an increase in the number of nodes between the apex and the first node with a branch (Newton 1986; Thomas 1987b).

Limitations in P supply only substantially reduce node appearance rates when the deficiency is extreme (Sackville Hamilton 1980). Strong correlations between soil temperature and node appearance rate, except where drought was associated with high summer temperatures, across a range of soil fertilities (Wilman & Asiegbu 1982a; Chapman *et al.* 1983) suggest that, in general, nutrient supply has a minor role in determining node appearance rate in pastures. Studies are required to quantify how apical dominance, proportion of nodes branching and axillary branch survival and growth interact to give the positive relationship between branching and P supply in clonally growing white clover.

Limitation in P supply also alters, both quantitatively and qualitatively, the allocation of dry matter to metamers in that total dry matter allocation is reduced and distribution favours root at the expense of shoot (Mouat 1983). Reductions in petiole length and area of leaflets decrease leaf size, another important factor contributing to the reduction in yield of white clover under conditions of low P supply (Levy 1970; Caradus 1984).

2.3.2.4 MOISTURE

Soil moisture deficits are key determinants of the persistence and productivity of white clover in the field (Lazenby & Swain 1972; Hoglund *et al.* 1979) and can limit white clover more severely than the associated grasses in swards (Snaydon & Baines 1981; Thomas 1984; Stevenson & Laidlaw 1985). Previous work has focused on the negative effects of drought on nodal root initiation (Ueno & Yoshihara 1968; Ueno 1982; Stevenson & Laidlaw 1985), nodal root development (Thomas 1984; Stevenson & Laidlaw 1985) and rate of nitrogen fixation (Engin & Sprent 1973; Stevenson & Laidlaw 1985). There has been relatively little detailed work examining the mechanism of response of shoots of clonal plants of white clover to soil moisture deficits. However the appearance rate, dimensions and longevity of leaves decrease with increasing moisture deficit so that the allocation of DW to leaf decreases (Thomas 1984). Associated with this primary response is a reduction in branching (Thomas 1984) although whether this results solely from an increase in apical dominance, a reduction in the proportion of axillary buds

developing, a reduction in survival/establishment of branches or a combination of these features has not been established. J.L. Brock (personal communication) has repeatedly observed very high mortality rates in young branches of two emerged nodes or less to occur suddenly, within a two to three day period, as drought conditions intensify in pastures. Given the high frequency of occurrence of soil moisture deficits over most of the improved pastures of New Zealand during summer and autumn (Soil Bureau Bulletin 26, 1968) there is a requirement to further assess the physiological and morphological responses of white clover to drought.

2.3.2.5 DEFOLIATION

The influences of various aspects of defoliation (frequency, intensity, timing and uniformity) on the content of white clover in swards have been reviewed (Curll 1982; Harris 1987) and responses found to vary according to current environmental conditions, previous defoliation history and growth rates of associated species. The seemingly conflicting literature on the effects of defoliation on clover content in swards has been attributed to the interaction of defoliation on that process which most limits growth of white clover relative to companion species at the time (Harris 1987). The complexity of the white clover response to a defoliation event arises because defoliation influences growth both directly (through wounding, changing potential for photosynthate and plant hormone production, alteration of the transpiration stream) and indirectly (through altering the microenvironment at the sward base i.e. light intensity and quality, temperature regime, soil moisture and exposure to wind, growth of associated species in the pasture and carbohydrate status of plants) with the relative importance of variation in any individual factor varying with conditions specific to a site at the time. Improvement in the understanding of the effects of defoliation on white clover growth in swards will require quantifying the direct effects of defoliation before the more variable impacts of the indirect effects can be assessed. This will require incisive experimentation utilising an approach based on defoliation, wounding or petiole girdling of individual leaves of different ontogeny on stolons in canopies of differing leaf area index at time of treatment.

Defoliation of single plants will, depending on severity, reduce node appearance rate (Carlson 1966b; Sanderson 1966; King *et al.* 1978), and markedly reduce petiole length and leaf area of subsequently produced leaves (Carlson 1966b) with the effects most severely expressed on lateral branches (King *et al.* 1978). Severe defoliation regimes

maintaining either one or no fully expanded leaves per stolon apex reduced branching frequency 25 and 40% respectively (Jones & Davies 1988) although whether this was due to increased apical dominance or reduced frequency of branching at nodes basipetal to the first node with a branch was not identified.

Defoliation events can also involve removal of the stolon apex along with variable amounts of stolon. The frequency of ingestion of stolon increases as grazing intensity increases (Lancashire & Keogh 1968; Curll & Wilkins 1982). In pastures intensively grazed by sheep 13% of stolons had no apex (Brock *et al.* 1988). In the field, apex removal can result in rapid development of the youngest remaining axillary bud so that stolon growth is indistinguishable from that of growth of a main axis apex (Sackville Hamilton 1982). In contrast Thomas (1987b) found excision of the apex or the oldest three leaf primordia within the stolon apex allowed rapid growth of the youngest one, two or three axillary buds of glasshouse grown plants. The difference in number of axillary buds stimulated to grow by apical excision between glasshouse and field plants possibly relates to abundance of resource within stolons.

2.3.2.6 PHYSICAL IMPEDANCE

In pastures the growth of apices of white clover stolons is often physically impeded by the presence of other plants, uneven soil surface or soil when the stolon apex is buried. White clover (a plagiotropic species) shows in common with most orthotropic species characteristic responses to physical impedance of in particular a decrease in elongation rate and an increase in radial growth (Newton 1986). These responses have been termed thigmomorphogenetic (Jaffe 1973). However in white clover the most striking response to obstruction of the primary stolon apex was an increase in the frequency of branching and an increase in dry weight of the branches (Newton 1986).

In other species, obstructed stems evolve increased levels of ethylene and thigmomorphogenetic responses are induced by ethylene applications (Goeschl *et al.* 1966; Eisinger & Burg 1972; Pooviah 1974). Thus it has been concluded that ethylene is the active agent initiating thigmomorphogenesis (Biro *et al.* 1980). As ethylene is implicated in the inhibition of auxin synthesis and transport (Eisinger 1983) it may also modify apical dominance and be responsible for the increase in axillary bud development observed in white clover (Newton 1986).

2.3.2.7 BIOTIC INFLUENCES

Erith (1924) grew white clover either as single plants or thickly sown swards and found high plant density to reduce development of individual plants by reducing node appearance rate, numbers of branches and stolon elongation. Solangaarachchi & Harper (1989) suggest that intra-specific effects by plants of the same or differing genotypes suppress the development of a plant via the strongly localised control of axillary bud development. A suggested mechanism is that plants affect each other by altering the ratio of red to far red radiation within the canopy and this acts on phytochrome (Smith 1975) which induces an alteration in the branching pattern (Solangaarachchi & Harper 1987).

Interspecific effects of an associated grass species on white clover development vary with species of grass. Studies of neighbourhood associations consistently indicate a white clover preference for *Lolium perenne* L. as the neighbouring grass species (Kershaw 1959; Harris & Brougham 1968; Harris 1973; Turkington & Harper 1979a) and are supported by measures of white clover content that indicate *Lolium perenne* and *Festuca pratensis* are the most compatible and *Dactylis glomerata* the least compatible grass species (Chestnutt & Lowe 1970). In terms of dry matter production of white clover transplants Turkington *et al.* (1979) found grasses to rank: *Cynosurus cristatus* > *Lolium perenne* > *Agrostis tenuis* > *Holcus lanatus.* The compatibility of a grass species with white clover increases when it colonises deeper soils (Kershaw 1959), has an asynchronous growth rhythm (Turkington & Harper 1979b; Harris & Hoglund 1980), has an open rather than dense or mat-like habit (Jackman & Mouat 1972; Schmid & Harper 1985) and a high red to far red ratio of light transmitted or reflected by its foliage (L.A. Mehrhoff pers. comm.). There is also increasing evidence from established swards that a particular genotype of white clover will grow most vigorously when growing with a specific genotype of *Lolium perenne* (R. Turkington, pers. comm.). Stolon extension confers mobility to white clover clones and provides them with the opportunity to sample several different neighbouring species and/or genotypes. Selective pressures will act to favour the presence of a clone in association with the species/clone which permits most vigorous growth.

Other important biotic influences on white clover growth accrue from the symbioses formed with *Rhizobium trifolii* (review Crush 1987) and vesicular-arbuscular (V-A) mycorrhizas (Dunlop & Hart 1987) which improve growth when soil supply of nitrogen or other nutrients (e.g. phosphorus, sulphur) is low. These symbioses, through alleviating

nitrogen or other nutrient deficiency (e.g. phosphorus) in plants, decrease apical dominance effects (see Section 2.3.2.3) thereby increasing outgrowth of axillary buds, so increasing growth. On the other hand negative influences on white clover growth can result from acute pathogenic attack by fungal, bacterial, viral, mycoplasma-like organisms (review Latch & Skipp 1987) and/or nematodes (Skipp & Gaynor 1987) as well as from damage through pest attack (Gaynor & Skipp 1987). Pest attack and disease may directly, by damaging axillary buds, or indirectly, by depleting reserves within plants and hence functioning of axillary buds, affect branching and thus growth of white clover in the field.

2.4 ASSESSMENT OF BRANCHING

Commonly, assessment of branching of white clover in pastures utilises the pasture plug technique (Mitchell & Glenday 1958) to estimate the number of apices (growing points) per unit area or less commonly the number of apices per unit length of stolon e.g. Hay (1985). Where the number of nodes are counted the ratio of apices to nodes (% of nodes with a branch) can be calculated.

Observations of individual stolons repeated over time can be used to delineate the probability of axillary bud activity over the lifespan of the associated node (Chapman 1983; Sackville Hamilton 1987a). A major advantage of this technique is the recording of the development of short-lived small branches which may be underestimated using destructive point in time sampling methods. However the effects of disturbance during observations on growth and development of stolons are unknown inaccuracies of this technique. A variation of this technique was utilized by Pascoe (1973) whereby the above-ground stolon systems of clonal fragments (plants) of white clover in pasture were mapped before and after each of three rotational grazings by sheep during summer (December to February). Results indicated the effect of grazing was very variable both from plant to plant and from grazing to grazing and that grazing could enhance the fragmentation of stolon systems.

An instantaneous assessment of the net result of branching frequency and rate of clonal fragmentation can be obtained by sampling plants comprising the white clover population, categorising branching structure of plants and hence the population. However close examination of individual nodes reveals the history of previous growth enabling the past history of branching of the clonal fragment to be determined at a single point in time (Callaghan 1976, 1984). Kershaw (1959) found differences between sites in the mean plant weight and branching structure of white clover populations in pastures in Wales.

2.5 STOLON BURIAL IN PASTURES

Barley (1953) provided photographic evidence that white clover stolons were present above and below ground level in Australian pastures. The distribution of white clover stolon above and below ground within New Zealand pastures has been quantified in relation to season, site, system of grazing management and effective rainfall (Hay 1983, 1985; Hay & Chapman 1984; Hay *et al.* 1983, 1987). There is, in most New Zealand pastures, a strong seasonal cycle of burial of most stolon (> 80%) during winter, re-emergence of growing points on the soil surface by spring and stolon development on the soil surface over summer so that by autumn the proportion of stolon buried is about 40% (Hay *et al.* 1987). A similar seasonal cycle has been measured in a Welsh pasture (Sackville Hamilton 1982) and in Scottish pastures (Grant *et al.* 1986). This seasonal pattern parallels that of the moisture content of the surface horizon of most New Zealand soils. Soil moisture contents tend to increase from minimal values in early autumn up to field capacity for the late winter-early spring period and then decline over summer (Cox 1968).

The major mechanisms of burial of stolon have been ascribed to earthworm casting (Hay 1983) or earthworm casting and stock treading (Hay *et al.* 1987). Both mechanisms are maximal when the surface soil horizon approaches field capacity with Hay *et al.* (1987) finding that earthworm casting was the more important process prior to soils becoming saturated but that on saturated soils earthworm casting and stock treading are equally important. High soil moisture content in any season increases the proportion of stolon buried (Grant *et al.* 1986; Hay *et al.* 1987).

Conversely any prolonged atypically low rainfall period may modify the expected seasonal pattern of stolon burial at a site (Hay & Chapman 1984; Hay *et al.*, 1987). A summer dry period can increase the proportion of stolon buried above expected values (Hay & Chapman 1984; Hay *et al.* 1987) when intensive grazings lower residual biomass and increase the amount of above-ground stolon consumed by stock (Lancashire & Keogh 1968; Curll & Wilkins 1982) or not affect the proportion of stolon buried (Hay *et al.* 1987). On the other hand a dry period over winter, by limiting the effectiveness of both earthworm casting and stock treading as burial processes, can result in a much lower than normally expected proportion of stolon buried in spring (Hay *et al.* 1987).

Burial immediately alters the microenvironment of the stolon in that the light, temperature and moisture regimes differ from those of surface growing stolons and additionally the soil exerts a physical pressure especially when any expansive development of the stolon takes place. In pastures, the depth of burial of stolon is usually not great and under conditions at Palmerston North varies from 0 to 20 mm (Hay 1983). Thus the buried stolon is usually associated with either leaf or other stolons of the plant that are above ground and sensing light. The response of a stolon to burial is therefore usually rapid and follows a description given by Thomas (1987b). Curvature of the stolon occurs at the internodes either side of the node with the youngest unfolded leaf enabling the stolon to grow vertically to the soil surface. When a stolon resurfaces it curves again to assume its characteristic plagiotropic growth form and this region of curvature is often associated with high branching frequency (Hay 1983). This increased branching frequency may reflect reduced apical dominance resulting from an inhibition of auxin synthesis by high endogenous levels of ethylene induced by the thigmomorphogenetic response to burial (Section 2.3.2.6) or changes in the balance of growth hormones as gibberellic acid levels readjust from the relatively higher levels maintained when stolons grow vertically (Thomas 1987b).

Given the extent and widespread occurrence of burial of stolon in New Zealand pastures (Hay *et al.* 1987) as well as the importance of branching for the persistence and productivity of white clover (Section 2.2), this study was undertaken to examine the effect of burial of stolon on the processes of branching so as to improve knowledge of the autecology of white clover in grazed pastures.

CHAPTER 3 SEASONAL VARIATION IN GROWTH, BRANCHING AND BURIAL OF STOLONS OF THREE WHITE CLOVER CULTIVARS IN PASTURE GRAZED BY SHEEP

3.1 INTRODUCTION

Trifolium repens is a most variable species and this variability has been utilised in the production of 232 cultivars worldwide (Caradus 1986) as breeders attempt to extend the range and performance of this agronomically useful species. New Zealand has a general purpose cultivar (Grasslands Huia) and a range of cultivars targeted for increased growth under particular environmental/management conditions. They vary from Tahora, a small-leaved typed suitable for sheep grazing on hill country, to larger-leaved lax types (Pitau and Kopu) which are more suited to rotational grazing by cattle on high fertility lowland pastures (Brock 1988). In the present experiment three new cultivars were incorporated in a field trial laid down to provide normal grazed swards within which studies of burial of white clover stolon and branching of white clover could be studied. The field trial provided an opportunity in which several objectives could be met.

The first objective was to measure the seasonal pattern of stolon burial, under the environmental conditions of the site, and to monitor factors likely to influence stolon burial such as cultivar growth pattern, earthworm surface casting and stock grazing effects.

The second objective was to obtain a measure of the seasonal pattern of branching of each cultivar and to relate this to their respective patterns of stolon burial.

3.2 MATERIALS AND METHODS

3.2.1 SITE

The trial was situated on the Pasture and Crop Research Unit of Massey University (40° 24'S, 175°36'E) (Grid reference, New Zealand Topographical Map, Palmerston North 010307). The soil of the trial area is Tokomaru silt loam (Typic Fragiaqualf) which was mole- and tile-trained in 1978. In March 1983 the trial area was

sown to permanent pasture with 18 kg Ellet perennial ryegrass (*Lolium perenne* L.), 2 kg Grasslands Pitau white clover (*Trifolium repens* L.) and 2 kg Grasslands Pawera red clover (*Trifolium pratense* L.) per hectare. A dressing of 200 kg ha^{-1} diammonium phosphate fertiliser was applied at sowing. Annual autumn dressings of 200 kg ha^{-1} of 15% potassic superphosphate had been applied since 1977. In September 1985 the site was supporting a vigorously growing white clover/ryegrass sward.

Uniformity of soil fertility across the site was examined by taking soil samples from each of the four replicate areas (20 cores of 5 cm diameter, 0-7.5 cm depth were bulked together from each replicate) and analysing the bulked sample for Olsen P, total N content and soil pH. All three indexes of soil fertility were uniform within the trial site (Table 3.1).

Table 3.1 Olsen P and total N% (on an airdry soil basis) and soil pH values for soil samples bulked from 20 cores of 2.5 cm diameter (0-7.5 cm depth) taken from each replicate on 10 March 1986.

	Replicate			
	1	2	3	4
* Olsen P (ppm)	15	15	15	15
* Total N content (%)	0.32	0.31	0.32	0.33
+ Soil pH	5.90	5.89	6.00	5.93

* Duplicate analyses

\+ Triplicate analyses

3.2.2 CLIMATE

Climatic data were collected at the New Zealand Meteorological Service Climatological Station, situated 1.5 km from the trial site, at DSIR Grasslands, Palmerston North.

3.2.3 TRIAL ESTABLISHMENT AND DESIGN

3.2.3.1 ESTABLISHMENT

The trial area of 0.70 ha was sprayed with Dicamba (2 litres ha^{-1}) on 30 September 1985 to kill off the resident white clover in the sward. On 29 and 31 October 1985 the pasture was sprayed with paraquat at 1.5 and 1 litre ha^{-1}, respectively, to check ryegrass growth. On 1 November 1985, white clover seed was direct drilled into the ryegrass sward using a Massey Direct Drill with Bio-blade openers set to sow at 1 cm depth and deliver seed at 4.5 kg ha^{-1}. Immediately following drilling Blitzem slug pellets were broadcast at 10 kg ha^{-1}. Further details of management during the establishment phase (1 November 1985 to 25 March 1986) are given in Appendix 3.1.

3.2.3.2 DESIGN

Three white clover cultivars, Grasslands Kopu, Grasslands Pitau and Grasslands Tahora were sown so that their performance in productive lowland white clover/ryegrass pasture under on-off mob-stocking by sheep could be evaluated. There were four replicates each 58 x 30 m which were fenced individually. Each replicate contained four blocks each of three plots (one plot of each cultivar). Each plot was two drill widths wide, and measured 4.8 x 30 m. Within blocks cultivars were randomly allocated to each plot, but to assist with drilling this allocation was the same for replicates one to three. There was a different allocation to the fourth replicate.

3.2.4 GRAZING MANAGEMENT

The system of grazing management was on/off mob-stocking. Swards were grazed when herbage mass approached 2000 kg ha^{-1}. Grazings were usually severe with residuals of 500-750 kg DM ha^{-1}. Within the constraint of number of stock available,

grazings were over as short a period as possible, the grazing period ranged from 1 to 6 days at different grazings. The trial commenced with the grazing on 5 May 1986 of the regrowth made following topping of pastures on 25 March. Intensive measurements on the trial continued for 2 years finishing 14 April 1988 and involved nine grazings per year.

3.2.5 MEASUREMENTS

3.2.5.1 DRY MATTER PRODUCTION

Mean daily growth rates were obtained from calculations based on measurements of herbage biomass immediately before and after grazings (pre- and post-grazing harvests). To assess herbage biomass, four strips of 78 mm x 3 m were cut at ground level from each plot with electrically-powered hand-shears (comb width 78 mm), bulked, subsampled for botanical dissection and then oven-dried and weighed.

3.2.5.2 SWARD DENSITY MEASUREMENTS

3.2.5.2.1 GRASS TILLER AND CLOVER GROWING POINT DENSITIES

Grass tiller and white clover growing point densities were monitored by taking three pasture cores (5 cm diameter) from each plot just prior to each grazing. The number of grass tillers and white clover growing points were recorded for each pasture core. A white clover growing point was defined as a bud which had produced a leaf of development 0.7 or greater on the Carlson (1966a) scale of leaf development.

Grass tiller densities were not recorded for the initial two samplings. From the ninth grazing pasture cores were also taken immediately following grazing, in the same manner as the pre-grazing cores, and white clover growing point density measured.

3.2.5.2.2 CHARACTERISATION OF WHITE CLOVER MORPHOLOGY

For both pre- and post-grazing samplings, following counting of growing points, the pasture cores from the four plots of each white clover cultivar within a replicate were bulked together (12 pasture cores). The white clover material from bulked cores was then dissected so that leaf dry weight (DW) m^{-2} and above- and below-ground stolon length and DW m^{-2} could be measured.

3.2.5.3 WHITE CLOVER PLANT SAMPLING

This work involved the initial development of a sampling technique which was subsequently refined and used by Hay *et al* (1991) to investigate the nodal structure and branching of white clover plants in grazed pastures.

Prior to the seventeenth (9 March 1988) and eighteenth (12 April 1988) grazings, swards of each of the white clover cultivars were sampled by removing two randomly located 300 x 300 mm turves by steel-edged quadrat and spade to 50 mm depth from each of the four replicate paddocks. Similarly on 3 November 1988 there was a third sampling which involved uplifting one turf of each cultivar per replicate.

Soil was washed from each turf and all clover plants separated intact from the vegetation mat. Plants with stolons cut by the quadrat edge were rejected. The number of uncut plants per turf was recorded and a maximum of 20 plants (randomly selected when more than 20 were present) were assessed as follows. Plants were classified as 1st, 2nd, 3rd order, etc depending on the degree of stolon branching present (1st order plants were unbranched, single stolon plants). The number of growing points per plant was counted before roots were cut to waste and the stolon and leaf plus petiole fractions dissected, dried and weighed. In addition a count was made of numbers of stolons and nodes per plant of plants sampled from replicate 4 in the March sampling and plants from one turf of each cultivar per replicate in the April and November samplings.

3.2.5.4 EARTHWORM POPULATION AND ACTIVITY

3.2.5.4.1 EARTHWORM POPULATION SAMPLING

The earthworm population was sampled in mid-July each year (23/07/86 and 16/07/87) by removing the earthworms from a randomly located turf, 25 x 25 cm in area and 20 cm in depth, from each of the white clover cultivar swards in each replicate, ie 12 turves were sampled. Earthworms were preserved in 5% formaldehyde solution and later sorted by species before recording numbers and fresh weights.

3.2.5.4.2 EARTHWORM SURFACE CASTING

Surface casting of earthworms was measured over the autumn to spring period during 1986 and 1987. Surface casts of earthworms were collected weekly from two randomly located 900 cm^2 permanent plots in each replicate. Casts were dried at 105°C and weighed to determine the weekly production of casts on an oven-dry basis.

3.2.6 STATISTICAL ANALYSES

All statistical analyses were carried out using SAS (SAS Users Guide, 1988).

3.2.6.1 DRY MATTER PRODUCTION

Data were tested by analysis of variance with the blocks nested within replicates and the analysis split for harvests.

3.2.6.2 GRASS TILLER AND CLOVER GROWING POINT DENSITIES

Data from individual plugs were tested by analysis of variance for differences among cultivars and samplings. These data were also tested by Chi-square analysis for changes, with date of sampling and white clover cultivar, in frequency distribution among the five categories of tiller (growing point) density given in Figure 3.3.

3.2.6.3 WHITE CLOVER MORPHOLOGY

The measurements were obtained from bulked samples (12 pasture plugs per cultivar per replicate) and the data were grouped by sampling date, cultivar and replicate for testing by analysis of variance.

3.2.6.4 WHITE CLOVER PLANTS

In the sampling process, edges of quadrats were taken as boundaries and plants which intersected edges were eliminated from the sample. Thus the sample was biased against larger plants since they were more likely to intersect the quadrat boundary. In a study where quadrat size was 625 cm^2, Brock *et al.* (1988) used measurements made of

length of stolons of plants to derive a probability that a plant of similar size would have been incorrectly excluded from the sample. This probability was then used to calculate a weighting factor for each plant and weighted data was then analysed. In this study lengths of stolons of plants were not measured so data were not weighted. However as quadrat size at 900 cm^2 was 44% larger than that used by Brock *et al.* (loc. cit.) and mean stolon DW per plant of Kopu and Pitau at 100 mg approximated the weighted values in rotationally grazed swards (Brock *et al.* 1988), the larger quadrat size, under these conditions, substantially reduced the biassing effect of the sampling method.

Individual plants were the experimental unit and analysis of variance was used to analyse for the effects of cultivar, sampling date and branching order on all the plant parameters measured. The influence of sampling date, and white clover cultivar on the distribution of plants in the populations among the branching orders was tested by Chi-square analysis.

3.3 RESULTS

3.3.1 CLIMATE

Mean daily soil 10 cm temperatures over the trial period were close to those of the long term mean Fig. 3.1a.

Comparison of monthly rainfall over the trial period with the 30 year monthly means indicates that rainfall from November 1986 to January 1987 was 70% below normal (Fig. 3.1b) but from March 1987 to mid-May was 50% above normal. Rainfall from July 1987 to January 1988 was 35% below normal.

A graph (Fig. 3.1c) of rainfall minus evapotranspiration (Pan Evaporation x 0.8) indicates that soil moisture deficits probably limited plant growth in the periods December 1986 to January 1987 and February 1988 to April 1988.

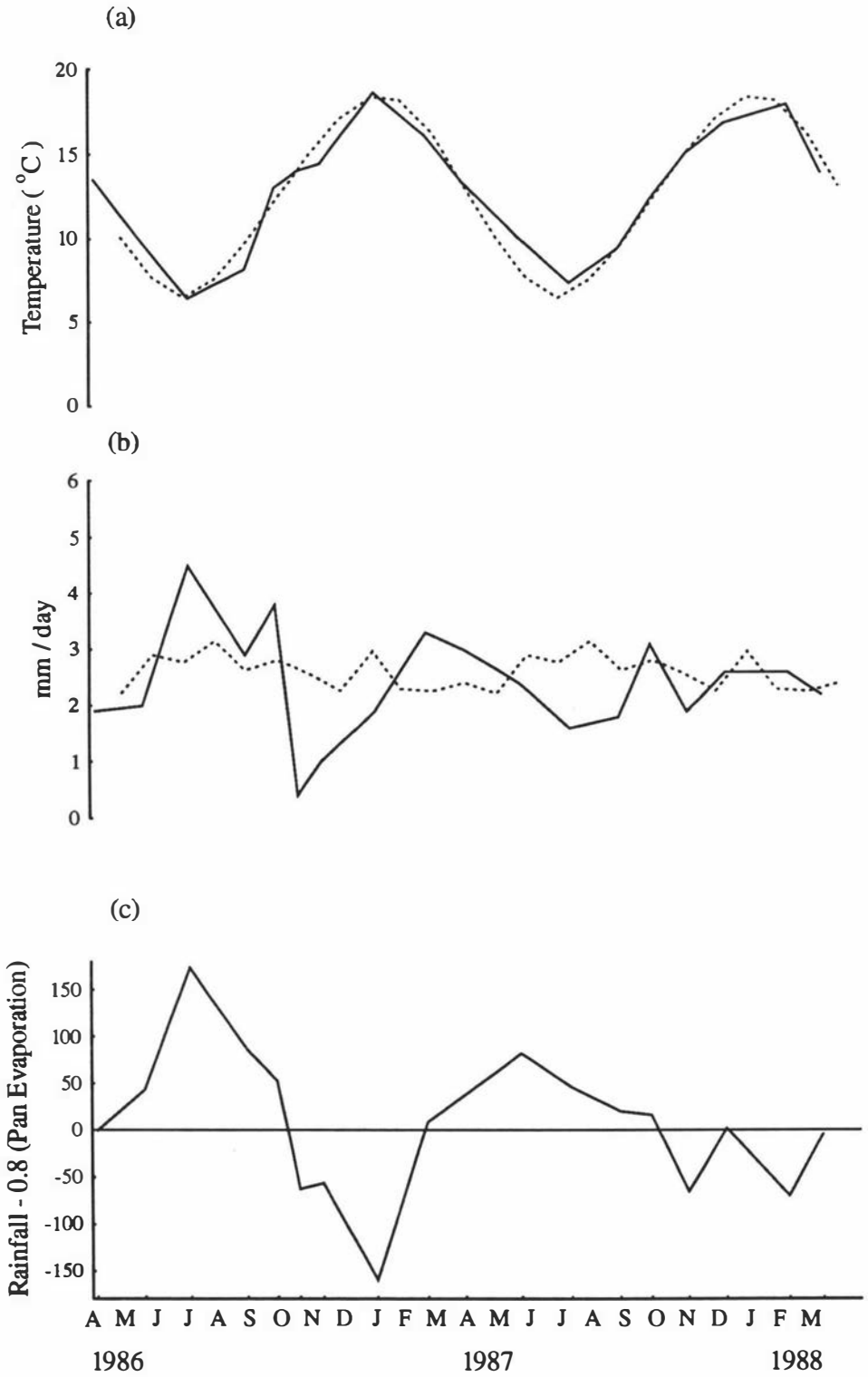


Figure 3.1 Climate data for the experimental period (1986 - 1988) (—) compared with longer term 30 year means (---) for (a) 10cm soil temperature (b) average daily rainfall (c) evapotranspiration.

3.3.2 DRY MATTER PRODUCTION

3.3.2.1 TOTAL YIELD

The mean daily rate of net DM accumulation for each regrowth period (see Appendix 3.2) showed that differences among swards of the different clover cultivars were small. On an annual basis Tahora swards (14610 ± 443) were 4% more productive ($P<0.025$) than Kopu (14030 ± 438) and 6% more productive ($P<0.0014$) than Pitau (13770 ± 439) swards. The seasonal change in daily rate of net DM accumulation was three fold (Table 3.2) but the season by cultivar sward type interaction was not significant ($P>0.44$).

3.3.2.2 GRASS YIELD

The mean daily rate of net accumulation of grass DM (see Appendix 3.2) did not differ ($P>0.21$) among swards of the white clover cultivars. On an annual basis net grass DM accumulation in the Kopu, Pitau and Tahora swards was 11940 ± 156, 12010 ± 156 and 12310 ± 156 kg ha^{-1}, respectively. The seasonal change in rate of DM accumulation was three fold (Table 3.2) and the season by clover cultivar sward type was not significant ($P>0.83$).

3.3.2.3 WHITE CLOVER YIELD

The mean daily rate of net accumulation of white clover DM differed ($P<0.0002$) among cultivars (Table 3.2). On an annual basis Pitau (1750 ± 74 kg ha^{-1}) net production was significantly less than that of Kopu (2130 ± 74 kg ha^{-1}; $P<0.001$) and Tahora (2340 ± 74 kg ha^{-1}; $P<0.0001$). Kopu and Tahora production did not differ ($P<0.053$).

For daily rate of net accumulation of white clover DM the season by white clover cultivar interaction was highly significant ($P<0.0001$). Values for Tahora were significantly ($P<0.05$) less than for Kopu in autumn and winter but greater than Pitau in spring and summer and for Kopu in summer (Table 3.2). Whereas the daily rate of net accumulation of clover DM of Kopu and Pitau doubled from winter to spring that of Tahora quadrupled (Table 3.2).

Table 3.2 Mean (± SEM) rate of daily net DM accumulation (kg DM ha^{-1} d^{-1}) of total, grass and clover herbage for each season in the Kopu, Pitau and Tahora swards.

	Autumn	Winter	Spring	Summer
Total				
Kopu	35.1 ± 2.18	18.6 ± 0.66	55.1 ± 1.67	35.7 ± 1.85
Pitau	33.5 ± 2.07	18.6 ± 0.67	54.2 ± 1.68	34.8 ± 1.98
Tahora	34.3 ± 1.38	18.6 ± 0.61	57.8 ± 1.58	38.4 ± 1.85
Grass				
Kopu	29.9 ± 2.49	14.9 ± 0.61	47.4 ± 1.70	30.4 ± 1.60
Pitau	29.2 ± 2.22	15.9 ± 0.65	48.5 ± 1.65	29.4 ± 1.57
Tahora	30.6 ± 1.67	16.4 ± 0.56	49.4 ± 1.48	29.8 ± 1.38
Clover				
Kopu	5.2 ± 0.77	3.9 ± 0.31	7.8 ± 0.54	5.3 ± 0.48
Pitau	4.3 ± 0.45	2.9 ± 0.26	5.6 ± 0.44	5.6 ± 0.66
Tahora	3.7 ± 0.52	2.2 ± 0.21	8.3 ± 0.38	8.5 ± 0.80

3.3.3 RESIDUAL YIELD POST-GRAZING

The mean values for residual DM following grazing for grass, clover and total herbage over all harvests (Table 3.3) showed that total herbage residuals were not significantly different for the different sward types. However the mean residual DM of white clover differed ($P<0.0001$) among the sward types with the Tahora residual 83% and 56% greater than the Pitau and Kopu residuals respectively. The grass residual DM in Tahora swards was significantly lower than that of Kopu and Pitau swards (Table 3.3).

The residual DM of white clover after grazing expressed as a percentage of clover DM before grazing (Table 3.3) indicated that Tahora was less severely grazed than Kopu ($p<0.006$) and Pitau ($P<0.05$).

Table 3.3 Mean overall harvests of post-grazing residual DM (kg ha^{-1}) for total, grass and clover herbage; and white clover residual DM as a percentage of pre-grazing clover DM.

	Total	Grass	Clover	Clover DM % Residual/Pre-grazing
Kopu	654	592	62	29.3 (0.294)*
Pitau	657	604	53	31.7 (0.321)
Tahora	637	540	97	35.5 (0.374)
LSD 5%	37.3	35.5	5.2	(0.0496)

* Arcsin transformation was required to satisfy the assumptions for analysis of variance.

3.3.4 SWARD DENSITY

3.3.4.1 RYEGRASS TILLER DENSITY

Ryegrass tiller density varied with harvest date ($P<0.0001$) but was not significantly affected by the associated cultivar of white clover and the harvest by white clover sward type interaction was non-significant. Mean ryegrass tiller densities for the Kopu, Pitau and Tahora swards were 5940 ± 216, 6630 ± 263 and 5960 ± 222 respectively. The ryegrass tiller density (mean of the three white clover sward types) at each harvest is presented in Figure 3.2. The major seasonal feature is the sharp decrease from September to October in ryegrass tiller density in both years. In both years, the October to November period had minimal density of tillers.

The frequency distribution of ryegrass tillers among five density categories did not differ with associated cultivar of white clover (Fig. 3.3). Results of samplings near the beginning (Harvest 3) and at the end (Harvest 18) of the trial were typical of those encountered throughout and indicated that the frequency distribution of ryegrass tillers was not significantly affected by the associated white clover cultivar over this time period (Fig. 3.3).

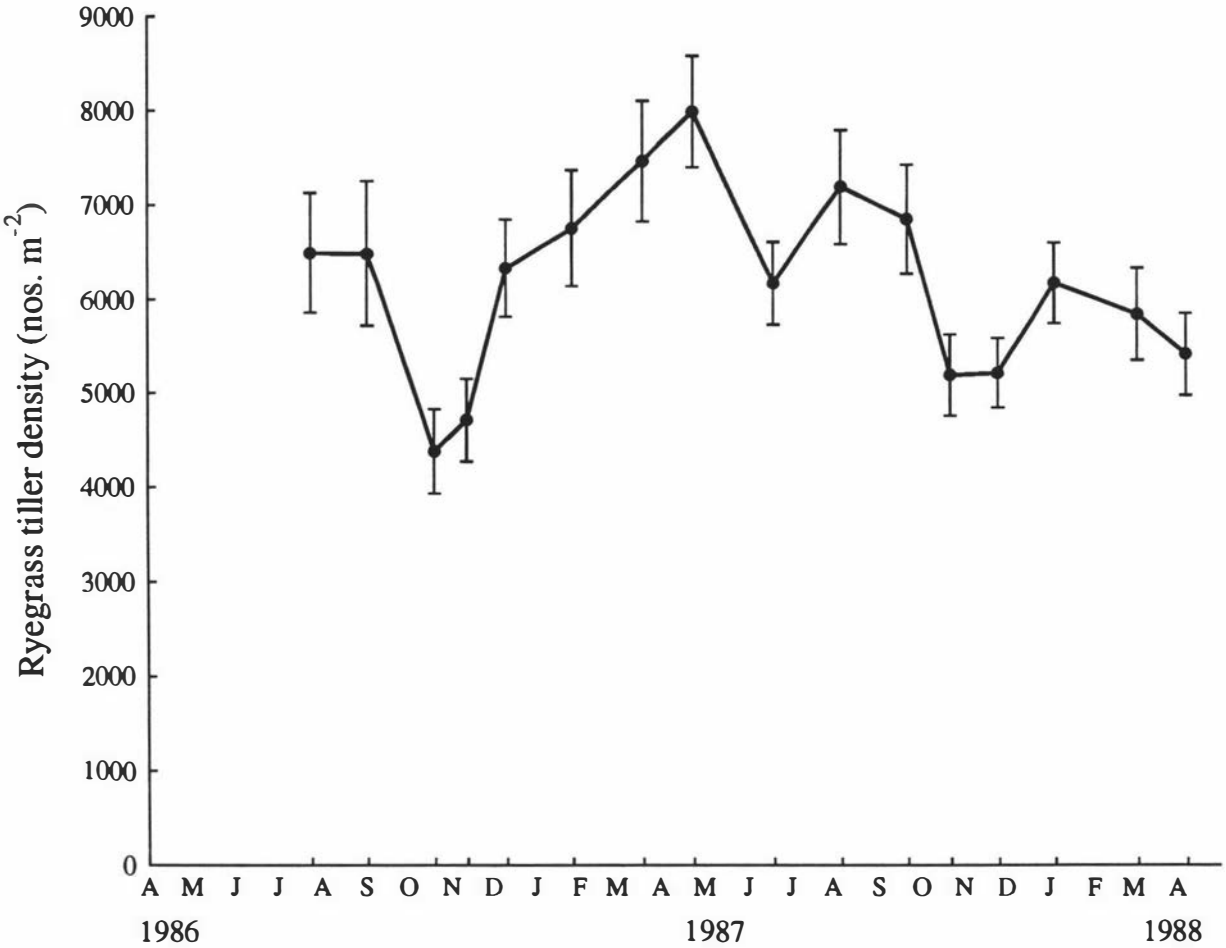


Figure 3.2 Main effect of sampling date on the density of ryegrass tillers. (Error bars indicate ± SEM.)

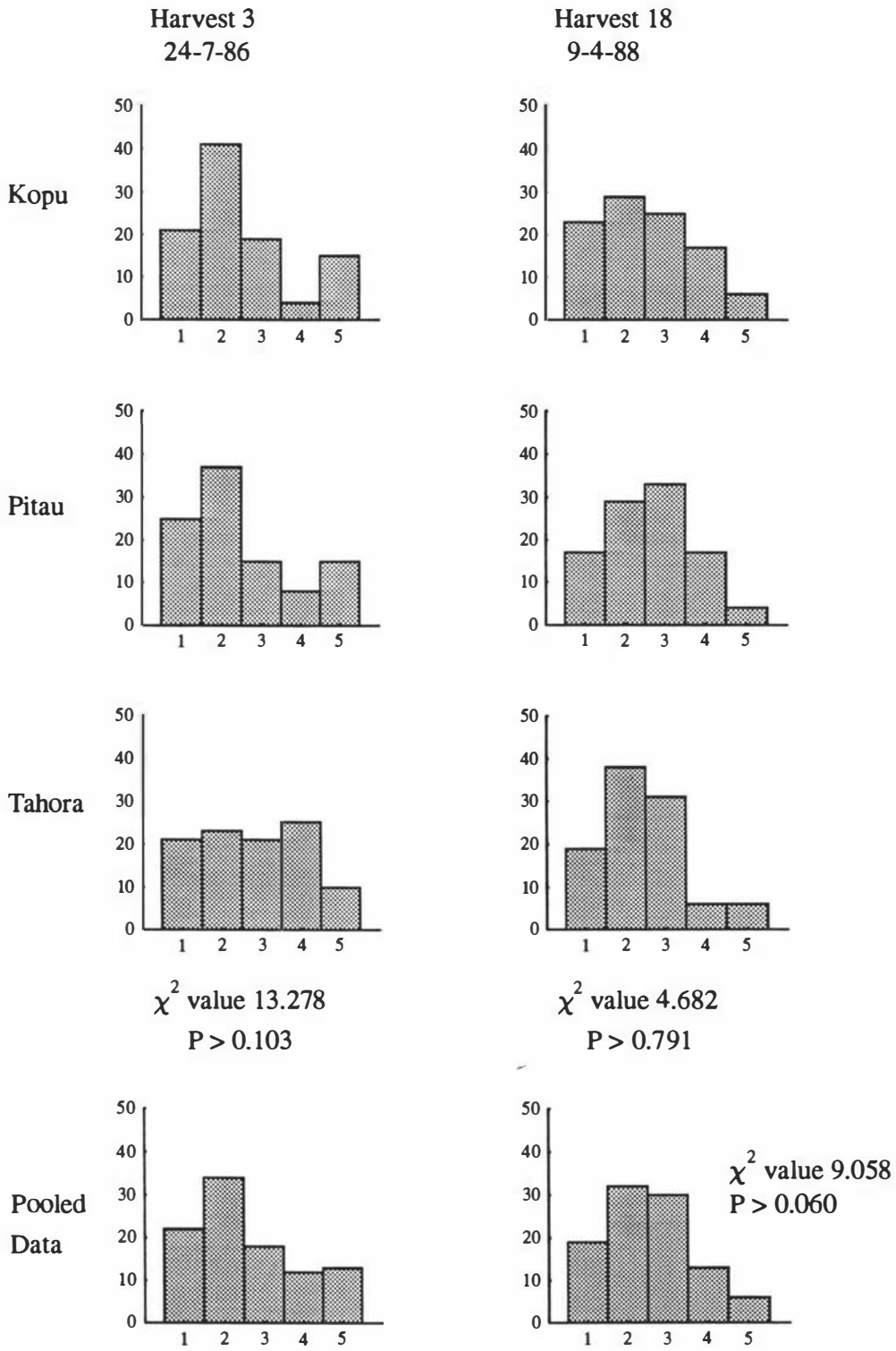


Figure 3.3 Relative frequency distribution of ryegrass tillers among five tiller density categories in Kopu, Pitau and Tahora swards and of data pooled over sward type at the third and eighteenth harvests. The density categories (1- 5) correspond to; 0, < 5000, 5 - 10,000, 10 - 15,000 and > 15,000 tillers m^{-2}, respectively. χ^2 values with level of significance are given for comparisons within harvests and between harvests for the pooled data.

3.3.4.2 WHITE CLOVER GROWING POINT DENSITY

3.3.4.2.1 PRE-GRAZING

The density of white clover growing points varied with white clover cultivar and sampling date ($P<0.0001$). Mean densities (No. m^{-2}) of growing points in the Kopu, Pitau and Tahora swards were; 3030 ± 114, 2830 ± 126 and 6460 ± 182, respectively. A significant ($P<0.0001$) cultivar by sampling date interaction (Fig. 3.4) was driven by the exaggerated seasonal fluctuations and relatively high September and October 1987 values in Tahora swards compared to those of Pitau and Kopu swards. The major features of the seasonal variation in density of white clover growing points were the increase during winter of 1986, the large decrease to low values for October and November, the increase over summer and winter 1987, the decrease in late winter early spring 1987 and a recovery over summer 1988 before a fall in autumn 1988.

The frequency distribution of white clover growing points differed among the swards (Fig. 3.5) in that Tahora swards had a lower frequency of zero density and higher frequencies in the density classes greater than 5000 m^{-2} than had Kopu and Pitau swards. Within swards of each white clover cultivar, the frequency distribution of growing points for the first and last sampling were not significantly different (Fig. 3.5) which indicates that the spatial distribution of white clover was similar in the swards at these dates.

3.3.4.2.2 POST-GRAZING

Mean densities of growing points (No. m^{-2}) post-grazing, in the Kopu, Pitau and Tahora swards were 2310 ± 128, 2130 ± 115 and 5110 ± 165 respectively which were 76.2, 75.3 and 79.1% of pre-grazing values respectively. Analysis of the difference between pre- and post-grazing values of growing point density indicated that only the effect of different grazings was significant.

3.3.4.3 STOLON BIOMASS

For pre-grazing samplings, total stolon biomass varied ($P<0.0001$) with cultivar and season but the cultivar by sampling date interaction was non-significant. Mean cultivar stolon biomass values (g m^{-2}) were 47.6 ± 2.18, 38.7 ± 2.20 and 71.8 ± 2.83 for Kopu, Pitau and Tahora, respectively. The most striking seasonal effect was the decrease in

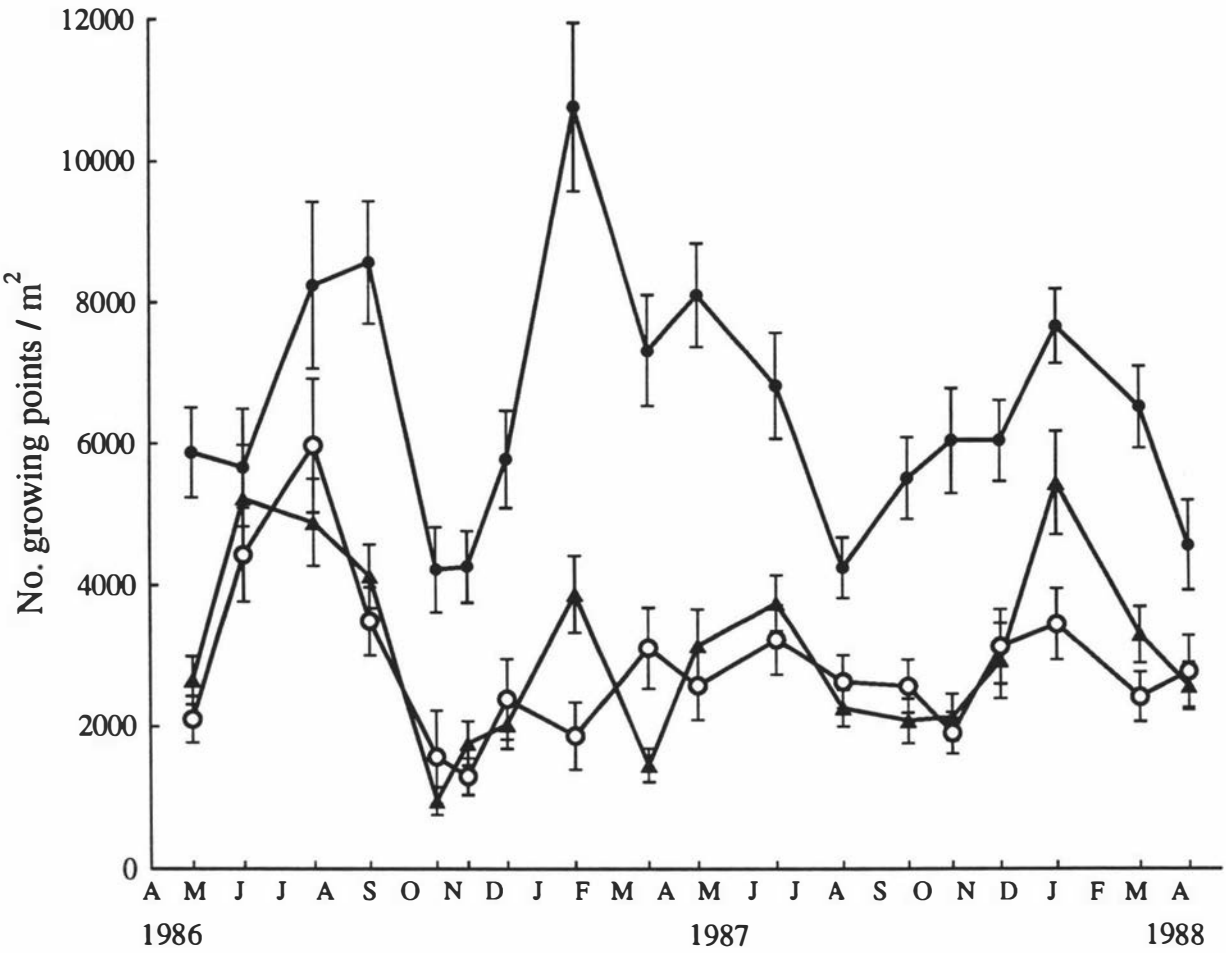


Figure 3.4 Mean density of white clover growing points in the Kopu (▲), Pitau (○) and Tahora (●) swards at the pre-grazing samplings. (Error bars indicate ± SEM.)

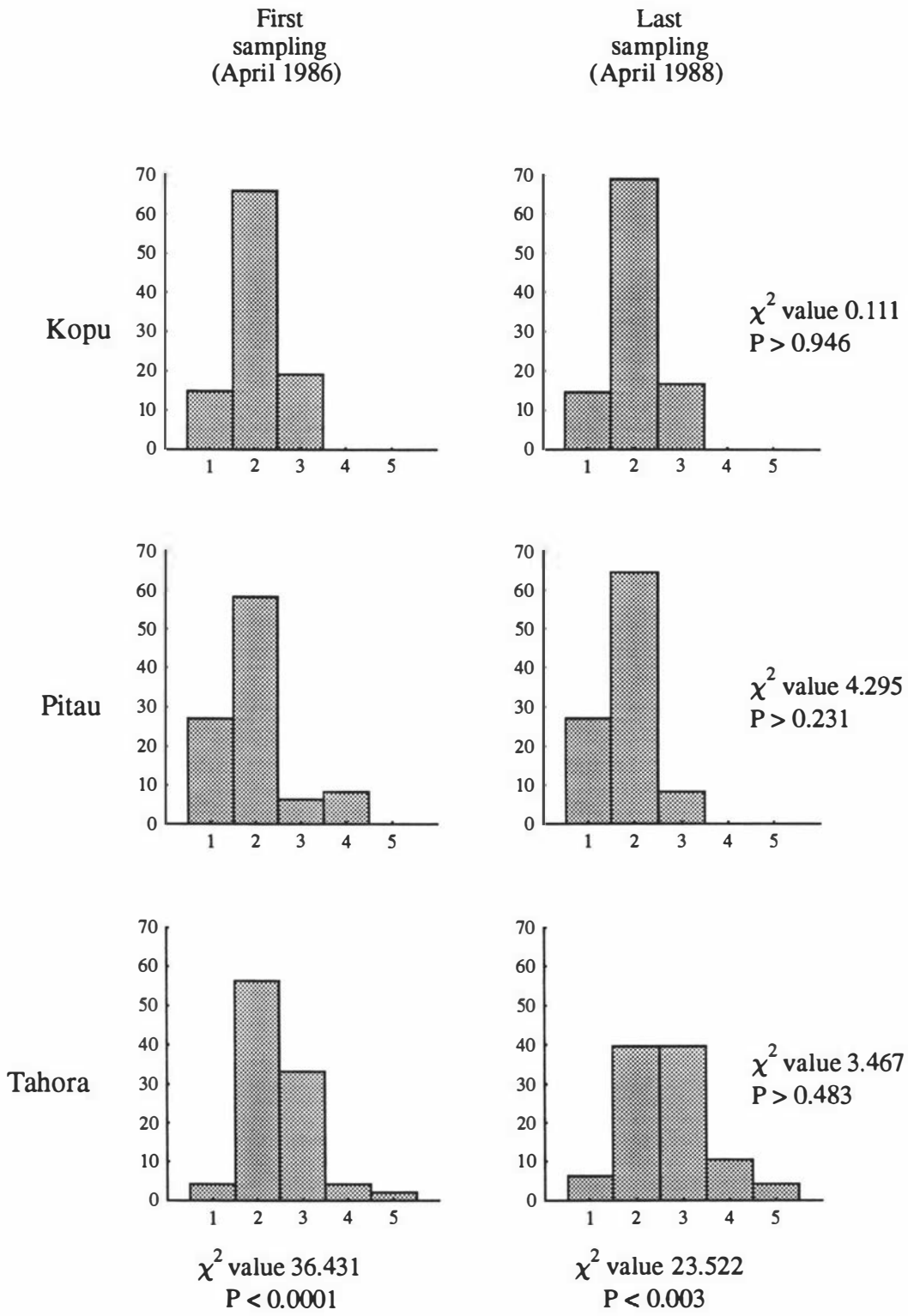


Figure 3.5 Relative frequency distribution of white clover growing points among five density categories in Kopu, Pitau and Tahora swards at the first (29-4-86) and last (7-4-88) pre-grazing samplings. The density categories (1-5) correspond to; 0, <5000, 5-10000, 10-15000 and >15000 growing points m^{-2}, respectively. (χ^2 values with level of significance are given for comparisons of cultivars within samplings and samplings within cultivars.)

spring of both years followed by a rapid recovery by January in total stolon biomass (Fig. 3.6). Stolon biomass from post-grazing samplings followed the same trends (Fig. 3.6). The mean percentage decrease of 9.6% in stolon biomass between pre- and post-grazing samplings was significant ($P<0.01$) and differed among cultivars ($P<0.01$) at 11.8, 17.5 and 3% for Kopu, Pitau and Tahora respectively.

