ESTABLISHMENT AND EARLY REGROWTH OF SHEEP'S BURNET

(*SANGUISORBA MINOR* SSP. *MURICATA* (SPACH) BRIQ.)

EXAMINED MULTIVARIATELY

A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy at

Massey University
Palmerston North
New Zealand

GRANT BRODIE DOUGLAS

February, 1991

Typical foliage and inflorescences of sheep's burnet.

ABSTRACT

Factors affecting establishment and early regrowth of the low growing perennial herb, sheep's burnet (*Sanguisorba minor* ssp. *muricata* (Spach) Briq.), were investigated in field and controlled environment studies. Lucerne (*Medicago sativa* L.) and sometimes birdsfoot trefoil (*Lotus corniculatus* L.), were included as dryland standards in the establishment studies.

Sheep's burnet emerged more slowly than lucerne and birdsfoot trefoil, but early vegetative growth was similar to that of lucerne and faster than birdsfoot trefoil. Under lower North Island field conditions, spring sown sheep's burnet established and tolerated three partial defoliations (5-7 cm stubble) as well as the legumes and averaged a total of 6.3 t DM ha^{-1}. Regrowth in the autumn indicated that a defoliation frequency of four weeks was suitable.

Field emergence of sheep's burnet was influenced markedly by temperature and was 66% on relatively warm, sandy soils at Flock House compared with 27% at Riverside. Seedlings emerged approximately 3-4 days earlier at Flock House. At constant temperatures of 10, 15, 20 and 25°C, final emergences of sheep's burnet were similar and averaged 70.4% but rates of emergence were again faster at higher temperatures. The minimum temperature for satisfactory (50%) emergence of sheep's burnet was 4.9°C and this was discussed in relation to sowing time. Temperature also had a pronounced effect on times to reach various seedling growth stages.

Large (>2.8 mm) seeds occasionally provided faster seedling emergence than small (<2.0 mm) and medium (2.0-2.8 mm) seeds, and at constant temperatures, large seeds gave greater emergence (81%) than small seeds (62%). Large and medium seeds also produced a greater proportion of seedling pairs (>50%) per hypanthium ("seed") than small seeds (9%), which may have advantages for rate of ground cover and perhaps earlier provision of forage. Field sowings of unseparated seed averaged 30% seedling pairs. Large seeds frequently produced superior seedlings and seed growers should be encouraged to produce similar seed. Material from Oregon, USA was

generally superior to that evaluated in early New Zealand trials but this depended on the evaluation environment, particularly temperature.

Foliar regrowth from a range of partially defoliated glasshouse grown plants was superior to that of plants defoliated completely. Reduction in root mass was the most important morphological effect of complete defoliation. The results indicated that current photosynthates from residual leaves were important in supplying energy for regrowth and this was discussed in relation to possible stand management. Osmotic adjustment was suggested as accounting for satisfactory growth of sheep's burnet in dry environments.

ACKNOWLEDGEMENTS

A full-time study award from the New Zealand government (Ministry of Works and Development/Department of Scientific and Industrial Research (DSIR)) to conduct these studies is gratefully acknowledged. Without this financial assistance, and the opportunity to study full-time on one topic, this research would not have been possible.

I am deeply indebted to my supervisors Mr A G Robertson (Chief) and Drs I L Gordon (later Chief) and A C P Chu for their encouragement and invaluable criticism during these studies and in compilation of the manuscript. Dr N J Withers provided valuable guidance during the early experiments and Dr I L Gordon considerably strengthened my use and understanding of several multivariate analysis procedures.

Land for the field trials was kindly provided at Flock House by MAFTech and at Riverside by Massey University. Messrs W Stiefel (Flock House) and W J Parker (Supervisor of Riverside) provided valuable discussions and advice on conducting trials at the respective sites.

I am most grateful for the use of controlled climate facilities at DSIR Fruit and Trees, Palmerston North and wish to thank Dr I J Warrington and Mrs D M Jansson and other members of the Climate Room Support Group for their assistance.

Staff and post graduate students of the Agronomy Department provided a stimulating environment for discussing my research and gave assistance when required. I wish to thank Mrs F P Brown and Mr D T Sollitt especially, for their instruction and support in conducting the nonstructural carbohydrate determinations.

Expert technical assistance was provided on numerous occasions by Mr A G Foote, particularly in the setting up and execution of the field trials at Riverside and at Flock House. I wish to thank him and other plant materials staff at DSIR Aokautere (formerly Plant Materials Group, Soil Conservation Centre, Aokautere, of the Ministry of Works and Development) for ensuring that the grass/legume/herb

programme was continued satisfactorily during my absence. Also, many thanks to Mr C W S van Kraayenoord for support during my applications for funding for the research.

In addition to my supervisors, several personnel critically appraised drafts of the manuscript and I wish to acknowledge them individually. They were:

- Dr B J Wills, DSIR Fruit and Trees, Alexandra who read all chapters except Chapter 10;

- Dr J S Sheppard, DSIR Fruit and Trees, Christchurch who read Chapters 1, 2 (excluding Section 2.4), 3 and 11;

- Dr N C Lambrechtsen, DSIR, Wellington who read Chapter 4;

- Dr W R N Edwards, DSIR Fruit and Trees, Palmerston North who read Chapter 9; and

- Dr D Scott, DSIR Grasslands, Lincoln who read Chapter 10 and the sections on competition in Chapters 2 and 4.

However, the final manuscript presented is my responsibility.

Mrs J M Warr