The seasonal pattern of variation in pre-grazing biomass of buried stolon differed from that of total stolon biomass (Fig. 3.6). The main differences were that the decrease in buried stolon biomass in spring was greater and that the minimal values reached in December were maintained through the summer and early autumn months, before a rapid, large increase occurred in late autumn-early winter.

3.3.4.4 STOLON LENGTH PER SQUARE METRE

Mean stolon lengths per unit area (m m^{-2}) for Kopu, Pitau and Tahora swards were 65.2 ± 3.08, 60.3 ± 3.59 and 142.2 ± 5.39 respectively for the pre-grazing samplings. Seasonal trends in stolon length m^{-2} were similar to those for stolon biomass (Fig. 3.6) except that the difference between Tahora and the other two cultivars, which had similar length m^{-2}, was increased. This reflected the differences ($P<0.0001$) among the cultivars in dry weight per unit length (g m^{-1}) of stolon which decreased in the order, Kopu (0.69 ± 0.026), Pitau (0.61 ± 0.025) to Tahora (0.52 ± 0.020).

3.3.5 VERTICAL DISTRIBUTION OF STOLONS IN SWARDS

The proportion of total stolon DM below ground level (buried stolon) in swards was not affected by white clover cultivar ($P>0.610$) but varied ($P<0.0001$) with date of sampling in both pre- and post-grazing samplings. Features of the strong seasonal pattern of change in the proportion of stolon in the buried category (Fig. 3.7) were values of over 90% in September of each year and summer values tending to a 40-45% range although a major exception was the minimum value of 18% of March 1987.

Over the period when pre- and post-grazing samplings were taken, the mean proportion of stolon DM buried was greater ($P<0.0001$) post-grazing (65.9 ± 0.86%) than pre-grazing (59.8 ± 0.86%).

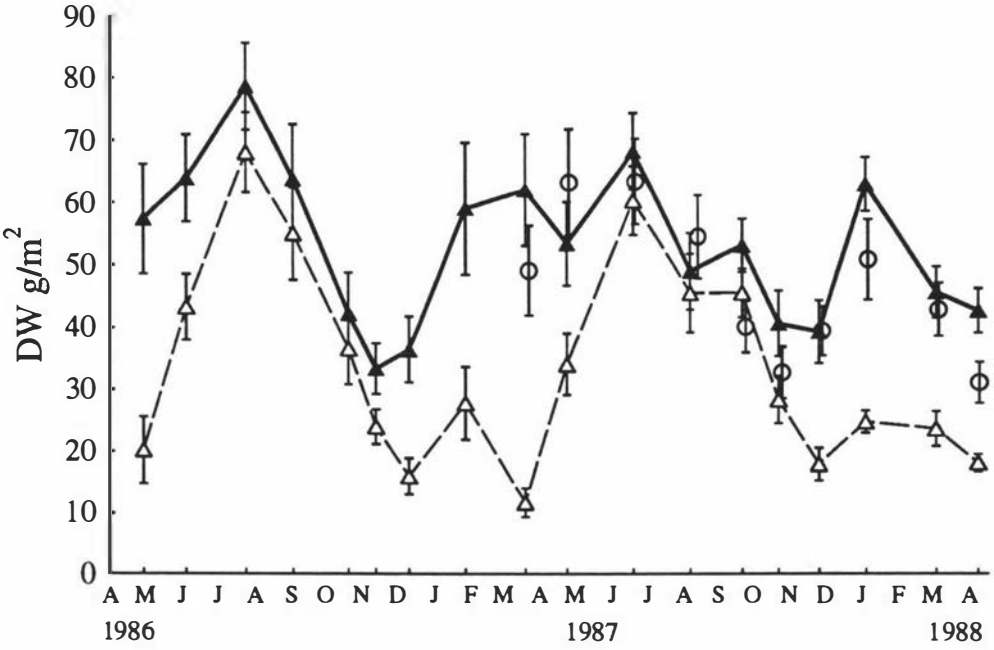


Figure 3.6 Main effect of sampling date on mean total (▲—▲) and buried (△---△) stolon biomass for pre-grazing samplings and total biomass of post-grazing (o), samplings. (Error bars indicate ± SEM.)

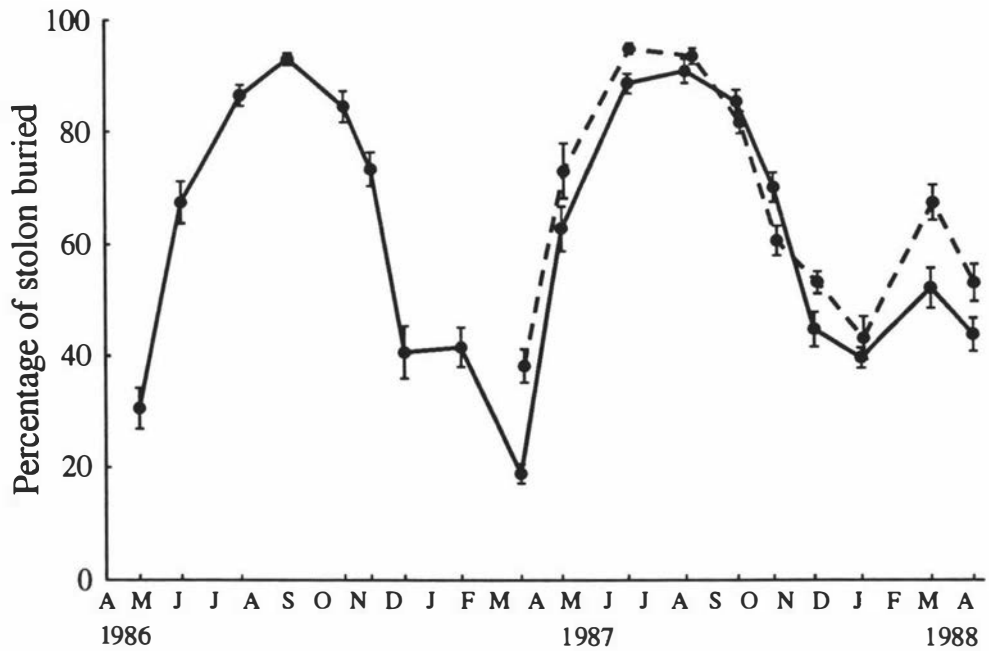


Figure 3.7 Proportion of total stolon biomass below ground (buried) at each sampling date for pre- (—) and post- (---) grazing samplings. (Error bars indicate ± SEM.)

3.3.6 CHARACTERISTICS OF WHITE CLOVER PLANTS

3.3.6.1 STOLON AND LEAF DW PER PLANT

Mean stolon and leaf (leaflets plus petioles) DW per plant (Table 3.4) both varied with cultivar ($P<0.005$) and sampling date ($P<0.002$). Kopu and Pitau had similar means for leaf and stolon DW per plant at each sampling which were greater than the values for Tahora. Whereas leaf DW per plant decreased between March and April samplings, stolon DW per plant did not. By the spring (November) sampling, mean dry weight per plant had decreased such that means of leaf DW and stolon DW per plant were 36 and 51% respectively, of values for the March sampling.

Table 3.4 Mean (± SEM) dry weight per plant (mg plant^{-1}) of leaf and stolon and leaf to stolon dry weight ratio of Kopu, Pitau and Tahora populations at three sampling dates.

Sampling Date		Cultivar		
		Kopu	Pitau	Tahora
09/03/88	Leaf DW	123 ± 15.6	124 ± 13.3	75 ± 7.7
	Stolon DW	106 ± 16.2	96 ± 10.8	60 ± 5.8
	Leaf:stolon	1.52 ± 0.092	1.50 ± 0.070	1.30 ± 0.047
12/04/88	Leaf DW	74 ± 6.9	69 ± 6.7	34 ± 3.9
	Stolon DW	99 ± 8.3	96 ± 10.2	68 ± 7.5
	Leaf:stolon	0.85 ± 0.041	0.87 ± 0.043	0.60 ± 0.024
03/11/88	Leaf DW	50 ± 5.0	50 ± 8.8	25 ± 3.3
	Stolon DW	45 ± 5.7	56 ± 10.6	33 ± 4.2
	Leaf:stolon	1.01 ± 0.062	1.08 ± 0.074	0.81 ± 0.046

The mean leaf:stolon DW ratio of plants also varied significantly with cultivar ($P<0.02$) and date of sampling ($P<0.0001$). The reported leaf:stolon DW ratios were calculated by taking the mean of values for each individual plant. Ratios for Kopu and Pitau plants were similar at each of the sampling dates and greater ($P<0.0001$) than Tahora

ratios (Table 3.4). However the leaf:stolon ratios were lowest at the April sampling being about half those of the March sampling.

3.3.6.2 PLANT BRANCHING OF WHITE CLOVER POPULATIONS

The highest hierarchy of plant branching found was 7th order and was observed in only one individual while 19 plants were recorded as showing 5th order branching and none as showing 6th order branching. These higher branching orders had insufficient data for analysis and as, collectively, they accounted for < 1.75% of the population, they were included within the 4th plant branching order group in this study.

Table 3.5 Relative frequency distributions of white clover plants among four plant branching orders on three sampling dates. (Data pooled for white clover cultivar).

Sampling Date	Branching Order				Sample Number (n)
	1	2	3	4	
09/03/88	17.9	43.2	26.0	12.9	458
12/04/88	17.1	43.0	28.5	11.4	467
03/11/88	37.8	42.2	13.5	6.5	230
					1155

X^2 value 55.91, Degrees of Freedom 6, P<0.001

At any one sampling date, the relative frequency distributions of plants among the four branching orders did not differ significantly between the populations of the three clover cultivars (Fig. 3.8). However, date of sampling had a large overall effect (P<0.0001) on relative frequency distributions (Table 3.5), although for Pitau the effect was not significant (Fig. 3.8). The similar distributions of the first two samplings contrasted with the higher proportion of 1st order and lower proportions of 3rd and 4th order plants in populations at the third (spring) sampling.

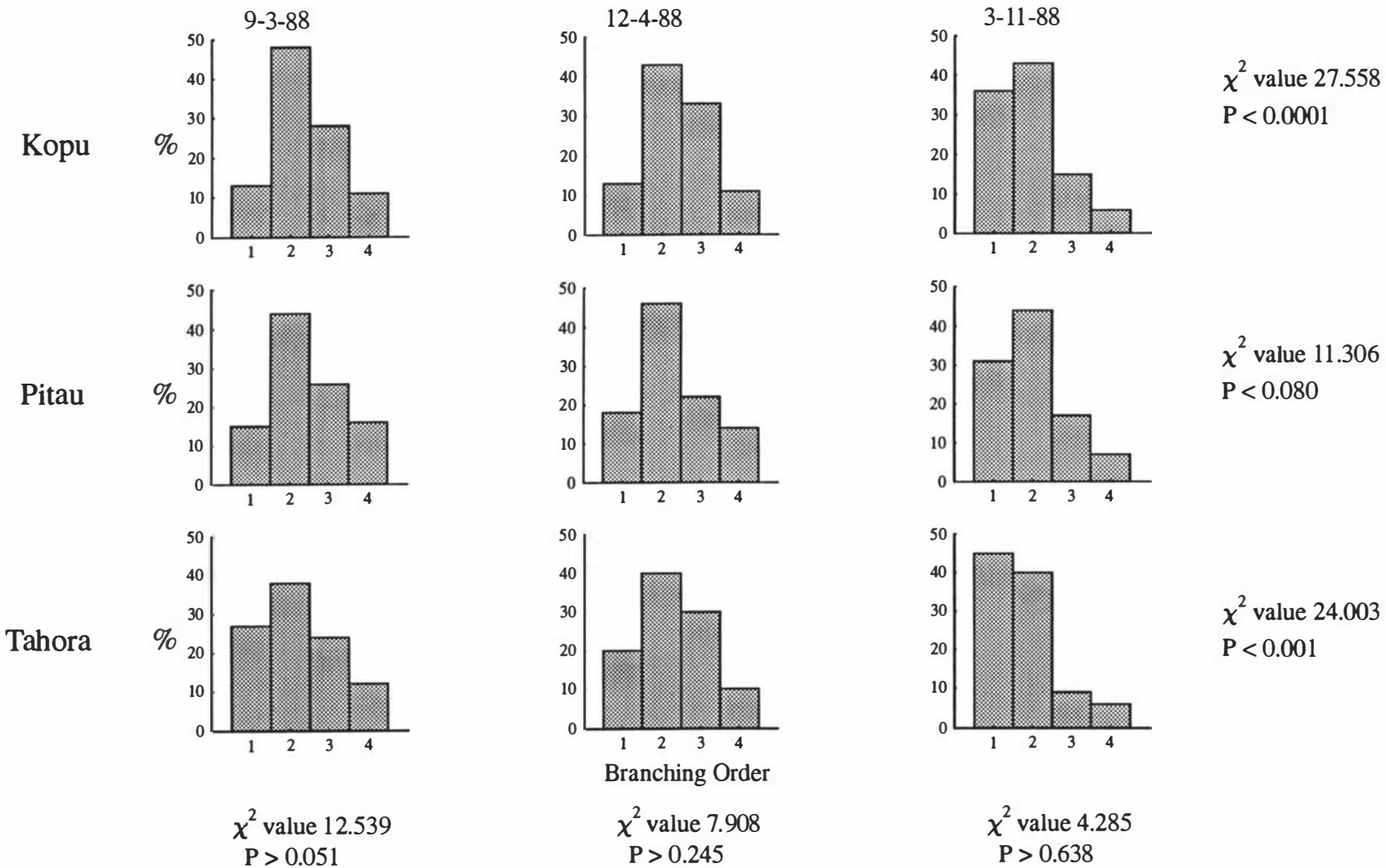


Figure 3.8 Relative frequency distributions of white clover plants among plant branching orders in the clover populations from Kopu, Pitau and Tahora swards at three sampling dates. (χ^2 values with level of significance are given for comparisons of cultivars within samplings and samplings within cultivars.)

3.3.6.3 NUMBERS OF GROWING POINTS, STOLONS AND NODES PER PLANT

Plant branching order had the expected very large effect on numbers of growing points, stolons and nodes per plant (Table 3.6). Within branching orders there were no significant changes with sampling date in values of these parameters (Table 3.6) with the exception of lower numbers of growing points of 2nd order plants in the November (spring) sampling. These parameters were not influenced by cultivar.

Table 3.6 Mean number of growing points, stolons and nodes per plant in each branching order at three sampling dates (09/03/88, I; 12/04/88, II; 03/11/88, III). (Mean overall cultivars, SEM and sample number (n) are presented).

Order	Date	Growing Points		Stolons		Nodes	
		Mean	n	Mean	n	Mean	n
1	I	1.0 ± 0.03	82	1.0	4	16.0 ± 1.50	14
	II	1.0 ± 0.03	80	1.0	39	14.7 ± 1.27	39
	III	1.0 ± 0.01	87	1.0	87	15.7 ± 0.51	87
2	I	3.3 ± 0.23	197	4.2 ± 0.60	14	29.6 ± 2.35	36
	II	3.6 ± 0.20	201	3.6 ± 0.16	97	28.5 ± 1.21	97
	III	2.9 ± 0.17	97	3.4 ± 0.16	97	35.2 ± 1.71	97
3	I	6.3 ± 0.37	119	8.1 ± 0.72	18	66.6 ± 5.07	32
	II	7.3 ± 0.38	133	8.8 ± 0.57	68	64.5 ± 4.95	68
	III	6.2 ± 0.68	31	7.4 ± 0.66	31	70.7 ± 6.77	31
4	I	14.3 ± 1.44	59	15.9 ± 2.57	14	146.0 ± 25.5	20
	II	16.3 ± 1.59	53	17.1 ± 1.96	31	144.9 ± 16.3	31
	III	13.3 ± 3.62	15	15.1 ± 3.71	15	156.5 ± 35.0	15

The analysis inclusive of all plants showed that the overall mean numbers of growing points, stolons and nodes per plant varied among sampling dates ($P<0.001$), principally because of lower values at the spring sampling (Table 3.7). There were no differences between cultivars. Over all sampling dates the means of node number per plant for white clover populations from Kopu, Pitau and Tahora swards were 46.6 ± 3.85, 50.2 ± 4.87 and 50.5 ± 3.90 respectively.

Table 3.7 Mean number of growing points, stolons and nodes per plant at three sampling dates. (Mean [over branching orders and cultivars], SEM and sample number (n) are presented).

Date	Growing Points		Stolons		Nodes	
	Mean	n	Mean	n	Mean	n
09/03/88	5.09 ± 0.296	457	8.62 ± 1.043	50	62.2 ± 6.90	102
12/04/88	5.64 ± 0.304	467	6.45 ± 0.449	235	52.0 ± 3.72	235
03/11/88	3.30 ± 0.328	230	3.79 ± 0.353	230	40.5 ± 3.42	230

3.3.6.4 DENSITY OF WHITE CLOVER PLANTS

For the last two grazings of the field trial, estimates of the density of white clover plants in swards were calculated on a stolon DW basis by dividing the stolon biomass (weight per unit area), measured by pasture plug sampling, by the mean stolon DW per plant, measured in the plant samplings. Plant density in Tahora swards was higher than in Kopu and Pitau swards which had similar densities (Table 3.8). As the mean number of growing points per plant was similar for all cultivars (Section 3.3.6.3) the higher growing point density of Tahora swards must have resulted from a greater density of plants (Table 3.8).

Table 3.8 Mean (± SEM) density of white clover plants, in Kopu, Pitau and Tahora swards prior to grazing in March and April 1988 calculated from mean stolon biomass and stolon DW per plant.

		Stolon DW per plant (mg)	Stolon Biomass ($mg\ m^{-2}$)	Plant Density ($No.\ m^{-2}$)
March	Kopu	115 ± 25.9	43661 ± 8980.6	430 ± 118.6
	Pitau	96 ± 11.4	39269 ± 3906.5	416 ± 24.7
	Tahora	60 ± 10.6	54006 ± 7287.3	992 ± 230.5
April	Kopu	99 ± 16.8	35901 ± 4383.2	402 ± 90.9
	Pitau	100 ± 19.6	38813 ± 1854.2	435 ± 82.2
	Tahora	68 ± 4.8	53455 ± 7663.9	796 ± 135.3

3.3.6.5 FREQUENCY OF NODES BEARING A BRANCH

Analysis of data combined over the three sampling dates found the mean percentage of nodes bearing a branch to differ with sampling date ($P<0.001$) but not between cultivars ($P>0.087$). Within the April sampling, cultivar differences were significant ($P<0.01$) with the Pitau value greater than those of Kopu and Tahora (Table 3.9). The values at the spring sampling date were approximately half those of the autumn samplings (Table 3.9).

Table 3.9 The mean percentage of nodes that bore live branches for the Kopu, Pitau and Tahora white clover cultivars at three sampling dates. (Mean, SEM and number of plants sampled (n) are presented).

Date	Kopu		Pitau		Tahora		Sampling Date Means	
	Mean	n	Mean	n	Mean	n	Mean	n
03/03	10.8 ± 1.90	10	10.9 ± 0.87	20	11.4 ± 1.54	20	11.1 ± 0.79	50
12/04	7.7 ± 0.64	79	11.5 ± 1.04	75	9.1 ± 0.60	80	9.4 ± 0.45	235
03/11	3.7 ± 0.43	80	5.2 ± 0.54	70	6.3 ± 1.09	80	5.0 ± 0.44	230
Cult. Means	7.4 ± 0.85	169	9.3 ± 0.68	165	8.9 ± 0.66	180		

3.3.7 EARTHWORM POPULATION AND SURFACE CASTING ACTIVITY

3.3.7.1 EARTHWORM POPULATION

Measurements of the earthworm population showed the density was approximately 1000 m^{-2} and the biomass (fresh weight) 4 t ha^{-1} in both 1986 and 1987 (Table 3.10a). The cultivar of white clover in swards did not affect earthworm densities but biomass in Pitau swards (4.73 t ha^{-1}) was greater ($P<0.05$) than in Kopu and Tahora swards (3.52 and 3.66 t ha^{-1} respectively). In each year the earthworm biomass was equally dominated by *Allolobophora caliginosa* Sav. and *A. longa* Ude with only a minor presence of *Lumbricus rubellus* Hoff. and *Octolesium cyaneum* Sav. (Table 3.10b).

Table 3.10 Characteristics of the earthworm population in mid-July of 1986 and 1987 (a) mean (± SEM) earthworm density (No. m^{-2}) and biomass (fresh live weight t ha^{-1}) and (b) mean contribution (% by fresh weight) of each earthworm species to earthworm biomass.

(a)

	1986	1987
Density (No. m^{-2})	1140 ± 99	960 ± 138
Biomass (t ha^{-1})	3.88 ± 0.407	4.07 ± 0.287

(b)

	Lumbricus rubellus Hoff. (%)	*Allolobophora caliginosa* Sav. (%)	*Allolobophora longa* Ude (%)	*Octolesium cyaneum* Sav. (%)
1986	6.0 ± 1.57	45.8 ± 3.17	47.3 ± 3.43	0.9 ± 0.87
1987	2.2 ± 0.47	46.1 ± 2.96	46.5 ± 2.63	5.2 ± 2.07

3.3.7.2 SURFACE CASTING ACTIVITY

The pattern of activity of surface casting by earthworms differed between the two years it was assessed (Fig. 3.9). Although some early season earthworm casting was not measured in 1986, there was little activity before 22 April as soil conditions were dry up

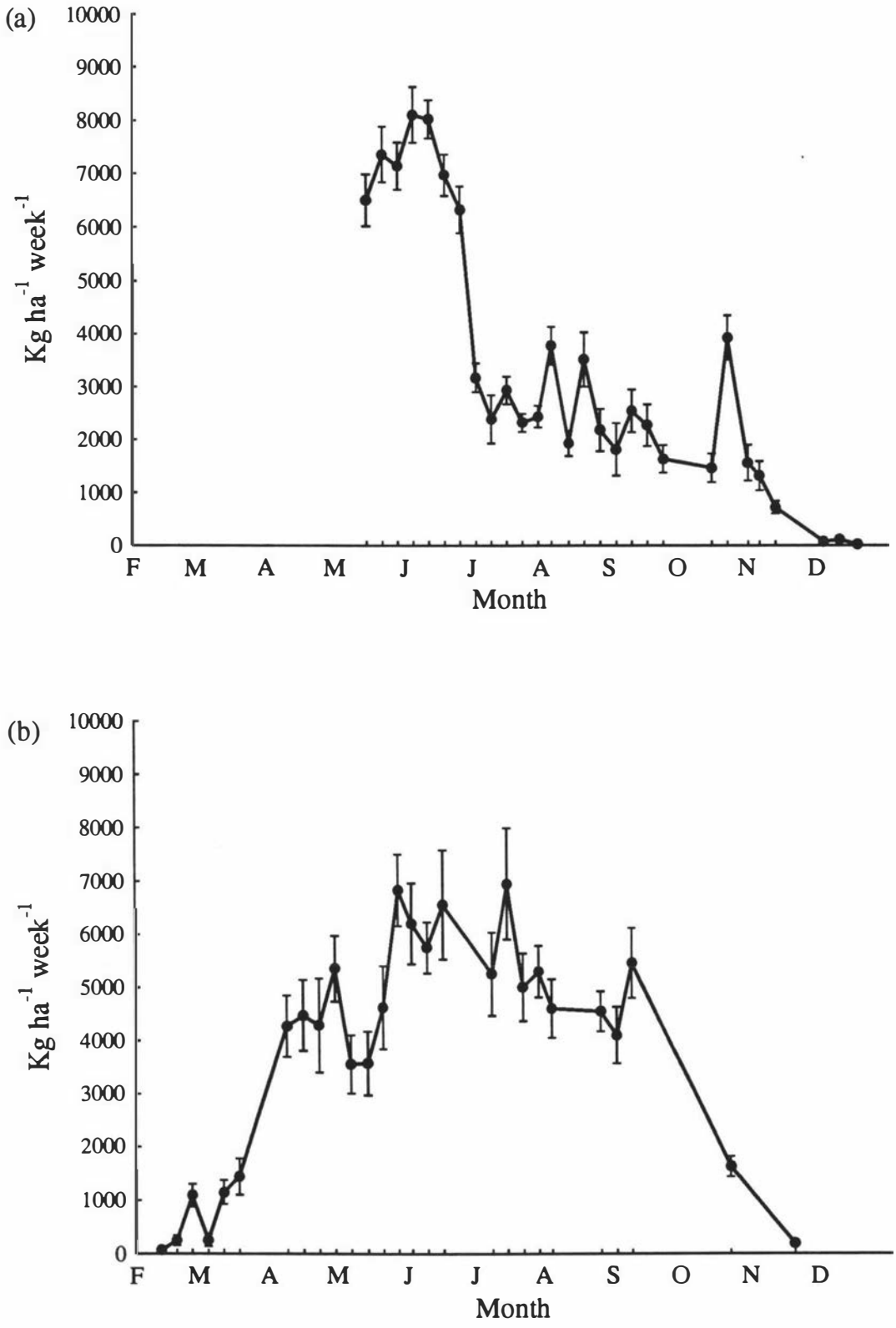


Figure 3.9 Seasonal variation in rate of surface casting by earthworms in (a) 1986 and (b) 1987. (Error bars indicate ± SEM.)

to this date. There was a flush of early winter activity in 1986, with a peak in mid-June of 8000 kg ha^{-1} $week^{-1}$ until activity halted in early November. In 1987, with the moist early autumn conditions, casting started earlier and had risen to about 5000 kg ha^{-1} $week^{-1}$ by mid-April and remained at this level, or slightly higher during June and July, until mid-September. The cumulative weights of surface earthworm casts measured in 1986 and 1987 were 95 and 119 t ha^{-1} respectively.

3.4 DISCUSSION

This discussion consists of three major sections. The first section begins by commenting on the similarity of the seasonal pattern of stolon burial measured in this trial with results of previous studies in New Zealand. Discussion of some of the factors that influence stolon burial follows, including comment on, surface casting by earthworms, treading and defoliation of stolon tissue by grazing sheep and the possible effect of white clover cultivar on stolon burial.

The second major section discusses branching of white clover in the swards. Limitations of the estimates of branching, as assessed by the tiller plug technique (Mitchell & Glenday 1958) and the plant sampling technique (Brock *et al.* 1988), are discussed.

The final section considers results in relation to the seasonal pattern of clonal growth of white clover in these grazed swards. Spring is identified as a period when large morphological and physiological changes occur and it is suggested that these changes influence population densities to such an extent that relationships between stolon burial and branching are obscured.

3.4.1 SEASONAL PATTERN OF STOLON BURIAL

The strong seasonal pattern of variation in the proportion of total stolon DW classified as below ground level (buried) at this site (Fig. 3.7), has been observed in other flat, highly productive swards in Manawatu (Hay 1983, 1985; Hay *et al.* 1983). The levels, in each year, of over 90% of stolon DW buried in winter and 30-50% in late summer are in the range previously reported (Hay 1985). The March 1987 value of 18% is within the range reported for New Zealand sites in March (Hay *et al.* 1987). In that the seasonal variation of vertical distribution of stolon in these swards is similar to that in other swards

both in Manawatu and elsewhere in New Zealand (Hay *et al.* 1987) it follows that the results of studies of seasonal variation in the growth process of white clover in these swards will be pertinent to field growth of white clover in many areas of New Zealand.

3.4.2 FACTORS INFLUENCING STOLON BURIAL

Several factors should be considered as regards their influence on the burial of white clover stolon. Surface casting of earthworms has been considered a major factor contributing to burial during autumn and winter (Hay 1983; Hay *et al.* 1987; Sackville Hamilton & Harper 1989) although Hay *et al.* (1987) found the contribution of other factors such as stock treading to be equally important when soil conditions were moist. The quantity of surface casting by earthworms is influenced by the species present and their abundance and activity which are in turn influenced by soil temperature and moisture, soil type and pH and the availability of organic matter substrate as a source of food (Waters 1955; Barley 1961; Edwards & Lofty 1977). Soil temperature and moisture also affect the quantity of organic matter debris in swards and so indirectly affect casting activity (Sharpley & Syers 1977). The levels of 95 and 119 t ha^{-1} of surface casts measured in 1986 and 1987 respectively are comparable with the highest levels recorded for temperate grassland soils as cited by Edwards & Lofty (1977) of 91 t ha^{-1} in Germany (Dreidax 1931) and 75-100 t ha^{-1} in Switzerland (Stockli 1928). Factors contributing to the very high level of earthworm casting at this site were the high worm biomass (4 t ha^{-1}), the dominance of this biomass (Table 3.10) by the surface casting species *Allolobophora longa* Ude and *A. caliginosa* (Sav.) (Edwards & Lofty 1977), moist soil conditions and mild soil temperatures over the autumn to spring period (Fig. 3.1), favourable silt loam texture and soil pH (5.9) (Edwards & Lofty 1977) and high levels of pasture productivity (net total DM production 14 t ha^{-1} yr^{-1}) which was grazed *in situ* so providing high levels of preferred food substrate in the form of dung (Barley 1959) and plant organic matter residues (Waters 1951). Whereas the bulk density of the 0-7.5 cm soil horizon at this site is 0.96 g cm^{-3} (J.H. Kirkman pers. comm.) the density of wormcasts will be slightly higher than that of the underlying parent soil (De Vleeschauwser & Lal 1981). Thus assuming a bulk density of 1.0 g cm^{-3} for the wormcasts, the 95 and 119 t ha^{-1} of casts in 1986 and 1987 represent a uniform depth of deposit of 9.5 and 12 mm respectively. The 1987 value corresponds well with another estimate of 15 ± 1.7 mm obtained in an adjacent paddock by measuring the depth to which 24 plastic rings of 100 mm diameter were buried over the period March to December 1987 (Matthew *et al.* 1989). Casting of 62 t ha^{-1} yr^{-1} in an English pasture was

calculated to deposit a soil layer of 6.1 mm on the surface (Evans 1948). As the largest value for diameter of stolon measured in these swards was 4.2 mm, the depth of soil deposited by wormcasts was more than adequate to bury any stolons that were on the soil surface as at March of both years.

Processes associated with activity of grazing stock also affect the burial of stolons in these swards. Grazing stock can alter the proportion of stolon material below ground through stock treading causing burial (Hay 1983; Hay *et al.* 1987; Matthew *et al.* 1989) or uncovering (Sackville Hamilton & Harper 1989) or by consumption of stolon tissue (Lancashire & Keogh 1968; Pascoe 1973; Curll & Wilkins 1982) disproportionately taken from the unburied stolon category (Hay & Chapman 1984). For the period when pre- and post-grazing pasture cores were taken there was a significant ($P<0.0001$) increase of 6% in the mean proportion of stolon material buried at the post-grazing over the pre-grazing samplings (Fig. 3.7). In autumn when the rate of increase in stolon burial was greatest it was possible to calculate the extent of the contribution of earthworm casting towards the difference between pre- and post-grazing samplings in the percentage of stolon buried. The daily rate of increase in the percentage of buried stolon material can be calculated for the period from a post-grazing sampling of a harvest to the pre-grazing sampling of the next harvest. Then if it is assumed that this rate of burial applied over the period stock grazed the pasture, the contribution due to earthworm casting can be estimated and any increase over this can be presumed to be caused by grazing. For autumn, these calculations indicated that 74% of burial resulted from earthworm casting and 26% from the process of stock grazing.

It is also possible to calculate an estimate of the relative contributions of treading and consumption of stolon material by stock to the overall effect of stock on stolon burial in the autumn period. To do this it must be further assumed that buried stolon increases only as a result of burial of unburied stolon, death of buried stolon is insignificant over the period stock graze pasture and changes in stolon biomass result from consumption of stolon. It is then calculated that 79% of the grazing effect is due to stock treading and 21% due to consumption of stolon material by stock.

It is noted that the overall average percentage decreases in growing point density and stolon biomass were 23.2 and 9.6, respectively from pre- to post-grazing samplings. This suggests that the apical region of stolons is preferentially consumed. Such a finding is consistent with observations that apices and young nodes are more likely to be above

ground than older nodes (Sackville Hamilton & Harper 1989), are not well rooted (Chapman 1983; Brock *et al.* 1988) and attract grazing as only young nodes support leaves (Brock *et al.* 1988). However on an annual basis preferential consumption of above-ground stolon material by stock only acts to increase the proportion of stolon in the buried category by *c.* 5%.

The third factor that could influence the proportion of stolon below-ground in a sward is the cultivar of white clover. Attributes of cultivars that might affect the proportion of stolon buried are stolon diameter, pattern of seasonal growth and interaction with the grass component of the swards. Tahora, a small-medium leaved variety (Williams *et al.* 1982; Caradus 1986), had smaller diameter stolons than Kopu, indicated by a 25% lower weight per unit length of stolon, and are more readily buried because height above the soil surface is reduced. The contrasts in cultivar morphology, especially between Tahora and the large-leaved cultivars, were most evident in autumn-winter (Plate 3.1) during the period when rate of stolon burial was greatest. On average Tahora compared with Kopu had a 210% greater growing point density, a 150% greater stolon biomass and seasonal growth rates 56% and 160% of that of Kopu in winter and summer, respectively. These large differences in the presence and activity of white clover cultivars had no effect on the ryegrass tiller density, the spatial distribution of tillers or the seasonal rate of growth of ryegrass. Thus a possible explanation of why there was no observed difference of cultivar on stolon burial is that the deposit of 10 mm swamped the possibility of measuring differences due to small differences in stolon diameter and that the structures of swards were similar as there were no cultivar by grass interactions.

3.4.3 BRANCHING

The second aspect of this study was concerned with assessing the branching of white clover in these swards. This was assessed by monitoring growing point density within swards and also by the sampling of plants and obtaining a measure of branching on a node basis within plants.

The clonal growth form of white clover in grazed swards makes assessment of the growth processes of a number of constantly changing individuals difficult, so assessment in the past has usually been by the pasture tiller plug technique (Mitchell & Glenday 1958) to generate data on a per unit area basis or by measuring the growth of individual apices (Hay *et al.* 1989). Tiller plug samplings were used in this study to

provide an indication of seasonal changes in the branching of white clover populations. Measures of growing point density do not include branches that have no growing point, but do include growing points of the primary stolon which are not branches. From tables 3.5 and 3.6 it can be calculated that the proportion of stolons without a growing point were 16.5, 5.3 and 9.2% for the March, April and November samplings, respectively. The average value of 10.3% is not dissimilar to the value of 13% found by Brock *et al.* (1988) averaged over monthly samplings for a year. A similar calculation indicates that primary stolons comprise 16.2, 16.2 and 26.4% of total stolons for the March, April and November samplings respectively. As the numbers of nodes and branches per plant in each of the branching orders varies little with season (Hay *et al.* 1991) and the percentage of the plant population in each of the branching orders is stable except for the spring early summer period (Brock *et al.* 1988; Hay *et al.* 1988), then it can be assumed that primary stolon will account for approximately 16% of stolons for most of the year, a value not much removed from the 16.8% mean value obtained by Brock *et al.* (1988). These two inaccuracies in the use of growing point density as an indication of branching in the population act in opposite directions, with the result that for much of the year growing point density will over estimate the number of branches per unit area by about 6%, but will more seriously overestimate (16%) in spring.

Given these limitations, the seasonal changes in growing point density are indicative of changes in the number of branches per unit area. The major cultivar effect is the substantially higher values for Tahora and the larger fluctuations over time for this cultivar (Fig. 3.4). The major seasonal influence, which for branches would be further exaggerated, was a marked spring depression. This occurred for all cultivars in October and November in 1986 but in 1987 the pattern was more diffuse as minimal values occured in August for Tahora and in early November for Kopu and Pitau (Fig. 3.4). Similarly for stolon biomass (Fig. 3.6) values fell in spring of both years but with the effect most pronounced in 1986. Although not as marked proportionately, ryegrass tiller density (Fig. 3.2) also showed a similar depression in October and November of both years but with the effect strongest in 1986.

The second estimate of branching in these populations was obtained by measuring intact plants sampled by removing turves from the sward (Brock *et al.* 1988; Hay *et al.* 1988). A problem with this technique is that there is likely to be a bias against plants of larger size (see Section 3.2.6.4). Data were not weighted in this study but the sampling quadrat was 44% larger than that used in the previously cited studies. Tahora had

less stolon DW per plant (Table 3.4) but higher stolon length per unit stolon DW (Section 3.3.4.4) than either Kopu or Pitau. Thus these effects operated in opposite directions and tended to cancel each other with respect to length of stolon per plant as stolon length of Tahora was within 10% of that of Kopu plants. Thus as sampling bias is determined by the length of stolon per plant (Brock *et al.* 1988) there should not have been significant sampling bias among the cultivars sampled. This conclusion is supported by the similarity of the distribution of plants among the branching orders of the three cultivars (Fig. 3.8) and the similarity of these distributions (Table 3.5) with those of the weighted data of Brock *et al.* (1988) and Hay *et al.* (1988). Also the similarity in mean number of nodes per plant among the three cultivars and the lack of seasonal effect on node number within branching orders (Table 3.6) is consistent with subsequent findings (Sackville Hamilton & Harper 1989; Hay *et al.* 1991) and a lack of sampling bias.

The mean frequency of nodes bearing a live branch was low (8.5 ± 0.29%) but similar to the mean values from sheep grazed pastures under set stocking or rotational grazing by sheep at DSIR, Palmerston North at 6.9 ± 0.24% and 8.1 ± 0.45%, respectively (Hay *et al.* 1991). These values represent only point in time measurements and the flux of birth and death of branches, not measured, could mean that the percentage of nodes that branch is considerably higher. In similarly grazed pastures, Chapman (1983) measured a mean frequency of branch initiation from nodes of 12 to 43%, depending on season. If similar branch initiation rates operated in these swards then considerable death or loss of branches must have occurred. The pattern of branching was similar in the three cultivars with respect to the distribution of plants among the branching orders (Fig. 3.8), the change in this distribution with season (Fig. 3.8) the number of stolons, growing points and nodes/plant in each of the branching orders (Table 3.6) and in the percentage of nodes with a live branch (Table 3.9). Thus it was the higher density of plants in Tahora swards (Table 3.8) rather than superior branching ability that conferred the greater growing point density (Fig. 3.4) in these swards compared to Kopu and Pitau swards. Hence one could argue that the use of any one of the cultivars within this field trial for studies of factors influencing branching would be representative of all three cultivars.

3.4.4 SEASONAL PATTERN OF CLONAL GROWTH

In common with previous studies (Brock *et al.*, 1988; Hay *et al.* 1988, 1990) plant characteristics associated with size of plants decreased markedly in spring (Tables 3.4, 3.7). This was caused primarily by changes within the population in distribution among

the branching orders (Table 3.5; Fig. 3.8) as change in structure of plants within branching orders with season was minimal (Tables 3.6, 3.7; Hay *et al.* 1991). The decrease in size characteristics of the mean plant in spring is accompanied by a decrease in total stolon biomass of up to 50% in spring (Fig. 3.6). This decrease in stolon biomass implies that the rate of death exceeds the rate of formation of stolon during spring. As biomass of buried stolon decreased by >70% over this period (Fig. 3.6) and that of surface stolon increased, it can be concluded that the accelerated rate of death of buried stolon drives the changes in total stolon biomass and plant morphology that occur during spring. Death of older nodes causes fragmentation of plants as death of a node bearing a branch releases that branch as an independent plant (Erith 1924; Kershaw 1959; Hollowell 1966; Chapman 1983; Sackville Hamilton & Harper 1989; Hay *et al.* 1990). Thus, when the rate of death of nodes exceeds that of formation, fragmentation of plants is not counter-balanced by development (growth) so the composition of the population changes in favour of plants of a lower branching order (Table 3.5). This change in branching structure of the population causes the proportion of nodes bearing a live branch to decrease by *c.* 50% in spring (Table 3.9). However it is not certain that branch initiation is necessarily reduced in spring as the method used to sample plants did not measure the flux of birth and death of branches. For instance, Chapman (1983) found that branch initiation from nodes appearing in June, July and August at 11.5% was very much lower than from nodes which appeared in spring (September, October and November) (32.5%). On the other hand a study assessing the potential for growth of axillary buds of white clover in grazed swards identified a 'spring dormancy' of buds (Newton *et al.* 1990, 1992). In white clover from rotationally grazed pasture the mean percentage of axillary buds with potential to branch decreased abruptly from 36% in August to 7% in September before recovering to 27% in November (Newton *et al* 1992). This 'spring dormancy' indicates that there are physiological changes as well as morphological changes occurring in white clover plants in spring, although the factors causing the 'spring dormancy' are as yet not known. It is clear that at present we lack a detailed understanding of branch initiation in white clover during the spring period when so much morphological and physiological change is taking place.

When the seasonal pattern of stolon burial was matched with that of growing point density results differed between the two years. Whereas in 1986 growing point densities increased in early winter before sharply declining in October, in 1987 densities declined from May through to minimum values by November for Kopu and Pitau while in Tahora they declined to a minimum in August (Fig. 3.4). Hence as the pattern of stolon burial was similar between years over the winter months, it is not possible to ascribe any

definitive role of burial on branching as estimated by growing point density. For pastures under rotational grazing by sheep the frequency of development of axillary buds into a branch or flowerhead from nodes appearing in winter (June, July, August) at 11.5% was a third of that in other seasons providing rainfall was non-limiting (Chapman 1983). As high frequency of branching occurs at low temperatures in growth cabinet studies (Beinhart 1963; Mitchell 1956; Hoglund & Williams 1984), in spaced plants in the field (Sanderson 1966) and in potted plants growing outside (Thomas 1981), factors other than temperature must cause the winter depression in branching frequency in pastures. One such factor could be that burial of nodes adversely affects axillary bud development into lateral branch stolons.

CHAPTER 4 EFFECT OF DEPTH, DATE AND DURATION OF BURIAL AND NODE POSITION AT BURIAL ON THE OUTGROWTH OF AXILLARY BUDS OF WHITE CLOVER IN PASTURE

4.1 INTRODUCTION

The previous chapter reported on net burial of stolon and branching of populations of three cultivars of white clover in sheep grazed swards. As changes in the density of branch numbers were greatly influenced by the fluxes in total stolon biomass in swards the effect of burial on branching of white clover could not be assessed accurately. The objective of this study was therefore, to investigate, in more detail under field conditions, the effect of burial of stolon on the outgrowth of axillary buds. Individual stolons were buried at different dates and depths and for various durations. The responses of individual axillary buds of similar ontogeny were compared. As all three cultivars examined in the field trial had similar patterns of stolon burial and plant branching (Chapter 3), it was decided to confine this study to a single cultivar, Kopu.

4.2 MATERIALS AND METHODS

4.2.1 SITE

These experiments were carried out within the field trial previously described (Chapter 3). Within each replicate, the four plots of Kopu were used for the various components of the experiments which were carried out over the period 16 June 1986 to 5 January 1987. All experiments were conducted on stolons of Kopu white clover.

4.2.2 ESTABLISHMENT AND DESIGN OF EXPERIMENTS

Four complementary experiments were run simultaneously in order to assess the effects of burial treatments. The timeframe of the work was related to the five grazings that followed the second grazing of the main field trial (Chapter 3).

In these experiments stolons were buried for a range of periods which corresponded with one to five regrowth (grazing) cycles of the main grazing trial (Table 4.1). The number of days between grazings varied as the time at which grazing should take place was determined by the net accumulation rate of swards (Chapter 3.2.4). For example, number of days between grazings ranged from 22 to 59 days for the fourth and first grazing cycles respectively. Despite this variation in time, the physiological development of white clover was similar for each regrowth cycle, as in each cycle approximately three nodes per stolon apex were initiated. Hence burial for a common number of regrowth cycles was considered an equivalent treatment, irrespective of when burial occurred or the total number of days buried (Table 4.1).

Table 4.1 Schematic presentation of dates of burial and harvest for the date-and-duration-of-burial experiment to illustrate how sequential harvests within each grazing cycle gave a patterned series of burial durations for each burial date.

	Harvest date				
	14/8	7/10	3/11	25/11	5/1/87
Date of Burial					
16/6	1	2	3	4	5
26/8	1	2	3	4	
14/10	1	2	3		
10/11	1	2			
2/12	1				

Burial treatments were imposed in the following manner. Following the recording of characteristics of marked stolons (4.2.3), galvanised iron rectangles (20 x 8 cm) of 1, 2 and 5 cm depth were placed so that stolons could be covered to the desired depth with topsoil (Fig. 4.1). Other herbage within the rectangle was defoliated to ground-level. Leaflets on petioles longer than the depth of burial were maintained in the light. The topsoil (0-3 cm) was obtained from adjacent pasture within the farm paddock in which the grazing trial was located.

The individual experiments are now described.

4.2.2.1 DEPTH OF BURIAL

This experiment, run in conjunction with the time-of-burial experiment, investigated the effect of depth of stolon burial on branching of buried nodes. The four treatments were burial of stolons by 0, 1, 2 or 5 cm of topsoil. The 1, 2 and 5 cm depth-of-burial treatments were imposed on stolons located within 1 x 2 m exclusion cages. The stolons buried to 2 cm depth also served for the first date of burial of stolons in the date-of-burial experiment (Section 4.2.2.2). The zero depth of burial treatment were the tagged stolons (see Section 4.2.4) from which comparable data were obtained only for the first and final harvests.

Within each replicate paddock there were two groups of five stolons buried at each of 1, 2 and 5 cm depths on 16 June 1986. One stolon from each group was subsequently harvested each grazing cycle giving a total of eight stolons harvested per treatment each grazing cycle.

Buried and tagged stolons were covered by grazing exclusion cages while stock were grazing. On 19 September 1987 and subsequently once per grazing cycle herbage around these experimental stolons was cut by hand and cleared away, but the experimental stolons were left undefoliated throughout.

4.2.2.2 DATE AND DURATION OF BURIAL

This experiment investigated the effect of date of burial of stolons on the outgrowth of axillary buds at buried nodes and the time course of any such effects. At each date of burial sufficient stolons were buried to allow sequential harvesting at each grazing cycle up to the date of the last harvest (7 January 1987).

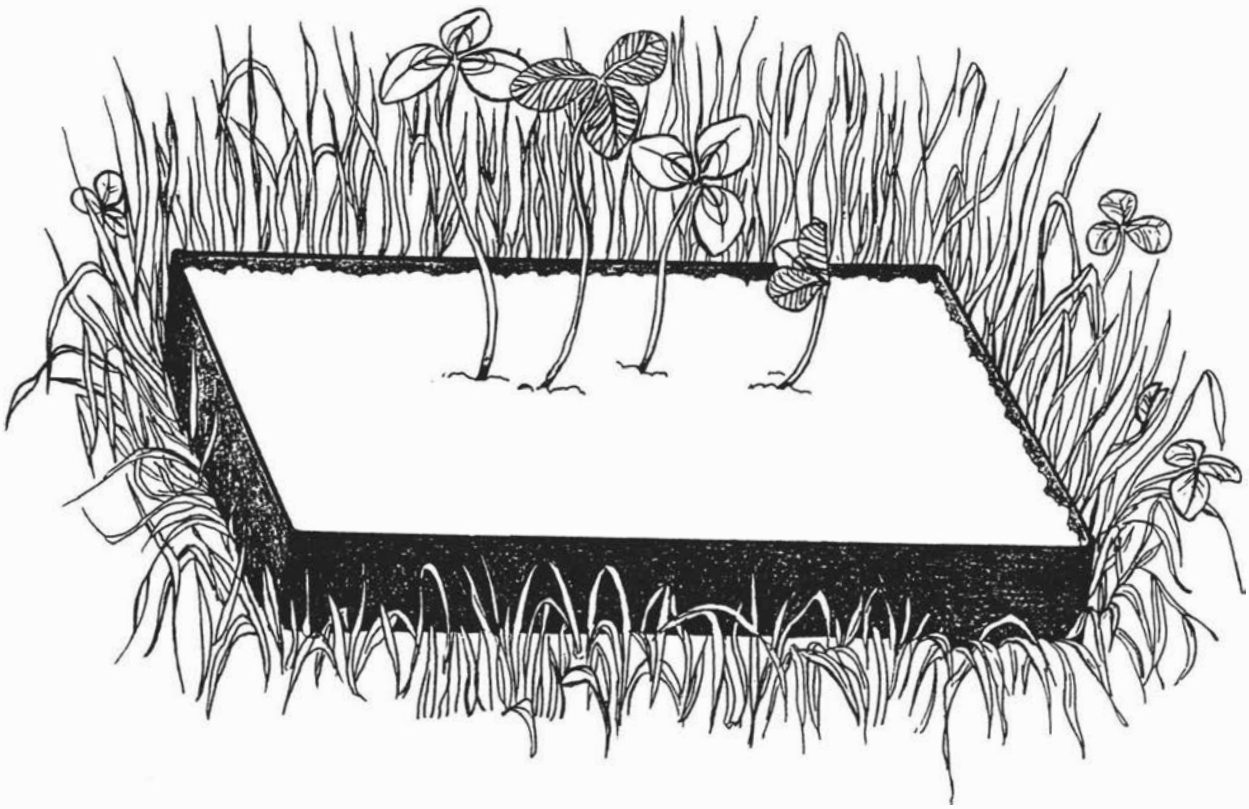


Figure 4.1 Drawing of a white clover stolon after imposition of a 2 cm depth of burial treatment.

Within each replicate of the field trial in each of two randomly located 2 x 1 m caged areas, five stolons were marked, measured and buried to a depth of 2 cm on 16 June 1986. After each subsequent grazing cycle (Table 4.1) one stolon from each caged area (2 per replicate) was harvested. Following each harvest a further two groups of stolons per replicate were buried. After the elapse of 1, 2, 3 or 4 grazing cycles from initiation of the experiment the number of stolons buried per group was 4, 3, 2 or 1 respectively.

4.2.2.3 ZONE OF STOLON BURIED

This experiment tested the effect of burial of different portions (zones) of stolon on subsequent branching of all nodes present on the stolon at the time of burial. For the purposes of this experiment the first ten nodes basipetal to the stolon apex were categorised into three developmental zones. The ***apical zone*** comprised the stolon apex (inclusive of nodes with leaf development <0.7 on the Carlson (1966a) scale) and the next three nodes. The node adjacent to the stolon apex with leaf development ≥ 0.7 was designated node position 1. Nodes of this zone supported almost all of the rapidly photosynthesizing leaves in the sward (Davies & Jones 1986, 1988; Jones & Davies 1988; Brock 1988; Brock *et al.* 1988). The second zone, the ***intermediate zone***, consisted of nodes in positions 4 to 7. Leaves at these nodes had usually been defoliated or were senescing. The third zone or ***mature zone*** consisted of nodes in positions 8 to 10. Some nodes in this zone supported well developed branches at the time of application of the treatments.

The different zones of each stolon were either buried or not with 2 cm of topsoil. Subsequently stolons were harvested sequentially, over following grazing cycles, so that the time course of treatment effects could be determined. Burial treatments were applied 16 July 1986 and stolons were harvested 28 August, 13 October, 6 November, 2 December and 8 January 1987 for grazing cycles 1, 2, 3, 4 and 5 respectively. The six treatments of burial of different zones of stolons were:

(a) the apical zone
(b) the intermediate zone
(c) the mature zone
(d) the apical and intermediate zones
(e) the apical and mature zones
(f) the intermediate and mature zones

Within each of the four replicates of the field trial, each treatment was imposed on five stolons, one of which was harvested at each of the harvest dates, (ie. 6 treatments x 5 harvests x 4 replicates = 120 stolons).

4.2.2.4 UNBURIED STOLONS

On 27 May 1986, 15 established stolons (with ≥ 10 nodes) were marked in each replicate with coloured plastic tubing a few nodes behind the apex. Stolons were subsequently observed on the following dates, 18 June, 21 July, 13 August, 27 August, 29 September, 15 October, 3 November and 4 December and where necessary uncovered by removing any soil or plant litter over stolons. On 27 August a second tag was placed six nodes behind the apex and subsequent descriptions included only those nodes distal to that tag. As of 3 November a third tag was positioned six nodes from the apex once stolons had produced 16 nodes forward of the second tag.

At each observation the number of nodes, number of leaves and axillary bud outgrowth were recorded. On 12 January 1987 tagged stolons were uplifted, described and measured.

4.2.3 MEASUREMENTS

4.2.3.1 RECORDINGS AT TIME OF STOLON BURIAL

Stolons were tagged in the following manner. A staple with a coloured plastic marker was inserted over the stolon immediately basipetal to the tenth node from the stolon apex. Nodes with leaf development of <0.7 on the Carlson (1966a) scale were included with the stolon apex. Stolons were then described, node by node, for presence or absence of a full leaf or grazed petiole and outgrowth of the axillary bud at the node. Axillary bud outgrowth from appearance of the first leaf beyond the stipule (0.2 Carlson (1966a) scale) up to the production of a fully expanded leaf was rated 0.5 and thereafter the number of emerged nodes on the axillary branch was recorded. The appropriate burial treatment was then applied to the stolon such that where petiole length was greater than the depth of burial, petioles bore leaflets above the soil.

4.2.3.2 RECORDINGS AT HARVEST

At harvest the marked stolon, plus tin rectangle inclusive of the treatment depth of soil, was removed in a small turf taken by spade. The soil applied as a burial treatment was then carefully washed away. The marked stolon was then removed by severing all roots and the stolon at the marked node.

The marked stolon was then described as detailed previously (Section 4.2.3.1).

4.2.4 STATISTICAL ANALYSES

All analyses were performed using SAS (SAS Users Guide, 1988). In general, data from these experiments were analysed in the following manner.

Axillary bud outgrowth at each node was assessed as present or absent at the dates of burial and harvest. Presence of outgrowth included ratings of 0.5 (Section 4.2.3.1). This assessment of bud development gave the following four categories of possible bud response over a treatment period:

(a) (1, 1) Outgrowth present at burial (1) and harvest dates (1)
(b) (1, 0) Outgrowth present at burial (1) but absent at harvest (0)
(c) (0, 1) Outgrowth absent at burial (0) but initiated after burial (1)
(d) (0, 0) No outgrowth at burial (0) or harvest (0)

Data were tested by Chi-square analysis for influence of burial treatments and node position on the proportions of buds in the response categories. As some Chi-square analyses had response cells with less than five counts per cell this method of analysis although useful for summarising treatment responses, required the supplementation of a further method of analysis. The same data were tested by analysis of variance. Axillary buds were grouped by node position on stolons and the effects of burial treatments and node position on the probability of bud outgrowth were separately assessed for data recorded at burial and harvest dates and for change between burial and harvest recordings.

4.2.4.1 DEPTH OF BURIAL

These data were grouped in several ways for analysis. As comparable data from unburied stolons (tagged stolons) were only available for burial intervals of one and five grazing cycles, a subset of data for these two burial intervals for 0, 1, 2 and 5 cm depth of burial was analysed. Node positions were grouped into apical (nodes 1 to 3) and intermediate (nodes 4 to 7) groups for this analysis. A further analysis grouped all the burial treatments (1, 2 and 5 cm depths of burial) together for comparison against unburied stolons (0 cm of burial).

A separate analysis of the 1, 2 and 5 cm depth of burial treatments for all five intervals of burial was performed.

4.2.4.2 DATE AND DURATION OF BURIAL

The effects of date and duration of burial treatments on the responses of axillary buds were compared using Chi-square analysis and analysis of variance as described above (Section 4.2.4).

4.2.4.3 ZONE OF STOLON BURIED

Data grouped by stolon, were tested by analysis of variance for burial treatment effects on probability of bud outgrowth at harvest and change in probability between burial and harvest. In an additional analysis, data were pooled across stolons, grouped by node position and analysed for effects of burial and duration of burial on probability of bud outgrowth at harvest and change in probability between burial and harvest.

4.3 RESULTS

4.3.1 DEPTH OF BURIAL

The probability of an axillary bud displaying outgrowth at the time of burial (BO), i.e. at the start of the experiment, increased markedly as its node position, relative to the stolon apex, increased from one to seven (Table 4.2). This meant that the node position of a bud influenced the range of responses possible to any applied treatment at

harvest (BH). Buds at positions 1, 2 or 3 had zero or very low probabilities of bud outgrowth at burial (Table 4.2) and so had zero or low potential to show response in the branch retained (1, 1) or branch died (1, 0) categories at harvest whereas for buds of positions 4 to 7 the opposite pertained (Table 4.2). To accommodate the difference in response with node position buds were grouped in apical (positions 1-3) and intermediate (positions 4-7) categories for subsequent analyses of variance (Table 4.3).

Table 4.2 Main effect of node position at burial on the probability of axillary bud outgrowth at burial (BO) and on the relative frequency distribution of buds (%) among four response categories at harvest. Chi-square value and level of significance for bud response are presented. Data is from the depth-of-burial experiment and pooled over all harvests and depths of burial.

Node Position	Probability of bud outgrowth at BO	% of buds in response categories at harvest			
		Branch retained (1, 1)	Branch died (1, 0)	Branch initiated (0, 1)	No branching (0, 0)
1	0.00	0	0	38	62
2	0.01	0	1	48	51
3	0.19	7	11	42	40
4	0.56	40	22	17	21
5	0.77	63	29	2	6
6	0.81	53	36	5	6
7	0.85	52	38	1	9
		Chi-square value 722.4			
		Df = 18, n = 1134			
		P<0.001			

Table 4.3 Main effects of depth of burial, duration of burial and node position on probability of axillary bud outgrowth at burial (BO) and harvest (BH) and change in probability of bud outgrowth (Change) in the depth-of-burial experiment. Change in probability of bud outgrowth = harvest probability of outgrowth - probability at burial. SEM and level of significance of main effects are given.

	BO	BH	Change
Depth of Burial (cm)			
0	0.51 ± 0.018	0.59 ± 0.017	+0.08 ± 0.022
1	0.53 ± 0.045	0.41 ± 0.047	-0.12 ± 0.059
2	0.44 ± 0.049	0.37 ± 0.046	-0.07 ± 0.060
5	0.50 ± 0.042	0.50 ± 0.046	+0.00 ± 0.062
	ns	P<0.001	P<0.001
Duration of Burial (no. grazing cycles)			
1	0.51 ± 0.018	0.65 ± 0.020	+0.14 ± 0.023
5	0.50 ± 0.018	0.44 ± 0.021	-0.06 ± 0.029
	ns	P<0.001	P<0.001
Node position groups			
1-3	0.06 ± 0.016	0.48 ± 0.022	+0.42 ± 0.025
4-7	0.84 ± 0.017	0.59 ± 0.019	-0.25 ± 0.021
	P<0.001	P<0.001	P<0.001

The main effect of depth of burial was highly significant for the probability and the change in probability of bud outgrowth at harvest (Table 4.3) as was the effect on the relative frequency distribution of buds among the four response categories (Table 4.4). This significance resulted in the main from the effect of any depth of burial (i.e. 1, 2 or 5 cm depths) against no burial (0 cm). This was evidenced by a significant (P<0.0001) contrast between unburied and the buried treatments grouped together for probabilities of bud outgrowth at harvest at 0.59 ± 0.022 and 0.42 ± 0.027 respectively and by the lack of significant difference (P>0.149) among the treatments of 1, 2 and 5 cm depth of burial in

Table 4.4 Main effect of depth of burial of stolons on the relative frequencies of axillary bud outgrowth among four response categories at harvest in the depth-of-burial experiment. Data pooled for duration of burial of one and five grazing cycles Chi-square values with level of significance are given for all treatments and for the three buried treatments only.

Depth of Burial (cm)	Bud Response Category				
	Branches present (1, 1)	Branches died (1, 0)	Branches initiated (0, 1)	No branching (0, 0)	n
0	33.8	17.5	23.7	25.0	819
1	25.7	26.7	13.3	34.3	105
2	20.5	25.0	17.0	37.5	112
5	24.5	25.5	16.3	35.7	98
					1134

Overall Chi-square Value = 32.51

P<0.001

For depths 1, 2 and 5 cm

Chi-square Value = 9.48

P<0.149

relative frequency distribution to the bud response categories (Table 4.4). Further detail of the nature of the axillary bud response to burial was obtained by reference to the time course of the response. Burial had a non-significant effect on the relative frequency distribution of axillary buds among response categories for a burial duration of one grazing cycle but pronounced (P<0.001) effects after burial for five grazing cycles (Table 4.5).

Table 4.5 Relative frequencies of axillary buds among four response categories from stolons either buried or unburied for durations of one or five grazing cycles. Data from the depth-of-burial experiment were pooled over node position and for buried stolons over 1, 2 and 5 cm depths of burial. Chi-square values and levels of significance are given.

Duration of burial (no. grazing cycles)	Burial Status	Bud Response Category				
		Branches present (1, 1)	Branches died (1, 0)	Branches initiated (0, 1)	No branching (0, 0)	n
1	Unburied	42.9	8.2	24.5	24.5	413
	Buried	36.3	13.7	23.2	26.8	168
		Chi-square Value = 9.70				
		P>0.375				
5	Unburied	24.6	26.9	22.9	25.6	406
	Buried	8.8	39.5	6.8	44.9	147
		Chi-square Value = 49.93				
		P<0.001				

Death of developed buds (branches) initially present increased and initiation of outgrowth of buds was decreased when stolons were buried for five grazing cycles. In this experiment burial affected both apical (node positions 1-3) and intermediate (node positions 4-7) buds to a similar extent (Table 4.6a). The time course of these effects within the buried stolons indicated that the major effects had occurred in both apical and intermediate categories after three to four grazing cycles (Table 4.6b).

Table 4.6 Effect of duration of burial on probability of axillary bud outgrowth at harvest (BH) and change in probability of bud outgrowth (Change) of buds from apical (node positions 1-3) and intermediate (node positions 4-7) zones of stolons at the time of burial; (a) buried (+) and unburied (-) stolons after one or five grazing cycles and (b) buried stolons at each of five durations of burial. Data from the depth-of-burial experiment and buried stolons pooled over 1, 2 and 5 cm depths of burial. SE of means are presented.

Duration of burial (no. grazing cycles)	Stolon node position zone	Burial treatment	BH	Change
(a) Buried and unburied stolons				
1	Apical	-	0.51 ± 0.037	+0.46 ± 0.040
	Interm.	-	0.80 ± 0.026	-0.05 ± 0.029
	Apical	+	0.49 ± 0.038	+0.44 ± 0.042
	Interm.	+	0.48 ± 0.033	-0.19 ± 0.035
5	Apical	-	0.52 ± 0.037	+0.47 ± 0.041
	Interm.	-	0.50 ± 0.032	-0.35 ± 0.039
	Apical	+	0.28 ± 0.053	+0.19 ± 0.065
	Interm.	+	0.24 ± 0.044	-0.52 ± 0.055
(b) Buried stolons				
1	Apical	+	0.49 ± 0.038	+0.44 ± 0.042
	Interm.	+	0.48 ± 0.033	-0.19 ± 0.035
2	Apical	+	0.44 ± 0.042	+0.36 ± 0.046
	Interm.	+	0.35 ± 0.035	-0.34 ± 0.043
3	Apical	+	0.34 ± 0.043	+0.28 ± 0.050
	Interm.	+	0.15 ± 0.028	-0.51 O 0.044
4	Apical	+	0.25 ± 0.044	+0.17 ± 0.053
	Interm.	+	0.27 ± 0.039	-0.43 ± 0.051
5	Apical	+	0.28 ± 0.053	+0.19 ± 0.065
	Interm.	+	0.24 ± 0.044	-0.52 ± 0.055

4.3.2 DATE AND DURATION OF BURIAL

Probability of axillary bud outgrowth at burial (BO) varied with date of burial (Table 4.7) in line with seasonal changes towards lower spring values for branching frequency (Chapman 1983; Wilman & Simpson 1988; Davies 1989; Chapter 3). At harvest, probabilities of bud outgrowth again reflected seasonal influences and also low rainfall during the period prior to the last harvest.

Table 4.7 Main effects, from analysis of variance of the date-and-duration-of-burial trial, for probability of axillary bud outgrowth at burial (BO) and harvest (BH) and change in bud status. Stolons were buried with 2 cm of soil. SE of means and levels of significance are presented.

	BO	BH	Change
Date of burial			
16 Jun	0.46 ± 0.052	0.32 ± 0.049	-0.14 ± 0.070
26 Aug	0.29 ± 0.057	0.23 ± 0.053	-0.06 ± 0.076
14 Sep	0.26 ± 0.057	0.31 ± 0.060	+0.04 ± 0.084
10 Nov	0.44 ± 0.085	0.56 ± 0.085	+0.12 ± 0.095
2 Dec	0.35 ± 0.144	0.04 ± 0.060	-0.31 ± 0.164
	P<0.023	P<0.001	P<0.027
Duration of burial (no. grazing cycles)			
1	0.35 ± 0.051	0.42 ± 0.053	+0.08 ± 0.066
2	0.39 ± 0.056	0.39 ± 0.056	+0.00 ± 0.074
3	0.35 ± 0.069	0.18 ± 0.055	-0.17 ± 0.093
4	0.35 ± 0.083	0.13 ± 0.059	-0.21 ± 0.097
5	0.46 ± 0.133	0.16 ± 0.098	-0.30 ± 0.159
	ns	P<0.001	P<0.005

Table 4.8 Relative frequencies of axillary buds among four response categories for buds on stolons buried on different dates for up to five durations of burial in the date-and-duration-of-burial trial. Stolons were buried with 2 cm of soil. The Chi-square value and level of significance are given for comparisons within each column or row.

	Duration of Burial (Number of grazing cycles)																			
Date of Burial	1 Response				2 Response				3 Response				4 Response				5 Response				Chi-square	Significance
	(1,1)	(1,0)	(0,1)	(0,0)	(1,1)	(1,0)	(0,1)	(0,0)	(1,1)	(1,0)	(0,1)	(0,0)	(1,1)	(1,0)	(0,1)	(0,0)	(1,1)	(1,0)	(0,1)	(0,0)		
16/6	33.3	6.3	31.3	29.2	12.5	25.0	33.3	29.2	8.3	31.3	14.6	45.8	8.3	29.2	6.3	56.3	8.3	31.3	8.3	52.1	48.69	P<0.001
26/8	12.5	8.3	31.3	47.9	4.2	22.9	14.6	58.3	0	19.6	17.4	63.0	0	22.9	10.4	66.7					23.51	P<0.005
14/10	8.3	16.7	33.3	41.7	10.4	12.5	27.1	50.0	2.4	23.8	16.7	57.1									7.30	P>0.294
10/4	29.2	2.1	29.2	39.6	31.1	13.3	22.2	33.3													4.62	P>0.200
2/12	0	28.6	4.2	66.7																		
Chi-square	54.69				26.16				7.46				5.28									
Significance	P<0.001				P<0.002				P>0.280				P>0.152									

As the duration of burial increased, probability of bud outgrowth at harvest decreased and the change in probability of bud outgrowth become increasingly negative (Table 4.7). This resulted from the death of an increasing proportion of the branches present at burial and from the death of branches initiated following burial which increased the proportion of buds categorised as showing no development (Table 4.8).The means for date of burial given in (Table 4.7) were confounded by effects of duration of burial as the unbalanced design of the trial (Table 4.8) has means of successively later dates of burial derived from fewer and successively shorter durations of burial. This effect accounts for the increasingly positive value for change in probability of bud outgrowth with later date of burial, with the exception of the last date of burial (2 December 1986). Given this, it is more appropriate to examine the effect of date of burial within each of the durations of burial (Table 4.8). Although date of burial had a significant effect on bud response for burial durations of one or two grazing cycles (Table 4.8), these could be considered minor. For burial of one grazing cycle the very different distribution of bud response for the last date of burial was responsible for the significance of the Chi-square test whereas for burial of two grazing cycles an inconsistent pattern occurred (Table 4.8).

4.3.3 ZONE OF BURIAL

Analysis of variance of data grouped by zone (nodes 1-3, 4-7, and 8-10) showed burial to have a significant ($P<0.001$) depressive effect on probability of bud outgrowth at harvest (unburied, 0.61 ± 0.020 and buried, 0.52 ± 0.020) and change in probability of bud outgrowth from date of burial to harvest (unburied, 0.00 ± 0.026 and buried -0.10 ± 0.024). The zone of stolon by burial interaction was significant for both probability of bud outgrowth at harvest ($P<0.001$) and change in bud outgrowth ($P<0.02$). This was driven by the pronounced negative effect of burial on outgrowth of buds at node positions 1 and 2 at time of burial and the relatively small effect on buds at positions >3 (Fig. 4.2). This effect in turn contributed to significant effects of treatments on probability of bud outgrowth at harvest ($P<0.001$) and change in probability of bud outgrowth ($P<0.005$) (i.e. burial of the tip zone (treatments 1, 4 and 5) induced a reduction in mean probability of bud outgrowth at harvest (treatments 1 and 4) and the greatest changes in probability of bud outgrowth (Table 4.9)).

Increased duration of burial was associated with decreased probability of bud outgrowth at harvest and increasingly negative values for change in probability of bud outgrowth (Table 4.10), results which are similar to those reported in sections 4.3.1 and 4.3.2. The major extent of these changes was evident after a burial duration of three grazing cycles.

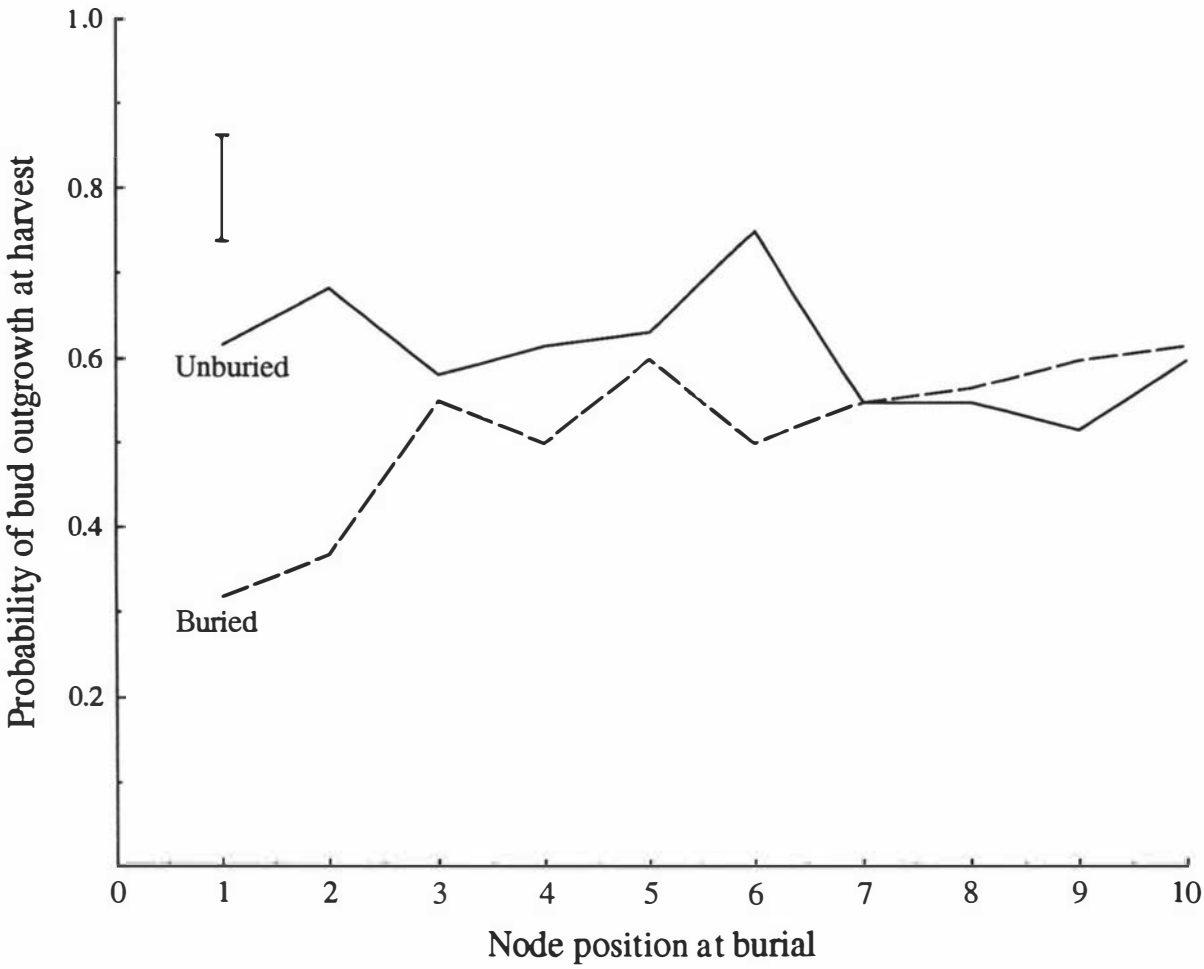


Figure 4.2 The effect of burial on the probability at harvest of axillary bud outgrowth at nodes of differing position (relative to the stolon apex (0)) at the time of burial. Data were from the zone-of-stolon-buried experiment and pooled across stolons such that all buried buds were compared with all unburied buds. Buried portions of stolons were covered with 2cm of soil. (Error bar indicates the SED.)

Table 4.9 Zone-of-burial experiment, (a) means for each burial treatment for probability of axillary bud outgrowth at burial (BO), harvest (BH) and change in probability of bud outgrowth (Change) and (b) probability of bud outgrowth at harvest (BH) and change in probability (Change) for buried and unburied buds of stolons in each of the burial treatments. Data grouped by stolon. Buried portions of stolons were covered with 2 cm of soil. Apical, intermediate and mature zones refer to node positions at burial of 1-3, 4-7 and 8-10 respectively.

Burial treatment (stolon zone buried)		BO	BH	Change
(a)	Treatment means (over both buried and unburied buds within treatments)			
1	Apical	0.57 ± 0.035	0.46 ± 0.035	-0.11 ± 0.040
2	Interm.	0.62 ± 0.034	0.60 ± 0.035	-0.02 ± 0.045
3	Mature	0.67 ± 0.033	0.69 ± 0.033	0.03 ± 0.046
4	Apical + Interm.	0.57 ± 0.035	0.49 ± 0.035	-0.09 ± 0.041
5	Apical + Mature	0.64 ± 0.034	0.58 ± 0.035	-0.06 ± 0.043
6	Interm. + Mature	0.64 ± 0.034	0.57 ± 0.035	-0.07 ± 0.047

Burial treatment (stolon zone buried)		BH		Change	
		Buried	Unburied	Buried	Unburied
(b)	Means for buried and unburied buds within treatments				
1	Apical	0.35 ± 0.062	0.51 ± 0.042	0.23 ± 0.073	-0.25 ± 0.042
2	Interm.	0.55 ± 0.056	0.63 ± 0.044	-0.24 ± 0.060	0.13 ± 0.060
3	Mature	0.68 ± 0.061	0.69 ± 0.039	-0.23 ± 0.069	0.14 ± 0.056
4	Apical + Interm.	0.45 ± 0.042	0.57 ± 0.065	-0.01 ± 0.049	-0.27 ± 0.067
5	Apical + Mature	0.52 ± 0.046	0.68 ± 0.053	0.00 ± 0.059	-0.14 ± 0.061
6	Interm. + Mature	0.56 ± 0.042	0.57 ± 0.065	-0.30 ± 0.049	0.47 ± 0.077

Table 4.10 Main effects, in the zone-of-burial experiment, of burial, node position and duration of burial on probability of axillary bud outgrowth at harvest (BH) and on change of probability over the treatment period (Change). Buried portions of stolons were covered with 2 cm of soil. Data grouped by node position zones, apical (1-3), intermediate (4-7) and mature (8-10). Change of probability of bud outgrowth = harvest probability - probability at burial. SEM and level of significance of main effects are given.

	BH	Change
Burial treatment		
unburied	0.61 ± 0.020	0.00 ± 0.026
buried	0.52 ± 0.020	-0.10 ± 0.024
	P<0.001	P<0.001
Node position zones		
apical	0.52 ± 0.034	0.39 ± 0.031
intermediate	0.59 ± 0.026	-0.22 ± 0.025
mature	0.57 ± 0.028	-0.27 ± 0.027
	ns	P<0.001
Duration of burial (no. grazing cycles)		
1	0.73 ± 0.029	0.14 ± 0.031
2	0.58 ± 0.032	-0.03 ± 0.040
3	0.50 ± 0.032	-0.10 ± 0.040
4	0.53 ± 0.032	-0.11 ± 0.041
5	0.48 ± 0.032	-0.15 ± 0.044
	P<0.001	P<0.001

4.4 DISCUSSION

The first of the two sections of this discussion considers aspects of the experimental methodology that need to be accommodated in any interpretation of the results of the study. The second section discusses the main findings of the study which centre on the negative influence of stolon burial on branching which is caused by burial decreasing the survival of young developing branches rather than decreasing initiation of outgrowth of axillary buds.

4.4.1 EXPERIMENTAL METHODOLOGY

The experiments all used similar methodology and were performed to study the effects of different aspects of stolon burial on the branching of treated stolons in the field. These experiments involved imposing burial treatments on stolons consisting of the apex and up to ten nodes and then after a time interval assessing the effect of treatments on outgrowth of axillary buds present at the time of burial.

Branching was registered once the axillary bud had produced a leaf of development stage 0.2 on the Carlson (1966a) scale. Although the node position at which axillary bud outgrowth commences varies with growth conditions (Davies & Evans 1990a), in grazed pastures in Manawatu nodes are positioned three or more from the apex before branch initiation occurs (Table 4.2; Hay *et al.* 1991) and under pastoral conditions most branch initiation ceases once node position exceeds eight (Chapman 1983; Davies & Evans 1990a; Hay *et al.* 1991). Hence interpretation of results must take account of this ontogenetic development of axillary buds. The response of buds at positions 1 to 3 therefore chiefly centres on the initiation of branches whereas that for positions ≥5 becomes increasingly centred on branch survival as with increasing node position the probability of initial branch presence increases (Table 4.2) and the potential for branch initiation decreases (Newton *et al.* 1992).

The system used to classify the response of buds was insensitive in that the category of no outgrowth before or after treatment (0, 0) contained two types of buds. The first type were the inactive buds and the second type reflected the net result of development over the burial period. This latter component comprised those buds that initiated outgrowth during the burial period but which then died before harvest date. As 50-75% of axillary buds of field populations of white clover may be inactive (Chapman 1983; Davies & Dutta 1988; Newton *et al.* 1990, 1992) the proportions of inactive buds after a burial period of one grazing interval (29-68%; Table 4.8) were not indicative of major distortion from this source. With an increase in the duration of burial the proportion of buds recorded as having initiated branches steadily decreased (Tables 4.5 and 4.8). Comparison of similarly treated stolons which had a range of durations of burial (i.e. along rows of Table 4.8) indicates that for longer durations of burial, branch survival following initiation is reduced and that this inflates the proportion of buds recorded in the inactive (0,0) category.

4.4.2 EFFECT OF BURIAL ON BRANCHING

The overall effect of burial on axillary bud outgrowth was to significantly reduce branching. This occurred regardless of whether the comparison was of burial of complete stolons (depth-of-burial and date-and-duration-of-burial experiments) or of portions of stolons (zone-of-stolon-buried experiment). Although in all experiments the effect of burial (of nodes) on axillary bud outgrowth was significant for nodes 1 to 3, results for nodes positioned >3 at the time of burial were less conclusive. Burial significantly reduced branch survival in the depth-of-burial experiment (Table 4.5) but had no significant effect in the zone-of-burial experiment (Fig. 4.2). A small impact of burial on branching of axillary buds of older nodes would not be a surprising result. Buds at these nodes have reduced potential to initiate branches (Dutta 1988; Davies & Evans 1990a; Newton *et al.* 1992) and the larger branches at these nodes have increased chances of survival (Chapman 1983). Both these factors act to limit the potential for response to burial under the assessment procedure used. A non-significant effect of burial on axillary bud outgrowth was reported in a glasshouse trial (Grant *et al.* 1991) although a stimulation of axillary branching was measured on the portion of stolon that resurfaced following burial. Such a comparison was not valid for this trial as resurfacing stolons grew into open conditions on the elevated soil surface in the metal containers, whereas unburied stolons were in the sward. On the other hand burial of nodes 1 to 3 had a significant depressing effect on initiation of branches from these buds (Table 4.6, Fig. 4.2). Work reported by Davies & Evans (1990a) and in Chapter 6 indicates that as these nodes had leaf or petiole above the soil, and in light, a depression in branching due to exclusion of light at the axillary bud site could not be expected. However there is some conflicting evidence (Harvey 1979; Newton & Hay 1992) that presence of a subtending leaf can act to depress axillary bud outgrowth. The depressive effect of burial on axillary bud outgrowth at nodes 1 and 2 (Fig. 4.2) suggests that buds at this ontogenetic stage are sensitive to an inhibitive effect of burial not alleviated by the presence of the subtending leaf in light. It is interesting to note that Grant *et al.* (1991) found a depression with burial of axillary bud outgrowth at nodes on the secondary branch stolons. A higher proportion of the nodes on these secondary stolons would have been of node position 1 to 3 at the time of burial. If buds at these nodes are sensitive to burial then this may have contributed to the result they obtained. Clearly then the major effects of burial centre on its effects on axillary bud outgrowth of the most recently formed nodes (positions 1, 2 and 3) and further work under more controlled conditions is required (see Chapter 6) to confirm these findings and possibly identify the processes responsible for the observed responses.

Duration of burial influenced survival of branches in both the depth-of-burial and the date-and-duration-of-burial experiments. A short duration of burial of one grazing cycle had no significant effects on axillary bud outgrowth (Table 4.5) whereas after two grazing cycles, trends were evident but not significant (Tables 4.6 and 4.7), and after three or more grazing cycles, responses were significant and of approximately maximal extent. Branches formed prior to and after the date of burial were both affected by burial (Tables 4.5, 4.8). In both cases burial decreased survival of branches. No evidence was obtained to suggest that burial treatments negatively affected the initiation of branches (see Table 4.5; also

compare response category values of unburied buds after one grazing cycle in Table 4.5 with those of the burial treatment of 16/6/86 after one grazing cycle in Table 4.8). Thus the response to burial developed over time as a result of burial reducing survival of branches, there being no effect on initiation of branches. Hence if the effects of burial on axillary bud outgrowth are to be observed the duration of burial should be such that the effects on branch survival can be detected. Under these trial conditions this corresponded with the elapse of three grazing cycles.

The effect of other factors such as depth and date of burial on outgrowth of axillary buds were generally small. During December 1986 and January 1987 very pronounced soil moisture deficits developed (see Chapter 3, Fig. 3.1). The normal response of white clover to moisture deficit includes the death of small daughter branches and inhibition of axillary bud outgrowth (Wang 1991). This response pattern is strikingly represented by the comparison (Table 4.8) of the first four dates of burial with the fifth date (2 December 1986) for frequencies of branch death and initiation of stolons buried for one grazing cycle. In the depth of burial trial (Section 4.3.2) this effect is not likely to make a large contribution to the observed difference in response between short and long intervals of burial as nodes and branches were considerably older at the time the drought developed and therefore beyond the zone where plant response to moisture stress was most marked (Wang 1991).

CHAPTER 5 EFFECT OF REDUCED INTRA-PLANT AVAILABILITY OF CARBON OR PHOSPHORUS ON INITIATION OF OUTGROWTH BY AXILLARY BUDS IN WHITE CLOVER

5.1 INTRODUCTION

The effect of stolon burial on the initiation of branch growth may be direct via an immediate sensing of the altered microenvironment at the axillary bud or indirect via a response to reduced plant carbon accumulation resulting from the effects of burial on other plant processes. Evidence presented in Chapter 4 suggests that alteration of the axillary bud microenvironment by stolon burial had little effect on initiation of axillary bud outgrowth but that the microenvironment associated with burial decreased the probability of survival of axillary meristems following initiation of outgrowth. However, when shading of axillary buds is also associated with defoliation of subtending leaves, initiation of outgrowth can be markedly suppressed when stolons have been previously pretreated by shading to exclude 84 or 94% of photosynthetically active radiation (Davies & Evans 1990a). Hence it appears that initiation of outgrowth may be strongly influenced by the general intra-plant level of photosynthate availability. That branching in white clover is depressed by limitation in photosynthate or phosphorus (P) supply and that these limitations increase apical dominance has long been recognised (Erith 1924; Levy 1970; Harvey 1979; Thomas 1987b; Caradus 1984; Davies & Evans 1990a; Caradus *et al.* 1993). However, development of the pattern of branching response to a limitation in resource supply has not previously been recorded within a framework which is fully descriptive of plant branching response. Such a framework should include measures of node appearance rate, position of first branching node relative to the stolon apex and the probability of axillary bud outgrowth at nodes proximal to and inclusive of the node with the first branch (Sackville Hamilton 1987a). As all these attributes may be assessed non-destructively it is possible to obtain a time course for the response of each branching attribute to a change in supply of an essential plant resource. Such information may enable branching attributes to be characterized as sensitive to either the external or internal plant environment. Attributes sensitive to the external environment would respond immediately to a change in resource supply whereas those sensitive to the internal plant environment would respond gradually as the intensity of the resource limitation increased within the plant.

In the field, factors may interact to severely reduce resource levels within plants. For instance, burial by preventing the relatively small contribution of stolon photosynthesis to plant carbon accumulation (c. 5%, Harris *et al.* 1983) may when coupled with simultaneous defoliation significantly increase the senescence rate of stolons (Chapman & Robson 1992), or when coupled with previous shading and defoliation suppress outgrowth of axillary buds (Davies & Evans 1990a). White clover plants, under New Zealand pastoral conditions, are commonly subjected to sub-optimal levels of phosphorus (P) supply which can severely restrict the lateral spread of white clover (Levy 1970; Jackman & Mouat 1972; Harvey 1979; Caradus 1984) with an altered branching pattern contributing to this result (Harvey 1979; Caradus 1984; Caradus *et al.* 1993). Plants in the field may be simultaneously subjected to limiting intraplant levels of more than one resource (Chapin *et al.* 1987) e.g., both photosynthate and P supply. Thus it is important to understand how, in general, white clover adapts branch initiation to a decline of resource availability within the plant.

The objective of the investigation described in this chapter was to secure information on the effect of limiting intraplant resource availability on the initiation of outgrowth of axillary buds in order to underpin understanding of the effect of stolon burial on initiation of bud outgrowth. In the first experiment conditions were manipulated to reduce the availability of photosynthate within plants and the effect of this on the initiation of axillary bud outgrowth recorded. A second experiment was performed in which the availability of the essential plant nutrient P was reduced in order to establish if the initiation of axillary bud growth was similarly affected. A final experiment involving application of burial and defoliation treatments to individual stolons within plants was undertaken to investigate whether the response of axillary bud outgrowth to burial conformed with responses obtained to resource depletion within plants in the above two experiments and to test for evidence of clonal integration within plants in the response patterns of attributes relating to outgrowth of axillary buds.

5.2 METHODS AND MATERIALS

Two separate glasshouse experiments were undertaken which will be referred to respectively as the shade and defoliation experiment and the phosphorus supply experiment.

5.2.1 SHADE AND DEFOLIATION EXPERIMENT

5.2.1.1 PLANT MATERIAL

Stolon cuttings from two genotypes of each of the white clover cultivars, Grasslands Kopu, Grasslands Pitau and Grasslands Tahora were taken in June 1985 from spaced plants of nucleus seed lines of each cultivar. These cuttings were vegetatively propagated in a heated glasshouse so as to provide clonal material for establishment of experimental plants. On 14 August 1985 experimental plants were established by planting stolon segments comprised a stolon apex and the first two emerged nodes. Leaves of morphological development > 0.7 on the Carlson (1966a) scale were removed so as to reduce transpiration demand. Six trays each with four cuttings were established for each genotype.

5.2.1.2 EXPERIMENTAL GROWTH CONDITIONS

Plants were grown in standard plastic propagating trays (40x30x5 cm) filled with a commercially obtained 60:40 peat moss: coarse sand mix amended with Osmocote fertiliser (1.5 kg m^{-3}), dolomite (3 kg m^{-3}), superphosphate (1 kg m^{-3}) and trace elements (125 g m^{-3}).

Daylength increased over the experimental period from 12 h 13 min on 25 September to 13 h 32 min by 25 October 1985. The photosynthetic photon flux density (PPFD), measured by a LiCor model 185A radiometer at midday in the glasshouse, on three days (10, 13 and 14 October) in bright sunshine was 1350, 1390 and 1480 μmol m^{-2} s^{-1} and on two days (11 and 15 October) under dark cloudy conditions was 470 and 420 μmol m^{-2} s^{-1}. The glasshouse had thermostatically activated heating and cooling systems. Mean daily maximum and minimum air temperatures were 28.9 ± 1.14°C (range 27.0 to 32.5°C) and 12.6 ± 0.57°C (range 12.0 to 14.5°C), respectively.

5.2.1.3 EXPERIMENTAL TREATMENTS

5.2.1.3.1 CULTIVAR

Each of the three cultivars, 'Grasslands Kopu', 'Grasslands Pitau' and 'Grasslands Tahora' were represented by two genotypes.

5.2.1.3.2 SHADE

The three shade treatments were:

1) natural daylight
2) 20% of daylight
3) 5% of daylight

The two shade treatments were obtained by placing trays within purpose-built shade-frames. The shade-frames fitted snugly over individual trays and were constructed by fixing the appropriate weave of shade cloth to light wooden frames. The sides and top of the frames were covered by shade cloth and the trays prevented light entering from below. The top of frames was 25 cm above the soil surface. The light intensity at the soil surface within shade frames was measured within a growth cabinet delivering a constant PPFD of 960 μmol m^{-2} s^{-1}. The PPFD measured, with a LiCor 185A radiometer, under the 80 and 95% shade cloth was 185 and 60 μmol m^{-2} s^{-1}, respectively. This indicated that the shade treatments were as indicated by the grading specifications of the respective shade cloths.

Temperatures at the soil surface of trays of each of the shade treatments were monitored hourly for five days (10-14 October) using thermocouples connected to a Campbell datalogger. Results indicated there was a diurnal pattern of response to the shade treatments in that the direction of response for the warmer daytime period (9.00 am to 5.00 pm) differed from that over the cooler night period (5.00 pm to 9.00 am). For the shade treatments of 100, 20 and 5% of daylight the mean temperatures in the daytime period were 24.3 ± 2.96°C, 22.0 ± 1.63°C and 22.1 ± 1.47°C whereas for nighttime, mean temperatures were 15.6 ± 2.28°C, 16.2 ± 2.53°C and 16.4 ± 1.96°C, respectively. The differing trends during night and day tended to cancel out treatment effects on mean daily temperatures which were 18.5 ± 2.53°C, 18.1 ± 2.27°C and 18.3 ± 1.81°C for the respective shade treatments of 100, 20 and 5% of daylight.

5.2.1.3.3 DEFOLIATION

Defoliation treatments were imposed once, at the start of the experimental period (25 September 1985). Treatments were either defoliation of all leaflets (L) or leaflets plus petiole (L+P) of all leaves on the plant at a morphological development stage greater than

0.5 on the Carlson (1966a) scale or leaving plants undefoliated (C). The L treatment involved severing the petiolules at the junction with the petiole. (This treatment left plants with intact petioles but no leaflets). For the L+P treatment the petiole was severed within 2 mm of the stolon.

5.2.1.4 EXPERIMENTAL DESIGN

Trial design was unbalanced with respect to defoliation treatments; normally defoliation treatments differed among trays but one plant in each tray (of four plants) had the L defoliation treatment. Thus each tray contained three plants subjected to the defoliation treatment designated to the tray (either L+P or C) plus one plant subjected to the L treatment. Genotypes were not replicated. Comparison of means in a preliminary analysis indicated non-significant genotypic effects within cultivars and hence forth genotypes of each cultivar were considered as cultivar replicates. The trial was of factorial design laid out as a single randomised block of 36 trays in the glasshouse with the following structure:

3 cultivars x 3 shade treatments x 2 designated defoliation treatments x 2 replicates (genotypes)
(L defoliation treatment)

Twice a week trays were systematically rotated by moving trays clockwise within the block.

5.2.1.5 MEASUREMENTS

At the beginning of the experimental period (25 September) the youngest node with an unfolded leaf on the main stolon of each plant was marked with nail varnish and the stage of morphological development (Carlson 1966a) of each partially unfolded leaf recorded. On four occasions, 2, 9 and 16 October during the experiment and at harvest, nodes on the main stolon distal to the marked node, on each plant, were assessed for stage of morphological development of the leaf and axillary bud. An axillary bud was defined as initiating outgrowth once its first leaf appeared beyond the stipule.

Plants were harvested from 21 to 25 October 1985 as follows:

21 October, Kopu replicate (genotype) 1

22 October, Kopu replicate 2

23 October, Pitau replicate 1

24 October, Pitau replicate 2; Tahora replicate 1

25 October, Tahora replicate 2

At harvest individual internode lengths and petiole length and leaf area per leaf were measured at all nodes distal to the marked node. Then each node of the main stolon was dissected such that all branch development originating from the axillary bud at a node was included with the nodal segment of the main stolon. Leaves were then dissected from stolon and the dryweight of each component measured after oven-drying for four days at 80°C.

5.2.1.6 ANALYSIS OF DATA

The following plant characteristics were derived from the measurements for statistical analysis;

node appearance rate (main stolon),
position of first branching node (main stolon),
probability of an axillary bud initiating outgrowth (main stolon),
internode length (main stolon),
petiole length of unfolded leaves (main stolon),
leaf area per leaf (main stolon),
leaf to stolon dry weight ratio (whole plant), and
total number of nodes produced per plant during the experimental period (whole plant).

The unbalanced experimental design meant it was only possible to include the third (L) defoliation treatment within the analysis if the analysis utilized individual plants as experimental units as opposed to trays. A preliminary analysis tested the variance between trays (using the paired L plants in the otherwise identical defoliated or undefoliated trays) against the variance within trays (using the three identical plants in each tray). No significant results were obtained, leading to the assumption that using individual plants as experimental units was justified.

Data were tested by an unbalanced analysis of variance using the GLM procedure in SAS (SAS Users Guide 1988). Data used to calculate node appearance rate, position of first branching node and probability of an axillary bud initiating outgrowth were obtained by assessing development of the main stolon of plants at approximately weekly intervals. Therefore the analysis of these characteristics utilized the Repeated Measures Option in the GLM procedure in SAS.

The harvesting procedure, successively harvesting replicates of cultivars over five days, did not compromise comparison of light and defoliation treatments but did, because of the allometric growth of white clover plants, invalidate comparisons among cultivars of parameters reflecting the overall growth of plants. However, it was valid to test for cultivar differences in daily growth rate of parts of plants, e.g. node appearance rate of the main stolon, and differences in point in time measures of characteristics of plant parts, e.g., petiole length and area of main stolon leaves, position of first branching node. The effects of shade and defoliation treatments, meaned over cultivars, on leaf: stolon DW ratio were tested, but possible ontogenetic influences due to the staggered harvest prevented valid comparison among cultivars for this parameter. For the parameter total number of nodes produced per plant, data were meaned between cultivars and tested for the effects of shade and defoliation.

5.2.2 PHOSPHORUS SUPPLY EXPERIMENT

5.2.2.1 PLANT MATERIAL

This experiment utilized the same two genotypes of 'Grasslands Tahora' that were used in the previously described shade and defoliation experiment (Section 5.2.1). Stolon cuttings, of two nodes plus the apical bud with root growth initiated at the proximal node, were taken on 29 January 1986 from a bank of clonal material of each genotype propagated in a temperature regulated glasshouse. Trays were planted with two cuttings of one of the genotypes, one at either end of the tray. Fifteen trays of each genotype were planted. Plants were allowed to establish for four weeks before treatments were applied on 25 February 1986. At this time, one of the two plants in each tray was selected for removal such that remaining plants were as uniform as was possible.

5.2.2.2 EXPERIMENTAL GROWTH CONDITIONS

Plants were grown in standard plastic propagating trays (40x30x5 cm) lined with paper towels and filled with 8 kg of dry quartz sand (Poutu sand; Winstone, North Shore, Auckland). Upon moistening, each tray absorbed 1.9 l of nutrient solution.

During the establishment period each cutting was supplied daily with 500 ml of a nutrient solution comprising (*mmol*) KNO_3 5.0, $Ca(NO_3)_2$ 1.5; $MgSO_4$ 1.5; $NaNO_3$ 2.0; KH_2PO_4 0.5 and minor elements (*μmol*) FeEDTA 9.22; H_3BO_3 9.22, $CuSO_4$ 0.16, $MnSO_4$

3.5, $ZnSO_4$ 0.77, KCl 14.5 and $(NH_4)_6$ Mo_7 O_{24} 0.016. The sand surface in the portion of the tray without plant material was covered with aluminium foil to reduce algal growth and evaporation losses. This practice continued throughout the experiment.

The experiment was performed in the same thermostatically temperature controlled glasshouse as the shade and defoliation experiment (Section 5.2.1.2). Mean daily maximum and minimum air temperatures were 27.1 ± 2.06°C (range 24.0 to 33.0°C) and 13.7 ± 1.23°C (range 12.1 to 16.3°C). Daylength decreased over the course of the experiment; 14 h 20 min at planting (29 January), 13 h 4 min at the beginning of the experimental period (25 February), 11 h 1 min at harvest (16 April).

5.2.2.3 EXPERIMENTAL TREATMENTS

5.2.2.3.1 GENOTYPE

The two genotypes of 'Grasslands Tahora' white clover used in the shade and defoliation experiment were compared.

5.2.2.3.2 PHOSPHORUS SUPPLY

The three treatment levels of phosphorus (P) supply were provided by applying nutrient solutions with P concentrations of 0.01, 0.20 and 1.0 *mM*. Phosphorus concentration was varied by altering the quantity of KH_2PO_4 in the nutrient solution previously detailed (Section 5.2.2.2). All other salts were maintained at their normal strength. This procedure resulted in the potassium (K) supply increasing from 5 to 6 *mM* as P supply increased from 0.01 to 1.0 *mM* P. Plant analyses performed in conjunction with previous experimentation using this nutrient solution (Hay *et al.* 1986) showed that the K content of white clover leaf DM was 3.9% when K was supplied at 5 *mM*. When leaf K contents are of this order, increases in K supply are not associated with growth responses in white clover (Andrew 1956; McNaught 1970). Hence in this experiment it was assumed that the responses in growth that occurred with variation in nutrient supply resulted from the changes in supply of P.

On 25 February prior to application of treatment nutrient solutions all trays were leached with five successive applications of one litre of distilled water. The P

concentration in the leachate collected after the fifth water application was 0.01 *mM* P. Then 2 l of the appropriate nutrient solution was applied to each tray. Thereafter daily, each cutting received 800 ml of the appropriate treatment nutrient solution.

5.2.2.4 EXPERIMENTAL DESIGN

The trial was of factorial design with five replicates; 2 genotypes x 3 P levels x 5 replicates. A single randomised block of 30 trays was put down along one side of a glasshouse and twice a week trays were systematically rotated by moving trays two positions within the block.

5.2.2.5 MEASUREMENTS

At the time when application of P supply treatments were started (25 February 1986) the youngest node with an unfolded leaf on the main stolon of each plant was marked with nail varnish and the stage of morphological development (Carlson 1966a) of each partially unfolded leaf recorded. Subsequently on six occasions at approximately weekly intervals and at harvest (16 April 1986) each plant was assessed for stage of morphological development of leaves and axillary buds at each node of the main stolon (Section 5.2.1.5).

At harvest plants were cut at the marked node and the distal part of the shoot system removed after severing of any roots. The length of the main stolon (marked node to node with youngest unfolded leaf), the petiole length and leaf area of the third unfolded leaf and the diameter of the internode distal to the third unfolded leaf were measured. Leaves were then dissected from stolons and the dry weight of both components measured after oven-drying for four days at 80°C. Leaf and stolon material was then ground and the P content measured by autoanalysis after acid digestion (Williams & Twine 1967).

5.2.2.6 ANALYSIS OF DATA

The following plant characteristics were derived from the measurements for statistical analysis; node appearance rate, position of first branching node, probability of an axillary bud initiating outgrowth and mean internode length, all relating to the main

stolon; petiole length and leaf area of the third unfolded leaf and diameter of the internode distal to it; leaf, stolon and total shoot dry weight of the plant fragment distal to the marked node and P contents of the leaf and stolon components.

The design for this experiment was balanced and data was analysed by analysis of variance using the GLM procedure in SAS (SAS Users Guide 1988), with the Repeated Measures Option used for the analysis of the characteristics of node appearance rate, position of first branching node and probability of axillary bud initiation of outgrowth (see Section 5.2.1.6).

5.3 RESULTS

5.3.1 SHADE AND DEFOLIATION EXPERIMENT

5.3.1.1 NODE APPEARANCE RATE

Only the main effects of shade, defoliation and growth interval were significant (Table 5.1). Node appearance rate (nodes produced per main stolon apex per day) was reduced by a third by shading to 20% sunlight and by two thirds by shading to 5% sunlight. The defoliation treatments (applied only once at the beginning of the experimental period) induced smaller differences (Table 5.1). Mean node appearance rate increased with growth interval during the experiment (Table 5.1).

Table 5.1 Main effects of cultivar, shade, defoliation and growth interval on node appearance rate (nodes produced per main stolon apex per day) of the main stolon of white clover plants. F test value and level of significance are presented, NS, not significant.

Cultivar		Shade (% full sunlight)		Defoliation		Growth Interval	
Kopu	0.178	100	0.273	None	0.205	1	0.130
Pitau	0.166	20	0.182	Leaflets	0.166	2	0.182
Tahora	0.178	5	0.067	Leaflets + petioles	0.149	3	0.190
						4	0.195
F value	2.44		445.98		39.32		18.05
P	NS		<0.0001		<0.0001		<0.0001

The shade by growth interval interaction was highly significant (P<0.0001) and resulted from an increase in node appearance rate with growth interval under the full and 20% sunlight treatments in contrast to unchanging rates under 5% sunlight (Fig 5.1).

The second order shade by defoliation by growth interval interaction was highly significant (P<0.0002) and indicated differing response patterns to defoliation at each shade level over the duration of the experiment (Fig 5.2). In the unshaded, 100% sunlight treatment the three defoliation treatments had induced differences in node appearance rates during the first growth interval, which had disappeared by the third growth interval. (The reason for the low value of the undefoliated treatment in the fourth growth interval is not known). Under the 20% sunlight treatment defoliation induced a more variable response which saw initial separation of the undefoliated treatment from the two defoliated treatments followed by no separation for intervals two and three but higher node appearance rates for undefoliated plants in interval four. Under 5% sunlight whereas the node appearance rate of undefoliated plants remained constant, that of each of the defoliation treatments decreased during the experiment.

5.3.1.2 POSITION OF FIRST BRANCHING NODE

For position of first branching node (nodes numbered basipetally from the stolon apex, 1 being the first node proximal to the stolon apex with unfolding leaf > 0.5 Carlson scale) the main effects of light, defoliation and growth interval were significant (Table 5.2). These results indicated that highest mean values for position of first branching node were associated with the 20% sunlight treatment and occurred towards the end of the experiment and that defoliation of leaf plus petiole (L+P) reduced values.

Table 5.2 Main effects of cultivar, shade, defoliation and growth interval on position of first branching node, relative to the apex of the main stolon. F test value and level of significance are presented, NS, not significant.

Cultivar		Shade (% full sunlight)		Defoliation		Growth Interval	
Kopu	3.73	100	3.22	None	3.85	1	2.80
Pitau	3.66	20	4.49	Leaflets	3.72	2	3.36
Tahora	3.55	5	3.21	Leaflets + petioles	3.39	3	3.76
						4	4.65
F	0.78		56.39		6.31		210.75
P	NS		>0.0001		>0.0025		<0.0001

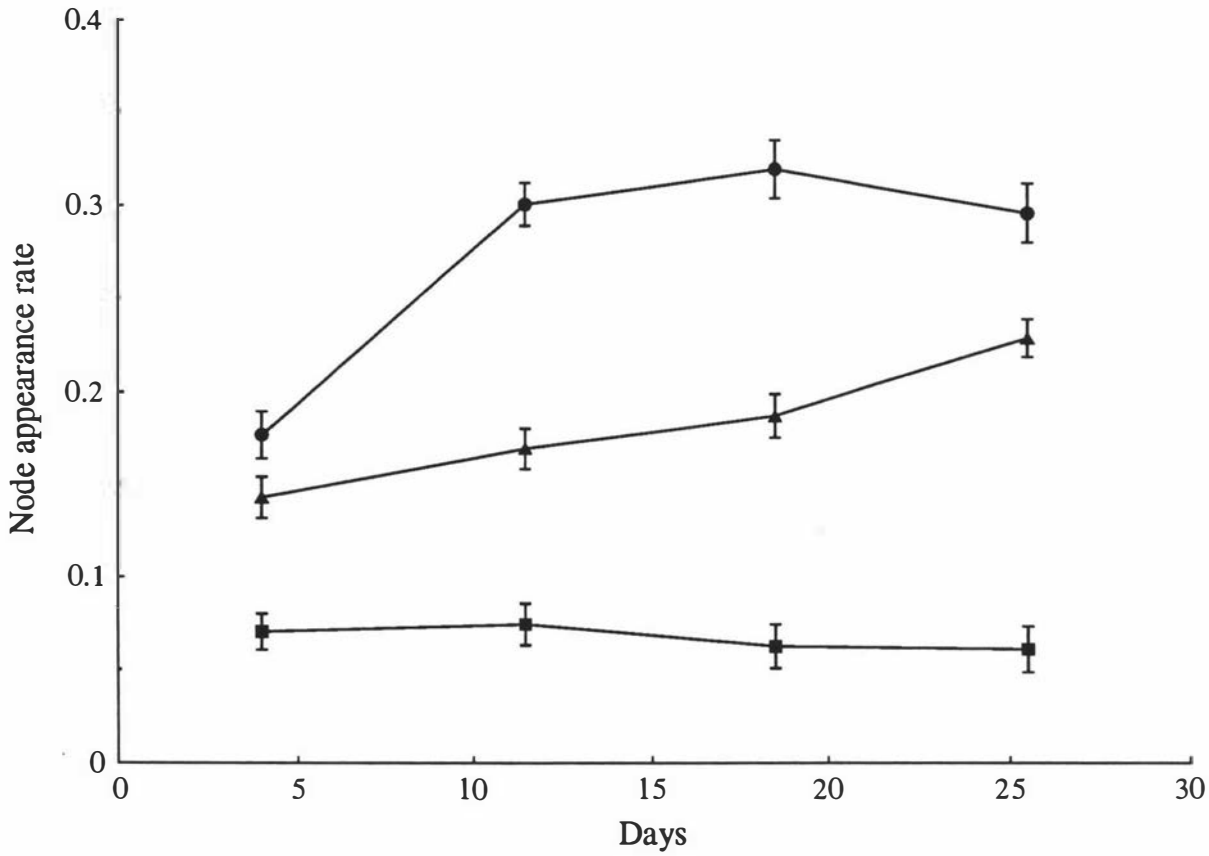


Figure 5.1 Effect of shade treatments, 100% sunlight (●), 20% sunlight (▲) and 5% sunlight (■), on node appearance rate of the main stolon of white clover plants for each of four growth intervals. Values presented at the midpoint of each growth interval and scaled from the start of the experimental period. (Error bars indicate ± SEM.)

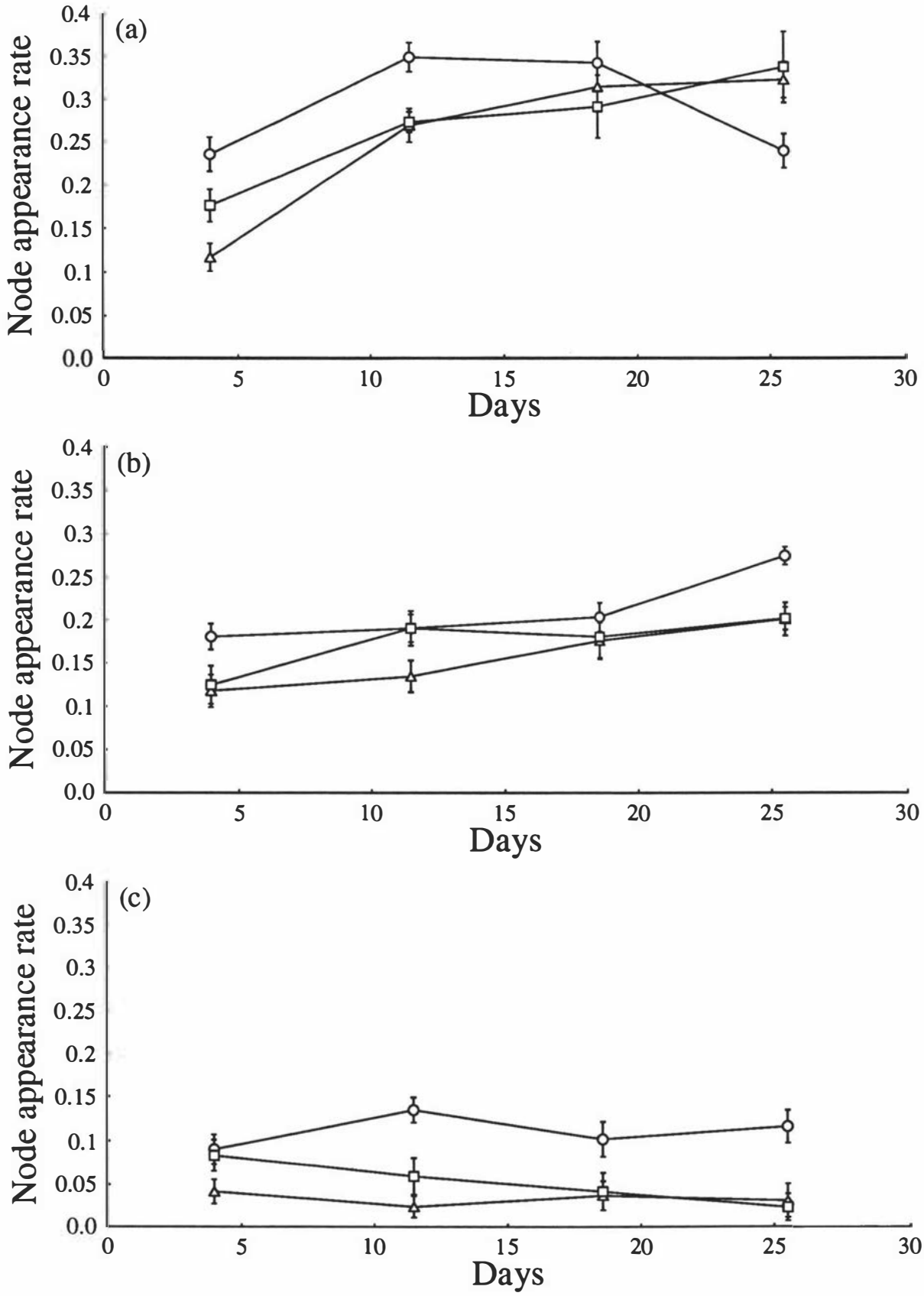


Figure 5.2 Effect of defoliation treatments, undefoliated (o), leaflets defoliated (▵) and leaflets plus petioles defoliated (□), within each of the shade treatments (a) 100% sunlight, (b) 20% sunlight and (c) 5% sunlight, on node appearance rate of the main stolon of plants at each of four growth intervals. Values presented at the midpoint of each growth interval and scaled from the start of the experimental period. (Error bars indicate ± SEM.)

A highly significant (P<0.0001) shade by growth interval interaction (Fig 5.3) resulted from a more rapid increase in position of first branching node with growth interval under 20% sunlight than under full or 5% sunlight, which responded similarly. The lack of response shown by this characteristic to the 5% sunlight treatment (Fig. 5.3), especially when plants were also defoliated (L or L+P treatments, Fig. 5.4c) resulted from the very low node appearance rates in these treatments (Fig. 5.2c) limiting the potential of these plants to express a response. Further evidence in support of this argument is that where plants of the 5% sunlight treatment were not defoliated the node appearance rate was slightly higher than for the defoliated treatments (Fig. 5.2c) and under these conditions the characteristic of position of first branching node showed increased responsiveness (Fig. 5.4c) to the extent that at harvest values were greater than those of the 100% sunlight treatments. The second order interaction, shade by defoliation by growth interval, (P<0.001) resulted in the main from the large separation among defoliation treatments for position of first branching node under the 5% sunlight treatment at growth intervals three and four (Fig 5.4).

5.3.1.3 PROBABILITY OF INITIATION OF OUTGROWTH OF AXILLARY BUDS

The probability of outgrowth of axillary buds at nodes proximal to (and inclusive of) the first branching node on the main stolon was not significantly influenced by any of the variables in this experiment. Branches were produced at almost every node (overall mean probability 0.974± 0.0068).

5.3.1.4 MORPHOLOGICAL CHARACTERISTICS AT HARVEST

The main effects of treatments on leaf and stolon characteristics of the main stolon are given in Table 5.3. Although cultivars differed as expected for petiole length and leaf area of main stolon leaves, the main effect of cultivar on mean internode length was non-significant. The main effect of cultivar on leaf to stolon dry weight (DW) ratio was also non-significant.

The main effect of shade treatments was significant for each of the four morphological characteristics examined (Table 5.3) although the direction of the responses differed among the characteristics. Petiole length was similar in both shaded treatments but greater than in the unshaded treatment. Leaf area per leaf was reduced 40% by the

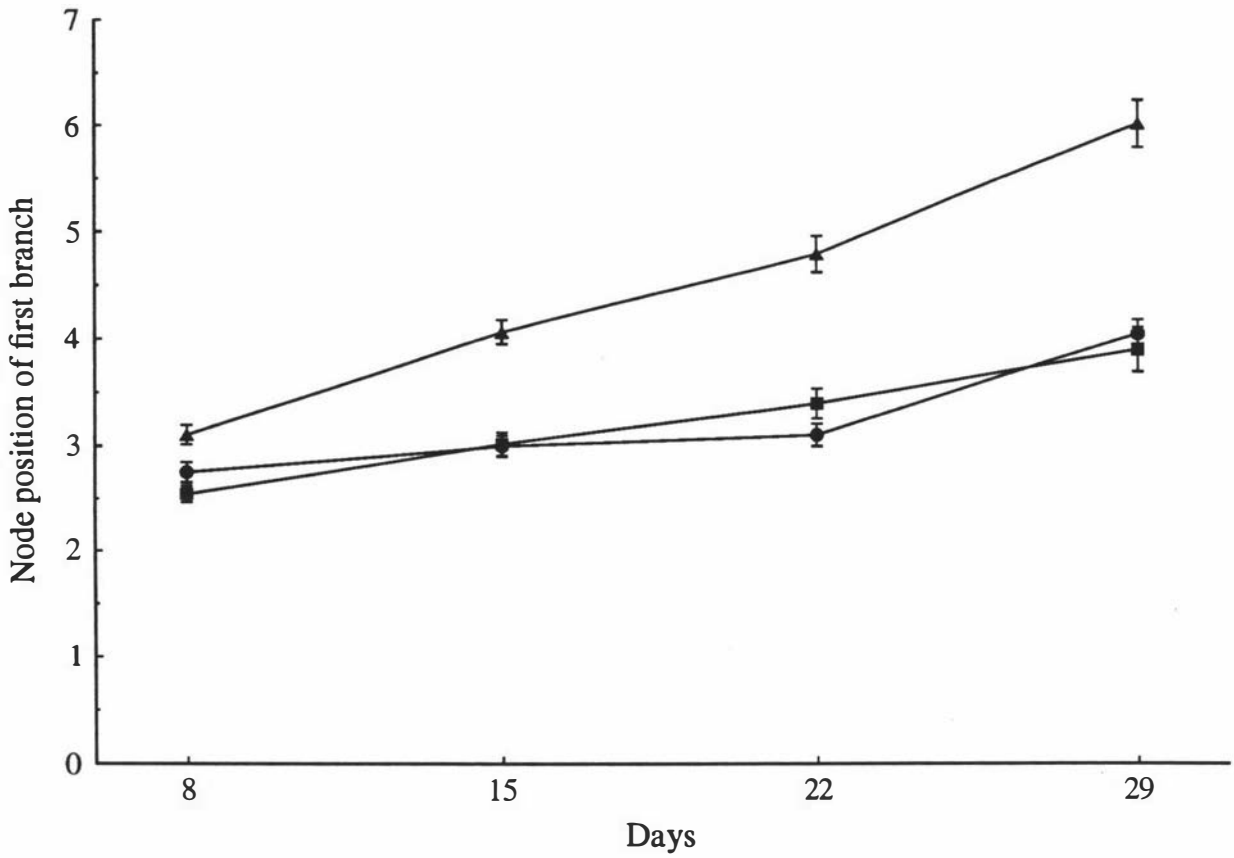


Figure 5.3 Effect of shade treatments, 100% sunlight (●), 20% sunlight (▲) and 5% sunlight (■), on position of first branching node relative to the apex of the main stolon at the end of each of four growth intervals. (Error bars indicate ± SEM.)

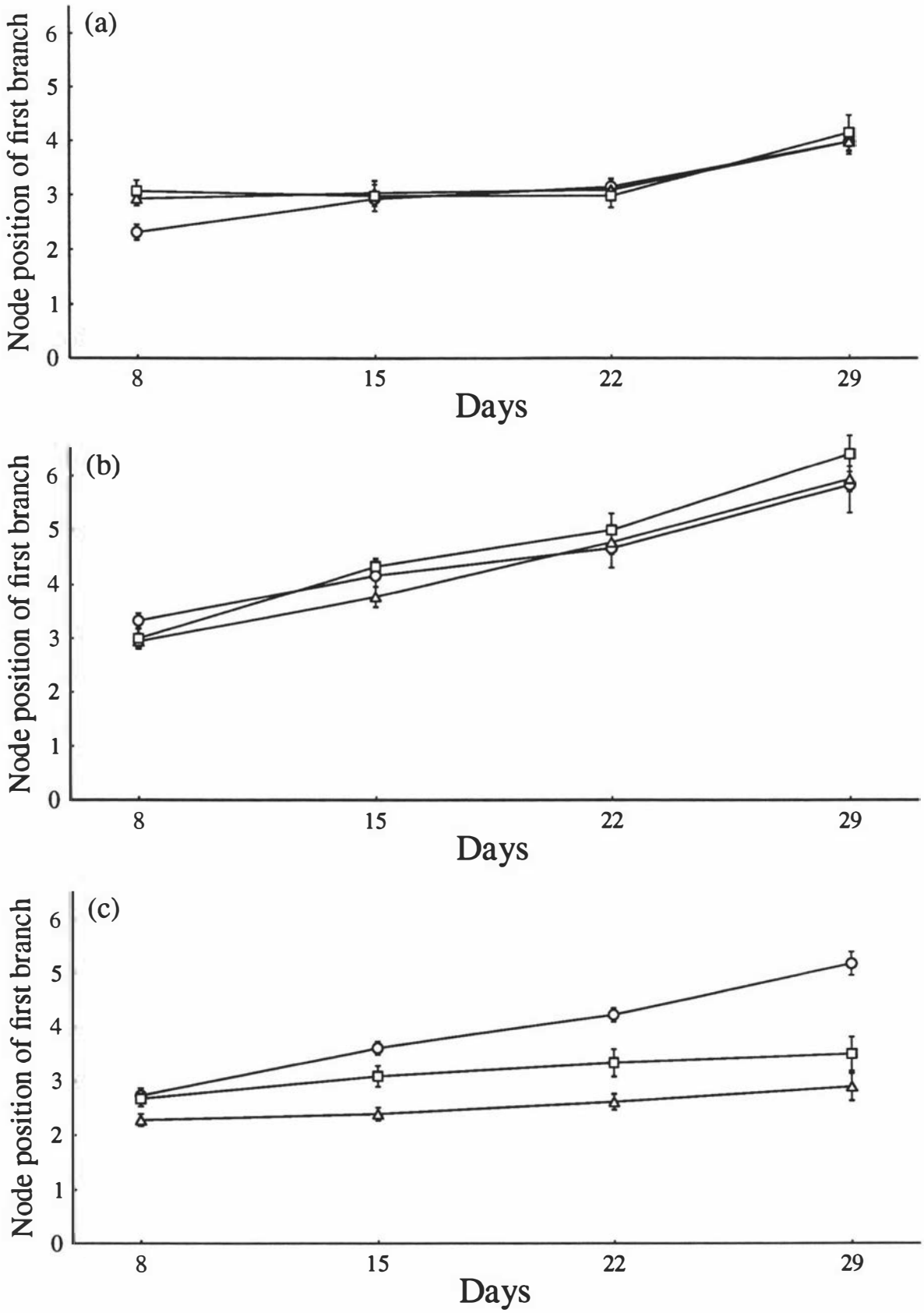


Figure 5.4 Effect of defoliation treatments, undefoliated (o), leaflets defoliated (▵) and leaflets plus petioles defoliated (□), within each of the shade treatments, (a) 100% sunlight, (b) 20% sunlight and (c) 5% sunlight, on position of first branching node relative to the apex of the main stolon at the end of each of four growth intervals. (Error bars indicate ± SEM.)

Table 5.3 Main effects of cultivar, shade and defoliation treatments, measured at harvest, on petiole length and area per leaf of main stolon leaves, mean length of main stolon internodes and leaf: stolon dry weight ratio of plant material grown during the experimental period. Main effects of shade and defoliation treatments on number of nodes produced per plant over the experimental period, meaned over cultivars are also provided. F test values and level of significance are given.

Plant Characteristic	Cultivar					Shade (% full sunlight)					Defoliation				
	Kopu	Pitau	Tahora	F	P	100	20	5	F	P	None	Leaflets	Leaflets + petioles	F	P
Petiole length (cm)	13.4	12.2	11.0	6.3	.003	10.3	13.6	12.8	29.4	.0001	14.8	10.5	9.9	74.0	.0001
Leaf area (cm^{-2})	8.8	5.6	3.3	74.1	.0001	6.4	6.5	3.9	26.1	.0001	7.1	5.0	4.7	33.3	.0001
Internode length (cm)	1.71	1.63	1.56	1.9	ns	2.06	1.56	1.00	37.4	.0001	2.03	1.42	1.24	36.3	.0001
Leaf:stolon ratio	5.23	6.05	6.37	1.2	ns	3.31	6.39	9.72	16.4	.0001	5.98	5.32	6.19	0.7	ns
Total nodes produced	Not applicable					32.2	8.9	4.0	156.3	.0001	18.9	14.8	15.1	9.4	.001

most severe shade treatment (5% sunlight) but not at all by the 20% sunlight treatment in comparison with the unshaded treatment. Increasing the severity of shade treatment progressively reduced mean internode length but progressively increased whole plant leaf to stolon DW ratio.

Both the L and the L+P defoliation treatments significantly and similarly reduced petiole length and leaf area of main stolon leaves and internode length on the main stolon as compared to the undefoliated treatment; leaf to stolon DW ratio of plants was not influenced by defoliation treatment.

5.3.2 PHOSPHORUS SUPPLY EXPERIMENT

5.3.2.1 NODE APPEARANCE RATE

The main effects of genotype, P supply and growth interval on number of nodes produced per main stolon apex per day were all highly significant (Table 5.4) but there were no significant interactions. However node appearance rates did not differ significantly with P supply until the second growth interval. Node appearance rate increased to a maximum for the second growth interval and then declined during the remainder of the experiment. This decline corresponded with a 2 h decrease in daylength and associated reductions in mean daily temperature. The irregularity of this trend over the fifth/sixth growth intervals could not be related with unusual fluctuations in glasshouse temperatures.

The difference in mean node appearance rate at the main stolon apex between genotypes was of the same order as the difference between values at 1 *mM* and 0.01 *mM* P supply (Table 5.4).

Table 5.4 Main effects of genotype, phosphorus supply and growth interval on node appearance rate (nodes produced, main stolon apex^{-1} d^{-1}) of the main stolon of white clover plants of the P supply experiment. F test value and level of significance are presented.

Genotype		P supply		Growth Interval	
1	0.272	0.01 *mM*	0.193	1	0.227
2	0.206	0.2 *mM*	0.244	2	0.293
		1.0 *mM*	0.279	3	0.266
				4	0.236
				5	0.200
				6	0.234
				7	0.214
F	77.2		44.0		7.2
P	<0.0001		<0.0001		<0.0001

5.3.2.2 POSITION OF FIRST BRANCHING NODE

For position of first branching node relative to the apex of the main stolon, analysis indicated that as well as highly significant (P<0.0001) main effects of genotype, P supply and growth interval there were also highly significant interactions.

The genotype by P supply interaction (P<0.0045) indicated that genotype 1 was more responsive for this characteristic as there was a 50% increase from the highest to lowest levels of P supply compared to a 25% change for genotype 2 (Fig 5.5). Genotype 1 had higher values than genotype 2 for this characteristic (P<0.0001).

The major feature of the P supply by growth interval interaction (P<0.0001) was the increase in separation in values for position of first branching node among P supply treatments as duration of treatment increased (Fig 5.6). Significant separation in values occurred at growth interval 2 (P<0.002) and subsequently. In particular, values increased for plants grown at the lowest level of P supply relative to those grown at the two higher levels of P supply. At the two higher levels of P supply the tendency for values to decrease towards the end of the experiment may have been related to the decline in daylength and the general environment for growth. The second order, genotype by P supply by growth interval, interaction was not significant.

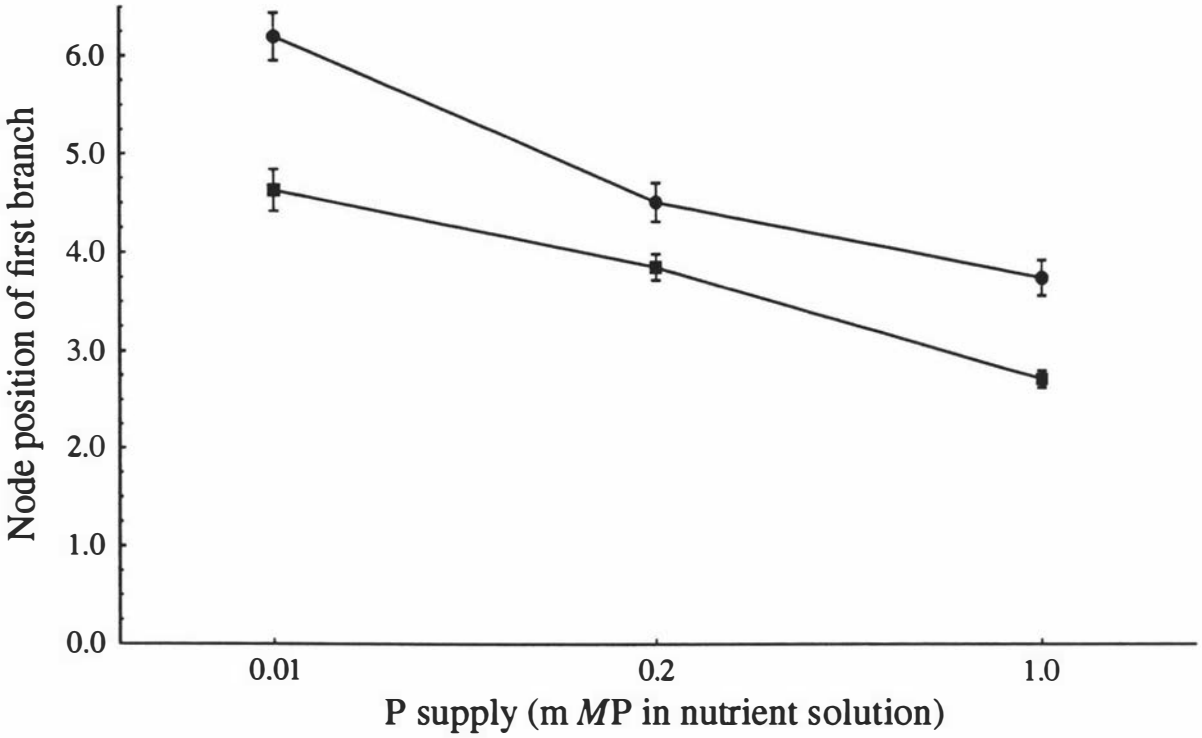


Figure 5.5 Effect of level of phosphorus supply on the position of the first branching node (relative to the stolon apex) on the main stolon of each of two Grasslands Tahora genotypes; genotype 1 (●), genotype 2 (■). Data is the mean of all growth intervals. (Error bars indicate ± SEM.)

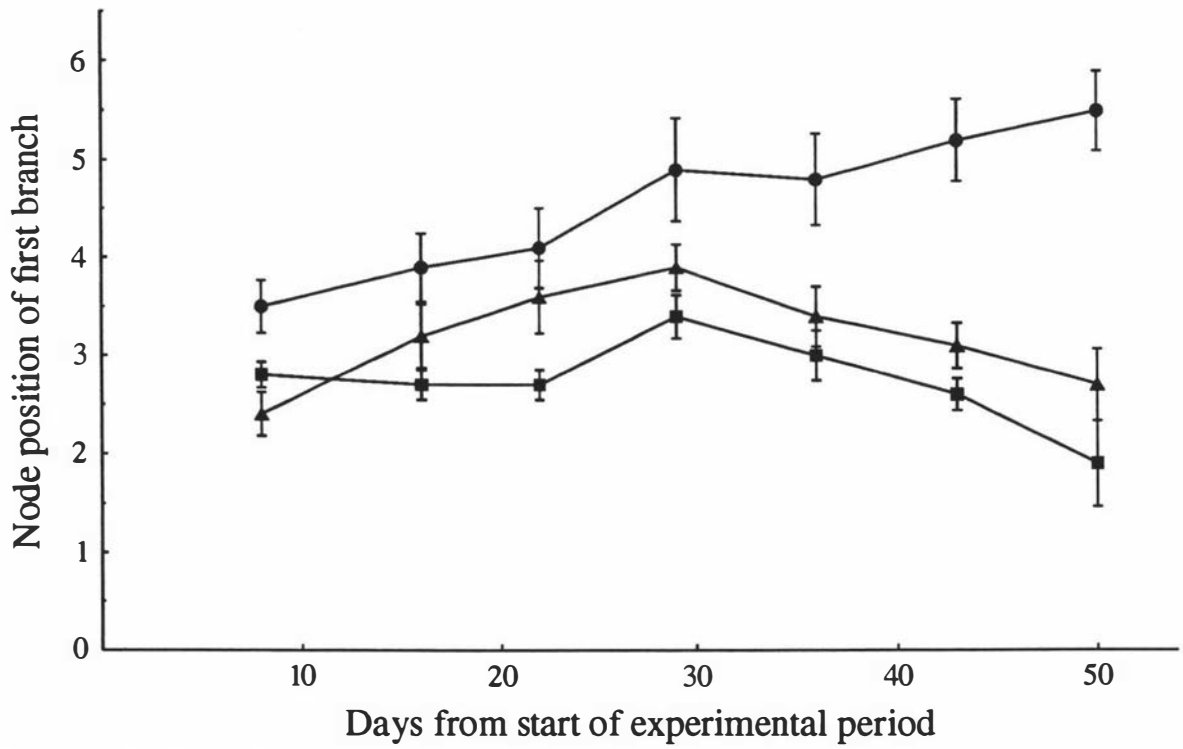


Figure 5.6 Effect of three levels of phosphorus supply, 0.01 m *M* P (●), 0.2 m *M* P (▲) and 1.0 m *M* P (■), on position of the first node with a branch (relative to the stolon apex) on the main stolon at each of seven growth intervals during the experiment. Data is the mean of both genotypes. (Error bars indicate ± SEM.)

5.3.2.3 PROBABILITY OF INITIATION OF OUTGROWTH OF AXILLARY BUDS

The probability of outgrowth of axillary buds at nodes proximal to (and inclusive of) the first branching node on the main stolon was not significantly influenced by any of the variables in this experiment. Initiation of outgrowth occurred at almost every node (overall mean probability was 0.965 ± 0.0058).

5.3.2.4 PLANT CHARACTERISTICS AT HARVEST

The effects of treatments on the selected plant characteristics of plants at harvest are presented in Table 5.5. Significant genotypic differences were recorded for the leaf characteristics of petiole length and area per leaf, stolon diameter with greater diameter associated with the larger leaved genotype 2, number of branches on the main stolon and total number of nodes per plant. Dry matter yields, mean internode length and P content of leaf and stolon did not differ between genotypes. All characteristics except stolon diameter increased with increasing P supply.

The genotype by P supply interaction was significant only for two characteristics, area per leaf and total node number per plant. The significance of the interaction for area per leaf was due to differential increases of 50 and 100% in values at 0.2 *mM* and 1.0 *mM* P supply for genotypes 1 and 2, respectively. The significance of the interaction for total nodes per plant reflected the larger percentage increase in values at 0.2 *mM* P supply for genotype 1 than for genotype 2.

5.4 DISCUSSION

The objective of the reported experiments was to further understanding of the effects of deprivation of resources, in particular photosynthate and P supply, on branch initiation in white clover. This has relevance as regards the effect of stolon burial on branching as at the time of grazing, defoliation and burial can occur simultaneously and act to substantially reduce the availability of photosynthates within plants. Also as supply of P is suboptimal in many New Zealand pastures the effects of deprivation of P supply were studied to establish if the directions of the branching responses to both resources were similar.

Table 5.5 Mean values of specified plant characteristics of two genotypes of Grasslands Tahora white clover grown at three levels of phosphorus supply as measured at harvest. F test values and significance are given for the main effects for genotype (G) and phosphorus supply (P) and for the genotype by phosphorus supply interaction (GxP) for each characteristic.

Plant Characteristic	Phosphorus Supply Treatment								
	0.01 mM		0.2 mM		1.0 mM		Significance levels		
	Genotype		Genotype		Genotype		G	P	GxP
	1	2	1	2	1	2			
First unfolded leaf Petiole length (cm)	4.3	4.6	7.0	8.3	9.3	11.3	F 9.3 P < 0.006	68.1 0.0001	1.9 ns
Area leaf^{-1} (cm^2)	1.08	1.43	2.08	3.16	2.95	6.31	F 14.1 P < 0.001	16.2 0.0001	4.5* 0.03
Stolon diameter proximal to first unfolded leaf (mm)	1.65	1.85	1.67	1.80	1.67	2.07	F 21.2 P < 0.0001	2.8 ns	2.2 ns
Internode length main stolon (cm)	1.25	1.13	1.47	1.59	1.69	2.14	F 3.3 P < ns	23.5 0.0001	1.4 ns
No. branches on main stolon	6.8	3.4	12.0	7.2	13.2	10.8	F 61.7 P < 0.0001	80.9 0.0001	2.4 ns
No. nodes plant^{-1}	29.0	17.6	66.6	33.8	91.6	67.0	F 63.0 P < 0.0001	125.3 0.0001	4.7 0.02
Dry Weight plant^{-1} (g)	0.139	0.175	0.555	0.438	1.496	1.975	F 1.2	62.5	2.2
Leaf							P ns	0.0001	ns
	0.088	0.085	0.264	0.202	0.721	0.992	F 1.0	44.8	2.1
Stolon							P < ns	0.0001	ns
Total	0.226	0.260	0.819	0.640	2.217	2.9	F 1.1	56.0	2.2
							P < ns	0.0001	ns
P content (% of DW)	.14	.13	.17	.19	.32	.33	F 0.6	62.1	0.4
Leaf							P < ns	0.0001	ns
Stolon	.14	.11	.15	.16	.26	.27	F 1.8	47.3	0.9
							P ns	0.0001	ns

This discussion consists of four sections followed by a conclusion. Firstly, aspects relating to the scope of interpretation of the study are considered before the second section discusses the effects of photosynthate and P supply on the plant attributes that determine branching of white clover. The third section discusses the effects of deprivation of photosynthate and P on the allocation of resources to metamers and to organs within metamers. Prior to the conclusion, the fourth section comments on the integration of clonal growth in relation to branching.

5.4.1 DELIMITATION OF INTERPRETATION

The study was not undertaken with the purpose of assessing the extent of genetic variation in response but included known genetic variation within experiments so as to ensure that the principles of response were more general to white clover than that specific to one genotype cloned for glasshouse experimentation. Hence experiments were inclusive of elements of genetic variation only for the purpose of monitoring for the possibility of large genetic influences on either the direction or the scale of responses to treatments. Within these constraints genotype was not found to significantly alter the direction or scale of branching responses to shade or defoliation but to influence the mode of response to limitation in P supply. Although genotypic variation in node appearance rate (Burdon & Harper 1980; Caradus & Chapman 1991) and position of first branching node (Erith 1924; Caradus & Chapman 1991; Turkington *et al.* 1991) has been established, the absolute values of these characteristics are dominated by environmental conditions in any given situation (Sackville Hamilton & Harper 1989) and this proved to be the case within the shade and defoliation experiment. However under the conditions of the P supply experiment significant and consistent genotypic differences were obtained for node appearance rate and position of first branching node (Table 5.4, Fig 5.5) although the direction response was the same for both genotypes.

Understanding of branching responses of plants requires information at the individual axillary bud level, rather than at the developed meristem level (Harper 1977; Sackville Hamilton & Harper 1989; Hay *et al.* 1991; Newton *et al.* 1992). Thus as there is an axillary bud at each node, the demography of nodes becomes an issue that is involved in the assessment of branching responses of plants to treatments. As, in these short-term glasshouse experiments, there was no plant fragmentation the response to imposed treatments can be described by the simple model proposed by Turkington *et al.* (1991); the

number of nodes per plant is a function of node appearance rate, position of first branching node (relative to the stolon apex) and frequency of branching at nodes proximal to the first branched node. These parameters define the recruitment and activity of axillary buds on a stolon basis which when summed over stolons provides whole plant data. However, the short-term nature of the experiments reported means that analysis has necessarily been restricted to the main stolon of plants. It is recognised that this may underrepresent the longer-term branching response of the plant to treatments as branching performance of secondary branches can be affected to a greater extent than the main stolon under some situations (King *et al.* 1978; Grant et al. 1991; see also Chapter 6). An advantage of use of the three parameters cited by Turkington *et al.* (1991) to assess branching response is that assessment is non-destructive which means that assessments can be repeated throughout an experiment and so allow identification of when a particular response occurs.

Defoliation treatments were applied as a single event at commencement of the experimental period. Thus the response to defoliation during the course of the experiment was one of recovery and so differed fundamentally from the response to shade treatments which imposed a regime of increasing intensity of deprivation of photosynthate. Hence the previously observed depressive effects of defoliation on node appearance rate (Mitchell 1956; Carlson 1966b; Sanderson 1966; King *et al.* 1978; Sackville Hamilton & Harper 1989; Grant *et al.* 1991) were most evident early in the experiment (Fig. 5.2). The major effect was an immediate halving of node appearance rate in the growth interval following defoliation; an effect which, for unshaded plants, had disappeared by growth period three (Fig. 5.2). A probable mechanistic explanation is that defoliation reduces photosynthate production and hence supply of carbon for new growth. The plant adapts by remobilizing photosynthate stored as starch in root and stolon (Moran *et al.* 1953; Vez 1961; Murphy 1982; see Sections 7.3.4 and 7.4 for description of starch depletion and replenishment in stolon following defoliation). However the rate of remobilization is insufficient to prevent a penalisation of new growth through reductions in leaf (Carlson 1966b) and internode (Thomas 1987b) dry weight and node appearance rate. However defoliation had a quantitatively small effect on position of first branching node which did not alter significantly with growth period, and indicated that large changes in this characteristic were associated with variation in the light rather than the defoliation environment (Fig. 5.4).

5.4.2 BRANCHING

In general, branching responses to deprivation of light (shade treatments) or P supply were similar. In both cases the largest response was a two- to three-fold increase in the number of nodes between the stolon apex and the first node with a branch. This is commonly referred to as an increase in apical dominance (Phillips 1975; Cline 1991; Murphy & Briske 1992) and such responses have often been noted in white clover in response to shade and low nutrient supply (Harvey 1979; Newton 1986; Thomas 1987b; Davies & Evans 1990a; Caradus & Chapman 1991). The lack of an increase in apical dominance in plants of the 5% sunlight treatment, relative to the 100% sunlight treatment, was due, at least in part, to the severe reduction in node appearance rate that occurred in these plants which limited their potential to express a response within the timeframe of the experiment. In addition there were reductions in node appearance rate by two thirds under the severe (5% sunlight) treatment, one third under the 20% sunlight and one quarter under the 0.01 *mM* P supply treatments (Tables 5.1, 5.4). As environmental factors other than temperature tend to have small effects on node appearance rate (Mitchell 1956; Sanderson 1966; King *et al.* 1978; Harvey 1979; Chapman 1983; Sackville Hamilton & Harper 1989; Caradus & Chapman 1991; Caradus *et al.* 1993) these results indicate that the imposed treatments were severely limiting resources within plants. Despite the severe consequences of deprivation of resources (reductions of > 90% in shoot DW or 75% in total node number per plant) none of the factors examined (shading, defoliation, P supply or burial (Appendix 5.1)) significantly reduced the probability of an axillary bud initiating outgrowth. This probability was calculated by dividing the number of branched nodes on a stolon by the total number of nodes proximal (but including) the first branching node proximal to the stolon apex. Such calculation excludes nodes that are ontogenetically without potential to branch (i.e., those in positions under apical dominance, such nodes are accounted for by the characteristic, position of first branching node) and gives further precise information on the branching activity of axillary buds. Under the conditions of these experiments, the severe treatments imposed did not significantly reduce the probability of initiation of outgrowth, i.e., all axillary buds eventually initiated outgrowth. These results suggest two interesting corollaries; 1) that resource levels within the plant have a minor influence on the viability of axillary buds and 2) that factors other than resource levels within plants must determine the reduced level of branching usually observed in field plants. These issues have not been clearly observed before because of the previous tendency to calculate the percentage of branching across all nodes, therefore confounding results by the inclusion

of nodes influenced by apical dominance thereby lowering values whenever treatments enhance apical dominance. For instance, Caradus & Chapman (1991) report shading (50% of sunlight) increased the position of the first branching node some 20% and decreased the percentage of nodes branching 67%. However recalculation of their data indicates that the decrease in percentage of nodes branching in shaded stolons can be wholly accounted for by the change in position of first branching node and the fact that unshaded plants produced more nodes for inclusion in the calculation.

The changes in branching form of plants with treatment in both experiments resulted solely from the effects of deprivation of resource acting to delay the outgrowth of axillary buds until they were at nodes positioned up to six nodes proximal to the stolon apex and from decreases in node appearance rate. As under these experimental conditions, virtually all (96+%) axillary buds eventually initiated outgrowth, probability of initiation of axillary bud outgrowth was not a factor contributing to the branching form or total node number of plants.

During the first two growth periods in both experiments node appearance rate of the designated unrestricted supply treatment increased (Figs. 5.1, 5.3). In both experiments an ontogenetic effect would contribute to this result because plants were established from stolon cuttings comprised of only two unfolded leaves plus the apex and node appearance would reach maximum rates only after cuttings had established effective branches contributing photosynthate to the main stolon (Chapman *et al.* 1992b). In the P supply experiment, this ontogenetic effect probably accounted for the initial slight increase in node appearance rate. However the considerably larger early response in node appearance rate observed in unshaded plants in the shade and defoliation experiment may also reflect the potential of these plants to respond to the rapidly improving growth conditions in late September - early October, a potential that was unrealised in shaded plants. The P supply experiment was conducted from 25 February to 16 April when growth conditions (temperature, light intensity and photoperiod) were declining rapidly as the experimental period straddled the autumnal equinox. This was reflected in a progressive decline in node appearance rate after the third week of the experiment regardless of P supply and may have been a factor contributing to the tendency for position of first branching node to decrease over the latter stages of the experiment at the two higher rates of P supply (Fig. 5.6).

Plants have ability, conferred via the photoreceptor phytochrome, to sense and alter morphology in response to changes in the red: far-red ratio (R:FR) of light even though total photosynthetically active radiation (PAR) remains unchanged (Ballaré *et al.* 1987, 1990). A decrease in the R:FR ratio of light incident just at the main stolon apex of white clover plants, along with no change in PAR, induced an increase in the position of the first branching node on the main stolon (Robin *et al.* 1994). This is evidence that in white clover initiation of branching is responsive to an external environmental signal even when resources within the plant remain unaltered at adequate levels. However in both experiments of this study differences among treatments for position of first branching node and to some extent for node appearance rate (shade experiment) increased over the course of the experiment. Such results suggest that the response pattern is driven by resource availability within the plant rather than by sensing of changes in the external environment. It has been previously postulated that reduction in availability of any resource at the apex of stolons enhances the expression of apical dominance in white clover (Harvey 1979; Newton 1986; Thomas 1987b); the results of these experiments do not contradict this suggestion.

5.4.3 ALLOCATION OF RESOURCES TO AND WITHIN METAMERS

The factors considered thus far, node appearance rate, position of first branching node and probability of branching, in combination determine the number of metamers (nodes) produced per stolon per unit time. However another important dimension of morphological response to deprivation of resource is modification of the size of metamers and relative changes in the size of organs (leaf, internode, root, axillary bud) within metamers (Tables 5.3 & 5.5). With less severe deprivation, adjustment occurs by preferential allocation of resource to organs that capture or maintain the scarce plant resource (Chapin 1980). In white clover, this response gives rise to well documented increases in root to shoot dry weight ratio with limitation of nutrient supply (see review by Caradus 1990), increases in petiole length and leaf area of leaflets with limiting light supply (see Hart 1987) and changes in leaf to stolon dry weight ratio to equilibrate with imposed defoliation regime (Briseno de la Hoz & Wilman 1981; Wilman & Asiegbu 1982b; Horikawa 1986b; Brock *et al.* 1988). The response patterns within these experiments were consistent with the above described principles in relation to shade, defoliation and nutrient supply treatments. As root dry weight was not measured, root:shoot ratios could not be ascertained in either experiment, however, as would be

predicted, leaf:stolon ratios increased three fold with increasing shade (Table 5.3) but remained constant when P supply decreased even though metamer size decreased (Table 5.5).

It is the ability of white clover to significantly modify both the number and size of metamers in response to available plant resources that confers the species with a high ranking for phenotypic plasticity (Hill 1977; Brougham *et al.* 1978; Forde *et al.* 1989; Caradus *et al.* 1993). This potential to adjust phenotype to the environment greatly assists the species to achieve a wide ranging colonization of habitats within the temperate zone of the world (Williams 1987) and is also a characteristic that contributes substantially to the robust success of the species as a forage legume (Forde *et al.* 1989).

Shoot dry weight per plant is a morphological characteristic that reflects the combination of responses of metamer number and size to treatments. Comparison of results from the P supply experiment well illustrate this principle. Comparison of the two genotypes involved indicates that as compared with genotype 2, genotype 1 had a greater node appearance rate, a more marked responsiveness of position of first branching node but smaller sized metamers. On the other hand genotype 2 produced fewer but larger metamers and responded to limitation of P supply through reducing the size of metamers rather than greatly changing the position of first branching node. The end result was that leaf, stolon and total shoot dry weight per plant were all similar for both genotypes at each level of P supply (Table 5.5).

The pattern of response of different plant characteristics of white clover to the shade levels selected differed. For instance at the end of the experiment, whereas internode length decreased with increasing shade level, petiole length was greatest at 20% sunlight and least at 100% and 5% sunlight and leaf area per leaf was similar at 100% and 20% sunlight but was reduced (40%) at 5% sunlight. Differing patterns of non-linear response to decreasing percentages of PAR have previously been reported for internode and petiole length and the patterns shown to differ between genotypes (Thompson 1993b). Such differences in non-linearity of response between plant characteristics reflect the balance between the changes in intraplant allocation as a resource becomes scarce, the relative sink strength of the region associated with a particular characteristic and changes in the total amount of resource available for allocation.

5.4.4 INTEGRATION OF CLONAL GROWTH

In both experiments plants were grown individually, so were uninfluenced by intra- or inter-specific competition for space or resource and any limitation of resource was applied to the whole plant. Thus the responses measured at both the plant and organ level could have represented either a highly integrated response mediated by 'global' sensing by the plant of the resource level or a non-integrated response which was the sum of similar but independently controlled responses mediated by sensing of resource level at a local level within the plant. Turkington *et al.* (1991) suggest that the activity of all apices (i.e., node appearance rate) within a clonal fragment is integrated whereas growth of individual metamers (i.e., dimensions of leaf and internode and axillary bud and root primordia activity) is not, being instead, responsive to local conditions at the site of the metamer. In pastures there is typically marked heterogeneity at the microsite level so that different portions of a clonal fragment (plant) may encounter a range of resource levels. One factor contributing to such microsite heterogeneity is burial of portions of the stolon system of plants (Forde *et al.* 1989; Sackville Hamilton & Harper 1989). In a small glasshouse experiment (see Appendix 5.1) the resources available to different branch stolons of a single plant were altered by imposing combinations of burial and defoliation treatments on individual stolons. Node appearance rate ($P<0.003$) and position of first branching node ($P<0.012$) were found to differ significantly among stolons of the same plant in response to burial but not with defoliation. Treatments had no significant effect on the probability of an axillary bud branching as virtually all buds initiated outgrowth. Both these results conflict with the theoretical framework of clonal integration within white clover put forward by Turkington *et al.* (1991). A difference between this experiment and that of Turkington *et al.* (1991) is that in the latter case the main stolon was maintained intact whereas in this experiment the main stolon was cut so that the apical region including the apex was removed. It is possible that presence of the main stolon apex is a prerequisite for the integration of apical activity within a plant. However while the model describing clonal integration within white clover proposed by Turkington *et al.* (1991) provides an adequate basis for accounting for observed plant responses in some situations (i.e., biotic patchiness) the results obtained in these experiments indicate the model does not have universal applicability. In fact these results suggest that stolons within plants are responsive to the microenvironment at the stolon and that there is no greater integration of growth of apices than there is of growth of individual metamers.

5.4.5 CONCLUSIONS

In conclusion, the results presented in this chapter confirm that deprivation of resource (light or phosphorus) has an important depressive effect on branching of white clover. The new information is the clear result that such resource deprivation, despite inducing large changes in the pattern of allocation of resources to plant organs, has a minor influence on the probability of an axillary bud initiating outgrowth but acts to reduce branching by very significantly delaying the outgrowth of buds. Not only is the position of the first branching node, relative to the stolon apex, increased two- to three-fold but node appearance rate is also significantly decreased and these factors combine to reduce the number of branches produced. The results reported relate solely to effects of treatments on the initiation of outgrowth of axillary buds; the short-term nature of these experiments precluded examination of survival of branches following initiation. These results have implications regarding the effect of burial of stolons in that the relatively minor costs to the plant of loss of photosynthetic capacity and increased energy costs associated with thigmomorphogenetic responses upon burial should have minor effects on the probability of initiation of outgrowth of axillary buds. Indeed the glasshouse trial (Appendix 5.1) found stolon burial to have no effect on the probability of initiation of outgrowth of axillary buds, a result similar to that previously reported in a field experiment (Chapter 4).

CHAPTER 6 EFFECT OF BURIAL OF NODES OF DIFFERENT POSITION WITHIN STOLONS ON THE PROBABILITY OF INITIATION OF AXILLARY BUD OUTGROWTH

6.1 INTRODUCTION

Results obtained in Chapter 4, when stolons received burial treatments in the field, showed there was an overall depressive effect of burial on branch presence which was attributed to a reduction in survival of young branches rather than to any reduction in the initiation of outgrowth of axillary buds. The effect of burial on branch presence was closely linked to ontogeny of axillary buds at the time of burial. In all the experiments in Chapter 4 burial had its most marked effect on the outgrowth of axillary buds at nodes positioned one or two from the stolon apex at time of burial whereas for nodes positioned further than three from the apex, results were more variable. However, these experiments only examined the response to burial of axillary buds at nodes that had emerged from the stolon apex on the soil surface before they were buried. Measurement of field populations show that presence of incipient branches (defined as the stage when the first leaf from axillary buds is within the 0.1-0.9 developmental stages of the Carlson (1966a) scale) is one third at node position 2 that of the maximal values occurring at node position 4 (Newton *et al.* 1992). This suggests that factors influencing the initiation of outgrowth of axillary buds probably exert an influence early in the development of a node. Thus there is a requirement for more detailed experimentation conducted under controlled conditions to examine the effect of burial, at early stages of axillary bud ontogeny (inclusive of bud development within the stolon apex) through to more mature stages (buds at nodes positioned six to eight from the stolon apex), on the initiation of outgrowth of axillary buds. In conjunction with this experimentation it is possible to obtain information as to whether burial limits outgrowth of axillary buds via inhibition mechanisms or through decreasing the viability of buds by imposing additional treatments and recording extra measurements.

This chapter will report on an experiment investigating the effect of period of burial and ontogeny of axillary buds at burial on the initiation of outgrowth of axillary buds, especially those buds at nodes recently emerged from the apex (< three nodes from the apex). The assumption that the major influence of burial on initiation of axillary bud

outgrowth was through its effect on the light environment at bud sites was implicit in the design and interpretation of results of this experiment.

6.2 METHODS AND MATERIALS

6.2.1 PLANT MATERIAL AND GROWTH MEDIUM

Stolon cuttings consisting of an apex and two nodes with subtending leaves of development >0.5 (Carlson (1966a) scale) were taken from a single genotype of Grasslands Kopu white clover. One stolon cutting was planted, at each end of 32 plastic trays (40 x 30 x 5 cm), into potting mix (Section 5.2.3.1) on 21 February 1989. Cuttings were allowed to establish for 22 days and had either three or four leaves by the time the experimental treatments were applied on 15 March 1989.

6.2.2 GROWTH CONDITIONS

The experiment was conducted in a glasshouse with thermostatically activated heating and cooling systems from 15 March through to 18 May 1989. During this period daylength decreased from 12 h 23 min in mid-March to 9 h 23 min in mid-May. At Palmerston North, the maximum recorded daily light intensity decreases from March to May from 1710 to 912 μM photons $m^{-2}s^{-1}$, respectively and mean daily light intensity from 1250 to 540 μM photons $m^{-2}s^{-1}$ (D.H. Greer, pers. comm.).

Mean daily maximum and minimum air temperatures in the glasshouse over the trial period in March, April and May were 30.1 ± 3.06°C and 15.0 ± 2.31°C, 29.4 ± 2.06°C and 14.4 ± 1.01°C and 26.3 ± 3.12°C and 13.3 ± 1.93°C respectively.

On 15 March 1989 when treatments were applied, all plants, except those of the undefoliated treatments, had leaves defoliated (petioles were cut within 2 mm of the stolon) such that each stolon had a maximum of two fully unfolded leaves plus any developing leaves. Throughout the trial stolons were maintained in this state by removing leaves as necessary.

6.2.3 EXPERIMENTAL

Burial treatments were applied to produce axillary buds of four differing backgrounds:

(1) axillary buds that emerge (from the apex) and then develop in light,

(2) axillary buds that emerge in light and then develop in dark,

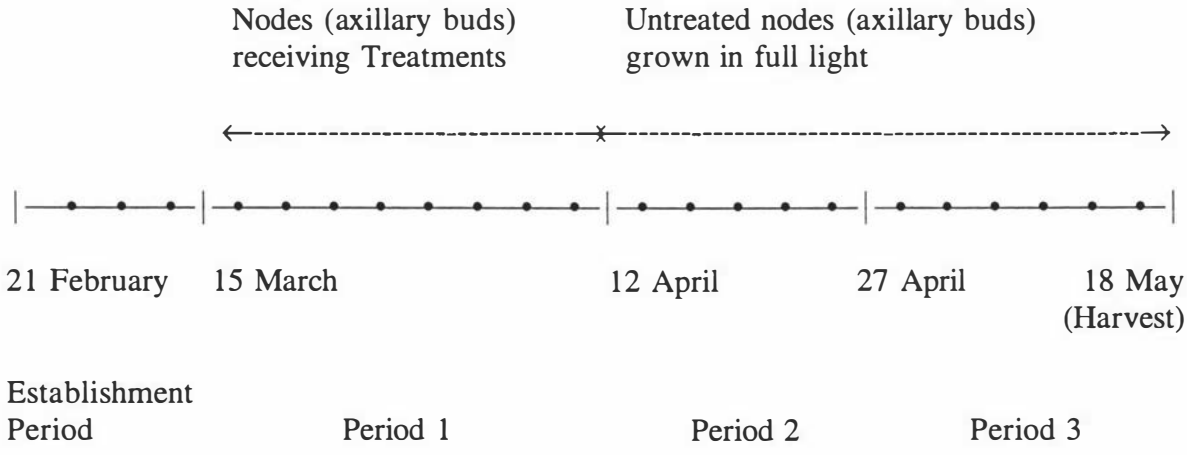
(3) axillary buds that emerge in dark and then develop in light,

(4) axillary buds that emerge and then develop in dark.

These four backgrounds were incorporated in the development of the eight treatments imposed and tested in the trial.

6.2.3.1 TREATMENTS

The trial consisted of three distinct time periods as indicated schematically below. These periods (1-3) corresponded with successive intervals which allowed the emergence of approximately eight, five and six nodes, respectively, on the experimental stolons.



The line above depicts the primary stolon at harvest (18 May) with the solid circles representing nodes (with associated axillary buds) emerging from the stolon apex during each of the time periods. Only the eight or so nodes (including the axillary buds thereof) that emerged during Period 1, that is between 15 March and 12 April, received treatments. Treatments were applied to these nodes during both Period 1 and Period 2. All nodes (axillary buds) that emerged after 12 April (i.e. during Periods 2 and 3), were untreated and grew normally on the soil surface in full light.

On 15 March 1989 the tips of stolons were either buried with 2 cm of potting mix or not buried. Where necessary during Period 1 stolons were restrained with staples to ensure they remained buried to the desired depth. On 12 April, stolons were tagged just forward of the first (youngest) node differentiated from the apex. Stolons that had received the burial treatment during Period 1 had those nodes formed in Period 1 either kept buried or uncovered for Period 2. Similarly for stolons not buried during Period 1, the nodes formed during Period 1 were either buried or not for Period 2.

At the end of Period 2 on 27 April, stolons were tagged just forward of the youngest emerged node. Then the potting mix was carefully scraped away to uncover those stolon segments which had received burial treatments during Period 2. At this stage all stolons were completely uncovered. Stolons were then grown on during Period 3, until 18 May, to provide an opportunity for treated axillary nodes to develop. On the 18 May stolons were harvested.

This gave four basic burial treatments of nodes (axillary buds) emerging in Period 1 during Periods 1 and 2:

Treatment 1, axillary buds emerged and developed in light (Periods 1 and 2), designated O/O;

Treatment 2, axillary buds emerged in light (Period 1) and developed in dark (Period 2), designated O/B;

Treatment 3, axillary buds emerged in dark (Period 1) and developed in light (Period 2), designated B/O;

Treatment 4, axillary buds emerged and developed in dark (Periods 1 and 2), designated B/B.

In addition, the following treatments were included in the trial to assist interpretation of results in relation to the growth of white clover in grazed pastures:

Treatment 5, polythene covering. The treated portion of stolon was buried with 2 cm of potting mix and then covered with black polythene to ensure the complete exclusion of

light from the buried portion of stolon. Developing leaves were given access through the polythene. The stolon portion emerging in Period 1 was buried for both Periods 1 and 2 and the treatment designated B/B P.

Treatment 6, excision of stolon apex. The apex was excised, by cutting just forward of the first emerged node from the apex on 12 April (i.e. at the end of Period 1), from stolons which received burial treatments over both Periods 1 and 2. This treatment was designated B/B EA.

Treatment 7, undefoliated O/B treated stolons. This treatment was included for comparison with the standard defoliation regime (O/B) and was designated O/B U.

Treatment 8, undefoliated B/O treated stolons. This treatment was included for comparison with the standard defoliation regime (B/O) and was designated B/O U.

The treatments are outlined in Table 6.1.

6.2.3.2 DESIGN

On 15 March 1989 plants were numbered and the eight treatments (Table 6.1) were randomly allocated to plants irrespective of the tray they were in. Each treatment was replicated eight times giving 64 plants which were grown two per tray (Plate 6.1). Trial layout was randomised by blocks (trays) and, twice weekly, systematic movement of trays minimised glasshouse positional effects.

6.2.4 MEASUREMENTS

At harvest each plant had the outgrowth of each axillary bud assessed as previously described (Section 4.2.3.1). Then plants were dissected such that the primary stolon was divided into those nodes that emerged in each of the three periods, with any branch formed from an axillary bud included with the node of origin. The lengths of the primary and branch stolons and the diameter of the central internode of the primary stolon that had emerged in each of the three time periods were measured before plant material was further dissected into primary stolon and leaf and branch stolon and leaf for dry weight determination of each component.

Table 6.1 A presentation of the treatments, design and treatment contrasts of the glasshouse experiment investigating burial/cultural effects on initiation of outgrowth of axillary buds. (B = buried, O = not buried).

Burial treatments during periods		Presence of black polythene covering	Application of defoliation regime	Occurrence of excision of apex	Designated abbreviation for each treatment
1	**2**				
O	O	-	+	-	O/O
O	B	-	+	-	O/B
B	O	-	+	-	B/O
B	B	-	+	-	B/B
B	B	+	+	-	B/B P
B	B	-	+	+	B/B EA
O	B	-	-	-	O/B U
B	O	-	-	-	B/O U

Treatments were replicated eight times using an incomplete block design with two plants per tray (block) with a total of 32 trays.

Treatments in specific contrasts:

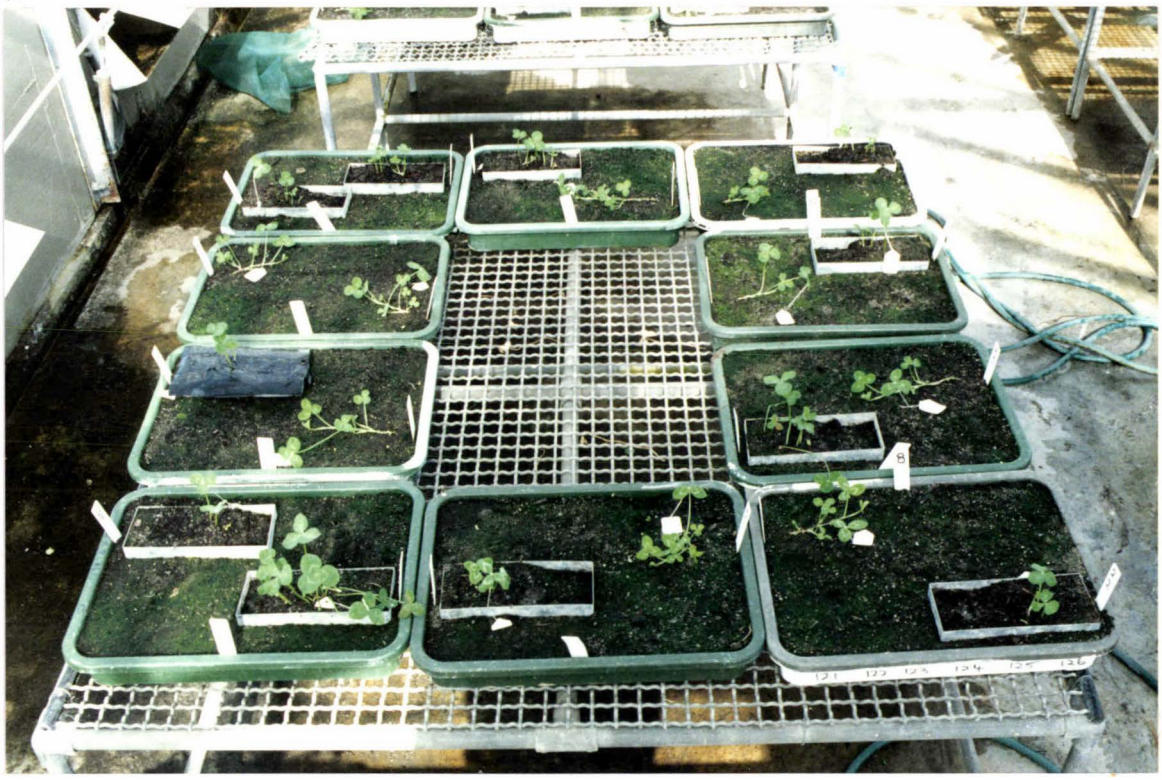
(1) Burial in Period 1 vs. no burial in Period 1
B/B, B/O, B/O U vs. O/B, O/B U, O/O

(2) Period 1 burial vs. Period 2 burial
B/O, B/O U vs. O/B, O/B U

(3) Defoliation vs. no defoliation
B/O, O/B vs. B/O U, O/B U

(4) Burial in Period 2 vs. no burial
O/B vs. O/O

(a)



(b)

Plate 6.1 Photographs taken during Period 1 illustrating (a) the layout of part of the trial within the glasshouse and imposition of the burial treatments, and (b) a close-up view of two undefoliated stolons, one of which was buried and the other which was subsequently buried during Period 2.

6.2.5 STATISTICAL ANALYSES

Analyses were performed using SAS (SAS Users Guide 1988). All data were analysed using the General Linear Models Procedure of SAS. Individual trays were treated as blocks and differences among treatments were tested by *t* tests. Where treatments with common elements were grouped, differences among groups were tested by the Contrast Option in the General Linear Models Procedure of SAS.

6.3 RESULTS

6.3.1 NODE APPEARANCE

The number of nodes appearing on the primary stolon of plants was significantly affected by treatment in Period 2 but not in Periods 1 and 3 (Table 6.2). In Period 2 treatment O/B U differed significantly from all other treatments except treatment O/B.

Table 6.2 Mean number of nodes ± SEM that appeared on the primary stolon of plants in each time period in each treatment. (Treatments as given in Table 6.1). Significance of treatment main effects are given.

Burial/cultural treatment	Experimental Time Period		
	1	**2**	**3**
O/O	8.50 ± 0.267	4.57 ± 0.297	5.86 ± 0.639
O/B	8.13 ± 0.227	5.13 ± 0.125	6.13 ± 0.599
B/O	8.14 ± 0.553	4.43 ± 0.297	6.00 ± 0.535
B/B	7.25 ± 0.313	4.50 ± 0.267	5.88 ± 1.166
B/B P	7.14 ± 0.769	4.14 ± 0.261	5.71 ± 0.452
B/B EA	8.43 ± 0.297	-	-
O/B U	9.50 ± 0.500	5.63 ± 0.375	6.38 ± 0.857
B/O U	9.00 ± 0.267	4.86 ± 0.261	6.14 ± 0.833
	ns (P<0.0929)	P<0.0104	

6.3.2 PROBABILITY OF BRANCHING AT PRIMARY STOLON NODES

The burial/cultural treatments significantly affected the probability of outgrowth of axillary buds on the primary stolon that emerged in both Period 1 and Period 2 (Table 6.3).

Table 6.3 The probability of axillary bud outgrowth from nodes that emerged on the primary stolon in each of the two treatment periods on stolons subjected to different burial/cultural treatments (refer to Table 6.1). Means and standard errors of means, significance of the treatment main effect and significance of specific treatment contrasts are presented.

	Period 1	Period 2
O/O	0.93 ± 0.032	0.80 ± 0.088
O/B	0.91 ± 0.029	0.85 ± 0.082
B/O	0.76 ± 0.028	0.55 ± 0.085
B/B	0.29 ± 0.105	0.76 ± 0.060
B/B P	0.32 ± 0.094	0.65 ± 0.109
B/B EA	0.73 ± 0.057	-
O/B U	0.97 ± 0.020	0.90 ± 0.052
B/O U	0.85 ± 0.028	0.75 ± 0.108
Specific contrasts:		
Period 1 burial vs. no burial	P<0.0001	P<0.0107
Period 1 vs Period 2 burial	P<0.0073	P<0.0008
Defoliation vs No defoliation	ns	ns
O/B vs. O/O	ns	ns

For axillary buds that had emerged during Period 1 the contrast between treatments with burial during the first period and treatments without (ie. B/B, B/O, B/O U vs. O/B, O/B U, O/O) was highly significant. With increasing severity of burial treatment, O/O, O/B, B/O and B/B the branching probabilities (0.93, 0.91, 0.76 and 0.29 respectively) significantly decreased ($P<0.05$) with each step except for the first step. For those stolons not buried during Period 1, burial treatments during Period 2 had no significant effect on the branching probabilities of the axillary buds that had emerged during Period 1.

The branching probability of axillary buds which emerged from the stolon apex during Period 2 was reduced ($P<0.0107$) for stolons which had been buried for Period 1 but was not affected by burial during Period 2.

Defoliation had no significant effect on the branching probabilities of axillary buds that emerged in either time period. However, whereas excision of the apex significantly ($P<0.0001$) increased the branching probability from 0.29 to 0.73 of axillary buds emerging in Period 1 and buried for both periods, covering with polythene had no significant effect (Table 6.3).

6.3.3 PROBABILITY OF BRANCHING AT SECONDARY STOLON NODES

The burial/cultural treatments imposed on stolons significantly ($P<0.0092$) affected the probability of axillary bud outgrowth from nodes on secondary stolons that originated from primary stolon nodes that had emerged during Period 1 (Table 6.4). There were insufficient numbers of axillary buds showing outgrowth on secondary stolons that originated from primary stolon nodes that had emerged during Period 2, to justify statistical analysis.

The contrast between treatments with burial and without burial during Period 1 (B/B, B/O, B/O U vs. O/B, O/B U, O/O) was significant (Table 6.4). Branching probabilities for the treatments B/B, O/B and B/O were not different although all were significantly less than for the O/O treatment. Defoliation regime had no influence on branching probabilities. Excision of the stolon apex had a positive and significant ($P<0.01$) effect on branching probabilities where the treated stolon segments were buried for both treatment periods.

Table 6.4 The probability of axillary bud outgrowth from nodes on secondary stolons originating from nodes that had emerged during Period 1 on stolons subjected to the different burial/cultural treatments described in Table 6.1. Means and standard errors of means, significance of the treatment main effect and significance of specific treatment contrasts are presented.

	Period 1
O/O	0.42 ± 0.060
O/B	0.18 ± 0.041
B/O	0.18 ± 0.069
B/B	0.09 ± 0.052
B/B P	0.02 ± 0.012
B/B EA	0.30 ± 0.034
O/B U	0.27 ± 0.057
B/O U	0.20 ± 0.057
Specific contrasts:	
Period 1 burial vs. no burial	P<0.0013
Period 1 vs Period 2 burial	ns
Defoliation vs No defoliation	ns
O/B vs. O/O	P<0.0187

6.3.4 MEAN TOTAL NUMBER OF BRANCH NODES PER PRIMARY STOLON NODE

For primary stolon nodes that emerged during each treatment period there was a significant (P<0.001) main effect of treatment on mean number of branch nodes per primary node (Table 6.5).

For primary stolon nodes that emerged during Period 1, burial during Period 1 depressed (P<0.0001) numbers of branch nodes per primary stolon node, although burial of these nodes during Period 2 had no significant effect (Table 6.5). Undefoliated stolons had more nodes per primary stolon than defoliated stolons. Excision of the stolon apex

significantly increased number of branch nodes per primary stolon node when treatment involved burial for both periods.

For primary stolon nodes that emerged during Period 2 burial of the treated nodes during Period 1 depressed number of branch nodes per primary stolon node whereas burial for Period 2 significantly (P<0.03) increased numbers of branch nodes (Table 6.5). Thus the contrast between treatments involving B/O and O/B was highly significant. Defoliation regime had no significant effect.

Table 6.5 Mean number of branch nodes per primary stolon node for primary stolon nodes that emerged during Periods 1 and 2 on stolons subjected to the burial/cultural treatments described in Table 6.1. Means and standard errors of means, significance of the treatment main effect and significance of specific treatment contrasts are presented.

	Period 1	Period 2
O/O	18.5 ± 1.86	2.9 ± 0.67
O/B	11.3 ± 1.34	3.2 ± 0.32
B/O	8.7 ± 1.78	1.7 ± 0.37
B/B	3.1 ± 0.90	3.0 ± 0.31
B/B P	2.5 ± 1.09	1.6 ± 0.27
B/B EA	12.2 ± 1.28	-
O/B U	17.9 ± 2.06	3.7 ± 0.55
B/O U	14.0 ± 1.72	2.1 ± 0.45
	P<0.0001	P<0.0005
Specific contrasts:		
Period 1 burial vs. no burial	P<0.0001	P<0.0021
Period 1 vs Period 2 burial	P<0.0115	P<0.0001
Defoliation vs No defoliation	P<0.0035	ns
O/B vs. O/O	ns	P<0.0219

6.3.5 EFFECT OF NODE POSITION ON BRANCHING PROBABILITY OF TREATED NODES

Probability of branch development from axillary buds decreased ($P<0.0002$) as node position from apex (as at the end of Period 1, 12 April 1989) increased (Fig. 6.1). The node position by treatment interaction was significant ($P<0.0035$) (Fig. 6.2) and low values at node position 6 of treatments buried during treatment Period 1 (B/B, B/B P, B/B EA and B/O) resulted in the low mean value for this position in Fig. 6.1.

6.3.6 TREATMENT EFFECTS ON YIELD CHARACTERISTICS OF STOLONS

6.3.6.1 PRIMARY STOLONS

The means for each treatment for stolon DW, internode length and diameter of primary stolon that emerged during each of the three periods of the experiment are presented in Table 6.6. Treatment main effects were significant ($P<0.001$) for all three variables in Period 1. Stolon DW per node varied significantly with defoliation regime ($P<0.001$) but not in response to burial treatments. For internode length the converse occurred, there was no response to defoliation but a depressive effect ($P<0.001$) of burial during Period 1. Stolon diameter was greater in undefoliated stolons ($P<0.0003$) and in treatments buried during Period 1 ($P<0.0001$).

For primary stolon tissue that emerged during Period 2, treatment main effects for the three variables were again significant ($P<0.01$). Stolon DW per node was greater in undefoliated stolons ($P<0.0025$) and may have been reduced ($P<0.0494$) by burial during Period 1. Internode length followed the same trends as for Period 1. Stolon diameter was greater in undefoliated than in defoliated stolons ($P<0.0001$) but was not affected by burial treatments.

For primary stolon tissue that emerged during Period 3, the only factor significantly influencing the three yield characteristics was the defoliation regime ($P<0.01$). Stolon diameter and internode length were both greater for undefoliated stolons and these combined to greatly increase (almost three-fold) the DW per node of undefoliated stolons over defoliated stolons.

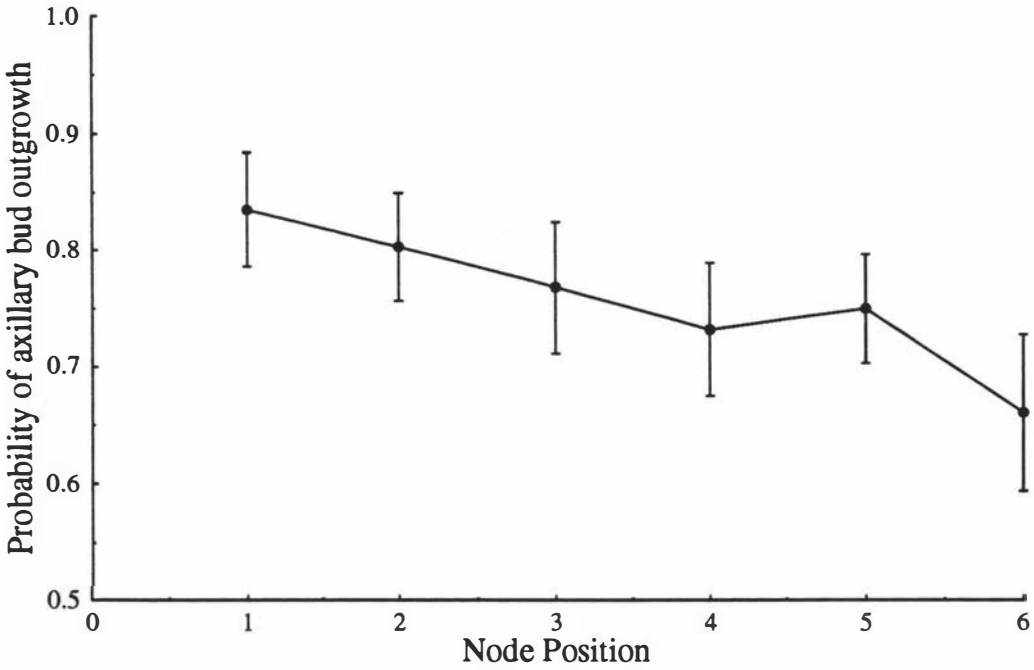


Figure 6.1 Mean probability of axillary bud outgrowth from nodes that emerged during Period 1: node position 1 represents the first (youngest) node differentiated from the apex as at April 12. Data meaned over treatments and replicates. (Error bars indicate ± SEM.)

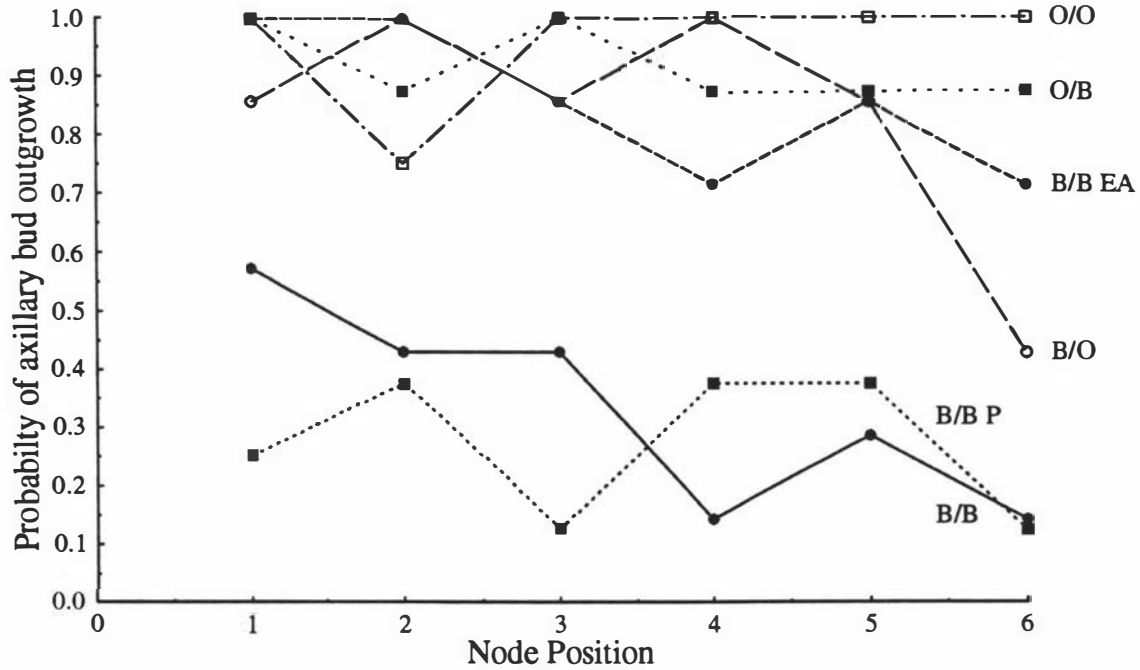


Figure 6.2 Mean probability of axillary bud outgrowth from nodes emerging during Period 1 on stolons of the various treatments (treatment abbreviations and descriptions are given in Section 6.2.3.1) in which the standard defoliation regime was applied, node position 1 represents the first (youngest) node differentiated from the apex as at April 12.

Table 6.6 Mean dry weight per node, internode length and stolon diameter at harvest of primary stolon that emerged during Periods 1, 2 and 3 in stolons subjected to burial/cultural treatments as described in Table 6.1. Means and standard errors of means are presented.

Treatment	Period 1	Period 2	Period 3
DW per node (mg)			
O/O	10.59 ± 0.566	10.24 ± 1.032	6.96 ± 0.677
O/B	9.51 ± 0.562	11.58 ± 0.637	6.74 ± 0.542
B/O	10.28 ± 0.804	6.07 ± 0.898	5.60 ± 0.493
B/B	10.04 ± 0.612	7.67 ± 0.478	5.70 ± 0.686
B/B P	7.66 ± 1.211	9.35 ± 1.972	5.73 ± 1.031
B/B EA	9.56 ± 0.631	-	-
O/B U	25.80 ± 1.671	19.68 ± 3.048	18.18 ± 0.940
B/O U	23.32 ± 3.309	14.73 ± 1.918	14.14 ± 1.240
Internode length (cm)			
O/O	1.45 ± 0.068	2.11 ± 0.081	2.19 ± 0.093
O/B	1.41 ± 0.080	2.10 ± 0.055	2.05 ± 0.066
B/O	1.05 ± 0.084	1.37 ± 0.077	1.81 ± 0.139
B/B	1.05 ± 0.066	1.36 ± 0.118	1.73 ± 0.186
B/B P	0.95 ± 0.058	1.47 ± 0.107	1.87 ± 0.212
B/B EA	1.06 ± 0.045	-	-
O/B U	1.45 ± 0.089	2.17 ± 0.146	2.77 ± 0.153
B/O U	0.93 ± 0.093	1.54 ± 0.194	2.42 ± 0.119
Stolon Diameter (cm)			
O/O	0.24 ± 0.008	0.24 ± 0.006	0.20 ± 0.006
O/B	0.24 ± 0.006	0.25 ± 0.008	0.20 ± 0.005
B/O	0.28 ± 0.015	0.21 ± 0.010	0.20 ± 0.006
B/B	0.31 ± 0.004	0.24 ± 0.007	0.19 ± 0.012
B/B P	0.26 ± 0.011	0.23 ± 0.008	0.21 ± 0.007
B/B EA	0.28 ± 0.010	-	-
O/B U	0.30 ± 0.006	0.31 ± 0.007	0.26 ± 0.007
B/O U	0.38 ± 0.022	0.27 ± 0.011	0.25 ± 0.012

6.3.6.2 BRANCH STOLONS

The means for each treatment for stolon DW and internode length of branch stolons originating from primary stolon nodes that emerged during Period 1 or 2 are given in Table 6.7 For branches from nodes that emerged during Period 1, DW and internode length were not affected by burial treatments but were greater ($P<0.01$) in undefoliated stolons than in defoliated stolons. For branches from nodes that emerged during Period 2 the only significant effect was that the DW per node of undefoliated stolons was greater than in defoliated stolons.

Table 6.7 Mean dry weight per node and internode length of branch stolons originating from primary stolon nodes that emerged during Periods 1 and 2 on stolons subjected to the burial/cultural treatments as described in Table 6.1. Means and standard errors of means are presented.

Treatment	Period 1	Period 2
DW per node (mg)		
O/O	3.29 ± 0.133	3.72 ± 0.563
O/B	3.40 ± 0.184	2.76 ± 0.351
B/O	2.47 ± 0.248	3.45 ± 0.843
B/B	3.57 ± 0.774	2.54 ± 0.761
B/B EA	5.47 ± 0.845	-
O/B U	8.69 ± 0.394	6.55 ± 0.691
B/O U	7.40 ± 0.612	6.69 ± 0.930
Internode length (cm)		
O/O	0.99 ± 0.031	0.65 ± 0.096
O/B	1.01 ± 0.059	0.97 ± 0.097
B/O	0.82 ± 0.071	0.64 ± 0.053
B/B	1.18 ± 0.148	0.88 ± 0.056
B/B P	1.30 ± 0.242	0.98 ± 0.087
B/B EA	0.84 ± 0.119	-
O/B U	1.30 ± 0.041	1.42 ± 0.162
B/O U	1.28 ± 0.063	0.99 ± 0.182

6.4 DISCUSSION

The discussion of results in this chapter begins by considering the effects of the various treatments on axillary bud outgrowth. In the next two sections results are interpreted so as to discuss aspects of the viability of axillary buds and the results previously obtained in Chapter 4. Then the fourth and fifth sections discuss, respectively, the observation that burial induces thigmomorphogenetic responses in stolons and burial as a factor contributing to the patchiness of the environment presented to white clover plants in the field. The final section presents conclusions.

6.4.1 RESPONSES TO EXPERIMENTAL TREATMENTS

Under these more controlled glasshouse conditions no inhibition in axillary bud outgrowth was observed when stolons were buried after emergence in the light (treatment O/B). Even when nodes emerged in the dark and were then subsequently exposed to light (treatment B/O) the probability of bud outgrowth at 0.76 compared reasonably with the control, full light treatment (0.93), especially when it is considered that the depression was largely accounted for by the low value of the oldest axillary bud (Fig. 6.2). A substantial depression of the probability of axillary bud outgrowth occurred only when axillary buds emerged and remained in the dark (under soil) until they were positioned 5 to 13 from the stolon apex, eg. treatment B/B. Thus buds could be stimulated to grow by exposure to light at any time whilst they were at node positions one to eight, but if buried until position was greater than eight such exposure failed to stimulate outgrowth. As mentioned previously (Chapter 4.4) under pastoral conditions there is also little branch initiation once node position exceeds eight (Chapman 1983; Davies & Evans 1990; Hay *et al.* 1991, Newton *et al.* 1992).

Burial of stolons plus apices also influenced the outgrowth of axillary buds of secondary stolons. The O/B treatment reduced branching probability of axillary buds of secondary stolons compared to the O/O control treatment (Table 6.4) even though for primary stolon the branching probabilities (Table 6.3), internode lengths and weights (Table 6.6) did not differ between treatments. Two factors contribute to this result. Firstly, at the time of burial of the O/B treatment, the secondary branches comprised five emerged nodes or less and axillary bud outgrowth from secondary stolons is normally suppressed until the stolon comprises four emerged nodes (M.J.M. Hay, unpublished data). Secondly the burial of the stolon and apex of secondary stolons would result in further inhibition of axillary bud outgrowth on these stolons in the same manner as was apparent in buried primary stolons. Upon removal of soil at the conclusion of Period 2 a proportion of the secondary buds would have passed beyond the zone of maximum responsiveness for axillary bud outgrowth thus resulting in a decrease in probability of axillary bud outgrowth.

The probabilities of outgrowth of axillary buds of primary or secondary stolons did not differ between the defoliation regimes studied (Tables 6.3, 6.4). However, defoliation so as to maintain two fully expanded leaves per stolon apex significantly reduced DW per node and stolon diameter but not internode length within the primary stolon in all periods compared to undefoliated stolons (Table 6.6). Given the lack of difference in internode length, calculations show that the decrease in internode volume associated with the reduction in stolon diameter of defoliated stolons could account for 57% and 82% of the recorded difference in DW per node between the defoliated and undefoliated stolons of the O/B and B/O treatments, respectively. Thus a change in the specific density of stolons would account the remainder of the difference in DW per node. The greater probability of axillary bud outgrowth of O/B compared to B/O treatments would provide more branch stolons with capability to export carbohydrate to the primary stolon (Chapman *et al.* 1992b). This would maximise the negative effects of defoliation on the supply of carbohydrate for starch storage, thus increasing the potential for difference in specific density between the two defoliation regimes of the O/B treatment.

The lack of response of internode length to defoliation regime is surprising as factors related to differences in defoliation intensity (stocking rate and number of mature leaves supporting an internode during development) were ranked two and three in importance out of ten factors considered likely to influence internode length (Sackville Hamilton 1990). Under these experimental conditions stolon diameter exhibited a plastic response to both defoliation (negative) and burial during Period 1 (positive) whereas internode length showed only a negative response to the burial treatment.

Excision of the stolon apex stimulated the outgrowth of proximally positioned axillary buds which, with the exception of those at the two most acropetal nodes, had no subtending leaves and so no possible light stimulus. Thus it is likely that it was removal of some control mechanism with excision of the apex that was responsible for this axillary bud outgrowth. There is the possibility that an ethylene wounding response may have precipitated outgrowth of the axillary buds (Harper 1977) although it is noted that excision of leaves did not precipitate such responses.

The presence of an apex on a stolon exerts an influence on axillary bud outgrowth usually referred to as apical dominance (Thomas 1987b). The number of nodes basipetal to the apex at which axillary bud outgrowth is inhibited varies according to growth conditions (Davies & Evans 1990a; Turkington *et al.* 1991). After nodes mature beyond the zone influenced by apical dominance, initiation of outgrowth can be stimulated by favourable conditions at the bud site. As the potential of an axillary bud to develop declines sharply with increasing node position (Davies & Evans 1990a), eg. potential is

40% and 20% at node positions 3 and 10 respectively (Newton *et al.* 1992), any delay in initiation of outgrowth by apical dominance will ultimately decrease the probability of branching from the bud. A sudden change in environmental conditions at the apex and along stolons (imposition of burial treatment) did not affect outgrowth of axillary buds already primed for growth, which implies that apical dominance effects are restricted solely to the initiation of outgrowth of axillary buds.

Results from this study are consistent with a model that the degree of apical dominance present is related to the sink strength of the apex for any limited endogenous plant resource and therefore does not reflect a direct sensing of the environment at the apex. Although not examined in this study others have found that shading of the stolon apex alone does not significantly influence stolon growth or frequency of branching at nodes (Harvey 1979; Newton 1986). This is evidence that sensing of the environment by the apex is not a factor driving the apical control mechanisms(s). Harvey (1979) and Thomas (1987b) have concluded that any one resource in short supply at the apex will increase the number of nodes at which axillary bud outgrowth is inhibited, a conclusion which is consistent with the results of this study.

Stolon burial by 2 cm of potting mix or burial plus covering with black polythene (treatment B/B P) gave similar probabilities of axillary bud outgrowth (Table 6.3) suggesting that the burial treatment effectively excluded light. On the other hand this result does not exclude the possibility that burial did not effectively exclude light and that lack of light was not responsible for the depressive effect of burial on branching. Given the depressive effects of shading of axillary buds on outgrowth of buds obtained by others (eg. Newton 1986; Davies & Evans 1990a) the former conclusion seems more likely.

6.4.2 VIABILITY OF AXILLARY BUDS

A factor contributing to the decrease in probability of axillary bud outgrowth when buds were buried for both periods (treatment B/B) may be a decrease in the viability of axillary buds associated with the normal course of ontogeny of axillary buds. Evidence in support of this suggestion is afforded in a recent study of axillary bud viability in white clover in pastures (Newton *et al.* 1992). By node position 4 axillary bud outgrowth had occurred at 20% of nodes with approximately 3% and 17% of nodes bearing live and incipient (first leaf at Carlson (1966a) developmental stage 0.1-0.9) branches, respectively. Incipient branch occurrence was appreciable at node position 2 and at peak levels (15-20% of nodes) for node positions 4-7 but declined at positions >7. Successful branch initiation was most likely to occur when axillary buds were positioned 4-8. In this context it is interesting to note that values for node positions 5 and 6 of the B/O treatment (Fig. 6.2)

had depressed branching probabilities indicating that they were probably approaching the critical stage of ontogeny as regards axillary bud development by the time of their exposure to light. Comparison of B/B and B/O treatments for branching probability of nodes formed during Period 1 (0.29 and 0.76, respectively) indicated that axillary buds were by and large viable at the end of Period 1 and that in the B/B treatment loss of potential to develop occurred during Period 2 when nodes developed to be in positions 5 to 12.

Additional evidence indicating that the axillary buds at nodes emerging in the dark are viable is afforded by comparison of B/B and B/B EA (excision of apex) treatments. The excision of the stolon apex at the conclusion of Period 1 induced an increase in probability of axillary bud outgrowth at nodes formed during Period 1 from 0.29 (B/B) to 0.73 B/B/EA. A stolon scoring at the conclusion of Period 2 (27 April) indicated that the response in bud outgrowth had chiefly occurred during Period 2 as at this time probabilities were 0.25 (B/B) and 0.63 (B/B EA) respectively. It is worthy of note that this response of axillary bud outgrowth to excision of the apex occurred in buds covered in soil, with no light stimulus and at nodes, except for the two most distal nodes, with no leaves.

6.4.3 INTERPRETATION OF CHAPTER 4 RESULTS

Explanation of the depression in branching probability of axillary buds at node positions 1 and 2 at the time of stolon burial in the zone of burial experiment (Section 4.3.3) leads to consideration of interesting possibilities. Firstly, the light regimes of the stolon apex differed in the field and glasshouse experiments (Table 6.8). At burial in the field experiment treated stolons had both stolon and apex buried whereas in the comparable treatment in the glasshouse trial (O/B) the stolon but not the apex was buried. This could be interpreted as indicating that the light regime of the apex has a direct effect on axillary buds which overrode the light conditions at individual axillary bud sites. However, this was ruled out by the lack of response of those axillary buds that emerged and developed under soil (B/B), to growth of the apex in light during Period 2. Either removal of soil (exposure of bud site to light) or excision of the apex was required to stimulate the outgrowth of axillary buds, light at the apex alone was not sufficient.

Table 6.8 Diagram illustrating the light regime at treated nodes and the apex of stolons subjected to various burial treatments in the glasshouse trial and in the zone of burial, field experiment along with the outgrowth response of axillary buds relative to non-buried, full-light treatments.

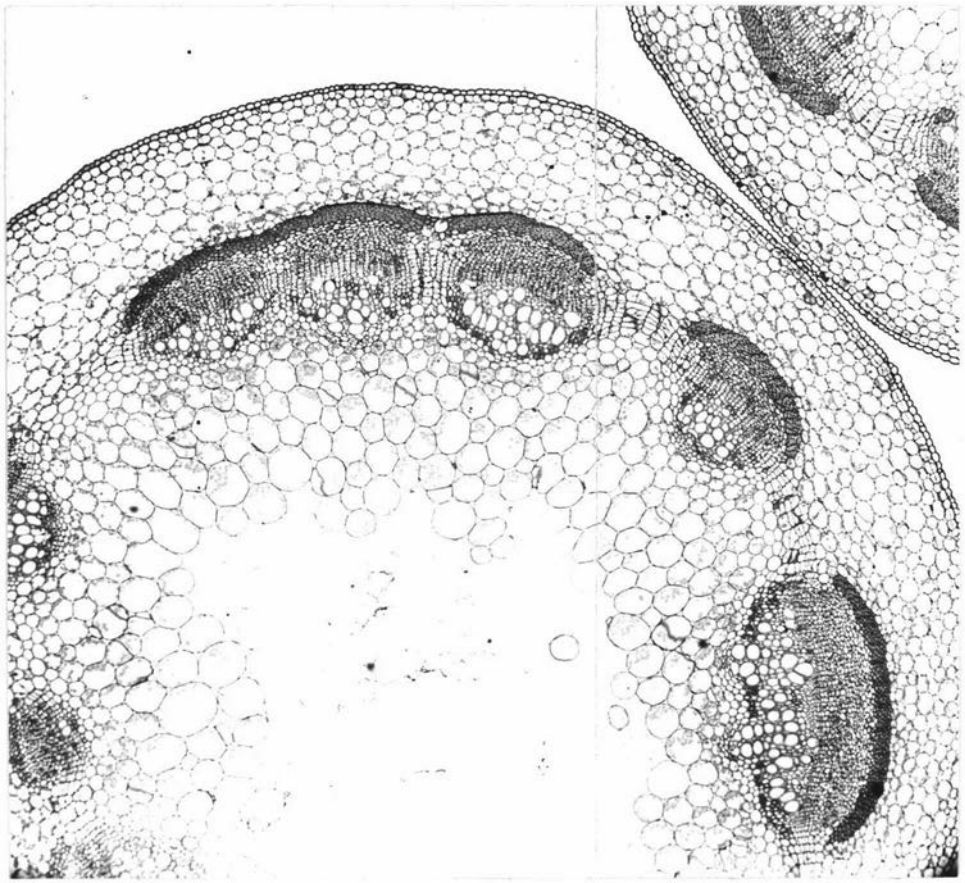
Experimental treatments	Light regime of treated nodes	Light regime of apex	Axillary bud outgrowth relative to control (full light) stolons
Field Experiment	Light → Dark	Light → Dark	50% Nodes 1 & 2
B/B	Dark → Dark	Dark → Light	31%
B/B EA	Dark → Dark	Dark → - (Excised)	78%
B/O	Dark → Light	Dark → Light	82%
O/B	Light → Dark	Light → Light	100%
O/O	Light → Light	Light → Light	100%

Secondly, there was a difference in growth of stolons following burial in that in the field experiment stolons were free to curve and make vertical growth to the soil surface whereas those in the glasshouse were restrained from making vertical growth. Gibberellic acid is involved in mediating vertical growth of stolons (Thomas 1987b) and has been shown to inhibit outgrowth of axillary buds in white clover (Fletcher & Martin 1962). Thus it is possible gibberellic acid levels rather than the light regime or apical control factors inhibited axillary bud outgrowth at nodes 1 and 2 in the field experiment.

6.4.4 THIGMOMORPHOGENETIC RESPONSES

Where the apex was buried, growth of stolon was characterised by a reduction in internode length and an increase in stolon diameter (Table 6.6). Such symptoms are typical of thigmotropic responses of white clover stolon where growth of the apex is offered a certain critical degree of physical impedance (Newton 1986). Increased ethylene concentrations are considered to be the trigger in thigmomorphogenesis (Biro *et al.* 1980) and this is likely to be the case for stolons growing through soil. A characteristic of such stolons is the presence of aerenchyma-like cavities in the pith (Plate 6.2). Ethylene is implicated in the formation of similar looking aerenchyma in roots of several species when grown under anoxic conditions (Jackson 1982). This strongly suggests that stolons growing through soil release increased concentrations of ethylene which trigger the thigmomorphogenetic changes observed.

(a)



(b)

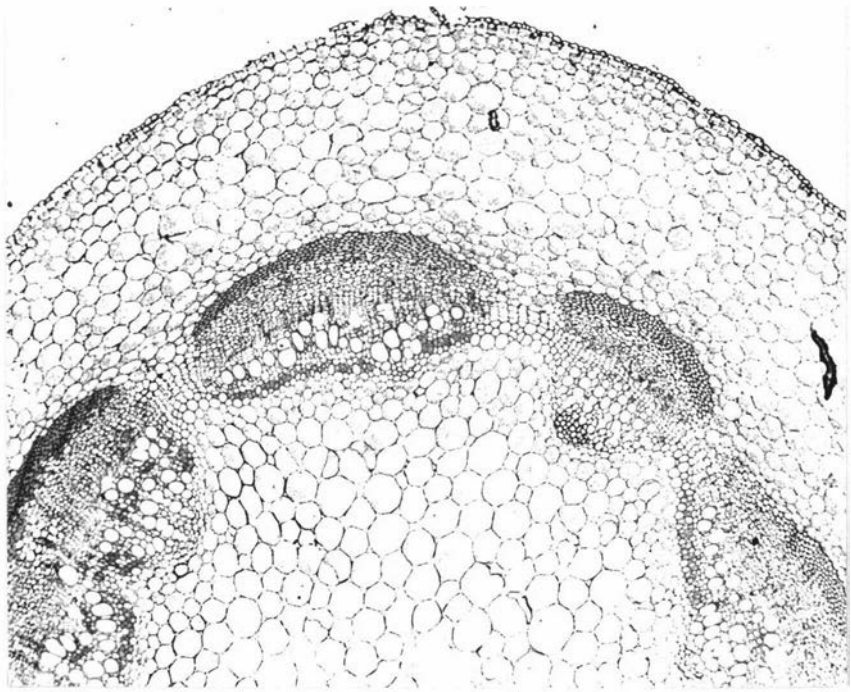


Plate 6.2 Transverse sections of (a) buried and (b) unburied stolon of 'Grasslands Kopu' white clover. The parenchymatous ground tissue at the centre of the buried stolon (a) has broken apart to form an aerenchyma-like cavity. The unburied stolon has normal parenchymatous ground tissue at the stolon centre (b). Magnification 50 X.

The burial treatment during Period 1 caused similar carryover effects on nodes produced during Period 2. The adjustments included decreased node appearance rate, branching probabilities, internode length and DW per node. However, for defoliated stolons the diameter of such treated stolon did not differ significantly from that of control O/O stolon produced in Period 2. This indicates that the thigmomorphogenetic processes ceased rapidly once the stolon apex was freed from the pressure of pushing through soil and that the observed carryover effects on other characteristics were probably caused by other factors. The carryover effects noted here may reflect the reduced photosynthate contribution to the primary stolon from the fewer branches on the stolons that were buried during Period 1 (Chapman *et al.* 1992a, b).

6.4.5 ENVIRONMENTAL PATCHINESS

Recently Turkington *et al.* (1991) hypothesised that node appearance rate was determined by whole plant sensing of the environment (chiefly 10 cm soil temperature (Sackville Hamilton & Harper 1989)) while module (node) development (size of organs, axillary bud outgrowth) was responsive to the environment at the site of the module. Their study was based on biotic patchiness (induced by varying the species of the companion grass) while the present study investigated environmental patchiness in relation to stolon burial. In common with the studies by with Turkington *et al.* (1991), the treatments studied here induced rather small differences in node appearance (27%) but larger differences in characteristics reflecting development of the node, eg. axillary bud outgrowth (three-fold), DW per node (three-fold), internode length (55%) and stolon diameter (58%). This suggests that development of nodes in field populations of white clover will be sensitive to variations in these physical conditions (stolon burial, defoliation regime, apical excision) as well as to climatic (Brougham 1962; Davies & Evans 1982; Chapman 1983, Sackville Hamilton 1990) and biotic influences (Turkington *et al.* 1979, 1991).

6.4.6 CONCLUSIONS

In conclusion the following points can be made in relation to the pattern of stolon burial occurring in grazed pastures and its effect on axillary bud outgrowth:

(1) stolon burial will reduce the probability of initiation of axillary bud outgrowth only when a bud has not already commenced outgrowth, and when it is buried at an early stage of ontogeny (at the time of emergence of its associated node from the apex) and the bud site remains buried until its associated node is positioned eight or more from the apex.

(2) as axillary buds at relatively few nodes are subjected to all these conditions, stolon burial has a relatively minor effect on initiation of axillary bud outgrowth when the whole population of axillary buds of white clover in pastures is considered.

(3) although stolon burial has little adverse effect on initiation of axillary bud outgrowth, it significantly decreases the survival of branches (see Chapter 4) and so ultimately decreases branching.

CHAPTER 7 VARIATION IN CARBOHYDRATE STATUS OF STOLON TISSUE WITH SEASON, INTERNODE POSITION, DEFOLIATION AND STOLON BURIAL AND ITS CORRELATION WITH AXILLARY BUD DEVELOPMENT

7.1 INTRODUCTION

Chapter 3 reported that large changes took place within the populations of white clover in grazed pastures during spring that resulted, by late spring, in a decrease in density parameters (stolon biomass m^{-2} and growing point numbers m^{-2}), minimal values of parameters reflecting mean plant size (plant dry weight, numbers of stolons and nodes per plant) and branching frequency of nodes. A reduction in spring of the frequency of branch formation of white clover in grazed pastures has been previously observed (Chapman 1983; Wilman & Simpson 1988; Davies 1989; Newton *et al.* 1992). Northern hemisphere studies show that by spring the carbohydrate status of white clover is at a minimum after steady depletion of starch reserves over the winter months (Moran *et al.* 1953; Ruelke & Smith 1956; Vez 1961; Murphy 1982; Harris *et al.* 1983). Charles-Edwards (1984a, b) suggests that a minimum intraplant flux of carbon to branch apices is required for their continued growth. Thus it was considered that the decrease in branching activity of white clover during spring might be related to a reduction in plant carbohydrate status at this time. For these reasons this chapter reports on measurements taken to assess seasonal variation in, and the effects of defoliation and stolon burial on, the carbohydrate content of white clover stolons from the field-trial pastures (Chapter 3) and the correlation of stolon carbohydrate content with branching activity.

White clover in grazed swards has approximately 20% of DW as root (Young 1958) and 55-60% as stolon (Cowling 1961; Hay *et al.* 1986). The carbohydrate content of roots is lower than that of stolons and root contents tend to mirror changes in stolon content (Moran *et al.* 1953; Vez 1961; Harris *et al.* 1983). For these reasons this study concentrated on stolon carbohydrate contents and no determinations of root carbohydrate content were performed.

As growth of white clover in moist temperate pastures is clonal (Turkington *et al.* 1979; Chapman 1987) it means older nodes are continually dying as new nodes form

(Chapman 1983; Sackville Hamilton & Harper 1989; Hay *et al.* 1991). This growth form suggested a hypothesis that the carbohydrate content of nodes would vary with the position of the node relative to the stolon apex. Examination of the carbohydrate content of stolons on a node by node basis was also logical from the view point of branch development as the axillary bud at each node has the potential to form a lateral branch or a flower (Erith 1924). Thus in this study all analyses of carbohydrate content were of individual internode segments of stolon.

This investigation of the carbohydrate status of white clover stolons in grazed swards had five major objectives:

(1) to establish the diurnal variation in carbohydrate content of stolons in order to facilitate the interpretation of results.

(2) to assess seasonal variation in carbohydrate contents.

(3) to assess variation in carbohydrate content of stolon tissue with nodal position relative to the stolon apex.

(4) to measure the effects of defoliation and stolon burial on carbohydrate contents.

(5) to investigate relationships between branching activity at nodes and carbohydrate content of adjacent stolon internodes.

7.2 METHODS AND MATERIALS

7.2.1 PLANT MATERIAL

White clover stolons were uplifted from the Kopu-perennial ryegrass swards of the grazing trial described in Chapter 3 over the time period 1 August 1986 to 25 October 1987. Stolons selected for sampling usually had 25 or more nodes. Stolons were described by recording for each node the presence or absence of a leaf and axillary bud outgrowth (as given in Section 4.2.3.1). Only the leading stolon was sampled, branches along with roots and petiole plus leaf were cut to waste. Stolons were then dissected by cutting through each node. Material acropetal to the first node bearing a leaf with development ≥ 0.4 on the Carlson (1966a) leaf development scale was designated stolon apex. Stolon tissue between the first and second leaves (nodes) was designated internode one. The first ten such internodes were sampled and thereafter each fifth internode through to the stolon base. Material was immediately freeze-dried.

7.2.2 CHEMICAL ANALYSIS OF CARBOHYDRATE FRACTIONS

The method of analysis for starch, sucrose and hexose contents followed that of Hart & Greer (1988) and an outline of essential features of the methodology follows.

Freeze-dried stolon segments were ground and a weighed quantity (approximately 2 mg) placed in a test tube. Two successive extractions were performed by digesting with 80% ethanol at 80°C for 10 min, centrifuging and aspirating the supernatant. The combined supernatant was taken to dryness and then taken up in water for sucrose and hexose sugar determinations.

Starch determination was performed on the residue pellet left after aspiration following the second ethanol extraction. Following addition of 0.2*M* KOH and incubation at 100°C for 30 min, 1*M* acetic acid was added to lower the pH to 5.5. A pellet of Polyclar AT and amyloglucosidase solution in 50*mM* sodium acetate were added and the mixture incubated at 50°C for 2h. After brief centrifugation, glucose oxidase-peroxidase reagent (Karkalas 1985) was added to aliquots of the supernatant. the tubes were stoppered and then incubated in the dark for 45 min at 35°C. After cooling for 10 min the absorbance at 505 nm was read. The concentration of glucose in the solution was multiplied by 0.9 to obtain the starch concentration.

The sucrose and hexose sugar contents were determined on aliquots of the aqueous solution, following clarification with $Ba(OH)_2$, $ZnSO_4$ and Polyclar AT. After centrifugation, equal aliquots of the supernatant were taken and one was incubated with invertase, the other with water. The solutions were then incubated with p-hydroxybenzoic acid hydrazide solution and the absorbances read at 415 nm. Sucrose content was estimated by subtracting the hexose content (obtained from water incubated aliquot) from the hexose content of the invertase incubated aliquot.

7.2.3 DESCRIPTION AND DESIGN OF EXPERIMENTS

7.2.3.1 DIURNAL VARIATION IN CARBOHYDRATE CONTENT

On 19 September 1987, 3 days prior to a grazing, stolons were sampled from the pastures. Stolons were uplifted at the following times; 5.30 am, 9.00 am, 12.00 (noon), 3.00 pm, 6.00 pm, 9.00 pm and 12.00 (midnight). At each time three stolons were sampled

as previously described (Section 7.2.1). The stolon apex, the first ten designated internodes and every fifth internode thereafter were analysed for starch, sucrose and hexose sugar content (Section 7.2.2).

7.2.3.2 SEASONAL VARIATION IN CARBOHYDRATE CONTENT

Immediately prior to each of the ten grazings during the period 1 August 1986 to 7 August 1987, four stolons selected from randomly chosen locations were uplifted between 10.30 am and 12.00 noon. Stolons were then described, dissected, freeze-dried and subsequently analysed for carbohydrate contents as previously given (Sections 7.2.1 and 7.2.2).

7.2.3.3 EFFECT OF DEFOLIATION AND STOLON BURIAL ON CARBOHYDRATE CONTENT

This experiment was designed to measure the time course of the effects of defoliation and burial treatments on the carbohydrate content of stolons. There were three treatments:

(1) defoliation; all leaves and petioles of leaf development > 0.7 (Carlson 1966a) were removed both from the leading stolon and its branches at the start of the experiment (day 0).

(2) burial; the leading stolon and its branches were buried at day 0 under 2 cm of soil, although leaves with petioles longer than 2 cm were above ground.

(3) control; stolons left undefoliated and unburied.

The three treatments were applied between 1.30 and 5.00 pm 25 September 1987 to six groups of three stolons of similar size. There were four replicates giving in total 72 stolons receiving treatments. Four stolons were also sampled on 25 September to monitor carbohydrate content at day 0. Subsequently every five days, *viz* 30 September, 5, 10, 15, 20 and 25 October, a group of three treated stolons was sampled from each replicate. On all occasions these samples were taken between 1.00 and 1.30 pm and immediately processed (Section 7.2.1). Carbohydrate contents were analysed using the previously given procedure (Section 7.2.2).

7.2.4 STATISTICAL ANALYSES

All statistical analyses were performed using SAS (SAS Users Guide, 1988).

7.2.4.1 DIURNAL VARIATION IN CARBOHYDRATE CONTENT

Data were tested by analysis of variance with data of internodes grouped by stolon and the effect of time of sampling conservatively tested by use of the effect of stolons nested within time as the error term.

7.2.4.2 SEASONAL VARIATION IN CARBOHYDRATE CONTENT

The effect of time (month) of sampling was tested using the procedure described in Section 7.2.4.1.

7.2.4.3 VARIATION WITH INTERNODE POSITION IN CARBOHYDRATE CONTENT

Analysis of variance was used to test the effect of internode position both across all samplings and for groupings of the October, November and December samplings against the remaining seven samplings. Curves were fitted using SAS and the method of least squares.

7.2.4.4 VARIATION WITH SEASON AND INTERNODE POSITION IN CARBOHYDRATE YIELD

Yield of carbohydrate per internode is the product of the dryweight and carbohydrate content of the internode. The effects of time of sampling and internode position on dryweight and carbohydrate yield were tested by analysis of variance in the manner previously given (Section 7.2.4.1 and 7.2.4.3 respectively).

7.2.4.5 EFFECT OF DEFOLIATION AND STOLON BURIAL ON CARBOHYDRATE CONTENT

Data were tested by analysis of variance with treatment and date of harvest effects tested by use of the combination of all the one way and two way interactions

involving replicates, harvest dates and treatments as the error term. Individual internodes were grouped by position into three categories (apical, young and basal) corresponding to positions 1-3, 4-8 and ≥ 9, respectively and the significance of the treatment x harvest date x node category interaction tested by analysis of variance.

7.2.4.6 STOLON INTERNODE CARBOHYDRATE CONTENT AND BRANCH DEVELOPMENT AT THE ACROPETAL NODE

Pearson correlation coefficients were tested against zero to examine the significance of relationships between axillary bud development at a node and starch, sucrose or hexose sugar content of the stolon internode basipetal to the node. Branch development of the axillary bud at a node was categorised as:

1) no development,
2) first leaf development 0.1-0.9 (Carlson (1966a) scale),
3) branch with ≥ 1 unfolded leaves.

Data sets from the seasonal sampling (Section 7.2.3.2) and the defoliation/burial trial (Section 7.2.3.3) were examined. Stolons of the seasonal sampling were grouped into a spring group comprising the October, November and December samplings and other samplings and each group independently analysed. Similarly, stolons of the control and buried treatments of the defoliation/burial trial were grouped and tested independently of stolons of the defoliated treatment.

7.3 RESULTS

7.3.1 DIURNAL VARIATION IN CARBOHYDRATE CONTENT

There was no significant diurnal variation of starch, sucrose or hexose sugar content in stolons of Kopu white clover (Fig 7.1). Even though the mean starch value at midnight was twice that of the three morning samplings the main effect of time of sampling was non-significant.

7.3.2 SEASONAL VARIATION IN CARBOHYDRATE CONTENT

Seasonal changes in the mean contents of the carbohydrate fractions of stolons were dominated by significant ($P<0.028$) variations in starch content (Fig 7.2). Starch

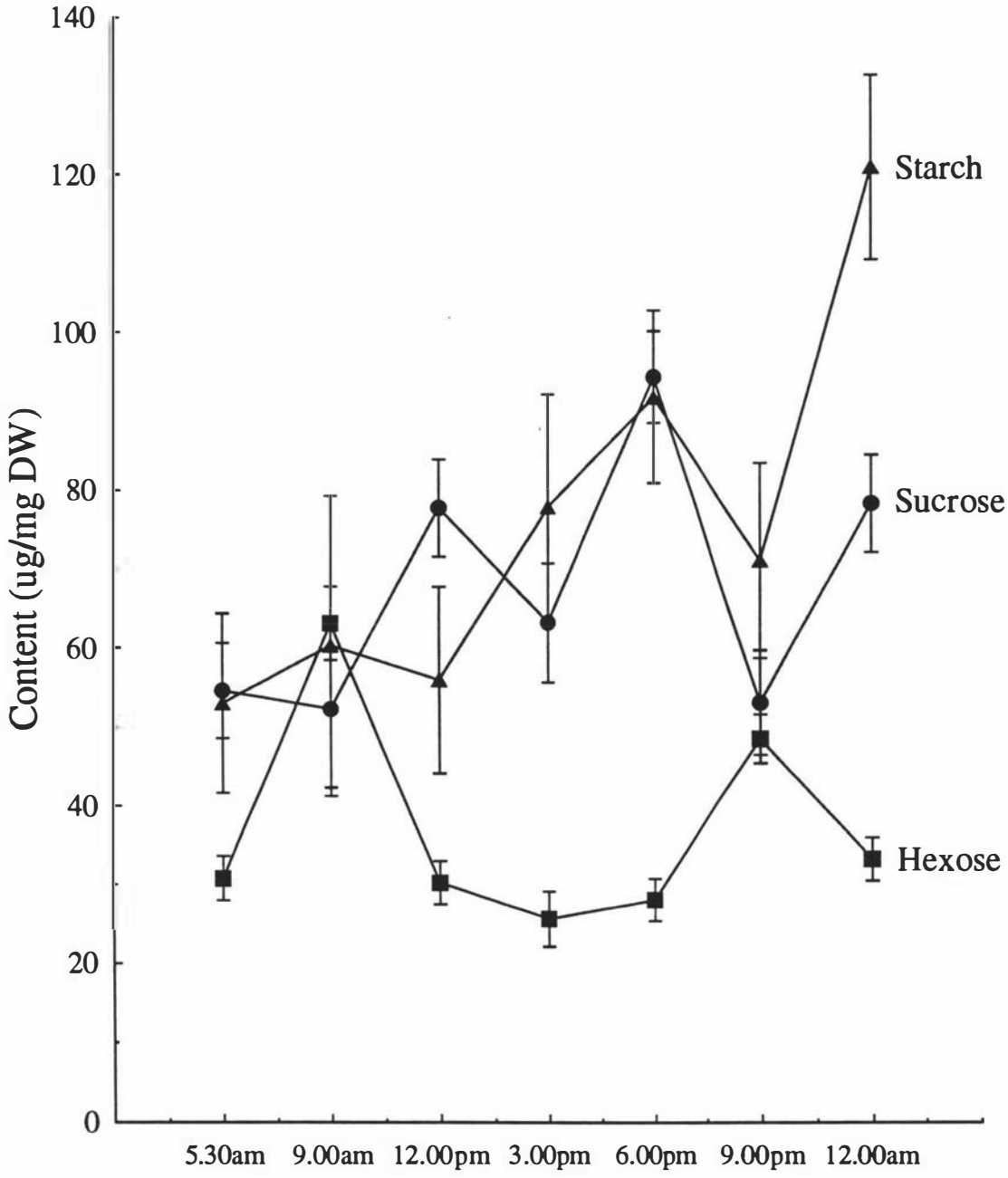


Figure 7.1 Diurnal variation in starch (▲), sucrose (●) and hexose (■) content in stolons of Kopu white clover sampled at seven times during 19 September 1987. (Sampling times are indicated. Error bars indicate ± SEM.)

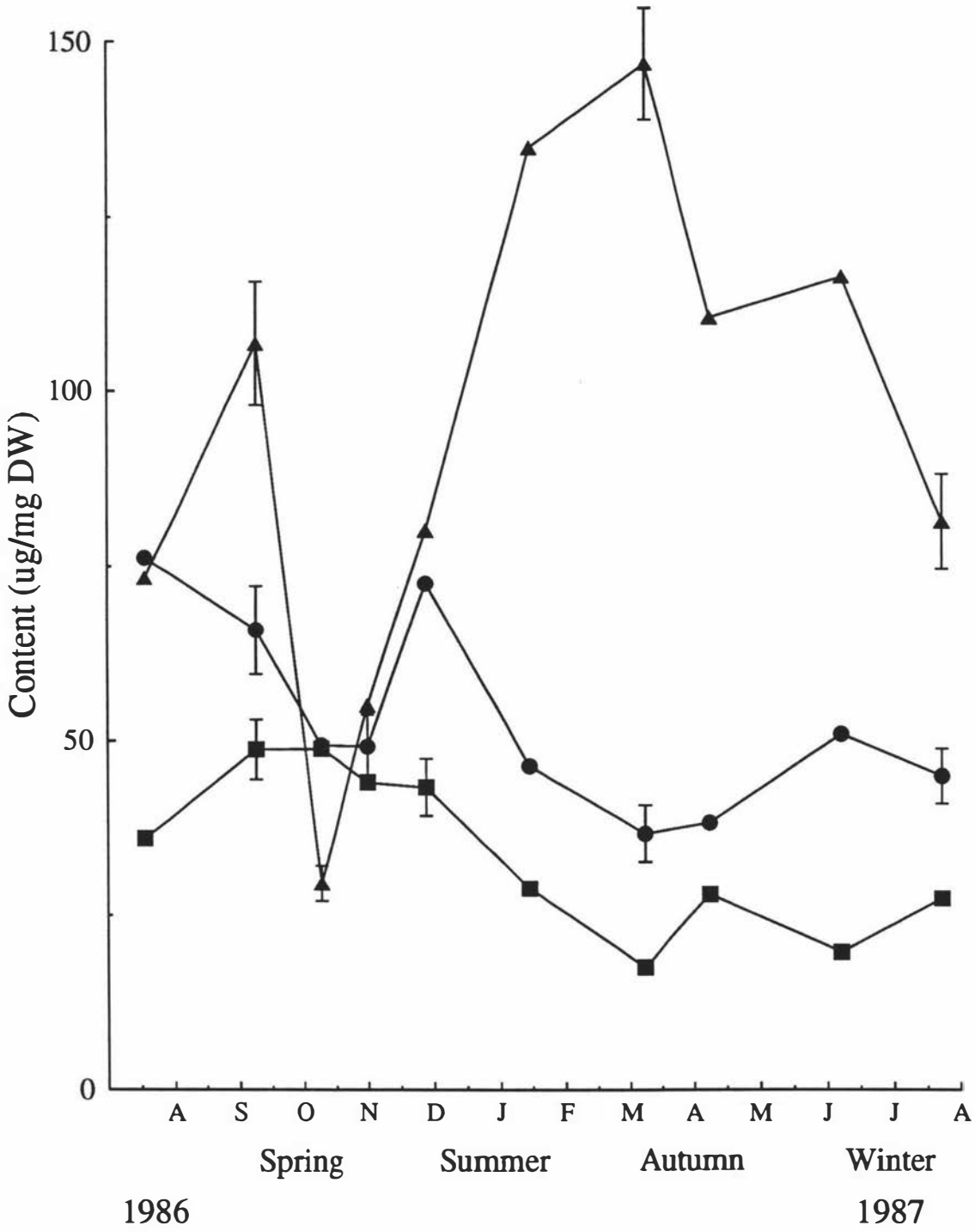


Figure 7.2 Seasonal variation in mean content of starch (▲), sucrose (●) and hexose (■) in stolons of Kopu white clover. (Error bars indicate ± SEM at representative months.)

content decreased abruptly in spring (October) to a minimum of 30μg mg^{-1} and increased over summer to a maximum of 146μg mg^{-1} in March. Starch content then steadily decreased during winter to a value of 82μg mg^{-1} by August 1987. The increase in starch content from August to September 1986 just prior to the large decrease in October was significant.

Mean contents of sucrose and hexose sugar did not vary significantly with season (Fig 7.2).

The overall mean contents of starch, sucrose and hexose sugar were, respectively, 102.9 ± 1.71, 57.0 ± 1.19 and 34.7 ± 0.84 μg mg^{-1} DW of stolon which gave a total non-structural carbohydrate content of 19.5% of stolon DW.

7.3.3 VARIATION WITH INTERNODE POSITION IN CARBOHYDRATE CONTENT

Mean starch, sucrose and hexose sugar contents of an internode all varied ($P<0.0001$) with the position of the internode in a stolon (Fig 7.3). Greatest changes were evident in the starch contents which increased rapidly from low values at the stolon apex (21 ± 2.2 μg mg^{-1}) to maximal levels (122 ± 9.5 μg mg^{-1}) by position 4. Starch contents remained high for internodes 4 to 10 but then declined with position. A fitted curve, Hoerl's special function, $y = ax^be^{cx}$, (a = 53.68, b = 0.722, c = -0.83), described the relationship of starch content with internode position ($r^2 = 0.898$).

The relationship of sucrose content with internode position was well described ($r^2 = 0.962$) by the asymptotic function, $y = ae^{b/x}$, (a = 82.27, b = -1.758). Sucrose content increased from a low value at the stolon apex (8 ± 1.7 μg mg^{-1}) so that by internode 8 (75 ± 7.4 μg mg^{-1}) content approached the asymptotic value (Fig 7.3).

The content of hexose sugars was highest in nodes nearest the stolon apex and decreased slightly as node position increased (Fig 7.3). The exponential function, $y = ae^{bx}$, (a = 41.14, b = -0.0247) best described ($r^2 = 0.787$) this relationship.

However when the late spring/early summer samplings of October, November and December were grouped for comparison with the other seven samplings, the season (group) by node position interaction for the starch and hexose sugar fractions was

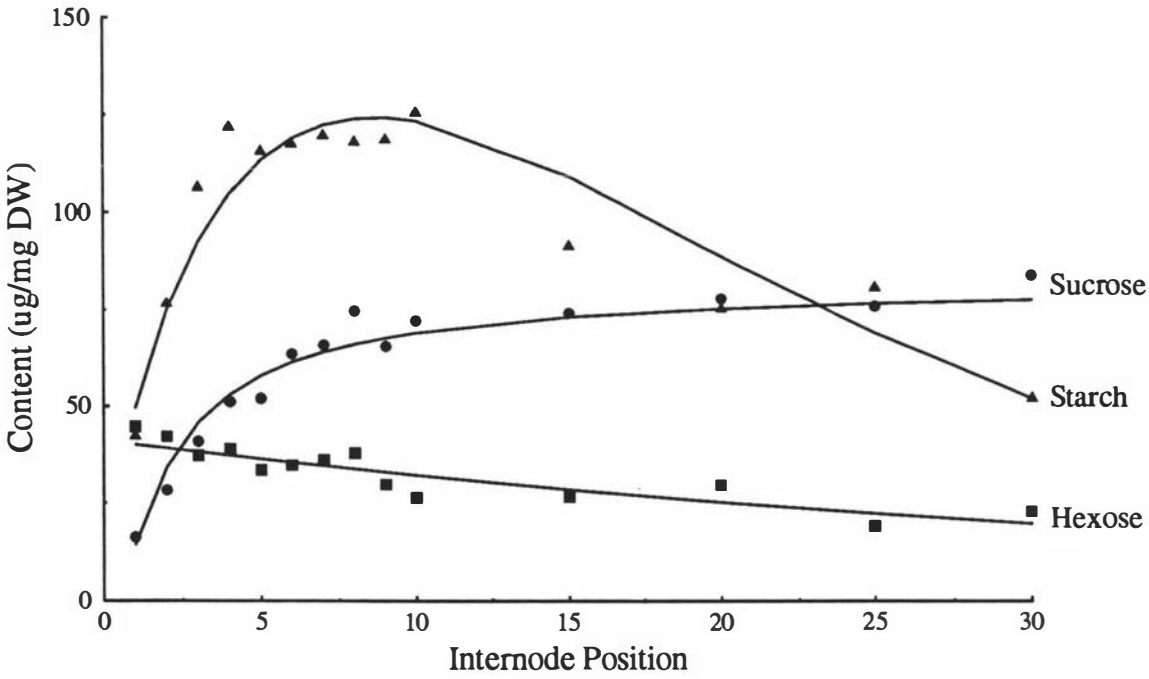


Figure 7.3 Variation in mean content of starch (▲), sucrose (●), and hexose (■) sugars in stolons of Kopu white clover with internode position (number of internodes from stolon apex). Each data point represents the mean across all samplings. Fitted lines as given in text.

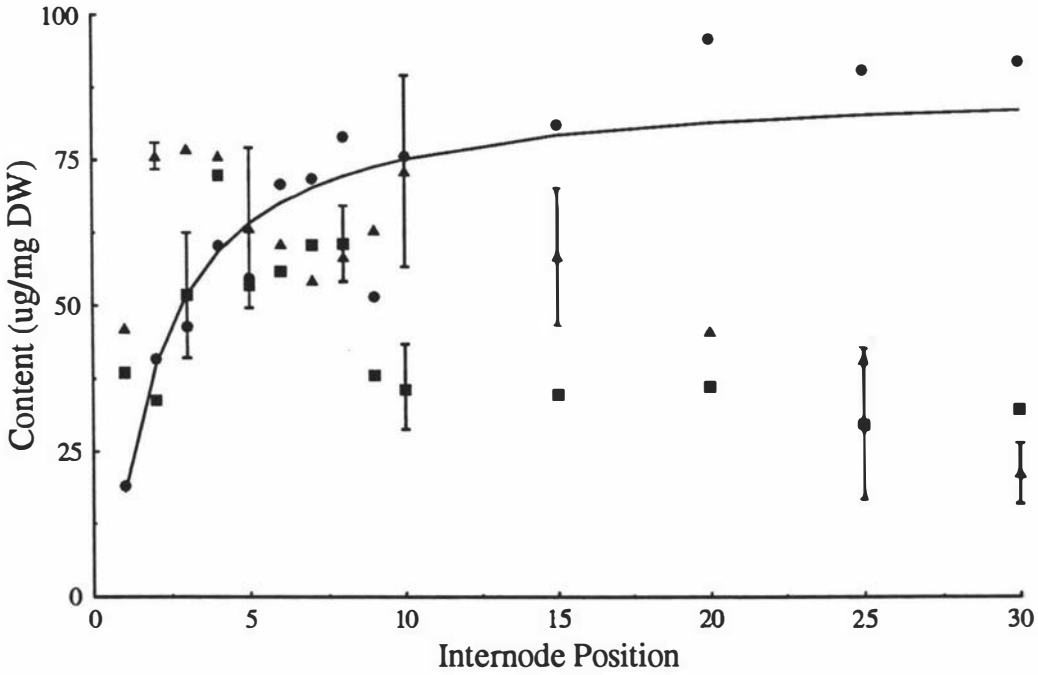


Figure 7.4 Variation in mean content of starch (▲), sucrose (●), and hexose (■) sugars in stolons of Kopu white clover with internode position (number of internodes from stolon apex) for the spring-early summer period (October, November and December) samplings. (Error bars indicate ± SEM for starch and hexose at representative nodes.)

significant ($P<0.001$) but that for sucrose non-significant. This reflected different patterns of distribution of starch and hexose sugar among nodes during late spring, early summer (Fig 7.4) while distribution of sucrose remained similar throughout the year.

7.3.4 VARIATION WITH INTERNODE POSITION IN CARBOHYDRATE YIELD

The two components that determine carbohydrate yield of each internode are carbohydrate content (previously described Sect. 7.3.3) and dryweight (DW) of the internode. Variation in internode DW with position (Fig 7.5) followed an asymptotic function, $y = a + b\,[1 - e^{-cx}]$, ($a = 4.79$, $b = 5.74$, $c = 0.258$). Mean internode DW increased rapidly from 4.8 mg at position 1 to 9.8 mg at position 9, a value which closely approached the asymptotic value (Fig 7.5).

Starch yield per internode increased five-fold from position 1 to 4 (Fig 7.6). This reflected the greater DW and starch content per internode at position 4 relative to position 1. Starch yield per internode did not vary with node position for positions ≥ 4.

In general, sucrose yield (Fig 7.6) followed the pattern of sucrose content (Fig 7.3). However the low sucrose content and DW per internode at position 1 resulted in a ten-fold difference in sucrose yield between positions 1 and 8.

Hexose sugar yield per internode was not influenced by position (Fig 7.6). Changes in hexose sugar content and DW per internode with position compensated and similar yield per internode resulted regardless of position.

7.3.5 SEASONAL VARIATION IN CARBOHYDRATE YIELD

Mean yield of starch per internode varied ($P<0.001$) with season (Fig 7.7) and had two peaks (September and March) and two troughs (October and August). The maximum occurred in March (2.21 ± 0.281 mg) and the minimum in October (0.26 ± 0.052 mg). Seasonal variation in mean sucrose or hexose yield per internode was not significant.

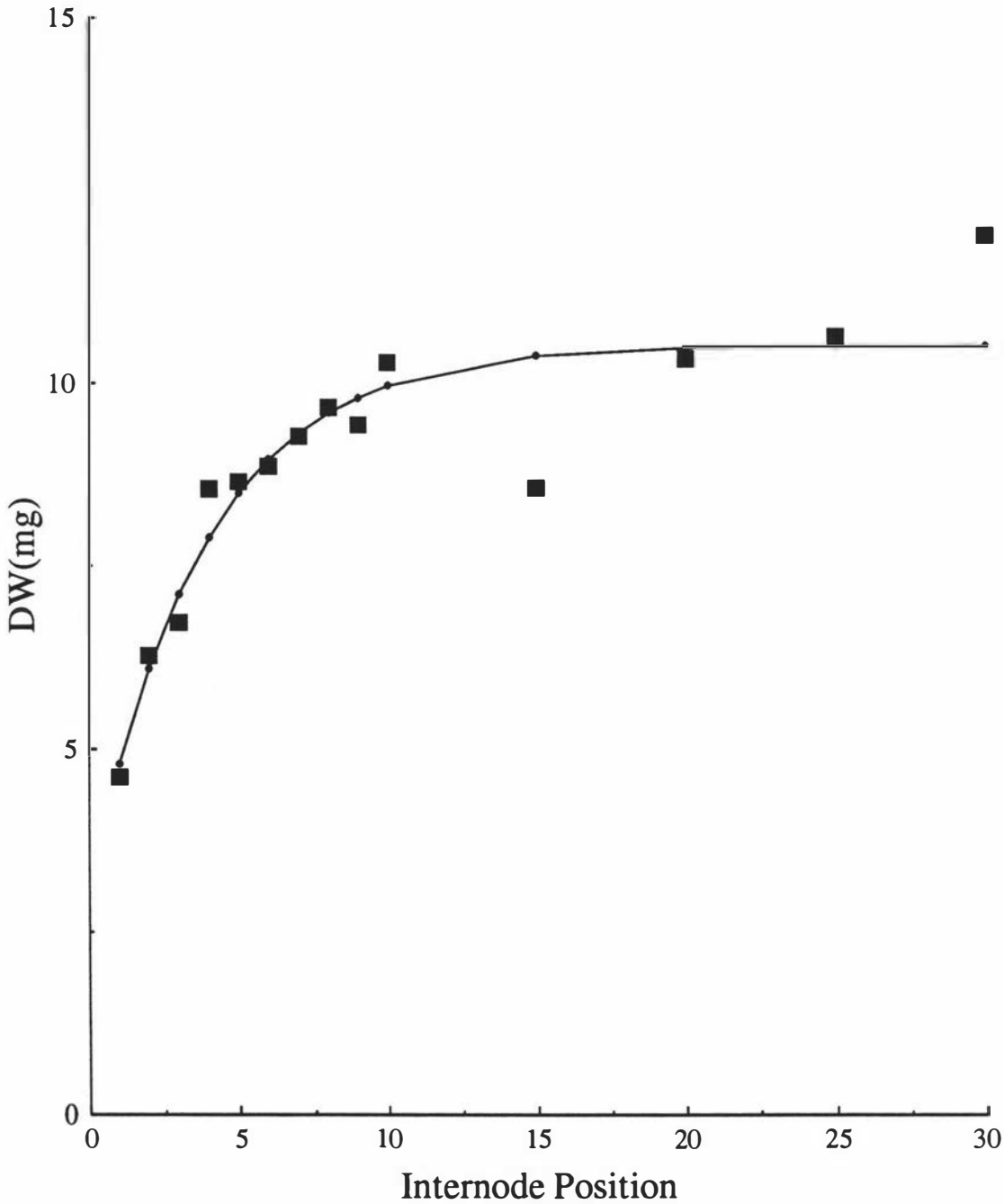


Figure 7.5 Variation with internode position (number of internodes from stolon apex) in mean dry weight per internode. Fitted line; $y = 4.79 + 5.74 [1 - e^{-0.258x}]$, ($r^2 = 0.994$).

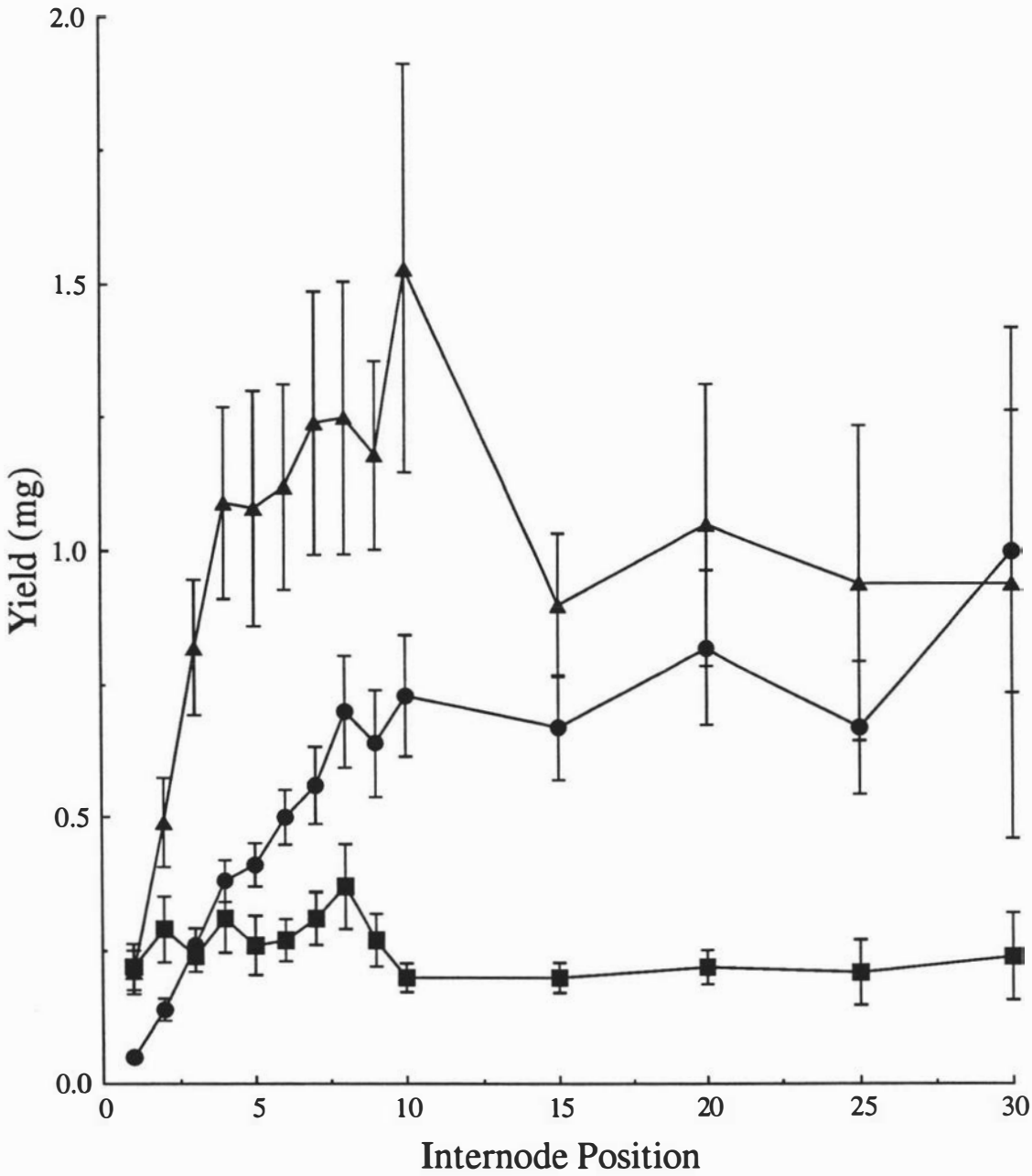


Figure 7.6 Variation in mean yield of starch (▲), sucrose (●), and hexose (■), in stolon internodes with internode position (number of internodes from stolon apex). (Error bars indicate ± SEM.)

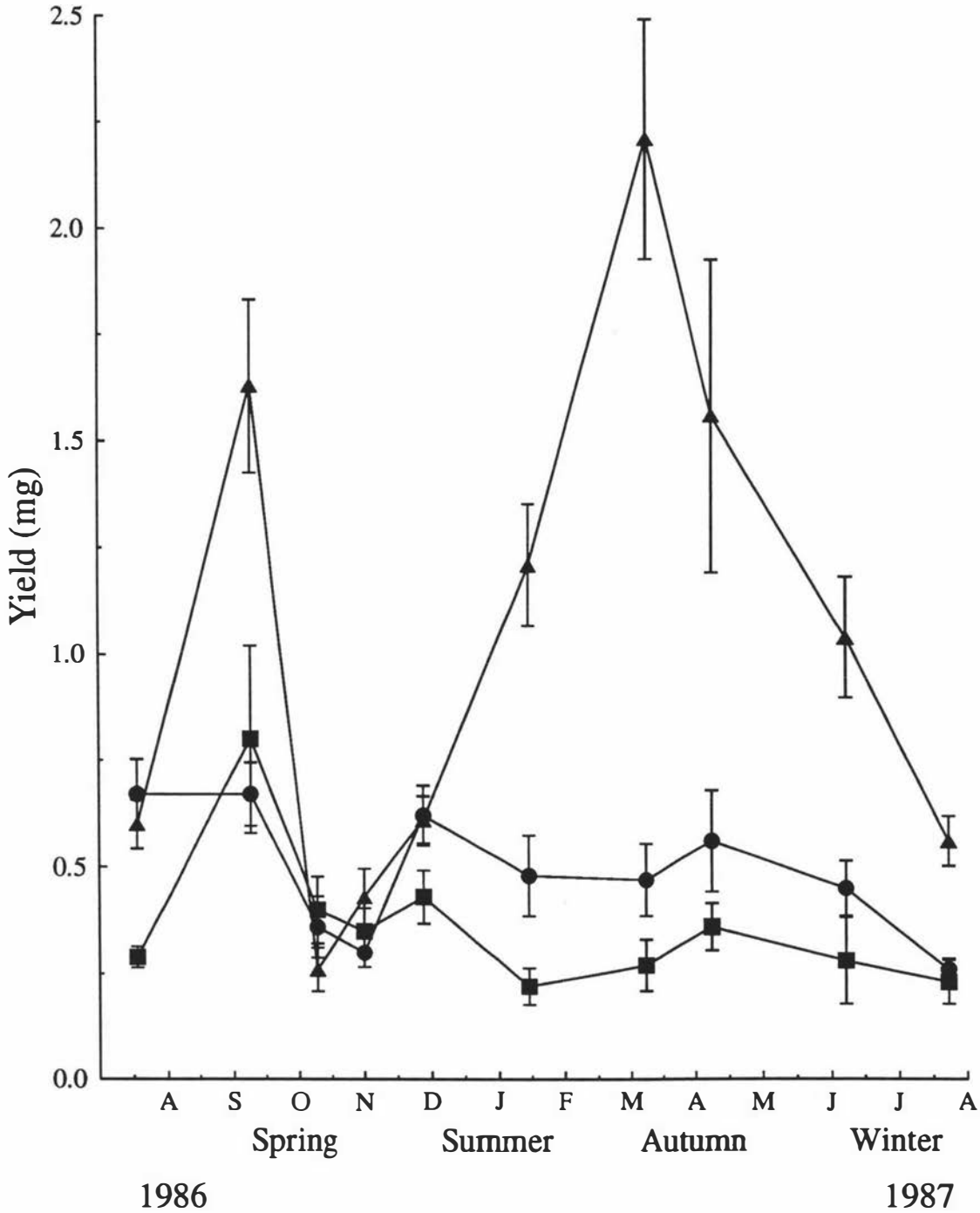


Figure 7.7 Seasonal variation in mean yield per internode of starch (▲), sucrose (●) and hexose (■) in stolons of Kopu white clover. (Error bars indicate ± SEM.)

7.3.6 EFFECT OF DEFOLIATION AND STOLON BURIAL ON CARBOHYDRATE CONTENT

The starch content of internodes varied ($P<0.0001$) with stolon treatment. Mean starch content per internode was similar for control (85 ± 3.4 μg $mg^{-1}DW$) and buried (86 ± 3.3μg $mg^{-1}DW$) stolons and higher than that of defoliated stolons (37 ± 2.6 μg $mg^{-1}DW$). The stolon treatments (control, buried and defoliated) did not influence mean content of sucrose at 47 ± 1.8, 49 ± 2.0 and 43 ± 2.0 μg $mg^{-1}DW$ or of hexose sugar at 34 ± 1.9, 28 ± 1.5 and 37 ± 1.7 μg $mg^{-1}DW$, respectively.

As stolon treatments only affected starch contents, the time courses for starch contents following treatment imposition are presented (Fig 7.8). A significant treatment x harvest x internode position interaction ($P<0.05$) was driven by the rapid initial depletion and subsequent replenishment of starch in the first three internode positions relative to that which occurred in positions ≥ 9 in stolons of the defoliation treatment. This contrasted with the control and burial treatments, in which there was little variation in starch content with harvest date and maintenance of a consistent relationship among internode positions with respect to starch content over time.

No significant difference ($P>0.543$) was found among the mean starch contents of defoliated stolons after 30 days (64.6 ± 9.04 μg $mg^{-1}DW$) and content at day 0 (64.4 ± 8.21 μg $mg^{-1}DW$).

7.3.7 STOLON INTERNODE CARBOHYDRATE CONTENT AND BRANCH DEVELOPMENT AT THE ACROPETAL NODE

Where data were pooled within the seasonal sampling study and the defoliation/burial experiment there were no significant correlations between branching status of axillary buds and the starch, sucrose or hexose content of the basipetal stolon internode. For both studies, treatments which substantially affected the contents of the carbohydrate fractions of stolons were identified and independently analysed. There was no indication at any internode position that starch, sucrose or hexose content in the stolon internode was correlated with branching status of the acropetal axillary bud in data from the seasonal sampling (Table 7.1) or the defoliation/burial experiment (Table 7.2).

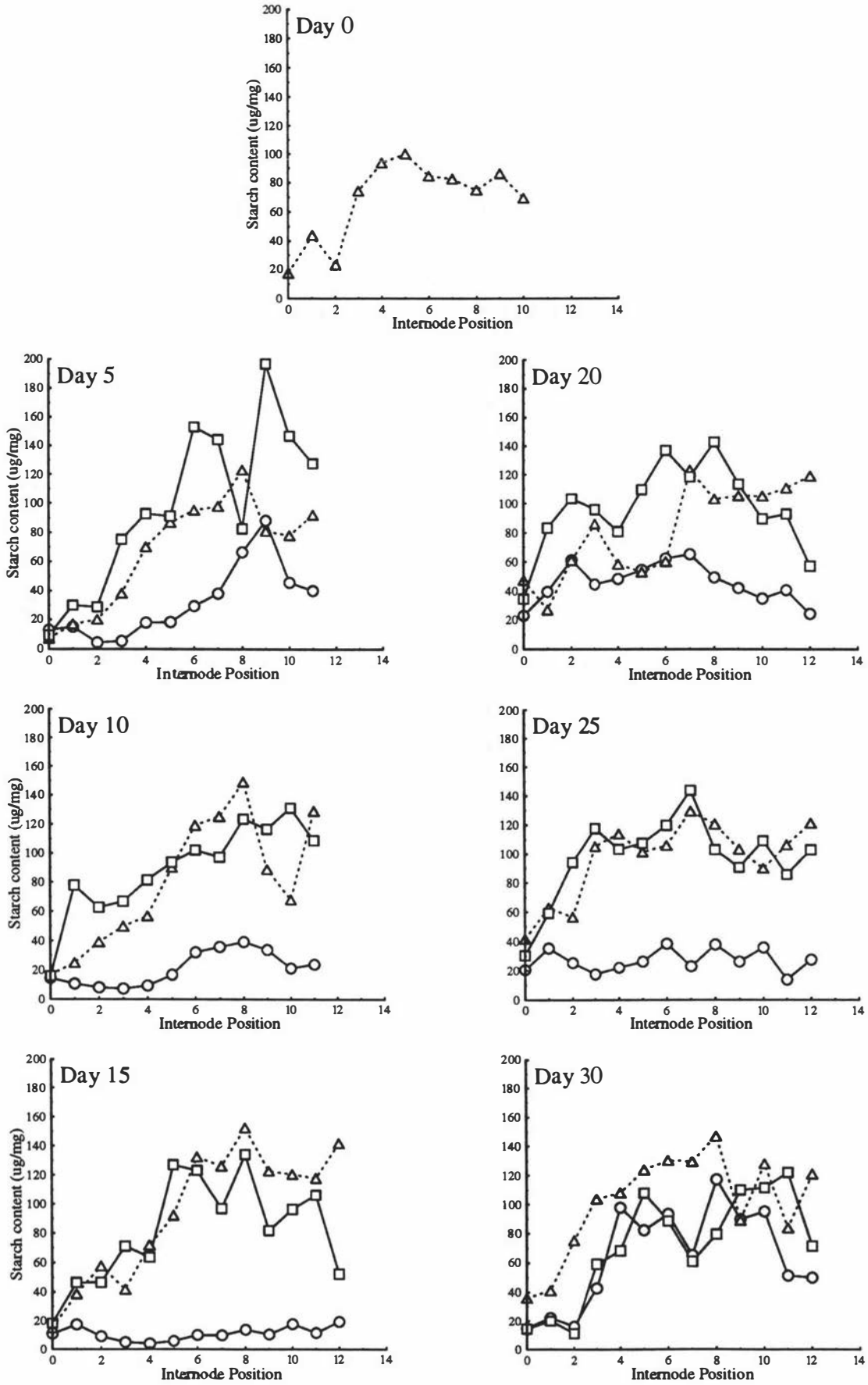


Figure 7.8 Starch content at each internode position (numbered from the stolon apex) in stolons of white clover sequentially harvested at five day intervals over a 30 day regrowth period following imposition of defoliation (○), burial (□) or control (no treatment, △) treatments on 25 September, 1987 (Day 0). Values for control stolons at Day 0 are presented.

Table 7.1 Correlation of branching status of the axillary bud at each of the first ten node positions with starch, sucrose or hexose content of its basipetal internode in stolons of Kopu white clover sampled in the seasonal variation study, (a) spring samplings pooled and (b) other samplings pooled. Pearson correlation coefficients (r), the probability of significance (p) and the number of observations (n) are presented.

	1	2	3	4	5	6	7	8	9	10
(a)	**Spring Samplings**									
Starch										
r	.056	-.259	-.306	-.310	.391	.050	.183	.439	-.175	.605
p	.871	.470	.359	.383	.298	.876	.569	.176	.607	.064
n	11	10	11	10	9	12	12	11	11	10
Sucrose										
r	-.235	-.290	.707	-.032	.561	.023	-.079	.070	.345	.645
p	.514	.386	.015	.934	.148	.946	.818	.848	.329	.061
n	10	11	11	9	8	11	11	10	10	9
Hexose										
r	.388	.167	-.048	-.184	.111	.032	.203	-.042	-.131	-.689
p	.268	.624	.888	.636	.794	.926	.549	.908	.718	.040
n	10	11	11	9	8	11	11	10	10	9
(b)	**Other Samplings**									
Starch										
r	.063	.113	.035	.118	.317	.197	-.207	-.106	.363	-.060
p	.721	.646	.883	.611	.151	.380	.356	.647	.106	.807
n	18	19	20	21	22	22	22	21	21	19
Sucrose										
r	-.168	-.039	-.232	-.358	-.320	-.126	.175	-.006	-.009	.147
p	.473	.875	.326	.111	.147	.578	.437	.979	.968	.549
n	18	19	20	21	22	22	22	21	21	19
Hexose										
r	-.135	-.124	-.243	-.364	-.050	.162	.167	.044	-.285	.191
p	.581	.612	.302	.105	.824	.471	.458	.849	.210	.433
n	18	19	20	21	22	22	22	21	21	19

Table 7.2 Correlation of branching status of the axillary bud at each of the first ten node positions with starch, sucrose or hexose content of its basipetal internode in stolons of the burial/defoliation experiment (a) control and burial treatments pooled and (b) the defoliation treatment. Pearson correlation coefficients (r), the probability of significance (p) and the number of observations (n) are presented.

	1	2	3	4	5	6	7	8	9	10
(a)	**Control and buried stolons**									
Starch										
r	.107	-.081	-.022	-.061	-.049	.040	-.034	.067	-.077	-.116
p	.533	.627	.890	.700	.797	.800	.831	.675	.635	.465
n	36	38	41	43	42	42	43	42	40	42
Sucrose										
r	.104	.247	.125	.080	.290	.108	.188	-.085	-.212	-.116
p	.558	.147	.445	.616	.066	.497	.240	.595	.184	.465
n	34	36	40	42	41	42	41	41	41	42
Hexose										
r	-.082	.041	.289	.125	.221	.084	.000	-.166	-.170	-.161
p	.646	.812	.070	.429	.165	.596	.999	.301	.289	.320
n	34	36	40	42	41	42	41	41	41	40
(b)	**Defoliated stolons**									
Starch										
r	.328	-.118	-.096	.033	.147	-.279	.409	-.378	.214	.354
p	.184	.640	.696	.893	.560	.248	.082	.110	.379	.137
n	18	18	19	19	18	19	19	19	19	19
Sucrose										
r	-.315	.005	-.170	-.182	-.469	-.114	-.109	-.181	-.365	.125
p	.203	.983	.485	.456	.057	.641	.657	.458	.136	.622
n	18	17	19	19	17	19	19	19	18	18
Hexose										
r	.050	.309	.049	-.033	.056	.441	.237	.207	.267	.344
p	.845	.227	.843	.895	.830	.059	.329	.396	.285	.163
n	18	17	19	19	17	19	19	19	18	18

7.4 DISCUSSION

The first five sections in this discussion relate to the five objectives of the work outlined in the introduction (Section 7.1) each of which is considered in turn. The effects of diurnal, seasonal and internode positional variation on the starch, sucrose and hexose contents of stolon tissue are discussed in that order. The fourth section considers the effects of defoliation and burial on the content of the three carbohydrate fractions. The fifth section discusses the lack of correlation between the content of starch, sucrose or hexose in an internode and the outgrowth of the axillary bud at the node distal to it. A conclusions section completes the chapter.

7.4.1 DIURNAL VARIATION

The results obtained have implications regarding the sampling of white clover stolons from pasture for assessment of carbohydrate status. The lack of large diurnal fluctuations in the carbohydrate fractions of stolons means that during the photoperiod time of sample collection is not an important constraint. Gordon *et al.* (1987) similarly reported no clear diurnal fluctuation in starch and hexose sugar content but found sucrose levels to increase during the photoperiod and decrease during hours of darkness in single plants grown in a growth cabinet. This diurnal variation in sucrose content was probably masked in the field-grown stolons by fluctuations in environmental conditions other than photoperiod. In retrospect, a diurnal sampling which included greater numbers of stolons, but with grouping of internodes within stolons for analysis, could have improved the precision of information on diurnal fluctuations without increasing the number of carbohydrate analyses. The highly significant variation between stolons in swards at any sampling indicates a requirement for several stolons (plants) in a sampling so that representative carbohydrate contents are obtained. The pronounced and consistent variations in the carbohydrate fractions with internode position means that field samplings must be representative of the distribution of nodes among node positions in the white clover population sampled. Another important factor affecting starch levels in stolons is defoliation (Tesar & Ahlgren 1950; Stewart & Bear 1951; Moran *et al.* 1953; Vez 1961; Murphy 1982; Gordon *et al.* 1986; Danckwerts & Gordon 1989; Baur-Hoch *et al.* 1990; Fig 7.8) and variation in severity of defoliation of stolons at previous grazings would be a factor contributing to the large variation among stolons in carbohydrate status at a sampling. Any sampling programme must accommodate all of the above effects.

7.4.2 SEASONAL VARIATION

In most respects seasonal variation in starch content of stolons followed the pattern observed in the northern hemisphere in that there was a pronounced early autumn buildup (Vez 1961; Boller & Nösberger 1983) followed by a steady decline through winter (Wood & Sprague 1952; Ruelke & Smith 1956; Vez 1961; Harris *et al.* 1983). Peculiar to these data was the abrupt decrease in starch content to low levels in mid-spring (October) (Fig 7.2). The lack of seasonal variation in hexose content is consistent with results of previous work (Ruelke & Smith 1956; Moran *et al.* 1953; Vez 1961); however, the increase in sucrose content in mid-winter (Wood & Sprague 1952; Ruelke & Smith 1956) was not apparent in this environment. The observed differences in results obtained between this study and the others cited can probably be related to the more favourable growth conditions for white clover in winter at Palmerston North (Fig 3.1) compared with the continental winters prevailing in Europe and North America. At Palmerston North active growth is maintained throughout winter (Table 3.2) which in turn limits the rate of depletion of starch in stolons. There was no increase in sucrose contents in winter as is found when osmotic adjustment increases the tolerance of frosting temperatures (Vez 1961). Whereas low carbohydrate status of stolons was associated with net loss of stolon biomass in winter in northern hemisphere studies (Wood & Sprague 1952; Vez 1961; Harris *et al.* 1983), low starch content and net loss of stolon biomass occurred in spring in this study (see Fig 7.2 and Fig 3.5, respectively). The sudden drop in starch content of stolons in October parallels other physiological changes within white clover populations at this time and these aspects will be considered in more detail in Chapter 8. As mean dryweight per internode is also at a minimum in spring the mean yield of starch per internode in October is only 11% that of the maximum measured for March (Fig 7.7) clearly indicating energy reserves are at a minimum at this time.

7.4.3 VARIATION WITH INTERNODE POSITION

Starch is the major component of total carbohydrate in stolon (Fig 7.2). The variation in starch yield with internode position has ramifications for the growth of white clover in grazed swards. If the yield of starch at each position is weighted by the proportion of nodes in the population at the position, the contribution of each node position to the overall starch storage can be calculated. The distribution of nodes at each node position has been calculated for white clover in nearby sheep-grazed swards (Hay *et al.* 1991) and if it is assumed that a similar distribution exists within this population the

contribution of each node position to total starch yield can be calculated (Table 7.3). The node positions 1-3 account for 25% of the node population but only 13% of starch yield while nodes 4-10 (39% of nodes) contribute 50% to starch yield. Hence nodes 1-10 account for approximately 64% of both nodes and starch yield. The lower yield of starch and sucrose in nodes 1-3 has ecological advantages for white clover. These nodes bear virtually all of the leaves in grazed pastures (Davies & Jones 1986, 1988; Jones & Davies 1988; Brock 1988; Brock *et al.* 1988), are not firmly anchored by roots (Brock *et al.* 1988) and are susceptible to loss by defoliation (10% of stolons were without apices, Chapter 3.4, in these swards). However, any defoliation of the first three internodes has a relatively small effect on the overall energy status of plants due to the low carbohydrate content of these internodes.

In the only comparable report found, Moran *et al.* (1953) measured variation in carbohydrate status of white clover stolon segments comprised of nodes 1-5, 6-10 and >10 and obtained similar results except that starch content of internodes >10 was higher than in nodes 5-10 in the autumn samplings in that environment.

7.4.4 EFFECT OF DEFOLIATION AND BURIAL

As predictable from previous work (Moran *et al.* 1953; Vez 1961; Murphy 1982), defoliation lowered the starch content of stolons to minimal values over days 10-15 following defoliation (Fig 7.8). The abrupt decrease by day 5 in sucrose and hexose contents and subsequent rapid recovery by day 10 after defoliation observed in glasshouse/growth cabinet grown plants (Moran *et al.*1953; Gordon *et al.* 1986; Baur-Hoch *et al.* 1990) was not detected in these field grown plants. Careful interpretation of Fig 7.8 provides insights into the controlled nature of the remobilisation of starch in white clover stolons following defoliation. The starch levels of defoliated stolons on day 5, 10 and 15 after defoliation (Fig 7.8) indicate that there is remobilisation initially from all nodes but that preferential exhaustion of internodes nearest the apex to minimal levels (0.4-1.0% of DM) occurred first and then with time internodes were successively depleted back along the stolon so that by day 15 the first 12 internodes were all similarly exhausted of starch. Similarly the replenishment of starch in stolons commenced with some storage in all internodes but with an indication that full replenishment initially occurred in the youngest internodes and then progressed back along the stolon. Replenishment was complete by day 30 at which time the pasture was ready for another grazing. The reason for the depressed values of defoliated stolons at day 25 (Fig 7.8) is unknown but does not appear to be due to unusual weather patterns, eg low radiation, temperature or rainfall.

Table 7.3 Table of calculation of the percentage of total starch in stolons at various internode positions in the Kopu white clover population utilising the distribution of nodes among node positions as given by Hay *et al.* (1991) for white clover populations in pastures grazed by sheep.

Internode position	Starch yield (mg)	% of total nodes at each node position (from Hay *et al.* (1991))	Weight of starch (mg per internode position) in a representative internode sample of 100	% of total starch
1	.211	8.30	1.75	1.87
2	.488	8.22	4.01	4.29
3	.818	8.18	6.69	7.17
4	1.093	7.69	8.41	9.01
5	1.084	6.51	7.06	7.56
6	1.116	5.83	6.51	6.97
7	1.241	5.38	6.68	7.15
8	1.252	5.19	6.50	6.96
9	1.178	4.63	5.45	5.84
10	1.526	4.10	6.26	6.70
11-20	0.95	25.01	23.76	25.45
> 20	0.94	10.95	10.29	11.02

Burial by 2 cm of soil (intact leaves maintained above soil) had no influence on the carbohydrate status of stolons, which implies that any influences of burial on branch development of axillary buds were via mechanisms other than carbohydrate supply.

7.4.5 INTERNODE CARBOHYDRATE CONTENT AND BRANCHING

In the grazing trial of this study the interval between grazings (defoliations) was constant in that plant development corresponding to approximately three node appearances occurred per grazing interval (Chapter 4.2.2). Thus an internode positioned 10 to 12 on a stolon has been subjected to three defoliation cycles and its starch content would have been depleted and replenished three times with the severity of the depletion dependent on initial status (Moran *et al.* 1953; Hartwig *et al.* 1990) which is dependent on season (Vez 1961; Fig 7.3) and severity of previous defoliations (Moran *et al.* 1953) particularly the

latest defoliation (Danckwerts & Gordon 1989; Baur-Hoch *et al.* 1990). In the regrowth cycle measured during October 1987, when mean starch levels were low (5.2% of DW) at the end of the regrowth period, 70 to 95% of starch in stolon internodes was remobilised following defoliation (Fig 7.8). Given the cyclic pattern of depletion and replenishment of starch content in stolons and that sucrose and hexose contents were relatively stable, the lack of any relationship between branching and internode starch, sucrose and hexose content immediately prior to defoliation (Sect. 7.3.6) is understandable. For white clover in these grazed swards it is concluded that the carbohydrate status of stolon internodes does not affect branch development from axillary buds. Recently Hartwig *et al.* (1990) demonstrated that carbohydrate supply was not the major factor involved in the decrease in nitrogen fixation in white clover immediately following defoliation. This study provided no evidence to suggest that carbohydrate supply within the plant limited plant performance in these swards.

7.4.6 CONCLUSIONS

In conclusion, although there were considerable responses in the starch content of stolons to both season and defoliation, burial had no effect and none of these factors significantly affected sucrose and hexose contents of stolons. The lack of relationship between branch development of axillary buds and carbohydrate status of the basipetal internode is explained by the non-significant variation in content of hexose sugars in stolons as these are the forms of carbohydrate directly metabolised (Butler & Bailey 1973) and there was no evidence to suggest that the level of supply was limiting.

CHAPTER 8 GENERAL DISCUSSION; ECOLOGY AND PHYSIOLOGY OF WHITE CLOVER WITH RESPECT TO BRANCH DEVELOPMENT AND BURIAL OF STOLON

The overall objective of this study was to undertake investigations into the effects of stolon burial on branching of stolons such that the knowledge gained could be utilised to further describe and understand the pattern of clonal growth of white clover in pastures. The following discussion, comprised of three sections, considers and develops the findings of the study as they relate to this objective. In the first section the results of investigations into the effects of stolon burial on branching are discussed before there is a discussion of how burial may affect the functioning of stolons and, thence, indirectly influence branching. The second section outlines how the study contributes to knowledge of the correlative control of branching. In the final section the new information arising from this study is integrated with previous knowledge so as to further understanding of, in particular, the spring period of the seasonal cycle of clonal growth of white clover in grazed swards.

8.1 STOLON BURIAL, BRANCHING AND STOLON FUNCTION

The strong seasonal cycle of stolon burial in winter measured in the field trial (Chapter 3) is consistent with previous measurements in Manawatu (Hay 1983, 1985; Hay *et al.* 1983), elsewhere in New Zealand (Hay & Chapman 1984; Hay *et al.* 1987) and in United Kingdom (Grant *et al.* 1986; Sackville Hamilton & Harper 1989). Although factors such as drought and overgrazing (Hay & Chapman 1984; Hay *et al.* 1987), low earthworm population density (Hay *et al.* 1987) and high rainfall (Hay *et al.* 1987), especially on heavy soils (Grant *et al.* 1986), can modify the seasonal trend in stolon burial, late winter-early spring is normally associated with maximal and late summer with minimal burial of stolon.

A framework for evaluating the branching response of white clover to environmental variables was proposed by Turkington *et al.* (1991), elements of which aid interpretation in this study. Three basic attributes of stolon development affect branching over time: (a) rate of node/axillary bud appearance, (b) position of first branching node (ie. position of the associated node in plastochrons when outgrowth of the axillary bud is observed) and (c) probability of branching (ie. probability of an axillary bud forming a branch).

Stolon burial has little effect on the node appearance rate of the primary stolon of plants (Grant *et al.* 1991; Chapter 6). However burial in association with defoliation significantly reduces node appearance rates of secondary (branch) stolons (Grant *et al.* 1991) and secondary stolon growth generally (Chapter 6). No significant effect of burial on the node position of first branch appearance was measured in the glasshouse trial (average node position of first incipient branch for buried and non-buried stolons, 3.6 ± 0.74 and 3.4 ± 0.52 respectively). However burial significantly depressed the probability of successful branch establishment both in the field (Sackville Hamilton 1982; Chapter 4) and in the glasshouse (Grant *et al.* 1991; Chapter 6).

The physiological basis for the negative effects of stolon burial on branching in white clover will now be discussed. Burial of stolons with soil alters the environment of stolons and axillary buds by influencing the temperature, light, moisture and atmospheric regimes and by exerting physical pressure or resistance to stolon surfaces particularly where meristems are actively expanding. Although this study has focused on the alteration of the light regime induced by burial some other effects of burial of stolon will also be considered in the following discussion.

When stolons or leaves grow through soil the pressure exerted on them induces thigmotropic responses which are expressed as thickening of stolons (Newton 1986; Chapter 6) or a thickening of the petiole. Field studies (Chapter 4) and glasshouse studies (Grant *et al.* 1991; Chapter 6) indicate that burial of formed stolon, without defoliation, has no significant effect on the probability of initiation of outgrowth of an axillary bud but results in a significant depression in the probability of establishment of a branch at a node (Sackville Hamilton 1982; Chapter 4). This implies that burial increases the mortality rate of axillary buds once outgrowth has commenced. The first leaves produced by axillary buds are smaller than parent stolon leaves (Wilman & Simpson 1988; Hay *et al.* 1993) and so have reduced chances of forcing their way through to the soil surface. Axillary buds developing in soil, in the dark, are totally dependent upon the parent stolon for photosynthate as they are unable to photosynthesize. Supply from the parent stolon is dependent upon growth conditions, with defoliation of the parent stolon resulting in cessation of supply (Chapman *et al.* 1992a, b). Thus axillary buds developing in soil not only have additional energy requirements imposed by thigmomorphogenetic responses but may have growth potential further reduced by a variable and limited supply of photosynthate. These factors combine to increase the mortality of axillary buds developing in soil over those developing above ground.

Burial alters the light regime by effectively reducing the light intensity to zero. Stolon internodes exposed to light photosynthesize at rates which are greater than stolon respiratory losses (Harris *et al.* 1983), contribute about 5% or more to total photosynthesis of white clover (Korte & Parsons 1984; Ryle *et al.* 1988), contribute significantly to carbon uptake during the first few days following defoliation (Davidson *et al.* 1990) and are 12-22% as efficient as leaves per unit surface area (Chapman & Robson 1992). When plants are severely defoliated and photosynthate is a scarce resource, shading (or burial) to prevent photosynthesis by stolons significantly impacts on the carbon economy of plants and a manifestation of this is an increase in death rate of older basal stolon, thus decreasing plant size (Chapman & Robson 1992). Burial of stolon systems of plants coupled with defoliation, although having little effect on the primary stolon, significantly affects secondary stolons by reducing node appearance and probability of branching and increasing mortality by 34% relative to unburied defoliated plants (Grant *et al.* 1991). This response is consistent with recently measured changes in intraplant partitioning of carbon following defoliation in that carbon supply from the primary stolon to young branches ceases and the sink strength of developing leaves on the primary stolon increases (Chapman *et al.* 1992a, b). The additional penalty of stolon burial in prohibiting stolon photosynthesis exacerbates the period of carbon shortage in young branches thereby increasing their mortality rate (Grant *et al.* 1991).

Probability of initiation of axillary bud outgrowth is not affected when undefoliated stolon tissue has emerged in light and then been buried (Grant *et al.* 1991; Chapter 4; Appendix 5.1; Chapter 6). However as the severity of defoliation of stolons increases, probability of initiation of axillary bud outgrowth decreases. This response is explicable in terms of sink-source theory in that defoliation limits carbon availability which is then preferentially allocated to re-establish developing leaves at the parent stolon apex (King *et al.* 1978; Chapman & Robson 1988; Chapman *et al.* 1991a, b). Davies & Evans (1990a) reported an all or nothing response to defoliation and shading on initiation of axillary bud outgrowth and suggested a photomorphogenetic response was involved. This interpretation is questionable as stolons were pretreated by shading (exclusion of 84 or 94% of photosynthetically active radiation) to the extent that reduced carbon availability increased the expression of apical dominance so as to inhibit axillary bud outgrowth at nodes one to five. Intraplant carbon availability was subsequently further reduced by defoliation and photosynthetic capacity available for recovery reduced by completely shading stolon at node positions 2 to 5. Hence this rather drastic response is also

explicable in terms of source-sink relationships in line with other studies (Grant *et al.* 1991; Chapter 6). Also, as the shading treatment of stolons was neutral with respect to light quality, photomorphogenetic responses over and above the effects of reduced photosynthetically active radiation would not be expected (Solangaarachchi & Harper 1987b; Thompson & Harper 1988). The sensing of light or a photomorphogenetic signal is not a prerequisite for initiation of axillary bud outgrowth as initiation occurs in the dark when certain correlative (eg. removal of apical dominance) (Newton & Hay 1992; Chapter 6) and environmental (Newton & Hay 1992) conditions are met. For example, probability of initiation of outgrowth of axillary buds formed under soil and maintained in soil increased from 0.29 to 0.73 when the stolon apex was excised (Chapter 6). It is possible that excision induces a wounding response which substitutes for a prerequisite light mediated signal although buds at nodes six away or approximately 6.4 cm from the site of apical excision showed greatly enhanced development in response to the excision (Fig 6.2) which means such a wounding response would have to be readily and widely translocated. If the latter possibility is discounted (excision of leaves at the base of petioles immediately adjacent to the axillary bud induced no such wounding response (Chapter 6)) it can be hypothesised that initiation of axillary bud outgrowth has no prerequisite for light stimulation at bud sites and that plant control of development is via activation of inhibitory mechanisms.

The known mechanisms of inhibition of axillary bud outgrowth are via apical dominance, phytochrome-mediated responses to light quality and a correlative mechanism inducing a period of dormancy in spring. Inhibition due to source-sink relationships has a general effect in determining the extent of apical dominance and rate of development (and mortality) of branches whereas phytochrome-mediated responses are more localised operating perhaps at the individual axillary bud level within plants. Findings of Moulia *et al.* (1989) were consistent with this concept of control by inhibition of initiation of bud outgrowth in that where total radiation supply was uniform but light quality was varied using end-of-day changes to red light supplied, probability of branching varied but number of nodes to first branch (apical dominance) remained unchanged. It is pertinent to note that changes in light quality induced by the presence of white clover leaves exaggerate the effects of reduced photosynthetically active radiation rather than induce a different range of plant responses (Solangaarachchi & Harper 1987) although changes in red to far-red ratio induced by canopies of grass species also significantly reduced node appearance rate as well as greatly decreasing branching frequency compared to neutrally shaded plants at

the same level of photosynthetically active radiation (Thompson & Harper 1988). The above hypothesis would suggest that the stimulation of branch development sometimes observed in white clover following defoliation of swards (Brougham 1958; Sanderson 1966; Davies & Evans 1990b; Grant & Barthram 1991) results from the altered light environment (increased photosynthetically active radiation and red to far-red ratio) removing the conditions which activate the processes involved in maintaining inhibition of bud development rather than from providing any direct stimulatory effect. The correlative processes that induce the dormancy of buds in spring are unknown but they affect all nodes in the stolon system of a plant and are not affected by excision of leaves and roots or separation of nodes and incubation under optimal conditions (Newton *et al.* 1990, 1992).

Stolon burial did not affect the carbohydrate status of stolons when leaves were positioned above the soil (Chapter 7). Defoliation history is the major determinant of starch content (refer to Chapter 7) but causes only minor fluctuations in sucrose and hexose contents (Moran *et al.* 1953; Gordon *et al.* 1986; Baur-Hoch *et al.* 1990; Chapter 7). However, burial in association with defoliation impacts heavily on the carbon economy of plants (Grant *et al.* 1991; Chapman & Robson 1992) and reduces initiation of outgrowth (Chapter 6) and development/survival (Davies & Evans 1990a; Grant *et al.* 1991; Chapter 6) of axillary buds on the main stolon and also on secondary stolons (Grant *et al.* 1991; Chapter 6). On average stolons are defoliated twice during the period an axillary bud is positioned three to eight from the stolon apex and hence with maximum potential for initiation of outgrowth. During this period starch contents are constantly changing within stolons in response to defoliation (Moran *et al.* 1953; Vez 1961; Murphy 1982; Chapter 7) while sucrose and hexose contents in stolon remain reasonably constant during regrowth (Chapter 7). There were no significant correlations of content of any carbohydrate fraction in internodes with probability of development of the associated acropetal or basipetal axillary bud (Chapter 7) which given the lack of variation in sucrose and hexose sugar content and the fluctuating starch content in response to defoliation is not an unexpected result. These findings were based on measures of total content of a carbohydrate fraction and so do not contradict explanations of patterns of axillary bud development based on fluxes of carbon, derived from current photosynthate or remobilisation of reserves, the direction and magnitude of which are determined by source-sink relationships.

Under New Zealand conditions maximal rates of initiation of new adventitious nodal roots per plant (Brock *et al.* 1988; Newton & Hay in press) and greatest rates of new root production and branching of new roots at depths below 5 cm (Caradus & Evans 1977) occur in white clover in late winter-early spring when burial of stolon is maximal, soil moisture is usually non-limiting for growth (Chapter 3) and temperatures are suboptimal for major pathogenic organisms (Skipp & Gaynor 1987). Successful establishment of nodal roots requires high humidity for initiation of growth (Knight 1953; Trautner & Gibson 1966; Ueno 1982; Stevenson & Laidlaw 1985; Thomas 1987b) and moist soil to aid root penetration into the soil (Ueno & Yoshihara 1968); stolon burial promotes both these conditions as the soil buffers stolons from the more extreme changes in moisture content or humidity that occur at the soil surface. Thus burial through aiding initiation and development of nodal roots reduces the probability of limitation of branch development through the lack of a nodal root at the node of origin (Knight 1953; Chapman 1983). However burial through reducing successful branch establishment will ultimately reduce the numbers of nodal roots on plants as roots at nodes that have no branch have lower survival rates (Newton & Hay, in press).

Absorption of nutrients is a further physiological function of stolons. Stolons actively absorb phosphate (Hay & Dunlop 1982; Dunlop & Hay 1985; Hay *et al.* 1986b) and plants may obtain more than 5% of total phosphorus in this manner (Hay *et al.* 1986b). Stolon burial assists nutrient uptake by exposing the whole surface area of stolons to the soil solution of the nutrient enriched upper zone of soil (Ozanne 1976) and improving access of the soil solution into stolons through increasing the potential for microbial lysis of the stolon cuticle and hypodermis (Hay *et al.* 1982). Hence stolon burial by encouraging both direct nutrient uptake by stolons and initiation and development of nodal roots acts to improve the nutritional status of plants and this in turn may improve branching by minimising the influence of apical dominance (Harvey 1970, 1979; Thomas 1987b; Chapter 5).

8.2 CORRELATIVE CONTROL OF BRANCHING

Results from this study extend knowledge of the correlative control of branching in white clover. Results from Chapter 6, in particular, lead to the development of some important concepts relating branching and axillary bud ontogeny. Two aspects very apparent in any study of branching of white clover are that initiation of branch development

occurs only at a limited number of node positions basipetal to the apex (Chapman 1983; Davies & Evans, 1990a, Hay *et al.* 1991; Newton *et al.* 1992; Chapter 6) and that branching is precluded at a variable number of nodes immediately basal to the apex (Erith 1924; Harvey 1979; Sackville Hamilton 1987a; Thomas 1987b; Sackville Hamilton & Harper 1989; Davies & Evans 1990a; Turkington *et al.* 1991; Caradus & Chapman 1991; Hay *et al.* 1991; Davies & Jones 1992 Chapters 5, 6), a characteristic generally considered to be an expression of apical dominance (Thomas 1987b). When axillary buds on stolons of the B/B treatment (Chapter 6) were exposed to light, their outgrowth occurred only at nodal positions of eight or less from the stolon apex and not at nodes positioned > 8 from the apex. The lack of outgrowth of axillary buds at the older nodes is unlikely to result from loss of viability as even in stolons sampled from pastures the viability of buds at node position 10 remained at 35-40% (Newton *et al.* 1992). Such a result suggests then, that correlative factors are operating within the plant which concentrate resources for the outgrowth of the more distally situated axillary buds. Hence it is hypothesized that within an intact stolon the ontogeny of an axillary bud includes a 'window of opportunity' for effective initiation of development. This 'window of opportunity' spans the developmental period of the bud from its release from apical dominance through to its development to a node position greater than eight. Although there is genetic variation (Erith 1924; Caradus & Chapman 1991) apical dominance normally inhibits vegetative axillary bud outgrowth at node positions 1 and 2 (Thomas 1987a; Hay *et al.* 1991) but its extent (the number of nodes basipetal to the apex affected) is sensitive to the availability of resources at the apex (Harvey 1979; Newton 1986; Thomas 1987b; Chapter 5). Severe resource limitations can result in effectively preventing the initiation of axillary bud outgrowth by extending the extent of apical dominance to node positions beyond eight thereby closing off the 'window of opportunity' for bud outgrowth (Caradus & Chapman 1991; Chapter 6). The basal limit to the 'window of opportunity' could not be rigidly defined. In Welsh pastures, initiation of axillary bud outgrowth is thought to cease after a bud passes on from node position 7 (Dutta 1988; Davies & Evans 1990a). Under New Zealand conditions Chapman (1983) found most branch initiation occurred within 4 to 8 weeks of node appearance, indicating a maximal node position of eight which is a result consistent with the findings of Newton *et al.* (1992). However Hay *et al.* (1991) estimated that some initiation of branching occurred up to node position 10 although most had taken place by node 7. In general under field conditions the 'window of opportunity' for branch initiation (defined as the production of a fully expanded leaf) is the period of node development inclusive of from node position 3 or 4 through to node position 7 or 8.

Severe reductions in levels of resource availability within plants were found to reduce plant branching by increasing markedly position of first branching node relative to the stolon apex and by decreasing node appearance rate. There was no change in probability of outgrowth of axillary buds that contributed to the decrease in plant branching (Chapter 5). Hence, as such severe resource limitations seldom occur in productive pastures it is unlikely that the observed low probabilities of a node branching in the field are the result of low levels of intraplant resource reducing the probability of outgrowth of axillary buds.

When nodes emerge from the stolon apex in soil and remain buried, probability of initiation of outgrowth of axillary buds decreased some 60-70% under the conditions of a glasshouse experiment (Chapter 6). This indicated that outgrowth of axillary buds is responsive to environmental treatments. If after the production of eight nodes the main stolon apex was excised, the level of outgrowth of the buried buds was largely restored. Hence the burial treatment reduced outgrowth of axillary buds through an inhibition process rather than by increasing the mortality of buds. These results also indicate that inhibition of axillary bud outgrowth was not caused by an absolute deficiency in photosynthate supply but was probably initiated by plant sensing of the light environment and the response mediated via an apical dominance mechanism which would involve alterations to the balance of plant growth hormones present (Thomas 1987b).

The small experiment reporting on the effects of burial and defoliation treatments applied to individual stolons within plants (Appendix 5.1) demonstrated the independence of action of node appearance rate and position of first branching node as stolon characteristics influencing branching and also the independence individual stolons had within plants for both these characteristics. Grant *et al.* (1991) reported differing node appearance rates between branch stolons and the main stolon of plants under burial and defoliation treatments. Such results clearly differ from the proposal that node appearance rate is common for apices within a plant (Turkington *et al.* 1991). Whereas plants in this study (Appendix 5.1) had no main stolon apex, those of Grant *et al.* (1991) did. Thus within-plant differences in node appearance rate were obtained regardless of the presence of the possible integrative role of the main stolon apex (Newton 1986). In pastures intensively grazed by sheep, calculations based on plant (Hay *et al.* 1991) and population (Hay *et al.* 1988) data indicate that 35% of apices are present in plants without a main stolon apex and hence any correlative/integrative effects such an apex may confer.

The number of nodes from the apex to the first branch (position of first branching node) is an attribute not of individual nodes but of the stolon and is integrated over the microenvironments of all nodes contributing resources to the apex. Thus nodes from other stolons that contribute resource to a particular stolon will also influence the characteristic, of position of first branching node, of that stolon. This property of the stolon (branch) was not fully addressed in the analysis by Turkington *et al.* (1991) which constitutes a significant gap in understanding as variation in this property (see earlier and also Chapter 5) can be an important mechanism of adaptation to an unfavourable microenvironment at the individual branch level. The characteristic, position of first branching node, varies independently of node appearance rate (Turkington *et al.* 1991) and probability of axillary bud outgrowth (Moulia *et al.* 1989) and is an attribute of white clover morphology/growth that is now receiving increasing attention (Sackville Hamilton 1987a; Sackville Hamilton & Harper 1989; Moulia *et al.* 1989; Davies & Evans 1990a; Hay *et al.* 1991; Turkington *et al.* 1991; Chapter 5).

In conclusion, the following model is suggested as controlling initiation of axillary bud outgrowth in white clover. Outgrowth of axillary buds is the norm and control of development is via mechanisms that inhibit this development. The first of these, often referred to as apical dominance, has the limits of its influence determined by the abundance of resource(s) at the stolon apex and is therefore a function of the collective acquisition of resources by all nodes supplying the apex. Apical dominance is manifested as a delay in initiation of axillary bud outgrowth seen as an increase in the position of first branching node relative to the apex or in extreme environments inhibition until a bud has passed through the 'window of opportunity' for bud development (see earlier discussion). The second inhibitory mechanism is activated by sensing of the light environment, possibly via phytochrome. Both these mechanisms are very responsive to changes in the environment. The third mechanism is a correlative inhibition operating in spring for a period of four to six weeks in response to unknown factors (Newton *et al.* 1990, 1992; J.R. Caradus & J. van den Bosch unpublished data). This model (hypothesis) requires testing by a series of experiments, involving examination of responses of both treated and untreated, nodes and stolons of plants which have individual nodes or stolons subjected to a range of treatments including variation in neutral light shading, light quality, defoliation regime and nutrient supply. This work must be designed to test for the independence of operation of the inhibitory processes and define the extent of independence of activity of each axillary bud within plants to these processes.

8.3 BURIAL AND THE AUTECOLOGY OF WHITE CLOVER IN PASTURES

The observations reported in this study have relevance for growth of white clover in grazed pastures. During autumn and early winter, surface casting by earthworms is the major mechanism of stolon burial (Hay *et al.* 1987). Stolons may be partially or completely buried and subject to various degrees of defoliation intensity at burial. In such cases the stolon tissue has emerged while on the soil surface before burial and is equivalent to the O/B treatment of the Chapter 6 experiment. This treatment had no effect on the probability of initiation of axillary bud outgrowth at nodes on primary stolon but halved that of nodes on secondary stolons. Although initiation of branch outgrowth on primary stolons is not affected by burial in this manner, successful branch establishment is significantly and consistently reduced (Sackville Hamilton 1982; Chapter 4) with the effect greatest at the youngest nodes (Chapter 4). In late winter-early spring factors such as treading by stock are equally important in the burial of stolons (Hay *et al.* 1987) and increased severity of defoliation often occurs in many farming systems at this time (Brock 1988) which acts to heighten the negative effects of burial (Grant *et al.* 1991; Chapter 6).

The most severe negative effects of burial on axillary bud outgrowth occur at nodes (axillary buds) which emerge and then develop in soil, ie. axillary buds which correspond to those of the B/B treatment in Chapter 6, as both the initiation of outgrowth (Chapter 6) and survival of developing buds (Chapter 4) are reduced under such conditions. However, growth of stolon maintained in the dark is negatively geotropic (Thomas 1987b) and apices of buried stolons respond to the darkness by curving and growing vertically to the soil surface (Maige 1900; Hay 1983; Thomas 1987b; Grant *et al.* 1991). This response of rapidly returning the apex to the soil surface therefore, minimises the number of nodes (axillary buds) in this category. Relatively few nodes emerge in soil and are then subsequently exposed to light although some re-exposure of nodes buried by earthworm casts occurred in early spring in Wales when stock reintroduced to pastures trampled down wormcasts (Sackville Hamilton & Harper 1989).

Approximately 65-70% of annual production of nodes per stolon apex occurs during the peak growing season (November to April) (Chapman 1983) when apices are on the soil surface and burial processes are inactive or active for short periods following heavy rainfall (Hay *et al.* 1987). Hence the majority of axillary buds developing in environmental conditions with potential for rapid growth, are on the soil surface and not subjected to the

effects of burial. During the off season (May to October) most stolons are subjected to some degree of burial (Hay 1983; Sackville Hamilton & Harper 1989) most of which occurs after formation of stolon on the soil surface. This form of burial has minor effects on the initiation of axillary bud outgrowth on mature stolon axes (Chapter 6) but major depressive actions on initiation of axillary bud outgrowth on secondary branch stolons and on the survival of developing branches (buds) on both primary and secondary stolons following initiation of outgrowth. There was a three-fold depression in the survival of developing buds in undefoliated stolons (Chapter 4) and defoliation is known to aggravate the problem (Grant *et al.* 1991). This decrease in axillary bud development due to burial of stolon contributes along with plant fragmentation, plant death and spring dormancy of axillary buds to the reduction in density of growing points in swards in spring (Chapter 3). These effects have also been observed as a reduction in spring of branching frequency of stolons (Wilman & Simpson 1988) and of populations (Hay *et al.* 1991; Chapter 3) as well as a low probability of branch establishment on nodes appearing during winter (Chapman 1983; Davies 1989). The consequences for the white clover population of lower growing point densities in spring is very dependent upon the particular environmental and managerial conditions that prevail during the spring early summer period (Jones 1933; Hay & Baxter 1984, 1989; Brock 1988; Hay *et al.* 1988, 1989b; Davies & Evans 1990b; Grant & Barthram 1991) although in some situations yield components are determined more by the density of growing points or stolon in spring than by subsequent environmental conditions during the regrowth period (Harris *et al.* 1983; Dennis *et al.* 1984; Collins *et al.* 1991).

This study also contributes some insight into the physiology underpinning the abrupt changes that occur in spring when fragmentation of stolon systems into smaller units results from accelerated death of older buried stolon (Brock *et al.* 1988; Hay *et al.* 1988, 1989a, 1989b, 1990; Chapter 3). In this environment a flush of initiation and development of adventitious roots occurs in late winter-spring (August to November) which doubles the number of root systems per plant (Brock *et al.* 1988) and increases the rate of new nodal root production and branching of new roots at soil depths >5 cm (Caradus & Evans 1977). The proportion of nodes supporting adventitious roots also increases from 30 to 56% over this spring period (Newton & Hay in press). Nitrogen fixation rates increase five- to seven-fold over winter rates to peak during September to November (Brock & Hoglund 1979; Crush 1989). These large increases in rates of root growth and nitrogen fixation both peak in October and result in a quantitatively significant increase in the demand for carbon.

In addition the sink strength of developing leaves also increases during early spring over winter values as dry weight per leaf increases four- to five-fold (Brougham 1962), and node appearance rate also increases two- to four-fold (Davies & Evans 1982; Chapman 1983) in response to increasing temperature at stolon apices (Sackville Hamilton & Harper 1989). Thus there is a considerable increase in sink demand in both roots and leaves for carbon which peaks in October. It is suggested that during September and early October the carbon demand for growth exceeds plant capacity to supply photosynthate (potential limited by the effects of low temperature (see Hart 1987)) and carbon is remobilised from reserves and this underlies the rapid decrease in starch content of stolon at this time (Hay *et al.* 1989c; Chapter 7).

Although not directly measured, it is suggested that the accelerated death rate of older buried stolon commences after depletion of starch to low levels in stolons has aided the establishment of new adventitious root systems at younger nodes. Also occurring in the spring period when the sink strengths of roots and developing leaves are high and the starch content of stolons is decreasing is a temporary seasonal dormancy of the axillary shoot buds (Newton *et al.* 1990, 1992) although a causal link between these changes and bud dormancy has not been established (Newton & Hay in press). Reduced shoot growth during periods of strong root growth is a response observed in many grassland species (Troughton 1957). A similar spring dormancy of buds has been identified in other clonal species such as *Elymus repens* (Leakey *et al.* 1977) and *Elymus farctus* (Harris & Davey 1986) when most rapid root and shoot growth and carbohydrate (*E. repens*) or nitrogen (*E. farctus*) are in short supply. It has been suggested that vegetative axillary bud development may also be influenced by physiological changes related to flowering that are expressed in spring as day length exceeds 12 h (Newton *et al.* 1992).

The information obtained on the carbohydrate contents of stolon (Chapter 7) can be utilized to significantly increase the depth of understanding of the processes underlying the observed seasonal growth pattern of white clover populations in grazed pastures in this environment. Characteristics of the white clover population in spring following the burst of plant fragmentation can be calculated. Mean stolon DW per plant is *c* 50% of that in autumn (Brock *et al.* 1988; Chapter 3). If it is assumed that the same processes occurred in the white clover population in spring 1986 as was measured in 1988, then the following calculations can be derived:

	October/November	March
Starch content (%DW) (Fig 7.2)	3.3	14 6
Mean stolon DW/plant (mg) (Table 3.4)	45	106
Mean stolon starch Wt/plant (mg)	1.5	15 5
Relative starch Wt/plant (%)	10	100

This value for the relative decrease in starch per plant in spring is comparable with the 89% reduction calculated on an internode basis (Chapter 7).

The above table indicates that in late spring white clover populations comprise, in the main, small plants with low reserves of carbohydrates. As outlined above, at this time the sink strengths of leaves and roots are very high and clover also faces competitive stress from the faster growing companion grasses such as perennial ryegrass (Mitchell 1956; Williams 1970; Rhodes 1981; Burdon 1983; Frame & Newbould 1986). The physiological status of the white clover population in spring, just described, has agronomic implications. Small plant size is associated with decreased survival and productivity in many plant communities (Harper 1977). Although plant density would be expected to increase in spring following fragmentation of larger plants into an increased number of smaller plants this, in general, does not occur (Hay *et al.* 1989a) which implies that the death rate of plants is such as to account for the lack of increase in density. It is worthy of note that any decrease in plant density in spring, if plants lost are small (first- or small second-order plants) may not be parallelled by an equivalent percentage decrease in growing point density (Hay *et al.* 1991). Overall, this evidence suggests that survival rates of small plants in white clover populations in spring are reduced even in populations which show little variation in growing point density.

Brock *et al.* (1988) suggest that in spring the white clover population may be particularly vulnerable to stress (drought, applications of nitrogenous fertilizer and lax defoliation regimes) and that this could be involved in the sudden, unexplained declines in white clover populations (Spedding & Diekmahns 1972; Stewart & Haycock 1984; Fame & Newbould 1986). The severe effects of spring droughts (Vartha & Hoglund 1983; Brock 1988) in reducing white clover stolon biomass by >70% supports this view. Brock (1888) ascribes reduced plant size and lack of development of root systems in spring as the reasons for the particularly adverse effects of spring as opposed to autumn droughts. White clover populations are considered to be particularly vulnerable to any stress until mid-summer (January) (Brock 1988) as during this period new root and stolon systems are

developing to re-establish plants back to the pre-spring levels of mean plant dry weight and branching complexity and starch reserves are being replenished.

CHAPTER 9 SUMMARY OF MAJOR RESULTS AND CONCLUSIONS

1) In the field trial, time of year (season) was the factor associated with major changes in the proportion of stolon buried, total stolon density, clover dry matter production and the branching structure of the white clover population. Although there were significant differences among the Tahora, Pitau and Kopu cultivars for seasonal growth rate, dry matter allocation to stolon and leaf, growing point density and plant density there were no differences in the mean node number per plant or branching structures of the populations.

2) Sequential sampling of undefoliated stolons either artificially buried or growing on the soil surface found probability of initiation of axillary bud outgrowth to be unaffected by burial but probability of survival of the outgrowing axillary bud to be significantly reduced which resulted in a three-fold lowering of probability of branch establishment at buried nodes. Axillary buds most affected by burial were at node positions 1 and 2 at the time of burial. Most of the negative effects of burial were evident after a burial period of three grazing cycles (approximately three months). Date and depth of burial of stolon had minor effects on the extent of the response.

3) Imposed treatments of neutral shading, defoliation and limitation in phosphorus supply significantly reduced node appearance rate (by 67%, 30% and 25% respectively), could increase node position of first branch (two- to three-fold), but had no significant effect on the proportion of nodes initiating branching. In general, where leaflets only were defoliated (petioles remained intact) values of assessed plant attributes were intermediate between undefoliated and defoliated stolons. Limitations of light and phosphorus supply influenced dry matter allocation to leaf and root in opposite directions.

4) Burial of already formed stolons had no influence on initiation of axillary bud outgrowth at nodes on the primary stolon but decreased outgrowth of buds on secondary stolons. This depressive effect in secondary stolons could be explained by the pattern of apical dominance in small branches and the ontogeny of axillary buds. Where axillary buds were on stolon tissue that emerged in soil

but was then exposed to light (after burial for a maximum of seven plastochrons) initiation of axillary bud outgrowth was significantly reduced (by 17%) although if burial continued for a further five plastochrons the reduction was 64%. Excision of the stolon apex, after burial for a period of seven plastochrons induced a probability of axillary bud outgrowth similar to that obtained by exposure to light at this time even though buds remained buried and received no light stimulus. These results indicate that burial of stolon decreased outgrowth of axillary buds by inhibition rather than by decreasing the viability of buds. Responses of stolon systems to burial were consistent with intraplant allocation patterns of carbon based on source-sink relationships.

5) Diurnal variation in contents of starch and hexose and sucrose sugars in stolons was not significant. Contents of hexose sugars and sucrose varied little with season or in response to burial or defoliation treatments. Starch contents varied five-fold with season (minimal and maximal contents in late spring and late summer respectively), ten-fold in response to defoliation but were unaffected by burial. Following defoliation starch contents in stolon were depleted preferentially from internodes immediately proximal to the apex and depletion progressed basipetally along the stolon. Starch contents were minimal about 15 days after defoliation. Subsequent repletion also occurred initially at internodes immediately proximal to the apex and progressed basipetally.

Starch, sucrose and hexose contents of stolon internode all varied significantly with position within the stolon. No significant correlations were found between any of the carbohydrate fractions in the internode basipetal to an axillary bud and initiation of outgrowth of the bud at any node position.

6) Results of this study were consistent with an hypothesis that in a stolon with a functioning apex there is a 'window of opportunity' for initiation of outgrowth of axillary buds which spans the interval of bud ontogeny from its release from inhibition by apical dominance until the bud is positioned more than eight nodes from the apex.

7) Expression of apical dominance (number of nodes from apex to first node position with axillary bud outgrowth) is determined by abundance of resource at the stolon apex. This is a function of the microenvironments of all metamers

contributing resource to the apex and this may include metamers from other stolons. Apical dominance is therefore a characteristic of a stolon but is influenced by all metamers that supply resource to the stolon. It is independent of other attributes such as node appearance rate and probability of successful branch establishment at metamers within the stolon.

8) It was hypothesised that in white clover initiation of axillary bud outgrowth occurred unless inhibitory processes (apical dominance, phytochrome mediated processes or spring dormancy) were activated to prevent initiation. All reported responses of initiation of branching to treatments could be interpreted within the confines of this model which indicated that initiation of bud outgrowth has no prerequisite for light stimulation.

9) It was suggested that in spring increased demand for carbohydrate initiated by greater growth and nitrogen fixation rates in roots and increased production of leaf tissue initiated changes in intraplant allocation patterns of carbon which resulted in the observed rapid decrease in starch content in stolon in October. This was accompanied by an acceleration in death of older, basal stolon in October which fragmented larger plants, so altering the plant population by increasing the proportion of plants of less complex branching structure and decreasing mean plant dry weight.

10) Following plant fragmentation in October, the physiological status of the population (decreased mean plant DW and carbohydrate reserves) was considered to have agronomic consequences in that it reduced ability to tolerate stress (environmental or managerial) and so increased the probability of large decreases in population density upon occurrence of adverse conditions. This period of increased vulnerability was considered to extend until January by which time mean plant DW and carbohydrate content had usually recovered to pre-spring values.

11) In regard to the population dynamics of white clover in grazed pastures the consequences of the effects of stolon burial on branch establishment in winter were not considered great because;

(a) several physiological and biotic factors act to significantly limit the branching potential of white clover in spring which means that the effect of burial of stolon is just one (physical) factor acting in the same direction as others.

(b) low node appearance rates over winter, when rates of stolon burial are greatest, acts to limit the formation of new nodes at this time and so reduces the number of axillary buds that are most susceptible to negative effects of burial. The most susceptible buds are those that emerge in and remain buried with soil.

(c) in the field, burial of stolon induces a negatively geotropic response that results in stolon curvature and a rapid return of the apex to the soil surface which in turn limits numbers of nodes that emerge in and remain buried with soil.

(d) other environmental and managerial regimes in spring usually have a major influence in determining the productive potential of the white clover population for the growing season; but burial in summer could have greater impact as node appearance rates are high which means that the reduction in branch survival on primary stolons coupled with a decrease in outgrowth of axillary buds on secondary stolons will lower total numbers of branches and nodes produced.

APPENDIX 3.1 DETAILS OF TRIAL MANAGEMENT DURING THE ESTABLISHMENT PERIOD (NOVEMBER 1985 - MARCH 1986)

- 10-12 December 1985. A hard grazing with a mob of approximately 300 sheep

- 6-9 January 1986. A similar grazing to above. It was observed that an infestation of penny royal (*Mentha pulegium* L.) was not defoliated.

- 15-16 January. A hard grazing to prepare for spraying of penny royal.

- 17 January. Trial sprayed for control of penny royal with a mixture of MCPB (6 *l* ha^{-1}) and Combine (2 *l* ha^{-1}).

- 10 February. Trial was topped with clippings returned.

- 10 March. Trial was topped and herbage removed.

- 25 March. Trial was topped and herbage removed.

- 27 March. Basal fertiliser at the following rates was applied; 800 kg ha^{-1} 30% potassic superphosphate; 5 kg ha^{-1} Copper sulphate; 5 kg ha^{-1} Zinc sulphate; 0.14 kg/ha^{-1} Sodium molybdate; 1 t ha^{-1} Dolomite.

APPENDIX 3.2 **Mean (± SEM) daily rate of net dry matter accumulation (kg DM ha^{-1} d^{-1}) for white clover, grass and total herbage in Kopu, Pitau and Tahora swards during each regrowth period. (Regrowth periods numbered by the harvest number of the pre-grazing cut).**

	Sward Type	Regrowth Period																
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
CLOVER	Kopu	4.6 ± 0.78	3.2 ± 0.33	10.8 ± 0.89	6.9 ± 1.1	5.3 ± 0.88	3.1 ± 0.96	1.3 ± 0.33	6.3 ± 0.98	8.4 ± 0.98	5.2 ± 0.51	2.4 ± 0.53	11.0 ± 1.21	10.0 ± 1.72	2.8 ± 0.74	10.0 ± 1.01	5.7 ± 0.44	2.1 ± 0.40
	Pitau	3.0 ± 0.57	3.0 ± 0.35	5.6 ± 0.83	1.9 ± 0.36	4.9 ± 1.1	1.7 ± 0.40	1.4 ± 0.37	4.3 ± 0.70	5.6 ± 0.64	3.9 ± 0.57	1.7 ± 0.42	8.9 ± 1.21	7.6 ± 0.98	4.9 ± 0.95	14.9 ± 1.34	5.1 ± 0.50	3.0 ± 0.45
	Tahora	2.1 ± 0.47	2.0 ± 0.33	7.7 ± 0.71	6.6 ± 0.89	8.2 ± 0.80	5.6 ± 0.72	2.3 ± 0.46	7.1 ± 0.89	5.7 ± 0.64	3.5 ± 0.35	1.00 ± 0.32	11.5 ± 0.92	9.0 ± 0.97	6.9 ± 0.77	20.5 ± 1.54	7.1 ± 0.29	1.7 ± 0.38
GRASS	Kopu	12.3 ± 1.11	14.5 ± 0.99	32.7 ± 1.36	42.6 ± 4.27	35.6 ± 2.70	30.3 ± 3.51	15.0 ± 1.10	27.7 ± 2.12	19.6 ± 1.91	17.3 ± 1.43	15.4 ± 1.03	49.3 ± 1.78	57.5 ± 3.30	66.9 ± 3.28	49.4 ± 2.49	29.6 ± 1.56	40.1 ± 2.82
	Pitau	17.0 ± 1.69	13.8 ± 1.44	34.9 ± 1.73	50.9 ± 4.63	32.9 ± 2.43	32.2 ± 2.59	14.7 ± 1.63	20.6 ± 1.53	19.9 ± 1.84	17.1 ± 0.85	15.7 ± 0.99	49.3 ± 2.55	60.9 ± 2.92	62.3 ± 2.50	48.5 ± 1.92	29.7 ± 1.90	38.4 ± 2.34
	Tahora	15.1 ± 0.96	14.2 ± 1.19	40.1 ± 1.61	44.5 ± 2.59	34.1 ± 2.26	36.2 ± 2.50	15.5 ± 1.1	28.7 ± 2.12	23.5 ± 1.51	18.1 ± 0.82	18.3 ± 1.16	54.2 ± 2.34	59.5 ± 3.04	64.9 ± 2.58	41.1 ± 3.12	27.6 ± 1.67	37.7 ± 1.61
TOTAL	Kopu	17.0 ± 1.05	17.7 ± 0.94	43.5 ± 1.37	49.6 ± 4.05	40.9 ± 2.37	33.5 ± 3.70	16.3 ± 1.08	34.0 ± 2.19	28.0 ± 2.11	22.5 ± 1.48	17.8 ± 1.38	60.3 ± 2.01	67.5 ± 4.03	69.8 ± 3.36	59.4 ± 2.14	35.3 ± 2.14	42.2 ± 2.92
	Pitau	20.0 ± 1.48	16.8 ± 1.42	40.5 ± 1.52	52.8 ± 4.61	37.8 ± 1.97	33.8 ± 2.70	16.4 ± 1.62	25.3 ± 1.63	25.5 ± 1.63	21.1 ± 1.01	17.4 ± 1.22	58.2 ± 2.46	68.5 ± 3.22	67.2 ± 2.57	63.4 ± 2.23	34.8 ± 1.60	41.5 ± 2.40
	Tahora	17.2 ± 1.19	16.2 ± 1.24	47.8 ± 1.32	51.1 ± 2.79	42.3 ± 2.52	41.8 ± 2.81	17.9 ± 0.91	35.8 ± 1.94	35.8 ± 1.94	21.6 ± 0.81	19.3 ± 1.25	65.8 ± 2.73	68.8 ± 3.73	71.8 ± 2.57	61.6 ± 2.98	34.7 ± 1.65	39.4 ± 1.52

APPENDIX 5.1 EFFECT OF BURIAL AND DEFOLIATION TREATMENTS APPLIED TO INDIVIDUAL STOLONS WITHIN PLANTS ON THE INITIATION OF OUTGROWTH OF AXILLARY BUDS

A5.1.1 INTRODUCTION

A small, short-term experiment was undertaken to investigate the extent of integration of the response of characteristics determining plant branching structure among stolons within plants where stolons were exposed to differing microenvironments by subjecting individual stolons within plants to differing burial/defoliation treatment combinations.

A5.1.2 METHODS

Ten stolon cuttings from a single 'Grasslands Pitau' genotype were planted, 21 June 1985, each into an individual plastic tray (30x40x5 cm) filled with potting mix (Section 5.2.1.2). Cuttings were maintained within a heated glasshouse and allowed to establish before treatments were applied on 5 September 1985. Four successive branch stolons along the main stolon of each plant were randomly assigned one of four treatments and the remainder of the plant was defoliated and the main stolon severed distal to the node bearing the youngest branch stolon assigned a treatment.

The burial treatment involved covering the stolon with 2 cm of potting mix such that where the petiole of a leaf was longer than 2 cm, the leaf was maintained in light. Stolons that were defoliated had the petioles of all leaves of morphological developmental stage > 0.3 (Carlson 1966a) severed within 2 mm of the junction of petiole and stolon.

The four treatments of branch stolons were:

1) stolon not buried or defoliated
2) stolon not buried but defoliated
3) stolon buried but not defoliated
4) stolon buried and defoliated

At this time plant location treatments were imposed by transferring five of the ten plants out of the glasshouse to a concrete apron (outside location) where mean daily maximum and minimum temperatures over the experimental period were 15.1 ± 1.72 and 6.8 ± 2.93°C, respectively and maintaining five plants within (inside location) the glasshouse (mean daily maximum and minimum temperatures 26.1 ± 3.84 and 12.2 ± 1.32°C, respectively).

Just prior to application of treatments the developmental stage of the leaf (Carlson 1966a) and the activity of the axillary bud (Section 5.2.1.5) at each node of each treated stolon was recorded. Then on each stolon the node bearing the first leaf proximal to the apex of Carlson (1966a) developmental stage > 0.6, was marked with nail varnish. At harvest, 23 September 1985, for each stolon, nodes distal to the marked node were assessed for leaf development and axillary bud activity as described above. Stolons were severed at the marked node and stolon length and dry weight of leaf and stolon measured for growth distal to the marked node.

Statistical analysis was by analysis of variance with data classified by location, plants within location, burial and defoliation. Main effects for these classifications were tested and the following first-order interactions calculated; location by burial, location by defoliation and burial by defoliation. All reported variables at harvest were thus analysed using the general linear model procedure in SAS. In addition, data for the number of nodes per stolon at the start of the experiment were similarly tested. The purpose of this analysis was to test for the possibility that results could have been strongly biased by the preferential allocation of particular treatments to larger or smaller stolons within plants. As there were no significant differences among treatments for number of nodes per stolon at the start of the experiment, this form of bias was considered not to influence the reported results.

A5.1.3 RESULTS

Table A5.1 presents results of the effects of treatments on measured plant characteristics.

Table A5.1 Treatment means (±SEM) of growth characteristics of stolons from the experiment in which stolons within plants were individually subjected to imposition (+) or not (-) of defoliation (D) and/or burial (B) treatments and where plants were grown either inside or outside a glasshouse.

Plant growth characteristic	Stolon Treatments				Location Treatments	
	1 -B-D	2 -B+D	3 +B-D	4 +B+D	Inside	Outside
Node appearance Rate (nodes week $^{-1}$)	1.4 (0.166)	1.28 (0.210)	1.13 (0.205)	1.04 (0.234)	1.78 (0.069)	0.68 (0.062)
Position of first branching node	2.2 (0.20)	2.5 (0.23)	1.8 (0.25)	1.9 (0.26)	2.2 (0.15)	2.0 (0.19)
Probability of axillary bud outgrowth	1.00	1.00	1.00	0.91	0.98	1.00
Mean internode length of treated stolon (cm)	1.40 (0.170)	1.16 (0.087)	1.76 (0.100)	1.39 (0.123)	1.64 (0.082)	1.23 (0.090)
Mean internode DW (mg)	6.9 (1.15)	3.7 (0.34)	10.6 (1.98)	5.9 (0.85)	7.4 (1.07)	6.2 (0.99)

The higher temperature regime in the inside glasshouse location as compared to the outside location was associated with a major ($P < 0.0001$) effect on node appearance rate and increased ($P < 0.001$) internode length but not stolon dry weight per node. Inside or outside location did not influence the position of the first branching node or the probability of initiation of outgrowth of axillary buds.

Node appearance rate was reduced ($P < 0.003$) by burial of stolon but not significantly affected by defoliation. Position of first branching node was significantly ($P < 0.012$) decreased by burial but not influenced by defoliation. As axillary bud outgrowth occurred at almost every node, treatment effects on probability of axillary bud outgrowth were non-significant.

Defoliation depressed both internode length ($P < 0.004$) and internode dry weight ($P < 0.0002$) whereas burial of stolon increased ($P < 0.005$ and $P < 0.004$, respectively) these values.

A5.1.4 DISCUSSION AND CONCLUSIONS

This experiment assessed the responses of individually and differently treated stolons which were interconnected within plants. The imposed treatments of stolon burial and defoliation are both events that can occur to parts of plants either alone or together when pastures are grazed.

The most interesting result was the finding that node appearance rate differed among stolons within a plant in response to the treatments applied to individual stolons within a plant. Turkington *et al.* (1991) examined plants which had branch stolons on either side of the main stolon growing into differing grass species and found that node appearance rate of all stolon apices within a plant were similar but that this common node appearance rate differed between plants grown with different combinations of grass species. On the basis of these results it was postulated that the plant was able to sense the whole of its environment, integrate this information and then modify the node appearance rate of all apices to a common value. In that the response of node appearance rate to treatments was found to occur at the individual stolon level within plants, the results of this experiment clearly do not support the hypothesis proposed above.

Defoliation and burial had minimal effects on either the probability or timing of outgrowth of axillary buds, once again suggesting that the negative effect of burial on branching observed in the field (Chapter 4) is likely to accrue from its effects on the survival of branches. There was a non-significant trend towards a lower probability of branching under the burial plus defoliation treatment such as was found by Davies & Evans (1990a).

The response of plants to the more favourable glasshouse location was predictable (Sackville Hamilton & Harper 1989) with much greater node appearance rates and significantly greater internode dimensions than in plants at the colder outside location. The less favourable colder outside location did not however, significantly affect either the position of the first branching node or probability of initiation of axillary bud outgrowth.

REFERENCES

ABELES, F.B. (1973). *Ethylene in Plant Biology.* London: Academic Press.

ANDREW, C.S. (1956). The critical range of phosphorus and potassium in *Trifolium repens* var. Irrigation White and their effects on other nutrients. *Proceedings of the 1st Australian Conference on Plant Nutrition 1*, 129-139.

ARNOTT, R.A. & RYLE, G.J.A. (1982). Leaf surface expansion on the main axes of white and red clovers. *Grass and Forage Science 37,* 227-233.

BAKER, M.J. & WILLIAMS, W.M. (1987). *White clover.* Wallingford: CAB International.

BALLARE, C.L., SANCHEZ, R.A., SCOPEL, A.L. CASAL, J.J. & GHERSA, C.M. (1987). Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. *Plant Cell and Environment 10*, 551-557.

BALLARE, C.L., SCOPEL, A.L. & SANCHEZ, R.A. (1990). Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. *Science 247*, 329-332.

BARLEY, K.P. (1953). The root growth of irrigated perennial pastures and its effect on soil structure. *Australian Journal of Agricultural Research 4,* 283-291.

BARLEY, K.P. (1959). The influence of earthworms on soil fertility. II. Consumption of soil organic matter by the earthworm *Allolobophora caliginosa. Australian Journal of Agricultural Research 10,* 158-179.

BARLEY, K.P. (1961). The abundance of earthworms in agricultural lands and their possible significance in agriculture. *Advances in Agronomy 13,* 249-268.

BARLOW, P.W. (1989). Meristems, metamers and modules and the development of shoot and root systems. *Botanical Journal of the Linnean Society 100,* 255-279.

BAUR-HOCH, B., MACHLER, F. & NOSBERGER, J. (1990). Effect of carbohydrate demand on the remobilisation of starch in stolons and roots of white clover (*Trifolium repens* L.) after defoliation. *Journal of Experimental Botany 41,* 573-578.

BEINHART, G. (1962). Effects of temperature and light intensity on carbon dioxide uptake, respiration and growth of white clover. *Plant Physiology 37,* 709-715.

BEINHART, G. (1963). Effects of environment on meristematic development, leaf area and growth of white clover. *Crop Science 3,* 209-213.

BEINHART, G. (1964). Free sugar concentrations in white clover, *Trifolium repens* L., grown at different temperatures and light intensities. *Crop Science 4,* 625-631.

BEINHART, G., GIBSON, P.B., HALPIN, J.E. & HOLLOWELL, E.A. (1963). Selection and evaluation of white clover clones. III. Clonal differences in branching in relation to leaf area production and persistence. *Crop Science 3,* 89-92.

BELL, A.D. (1984). Dynamic morphology: A contribution to plant population ecology. In: Dirzo, R. & Sarubhan, J. eds. *Perspectives on Plant Population Ecology.* Massachusetts: Sinauer, 48-65.

BIRO, R.L., HUNT, E.R., ERNER, Y. & JAFFE, M.J. (1980). Thigmomorphogenesis: Changes in cell division and elongation in the internodes of mechanically-perturbed or ethrel-treated bean plants. *Annals of Botany 45,* 555-664.

BLACKMAN, G.E. (1934). The ecological and physiological action of ammonium salts on the clover content of turf. *Annals of Botany 48,* 975-1001.

BLASER, R.E. & KILLINGER, G.W. (1950). Life history studies of Louisiana white clover (*Trifolium repens* L.). 1. Seed germination as related to temperature, pasture management and adaptation. *Agronomy Journal 42,* 215-220.

BOLLER, B.C. & NOSBERGER, J. (1983). Effects of temperature and photoperiod on stolon characteristics, dry matter partitioning and nonstructural carbohydrate concentration of two white clover ecotypes. *Crop Science 23,* 1057-1062.

BOOYSEN, P. deV. & LAUDE, H.M. (1964). Influence of flower initiation and development on internode growth in the Ladino clover stolon. *Crop Science 4,* 520-524.

BRISENO de la HOZ, V.M. & WILMAN, D. (1981). Effects of cattle grazing, sheep grazing, cutting and sward height on a grass-clover sward. *Journal of Agricultural Science, Cambridge 97,* 699-706.

BROCK, J.L. (1988). Evaluation of New Zealand bred white clover cultivars under rotational grazing and set stocking with sheep. *Proceedings of the New Zealand Grassland Association 49,* 203-206.

BROCK, J.L., HAY, M.J.M., THOMAS, V.J. & SEDCOLE, J.R. (1988). Morphology of white clover (*Trifolium repens* L.) plants in pastures under intensive sheep grazing. *Journal of Agricultural Science, Cambridge 111,* 273-283.

BROCK, J.L. & HOGLUND, J.H. (1979). Nitrogen fixation in pasture. VII. Recent alluvium, Palmerston North. *New Zealand Journal of Experimental Agriculture 7,* 27-29.

BROUGHAM, R.W. (1958). Leaf development in swards of white clover (*Trifolium repens* L.). *New Zealand Journal of Agricultural Research 1,* 707-718.

BROUGHAM, R.W. (1962). The leaf growth of *Trifolium repens* as influenced by the seasonal changes in light environment. *Journal of Ecology 50,* 449-459.

BROUGHAM, R.W., BALL, P.R. & WILLIAMS, W.M. (1978). The ecology and management of white clover based pastures. In: Wilson, J.R. ed. *Plant relations in pastures.* East Melbourne: CSIRO, 309-323.

BURDON, J.J. (1983). Biological flora of the British Isles, *Trifolium repens* L. *Journal of Ecology 71,* 307-330.

BURDON, J.J. & HARPER, J.L. (1980). Relative growth rates of individual members of a plant population. *Journal of Ecology 68*, 953-957.

BUTLER, G.W. & BAILEY, R.W. (1973). *Chemistry and biochemistry of herbage.* London: Academic Press.

CAHN, M.G. & HARPER, J.L. (1976). The biology of the leaf mark polymorphism in *Trifolium repens* L. 1. Distribution at a local site. *Heredity 37,* 309-325.

CALLAGHAN, T.V. (1976). Growth and population dynamics of *Carex bigelowii* in an alpine environment. *Oikos 27,* 402-413.

CALLAGHAN, T.V. (1984). Growth and translocation in a clonal southern hemisphere sedge, *Uncinia meridensis. Journal of Ecology 72,* 529-546.

CARADUS, J.R. (1984). *The phosphorus nutrition of populations of white clover* (Trifolium repens *L.*). Ph.D thesis, University of Reading, U.K.

CARADUS, J.R. (1986). World checklist of white clover varieties. *New Zealand Journal of Experimental Agriculture 14,* 119-164.

CARADUS, J.R. (1990). The structure and function of white clover root systems. *Advances in Agronomy 43*, 1-46.

CARADUS, J.R. & CHAPMAN, D.F. (1991). Variability of stolon characteristics and response to shading in two cultivars of white clover (*Trifolium repens* L). *New Zealand Journal of Agricultural Research 20,* 337-342.

CARADUS, J.R. & EVANS, P.S. (1977). Seasonal root formation of white clover, ryegrass and cocksfoot in New Zealand. *New Zealand Journal of Agricultural Research 20,* 337-342.

CARADUS, J.R., HAY, M.J.M., MACKAY, A.D., THOMAS, V.J. DUNLOP, J. LAMBERT, M.G. HART, A.L., VAN DEN BOSCH, J. & WEWALA, S. (1993). Variation within white clover (*Trifolium repens* L). for phenotypic plasticity of morphological and yield related characters, induced by phosphorus supply. *New Phytologist 123,* 175-184.

CARLSON, G.E. (1966a). Growth of clover leaves - developmental morphology and parameters at ten stages. *Crop Science 6,* 293-294.

CARLSON, G.E. (1966b). Growth of white clover leaves, after leaf removal. *Proceedings of the 10th International Grassland Congress, Helsinki*, 134-136.

CASAL, J.J., DEREGIBUS, V.A. & SANCHEZ, R.A. (1985). Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. vegetative and reproductive plants as affected by differences in red/far red irradiation. *Annals of Botany 56,* 553-559.

CHAPIN, F.S. (1980). The mineral nutrition of wild plants. *Annual review of Ecology and Systematics 11*, 233-260.

CHAPIN, F.S., BLOOM, A.J., FIELD, C.B. & WARING, R.H. (1987). Plant responses to multiple environmental factors. *Bio Science 37*, 49-57.

CHAPMAN, D.F. (1983). Growth and demography of *Trifolium repens* stolons in grazed hill pastures. *Journal of Applied Ecology 20,* 597-608.

CHAPMAN, D.F. (1986). Development, removal and death of white clover leaves under 3 grazing managements in hill country. *New Zealand Journal of Agricultural Research 29,* 39-47.

CHAPMAN, D.F. (1987). Natural reseeding and *Trifolium repens* demography in grazed hill pastures. 2. Seedling appearance and survival. *Journal of Applied Ecology 24,* 1037-1043.

CHAPMAN, D.F. (1989). *The assimilation and distribution of carbon in white clover* (Trifolium repens *L.*). Ph.D thesis, University of Reading, U.K.

CHAPMAN, D.F., CLARK, D.A., LAND, C.A. & DYMOCK, N. (1983). Leaf and tiller growth of *Lolium perenne* and *Agrostis* spp. and leaf appearance rates of *Trifolium repens* in set-stocked and rotationally grazed hill pastures. *New Zealand Journal of Agricultural Research 26,* 159-168.

CHAPMAN, D.F. & HAY, M.J.M. 1993. Translocation of phosphorus from nodal roots in two contrasting genotypes of white clover (*Trifolium repens*). *Physiologia Plantarum 89,* 323-330.

CHAPMAN, D.F. & ROBSON, M.J. (1988). Effects of defoliation on carbon assimilation and partitioning in white clover stolons. *Proceedings of the 12th General Meeting of the European Grassland Federation,* 277-281.

CHAPMAN, D.F. & ROBSON, M.J. (1992). The physiological role of old stolon material in white clover (*Trifolium repens* L.). *New Phytologist 122,* 53-62.

CHAPMAN, D.F., ROBSON, M.J. & SNAYDON, R.W. (1991a). The influence of leaf position and defoliation on the assimilation and translocation of carbon in white clover (*Trifolium repens* L.). 1. Carbon distribution patterns. *Annals of Botany 67,* 295-302.

CHAPMAN, D.F., ROBSON, M.J. & SNAYDON, R.W. (1991b). The influence of leaf position and defoliation on the assimilation and translocation of carbon in white clover (*Trifolium repens* L.). 2. Quantitative carbon movement. *Annals of Botany 67,* 303-308.

CHAPMAN, D.F. ROBSON, M.J. & SNAYDON, R.W. (1992a). Physiological integration in the clonal perennial herb *Trifolium repens* L. *Oecologia 89,* 338-347.

CHAPMAN, D.F., ROBSON, M.J. & SNAYDON, R.W. (1992b). The carbon economy of clonal plants of *Trifolium repens* L. *Journal of Experimental Botany 43,* 427-434.

CHARLES-EDWARDS, D.A. (1984a). On the ordered development of plants. 1. An hypothesis. *Annals of Botany 53,* 699-707.

CHARLES-EDWARDS, D.A. (1984b). On the ordered development of plants. 2. Self-thinning in plant communities. *Annals of Botany 53,* 709-714.

CHESTNUTT, D.M.B. & LOWE, J. (1970). Agronomy of white clover/grass swards: a review. In: Lowe, J. ed. *White clover research. Occasional Symposium of the British Grassland Society 6,* 191-213.

CHOW, H.S. (1966). *The structure and growth habit of white clover in relation to its persistence.* Ph.D Thesis, University of New Hampshire.

CLINE, M.G. (1991). Apical dominance. *The Botanical Review 57*, 318-358.

COLLINS, R.P., GLENDINING, M.J. & RHODES, I. (1991). The relationships between stolon characteristics, winter survival and annual yields in white clover (*Trifolium repens* L.). *Grass and Forage Science 46,* 51-61.

COOK, R.E. (1985). Growth and development in clonal plant populations. In: Jackson, J.B.C., Buss, L.W. & Cook, R.E. eds. *Population biology and evolution of clonal organisms.* New Haven: Yale University Press, 259-296.

COWLING, D.W. (1961). The effect of nitrogenous fertiliser on an established white clover sward. *Journal of the British Grassland Society 16,* 65-68.

COX, J.E. (1968). Evaluation of climate and its correlation with soil groups. In: Luke, J. & Heine, J. eds. *Soils of New Zealand. New Zealand Soil Bureau Bulletin 26, Part 1.* Wellington: New Zealand Soil Bureau, 33-44.

CRUSH, J.R. (1987). Nitrogen fixation. In: Baker, M.J. & Williams, W.M. eds. *White clover.* Wallingford: CAB International, 185-201.

CRUSH, J.R. (1989). Legume breeding strategy for increased nitrogen fixation in New Zealand temperate pastures. *Proceedings of the 16th International Grassland Congress, Nice,* 125-126.

CURLL, M.L. (1982). The grass and clover content of pastures grazed by sheep. *Herbage Abstracts 52,* 403-411.

CURLL, M.L. & WILKINS, R.J. (1982). Frequency and severity of defoliation of grass and clover by sheep at different stocking rates. *Grass and Forage Science 37,* 291-297.

DANCKWERTS, J.E. & GORDON, A.J. (1989). Long-term partitioning, storage and remobilisation of ^{14}C assimilated by *Trifolium repens* (cv. Blanca). *Annals of Botany 64,* 533-544.

DAVIDSON, I.A., CULVENOR, R.A. & SIMPSON, R.J. (1990). Effect of previous defoliation regime and mineral nitrogen on regrowth in white clover swards: photosynthesis, respiration, nitrogenase activity and growth. *Annals of Botany 65,* 665-677.

DAVIES, A. (1989). The structure of the grass/clover sward and its implications in sward management. *Proceedings of the XVI International Grassland Congress, Nice,* 1065-1066.

DAVIES, A. & DUTTA, M.J. (1988). Factors influencing the relationship between branching in white clover and tillering in grass in the field. *Proceedings British Grassland Society Research Meeting No. 1,* Session 3, 2-4.

DAVIES, A. & EVANS, M.E. (1982). The pattern of growth in swards of two contrasting varieties of white clover in winter and spring. *Grass and Forage Science 37,* 199-207.

DAVIES, A. & EVANS, M.E. (1983). Branching in white clover. *Welsh Plant Breeding Station Annual Report 1982,* 132.

DAVIES, A. & EVANS, M.E. (1990a). Axillary bud development in white clover in relation to defoliation and shading treatments. *Annals of Botany 66,* 349-357.

DAVIES, A. & EVANS, M.E. (1990b). Effects of spring defoliation and fertiliser nitrogen on the growth of white clover in ryegrass/clover swards. *Grass and Forage Science 45,* 345-356.

DAVIES, A. & JONES, D.R. (1986). Tissue fluxes in white clover varieties grown in swards continuously grazed by sheep. In: Pollot, G.E. ed. *Efficient sheep production from grass. Occasional Symposium of the British Grassland Society 21,* 149-152.

DAVIES, A. & JONES, D.R. (1988). Changes in the grass/clover balance in continuously grazed swards in relation to sward production components. *Proceedings of the XII General Meeting of the European Grassland Federation,* 297-301.

DAVIES, A. & JONES, D.R. (1992). The production of leaves and stolon branches on established white clover cuttings in relation to temperature and soil moisture in the field. *Annals of Botany 69,* 515-521.

DENNIS, W.D., WOLEDGE, J., CULHANE, K. & STOKES, J. (1984). Effect of clover morphology on growth and photosynthesis in mixed swards. In: Thompson, D.J. ed. *Forage legumes. Occasional Symposium of the British Grassland Society 16,* 188-199.

DEREGIBUS, V.A., SANCHEZ, R.A. & CASAL, J.J. (1983). Effects of light quality on tiller production in *Lolium* spp. *Plant Physiology 72,* 900-902.

DEREGIBUS, V.A., SANCHEZ, R.A., CASAL, J.J. & TRLICA, M.J. (1985). Tillering responses to enrichment of red light beneath the canopy in a humid natural grassland. *Journal of Applied Ecology 22,* 199-206.

DE VLEESCHAUWER, D. & LAL, R. (1981). Properties of worm casts under secondary tropical forest regrowth. *Soil Science 132,* 175-181.

DREIDAX, L. (1931). Investigations on the importance of earthworms for plant growth. *Archiv fur Pflanzenbau 7,* 413-467. (Not sighted).

DUNLOP, J. & HART, A.L. (1987). Mineral nutrition. In: Baker, M.J. & Williams, W.M. eds. *White clover.* Wallingford: CAB International, 153-183.

DUNLOP, J. & HAY, M.J.M. (1985). Electrophysiological evidence for the permeability of white clover stolons to nutrient ions. *Plant and Soil 84,* 153-165.

DUTTA, M.J. (1988). *Bud viability in white clover.* M.Sc. Thesis, University of Wales, Aberystwyth, U.K.

EDWARDS, C.A. & LOFTY, J.R. (1977). *Biology of earthworms.* (2nd edition) London: Chapman and Hall.

EISINGER, W.R. (1983). Regulation of pea internode extension by ethylene. *Annual Review of Plant Physiology 34,* 225-240.

EISINGER, W.R. & BURG, S.P. (1972). Ethylene-induced pea internode swelling: Its relation to ribonucleic acid metabolism, wall protein synthesis and cell wall structure. *Plant Physiology 50,* 510-517.

ERITH, A.G. (1924). *White clover* (Trifolium repens *L.) A monograph.* London: Duckworth.

EVANS, A.C. (1948). Studies on the relationships between earthworms and soil fertility. II. Some effects of earthworms on soil structure. *Annals of Applied Biology 35,* 1-13.

FLETCHER, W.W. & MARTIN, D.J. (1962). The effect of gibberellic acid on the germination, growth and development of *Trifolium repens* L. *Journal of Agricultural Science, Cambridge 58,* 235-241.

FORDE, M.B., HAY, M.J.M. & BROCK, J.L. (1989). Development and growth characteristics of temperate perennial legumes. In: Marten, G.C., Matches, A.G., Barnes, R.F., Brougham, R.W., Clements, R.J. & Sheath, G.W. eds. *Persistence of forage legumes.* Madison, Wisconsin: American Society of Agronomy Inc., 91-108.

GORDON, A.J., MITCHELL, D.F., RYLE, G.J.A. & POWELL, C.E. (1987). Diurnal production and utilisation of photosynthate in nodulated white clover. *Journal of Experimental Botany 38,* 84-98.

GORDON, A.J., RYLE, G.J.A., MITCHELL, D.F., LOWRY, K.H. & POWELL, C.E. (1986). The effect of defoliation on carbohydrate, protein and leghaemoglobin content of white clover nodules. *Annals of Botany 58,* 141-154.

GRANT, S.A. & BARTHRAM, G.T. (1991). The effects of contrasting cutting regimes on the components of clover and grass growth in micoswards. *Grass and Forage Science 46,* 1-13.

GRANT, S.A., BARTHRAM, G.T. & MARRIOTT, C.A. (1986). The influence of sward conditions and nitrogen supply on the performance of white clover in continuously stocked grass clover swards. In: Borba, F.M. & Abreu, J.M. eds. *Grasslands facing the energy crisis. Proceedings of 11th General Meeting of the European Grassland Federation, Troia, Portugal,* 352-356.

GRANT, S.A., TORVELL, L., SIM, E.M. & SMALL, J. (1991). The effect of stolon burial and defoliation early in the growing season on white clover performance. *Grass and Forage Science 46,* 173-182.

GRIME, J.P. (1966). Shade avoidance and shade tolerance in flowering plants. In: Bainbridge, R., Evans, G.C. & Rackham, O. eds. *Light as an ecological factor.* Oxford: Blackwell, 187-207.

HARPER, J.L. (1977). *Population biology of plants.* London: Academic Press.

HARPER, J.L. (1981). The population biology of modular organisms. In: May, R. ed. *Theoretical ecology.* Oxford : Blackwell, 53-77.

HARPER, J.L. (1985). Modules, branches, and the capture of resources. In: Jackson, J.B.C., Buss, L.W. & Cook, R.E. *Population biology and evolution of clonal organisms.* New Haven: Yale University Press, 1-33.

HARRIS, D. & DAVEY, A.J. (1986). Regenerative potential of *Elymus farctus* from rhizome fragments and seed. *Journal of Ecology 74,* 1057-1068.

HARRIS, W. (1973). Ryegrass genotype-environment interactions in response to density, cutting height and competition with white clover. *New Zealand Journal of Agricultural Research 16,* 207-222.

HARRIS, W. (1987). Population dynamics and competition. In: Baker, M.J. & Williams, W.M. eds. *White clover.* Wallingford: CAB International, 203-297.

HARRIS, W. & BROUGHAM, R.W. (1968). Some factors affecting change in botanical composition in a ryegrass-white clover pasture under continuous grazing. *New Zealand Journal of Agricultural Research 11,* 15-38.

HARRIS, W. & HOGLUND, J.H. (1980). Influences of seasonal growth periodicity and N-fixation on competitive combining abilities of grasses and legumes. *Proceedings of the 13th International Grassland Congress, Leipzig,* 239-243.

HARRIS, W., RHODES, I. & MEE, S.S. (1983). Observations on environmental and genotypic influences on the overwintering of white clover. *Journal of Applied Ecology 20,* 609-624.

HART, A.L. (1987). Physiology. In: Baker, M.J. & Williams, W.M. eds. *White clover.* Wallingford: CAB International, 125-147.

HART, A.L. & GREER, D.H. (1988). Photosynthesis and carbon export in white clover plants grown at various levels of phosphorus supply. *Physiologia Plantarum 73,* 46-51.

HARTWIG, W., BOLLER, B.C., BAUR-HÖCH, B. & NOSBERGER, J. (1990). The influence of carbohydrate reserves on the response of nodulated white clover to defoliation. *Annals of Botany 65,* 97-105.

HARVEY, H.J. (1970). Patterns of assimilate translocation in *Trifolium repens.* In: Lowe, J. ed. *White clover research. Occasional Symposium of the British Grassland Society 6,* 181-186.

HARVEY, H.J. (1979). *The regulation of vegetative reproduction.* Ph.D. thesis, University of Wales, U.K.

HAY, M.J.M. (1983). Seasonal variation in the distribution of white clover (*Trifolium repens* L.) stolons among 3 horizontal strata in 2 grazed swards. *New Zealand Journal of Agricultural Research 26,* 29-34.

HAY, M.J.M. (1985). Seasonal variation in the vertical distribution of white clover (*Trifolium repens* L.) stolons in 2 contrasting pastures. *Proceedings of the New Zealand Grassland Association 46,* 195-198.

HAY, M.J.M., BROCK, J.L. & FLETCHER, R.H. (1983). Effect of sheep grazing management on distribution of white clover stolons among 3 horizontal strata in ryegrass/white clover swards. *New Zealand Journal of Experimental Agriculture 11,* 215-218.

HAY, M.J.M., BROCK, J.L. & THOMAS, V.J. (1989a). Density of *Trifolium repens* plants in mixed swards under intensive grazing by sheep. *Journal of Agricultural Science, Cambridge 113,* 81-86.

HAY, M.J.M., BROCK, J.L. & THOMAS, V.J. (1989b). Characteristics of individual white clover plants in grazed swards. *Proceedings of the 16th International Grassland Congress, Nice*, 1051-1052.

HAY, M.J.M., BROCK, J.L., THOMAS, V.J. & KNIGHTON, M.V. (1988). Seasonal and sheep grazing management effects on branching structure and dry weight of white clover plants in mixed swards. *Proceedings of the New Zealand Grassland Association 49,* 197-201.

HAY, M.J.M. & CHAPMAN, D.F. (1984). Observations on the distribution of white clover stolons in hill swards. *Proceedings of the New Zealand Grassland Association 45,* 145-130.

HAY, M.J.M., CHAPMAN, D.F., HAY, R.J.M., PENNELL, C.G.L., WOODS, P.W. & FLETCHER, R.H. (1987). Seasonal variation in the vertical distribution of white clover stolons in grazed swards. *New Zealand Journal of Agricultural Research 30,* 1-8.

HAY, M.J.M., CHU, A.C.P., KNIGHTON, M.V. & WEWALA, S. (1989c). Variation with season and node position in carbohydrate content of white clover stolons. *Proceedings of the 16th International Grassland Congress, Nice,* 1059-1060.

HAY, M.J.M. & DUNLOP, J. (1982). Phosphate absorption by white clover stolons in pasture. *New Zealand Journal of Agricultural Research 25,* 211-216.

HAY, M.J.M., DUNLOP, J. & HOPCROFT, D.H. (1986a). Phosphate uptake and anatomy of unthickened and secondarily thickened adventitious roots of field grown white clover (*Trifolium repens* L.). *New Phytologist 103,* 659-668.

HAY, M.J.M., KIM, M.C., MCLEAN, R.W. & GONZALEZ-RODRIGUEZ, A. (1993). Defoliation of young branch and parent stolons of white clover in rotationally grazed pastures. *Proceedings of the 17th International Grassland Congress, Palmerston North,* 128-129.

HAY, M.J.M., NES, P. & ROBERTSON, M.R. (1985). Effect of grazing management and season on nitrogen and phosphorus content of leaves and stolons of white clover in mixed swards. *New Zealand Journal of Experimental Agriculture 13,* 209-214.

HAY, M.J.M., NEWTON, P.C.D. & THOMAS, V.J. (1991). Nodal structure and branching of *Trifolium repens* in pastures under intensive grazing by sheep. *Journal of Agricultural Science, Cambridge 116,* 221-228.

HAY, M.J.M., SAKER, L.R. & DREW, M.C. (1986b). Phosphate uptake and transport by roots and stolons of intact white clover (*Trifolium repens*). *Journal of Experimental Botany 37,* 577-588.

HAY, M.J.M., THOMAS, V.J. & BROCK, J.L. (1990). Frequency distribution of shoot weight of plants in populations of *Trifolium repens* persisting by clonal growth in grazed pastures. *Journal of Agricultural Science, Cambridge 115,* 41-47.

HAY, R.J.M. & BARKER, G.S. (1984). Spring management of pasture to increase summer white clover growth. *Proceedings of the Lincoln College Farmers Conference 34,* 132-137.

HAY, R.J.M. & BAXTER, G.S. (1989). Manipulating the plastic response of white clover through grazing in a cool temperate climate in New Zealand. *Proceedings of the 16th International Grassland Congress, Nice,* 1053-1054.

HILL, J. (1977). Plasticity of white clover grown in competition with perennial ryegrass. *Welsh Plant Breeding Station Annual Report 1976,* 24-25.

HILLMAN, J.R. (1984). Apical dominance. In: Wilkins, M.B. ed. *Advanced plant physiology*. London: Pitman Publishing Ltd, 127-148.

HILLMAN, J.R. (1986). Apical dominance and correlations by hormones. In: Bopp, M. ed. *Plant growth substances.* Berlin: Springer-Verlag, 341-349.

HOGLUND, J.H. & BROCK, J.L. (1973). Growth of Grasslands Huia and Grasslands 4700 white clovers. I. Effects of temperature and nitrogen. *New Zealand Journal of Agricultural Research 17,* 41-45.

HOGLUND, J.H., CRUSH, J.R., BROCK, J.L., BALL, P.R. & CARRAN, R.A. (1979). Nitrogen fixation in pasture XII. General discussion. *New Zealand Journal of Experimental Agriculture 7,* 45-51.

HOGLUND, J.H. & WILLIAMS, W.M. (1984). Genotypic variation in white clover growth and branching in response to temperature and nitrogen. *New Zealand Journal of Agricultural Research 27,* 19-24.

HOLLINGTON, P.A. & WILMAN, D. (1985). Effects of white clover and fertiliser nitrogen on clover and grass leaf dimensions, percentage cover and numbers of leaves and tillers. *Journal of Agricultural Science, Cambridge 104,* 595-607.

HOLLOWELL, E.A. (1966). White clover *Trifolium repens* L., annual or perennial? *Proceedings of the 10th International Grasslands Congress, Helsinki,* 184-187.

HOLMES, M.G. (1981). Spectral distribution of radiation within plant canopies. In: Smith, H. ed. *Plants and the daylight spectrum.* New York: Academic Press, 147-158.

HOLMES, M.G. & SMITH, H. (1977). The function of phytochrome in the natural environment. II. The influence of vegetation canopies on the spectral energy distribution of natural daylight. *Photochemistry and Photobiology 25,* 539-545.

HORIKAWA, Y. (1986a). Plastic allocation of photosynthetic product in white clover (*Trifolium repens* L.). *Journal of the Japanese Society of Grassland Science 32,* 225-234.

HORIKAWA, Y. (1986b). Reproductive strategy in white clover (*Trifolium repens* L.) of different habitats. *Journal of the Japanese Society of Grassland Science 32,* 235-242.

HOSHINO, M. (1974). Translocation and accumulation of assimilates and phosphorus in Ladino clover. *Bulletin of the National Grassland Research Institute 5,* 35-84.

HUNT, W.F. & HALLIGAN, G. (1981). Growth and developmental responses of perennial ryegrass grown at constant temperature. 1. Influence of light and temperature on growth and net assimilation. *Australian Journal of Plant Physiology 8,* 181-190.

HUNT, W.F. & THOMAS, V.J. (1985). Growth and developmental responses of perennial ryegrass grown at constant temperature. II. Influence of light and temperature on leaf, tiller and root appearance. *Australian Journal of Plant Physiology 12,* 69-76.

JACKMAN, R.H. & MOUAT, M.C.H. (1972). Competition between grass and clover for phosphate. 1. Effect of browntop (*Agrostis tenuis* Sibth.) on white clover (*Trifolium repens* L.) growth and nitrogen fixation. *New Zealand Journal of Agricultural Research 15,* 653-666.

JACKSON, M.B. (1982). Ethylene as a growth promoting hormone under flooded conditions. In: Wareing, P.F. ed. *Plant Growth Substances.* London: Academic Press, 291-304.

JAFFE, M. (1973). Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation with special reference to *Bryonia dioica. Planta 114,* 143-157.

JELMINI, G. & NÖSBERGER, J. (1978). Einfluss der Lichintensität auf die Ertragsbildung und den Gehalt an nichtstruckturbildenen Kohlenhydraten und Stickstoff von *Festuca pratensis* Huds., *Lolium multiflorum* Lam., *Trifolium pratense* L. und *Trifolium repens* L. *Zeitschrift für Acker- und Pflanzenbau 146,* 154-163.

JONES, D.R. & DAVIES, A. (1988). The effects of simulated continuous grazing on development and senescence of white clover. *Grass and Forage Science 43,* 421-425.

JONES, M.G. (1933). Grassland management and its influence on the sward. II. The management of a clovery sward and its effects. *Empire Journal of Experimental Agriculture 1,* 122-127.

JONES, R.M. (1982). White clover (*Trifolium repens*) in subtropical southeast Queensland. 1. Some effects of site, season and management practices on the population dynamics of white clover. *Tropical Grasslands 16,* 118-127.

KARKALAS, J. (1985). An improved enzymic method for the determination of native and modified starch. *Journal of Science, Food and Agriculture 36,* 1010-1027.

KERSHAW, K.A. (1959). An investigation of the structure of a grassland community. 2. The pattern of *Dactylis glomerata, Lolium perenne* and *Trifolium repens. Journal of Ecology 47,* 31-43.

KING, J., LAMB, W.I.C. & MCGREGOR, M.T. (1978). Effect of partial and complete defoliation on regrowth of white clover plants. *Journal of the British Grassland Society 33,* 49-55.

KNIGHT, W.E. (1953). Interrelationships of some morphological and physiological characteristics of Ladino clover. *Agronomy Journal 45,* 197-199.

KORTE, C.J. & PARSONS, A.J. (1984). Persistence of a large-leaved white clover variety under sheep grazing. *Proceedings of the New Zealand Grassland Association 45,* 118-123.

LANCASHIRE, J.A. & KEOGH, R.G. (1968). Facial eczema and grazing management. *Sheep Farming Annual,* 29-35.

LATCH, C.G.M. & SKIPP, R.A. (1987). Diseases. In: Baker, M.J. & Williams, W.M. eds. *White clover.* Wallingford: CAB International, 421-460.

LAZENBY, A. & SWAIN, F.G. (1972). Pasture species. In: Lazenby, A. & Swain, F.G. eds. *Intensive pasture production.* Sydney: Angus and Robertson Pty Ltd, 67-96.

LEAKEY, R.R.B., CHANCELLOR, R.J. & VINCE-PRUE, D. (1977). Regeneration from rhizome fragments of *Agropyron repens.* II. The breaking of 'late spring dormancy' and the influence of chilling and node position on growth from single-node fragments. *Annals of Applied Biology 87,* 433-441.

LEVY, E.B. (1970). *Grasslands of New Zealand.* Wellington: Government Printer.

LOVETT DOUST, L. (1981). Population dynamics and local specialisation in a clonal perennial (*Ranunculus repens*). 1. The dynamics of ramets in contrasting habitats. *Journal of Ecology 69,* 743-755.

MÄCHLER, F. & NÖSBERGER, J. (1977). Effect of light intensity and temperature on apparent photosynthesis of altitudinal ecotypes of *Trifolium repens* L. *Oecologia 31,* 73-78.

MAIGE, M.B. (1900). Recherges biologique sur les plantes rampantes. *Annals of Science and Nature Series 8,* 11, 249.

MANGE, M. (1963). Action de l'acide gibberellique sur *Trifolium repens* L.: observations sur quelques modifications morphologiques, physiologiques et automiques. *Annales Scientifiques de L'Universite de Besancon, Serie Botanique 19,* 43-53.

MATTHEW, C., QUILTER, S.J., KORTE, C.J., CHU, A.C.P. & MACKAY, A.D. (1989). Stolon formation and significance for sward tiller dynamics in perennial ryegrass. *Proceedings of the New Zealand Grassland Association 50,* 255-259.

MCINTYRE, G.I. (1965). Some effects of the nitrogen supply on the growth and development of *Agropyron repens* L. Beauv. *Weed Research 5,* 1-12.

MCNAUGHT, K.J. (1970). Diagnosis of mineral deficiencies in grass-legume pastures by plant analysis. *Proceedings of the 11th International Grassland Congress, Brisbane,* 334-358.

MITCHELL, K.J. (1956). Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *New Zealand Journal of Science and Technology A38,* 203-216.

MITCHELL, K.J. & GLENDAY, A.C. (1958). The tiller population of pastures. *New Zealand Journal of Agricultural Research 1,* 305-313.

MITCHELL, K.J. & LUCANUS, R. (1962). Growth of pasture species under controlled environment. III. Growth at various levels of constant temperature with 8 and 16 hours of uniform light per day. *New Zealand Journal of Agricultural Research 5,* 135-144.

MORAN, C.H., SPRAGUE, V.G. & SULLIVAN. J.T. (1953). Changes in the carbohydrate reserves of ladino white clover following defoliation. *Plant Physiology 28,* 467-474.

MOUAT, M.C.H. (1983). Competitive adaptation by plants to nutrient shortage through modification of root growth and surface charge. *New Zealand Journal of Agricultural Research 26,* 327-332.

MOULIA, B., VARLET-GRANCHER, C. & JACQUES, R. (1989). Phytochrome control of white clover morphogenesis. Description and ecological significance. *Plant Physiology (Life Science Advances) 8,* 105-111.

MURPHY, J.S. & BRISKE, D.D. (1992). Regulation of tillering by apical dominance: Chronology, interpretive value and current perspectives. *Journal of Range Management 45,* 419-429.

MURPHY, P. (1982). Growth reserves in white clover. *An Foras Taluntais, Soils and Grassland Production Research Report,* 20.

NEWTON, P.C.D. (1986). *The establishment, growth and fate of white clover plants with special reference to the physiology of stolon growth.* Ph.D. thesis, University of North Wales, Bangor, U.K.

NEWTON, P.C.D. & HAY, M.J.M. (1992). Technique for evaluating the potential for growth of shoot and root buds of white clover (*Trifolium repens*). *Journal of Agricultural Science, Cambridge 119,* 179-183.

NEWTON, P.C.D. & HAY, M.J.M. (1994). Patterns of nodal rooting in *Trifolium repens* L. and correlations with stages in the development of axillary buds. *Grass and Forage Science,* in press.

NEWTON, P.C.D., HAY, M.J.M., THOMAS, V.J. & DICK, H.B. (1992). Viability of axillary buds of white clover (*Trifolium repens*) in grazed pasture. *Journal of Agricultural Science, Cambridge 119,* 345-354.

NEWTON, P.C.D. HAY, M.J.M., THOMAS, V.J., GLASGOW, E.M. & DICK, H.B. (1990). Patterns of axillary bud activity in white clover. *Proceedings of the New Zealand Grassland Association 52,* 247-249.

NÖSBERGER, J., MACHLER, F. & BOLLER, B.C. (1983). Photosynthesis and dry-matter distribution in altitudinal ecotypes of white clover. *Proceedings of the 14th International Grassland Congress, Kentucky,* 410-413.

OZANNE, P.G. (1976). Phosphate nutrition of plants - a general treatise. In: Khasawneh, F.E., Sample, E.C. & Kamprath, E.J. eds. *The role of phosphorus in agriculture.* Madison: American Society of Agronomy, 559-589.

PASCOE, W.B. (1973). *Effects of grazing on the growth and development of white clover* (Trifolium repens L.). M.Ag. Sc. thesis, Massey University, New Zealand.

PHILLIPS, I.D.J. (1975). Apical dominance. *Annual review of Plant Physiology 26,* 341-367.

POOVIAH, B.W. (1974). Promotion of radial growth by 2-chloroethylphosphoric acid in bean. *Botanical Gazette 135,* 289-292.

ROBIN, C., HAY, M.J.M., NEWTON, P.C.D. & GREER, D.H. (1994). Effect of light quality (red: far-red ratio) at the apical bud of the main stolon on morphogenesis of *Trifolium repens* L. *Annals of Botany*, (in press).

ROBSON, M.J. & POLNIASZEK, T. (1984). Effect of contrasting light regimes on the growth and morphology of individual plants of white clover and of different stolons on the same plant. In: Thomson, D.J. ed. *Forage legumes. Occasional Symposium of the British Grassland Society 16*, 189-190.

ROMBERGER, J.A. (1963). Meristems, growth and development in woody plants. *USDA Technical Bulletin 1293.*

RUELKE, O.C. & SMITH, D. (1956). Overwintering trends of cold resistance and carbohydrates in medium red, ladino and common white clover. *Plant Physiology 31,* 364-368.

RYLE, G.J.A., POWELL, C.E. & GORDON, A.J. (1981). Patterns of ^{14}C-labelled assimilate partitioning in red and white clover during vegetative growth. *Annals of Botany 47,* 505-514.

RYLE, G.J.A., POWELL, C.E. & GORDON, A.J. (1988). Responses of N_2 fixation-linked respiration to host-plant energy status in white clover acclimated to a controlled environment. *Journal of Experimental Botany 39,* 879-887.

SACKVILLE HAMILTON, N.R. (1980). *Variation and adaptation in wild populations of white clover* (Trifolium repens L.) *in East Anglia.* Ph.D. thesis, University of Cambridge, U.K.

SACKVILLE HAMILTON, N.R. (1982). Observations on the growth of white clover in a pasture. *Working Group on Detailed Sward Measurement Newsletter 10,* 15-17.

SACKVILLE HAMILTON, N.R. (1987a). An appropriate characterisation of branching. *Working Group on Detailed Sward Measurement Newsletter 19,* 3-4.

SACKVILLE HAMILTON, N.R. (1987b). Pseudo-taproots and module longevity in *Trifolium repens. Working Group on Detailed Sward Measurement 19,* 14-15.

SACKVILLE HAMILTON, N.R. (1990). The dynamics of *Trifolium repens* in a permanent pasture. II. Internode length and the movement of shoot axes. *Proceedings of the Royal Society, London B239,* 359-381.

SACKVILLE HAMILTON, N.R. & HARPER, J.L. (1989). The dynamics of *Trifolium repens* in a permanent pasture. I. The population dynamics of leaves and nodes per shoot axis. *Proceedings of the Royal Society of London, B237,* 133-173.

SALTVIET, M.E. & DILLEY, D.R. (1978). Rapidly induced wound ethylene from excised segments of etiolated *Pisum sativum* L. cv. Alaska. III. Induction and transmission of the response. *Plant Physiology 62,* 710-712.

SANDERSON, J.F. (1966). *Aspects of the growth of white clover with particular reference to the effects of defoliation and shade.* Ph.D. thesis, University of Reading, U.K.

SAS INSTITUTE INCORPORATED (1988). *SAS/STAT User's Guide, Release 6.03 Edition.* Gary, North Carolina: SAS Institute Inc.

SCHILLINGER, J.A. & LEFFEL, R.C. (1964). Persistence of Ladino clover, *Trifolium repens* L. *Agronomy Journal 56,* 11-14.

SCHMID, B. & HARPER, J.L. (1985). Clonal growth in grassland perennials. 1. Density and pattern-dependent competition between plants with different growth forms. *Journal of Ecology 73,* 793-808.

SCOTT, D., MENALDA, P.H. & BROUGHAM, R.W. (1968). Spectral analysis of radiation transmitted and reflected by different vegetations. *New Zealand Journal of Botany 6,* 427-449.

SEARS, P.D., HYDE, E.O.C. & GREENWOOD, R.M. (1955). Pasture establishment on pumice soils. *New Zealand Journal of Science and Technology, Section A37,* 110-140.

SHARPLEY, A.N. & SYERS, J.K. (1977). Seasonal variation in casting activity and in the amounts and release to solution of phosphorus forms in earthworm casts. *Soil Biology and Biochemistry 9,* 227-231.

SKIPP, R.A. & GAYNOR, D.L. (1987). Pests - nematodes. In: Baker, M.J. & Williams, W.M. eds. *White clover.* Wallingford: CAB International, 493-512.

SLADE, A.J. & HUTCHINGS, M.J. (1987). The effects of nutrient availability and foraging in the clonal herb *Glechoma hederacea. Journal of Ecology 75,* 95-112.

SMITH, H. (1975). *Phytochrome and photomorphogenesis.* London: McGraw-Hill.

SNAYDON, R.W. (1985). Aspects of the ecological genetics of pasture species. In: Haeck, J. & Waldendorp, J.W. eds. *Structure and functioning of plant populations.* Amsterdam: North Holland Publishing Company, 127-152.

SNAYDON, R.W. & BAINES, R.N. (1981). Factors affecting interactions between white clover and grasses. In: Wright, C.E. ed. *Plant physiology and herbage production. Occasional Symposium of the British Grassland Society 13,* 179-184.

SOLANGAARACHCHI, S.M. (1985). *The nature and control of branching pattern in white clover* (Trifolium repens *L.*). Ph.D thesis, University of North Wales, Bangor, U.K.

SOLANGAARACHCHI, S.M. & HARPER, J.L. (1987). The effect of canopy filtered light on the growth of white clover (*Trifolium repens*). *Oecologia 72,* 372-376.

SOLANGAARACHCHI, S.M. & HARPER, J.L. (1989). The growth and asymmetry of neighbouring plants of white clover (*Trifolium repens* L.). *Oecologia 78,* 208-213.

SPEDDING, C.R.W. & DIEKMAHNS, F.L. (1972). *Grasses and legumes in British agriculture.* Farnham Royal: CAB Bulletin No. 49.

STEVENSON, C.A. & LAIDLAW, A.S. (1985). The effect of moisture stress on stolon and adventitious root development in white clover (*Trifolium repens* L.). *Plant and Soil 85,* 249-257.

STEWART, I. & BEAR, F.E. (1951). Ladino clover - its mineral requirements and chemical composition. *New Jersey Agricultural Experimental Station, Bulletin 759.*

STEWART, T.A. & HAYCOCK, R.E. (1984). Beef production from low N and high N S24 perennial ryegrass/Blanca white clover swards - a six-year farmlet scale comparison. *Research and Development in Agriculture 1,* 103-111.

STOCKLI, A. (1928). Studien uber den einflus der regenwurmer auf die beschaffenheit des bodens. *Landwirtschuftlichen Jahrbuch der Schweiz 42,* (1) (not sighted).

TESAR, M.B. & AHLGREN, H.L. (1950). Effect of height and frequency of cutting on the productivity and survival of Ladino clover (*Trifolium repens* L.). *Agronomy Journal 42,* 230-235.

THOMAS, H. (1984). Effects of drought on growth and competitive ability of perennial ryegrass and white clover. *Journal of Applied Ecology 21,* 591-602.

THOMAS, R.G. (1962). The initiation and growth of axillary bud primordia in relation to flowering in *Trifolium repens* L. *Annals of Botany 26,* 329-344.

THOMAS, R.G. (1980). Growth of the white clover plant in relation to seed production. In: Lancashire, J.A. ed. *Herbage seed production.* Palmerston North, New Zealand: New Zealand Grassland Association (Inc.), 56-63.

THOMAS, R.G. (1981). Flower initiation in relation to cool season growth of four lines of white clover. *New Zealand Journal of Agricultural Research 24,* 37-41.

THOMAS, R.G. (1987a). The structure of the mature plant. In: Baker, M.J. & Williams, W.M. eds. *White Clover*. Wallingford: C.A.B. International, 1-29.

THOMAS, R.G. (1987b). Vegetative growth and development. In: Baker, M.J. & Williams, W.M. eds. *White Clover*. Wallingford: C.A.B. International, 31-62.

THOMAS, R.G. (1987c). Reproductive development. In: Baker, M.J. & Williams, W.M. eds. *White clover*. Wallingford: CAB International, 63-123.

THOMPSON, L. (1993a). The influence of the radiation environment around the node on morphogenesis and growth of white clover (*Trifolium repens*). *Grass and Forage Science 48,* 271-278.

THOMPSON, L. (1993b). The influence of natural canopy density on the growth of white clover (*Trifolium repens*). *Oikos 67,* 321-324.

THOMPSON, L. & HARPER, J.L. (1988). The effect of grasses on the quality of transmitted light and its influence on the growth of white clover *Trifolium repens. Oecologia 75,* 343-347.

TRATHAN, P. (1983). *Clonal interactions of* Trifolium repens *and* Lolium perenne. Ph.D thesis, University of North Wales, Bangor, U.K.

TRAUTNER, J.L. & GIBSON, P.B. (1966). Fate of white clover axillary buds at five intensities of sunlight. *Agronomy Journal 58,* 557-558.

TREWAVAS, A.J. (1981). How do plant growth substances work? *Plant, Cell and Environment 4,* 203-228.

TROUGHTON, A. (1957). *The underground organs of herbage grasses.* Farnham Royal, England: CAB.

TURKINGTON, R. & BURDON, J.J. (1983). The biology of Canadian weeds. 57. *Trifolium repens* L. *Canadian Journal of Plant Science 63,* 243-266.

TURKINGTON, R., CAHN, M.A., VARDY, A. & HARPER, J.L. (1979). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. *Journal of Ecology 67,* 231-243.

TURKINGTON, R. & HARPER, J.L. (1979a). The growth distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. I. Ordination, pattern and contact. *Journal of Ecology 67,* 201-218.

TURKINGTON, R. & HARPER, J.L. (1979b). The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. II. Inter- and intra-specific contact. *Journal of Ecology 67,* 219-230.

TURKINGTON, R., SACKVILLE HAMILTON R. & GLIDDON, C. (1991). Within-population variation in localised and integrated responses of *Trifolium repens* to biotically patchy environments. *Oecologia 86,* 183-192.

UENO, M. (1982). Root development and function of white clover in warmer regions of Japan. *Japan Agricultural Research Quarterly 16,* 198-201.

UENO, M. & WILLIAMS, R.D. (1967). Absorption of radio-active phosphorus from tap- and nodal roots of white clover (*Trifolium repens* L.). *Journal of the British Grassland Society 22,* 165-169.

UENO, M. & YOSHIHARA, K. (1968). Studies on the root of herbage legumes. 5. Effect of some environmental factors on the penetration of the adventitious roots of white clover to the soil. *Journal of the Japanese Society of Grassland Science 14,* 100-103.

VARTHA, E.W. & HOGLUND, J.H. (1983). What is the make-up of a dryland pasture? *Proceedings of the New Zealand Grassland Association 44,* 204-210.

VEZ, A. (1961). Étude du dèveloppement du trèfle blanc en relation avec les fluctuations de quelques substances organiques. *Berichte der Schweizerischen Botanischen Gesellschaft 71,* 118-172.

WANG, X. (1991). *A study of growth, development and N-fixation of several white clover* (Trifolium repens *L.) cultivars under different water deficit and phosphorus levels.* Ph.D thesis, Massey University, New Zealand.

WARING, P.F. (1964). The developmental physiology of rhizomatous and creeping plants. *Proceedings of the Seventh British Weed Control Conference,* 1020-1030.

WARRINGTON, I.J. & MITCHELL, K.J. (1976). The influence of blue- and red-biased light spectra on the development of plants. *Agricultural Meteorology 16,* 247-262.

WATERS, R.A.S. (1951). Earthworms and the fertility of pasture. *Proceedings of the New Zealand Grassland Association 13,* 168-175.

WATERS, R.A.S. (1955). Numbers and weights of earthworms under a highly productive pasture. *New Zealand Journal of Science and Technology 36,* 516-525.

WATKINSON, A.R. (1978). Discussion section. In: Freysen, A.H.J. & Woldendorp, J.W. eds. *Structure and functioning of plant populations.* Amsterdam: North Holland, 48.

WILKINSON, S.R. & GROSS, C.F. (1967). Macro- and micro-nutrient distribution within Ladino clover (*Trifolium repens* L.). *Agronomy Journal 59,* 372-374.

WILLIAMS, C.H. & TWINE, J.R. (1967). Determination of nitrogen, sulphur, phosphorus, potassium, sodium, calcium and magnesium in plant material by automatic analysis. *CSIRO, Division of Plant Industry, technical paper 24.*

WILLIAMS, W.M. (1983). Exploitation of the genetic resource through plant breeding. *Trifolium repens.* In: McIvor, J.G. & Bray, R.A. eds. *Genetic resources of forage plants.* East Melbourne: CSIRO, 261-274.

WILLIAMS, W.M. (1987). Adaptive variation. In: Baker, M.J. & Williams, W.M. eds. *White Clover.* Wallingford: CAB International, 299-321.

WILLIAMS, W.M. & HOGLUND, J.H. (1978). Temperature responses of New Zealand, Spanish, and New Zealand x Spanish white clover populations. *New Zealand Journal of Agricultural Research 21,* 491-497.

WILLIAMS, W.M., LAMBERT, M.G. & CARADUS, J.R. (1982). Performance of a hill country white clover selection. *Proceedings of the New Zealand Grassland Association 43,* 188-195.

WILMAN, D. & ASIEGBU, J.E. (1982a). The effects of clover variety, cutting interval and nitrogen application on herbage yields, proportions and height in perennial ryegrass - white clover swards. *Grass and Forage Science 37,* 1-13.

WILMAN, D. & ASIEGBU, J.E. (1982b). The effects of clover variety, cutting interval and nitrogen application on morphology and development of stolons and leaves of white clover. *Grass and Forage Science 37,* 15-27.

WILMAN, D., FISHER, A. & MARSHALL, A.H. (1991). Effects of sowing a companion grass (*Lolium perenne*), application of grass suppressant and choice of clover variety on the growth and seed production potential of white clover. *Journal of Agricultural Science, Cambridge 117,* 47-56.

WILMAN, D. & SIMPSON, D. (1988). The growth of white clover (*Trifolium repens*) in five sown hill swards grazed by sheep. *Journal of Applied Ecology 25,* 631-642.

WOOD, G.M. & SPRAGUE, M.A. (1952). Relation of organic food reserves to cold hardiness of ladino clover. *Agronomy Journal 44,* 318-325.

YOUNG, D.J.B. (1958). A study of the influence of nitrogen on the root weight and nodulation of white clover in a mixed sward. *Journal of the British Grassland Society 13,* 106-114.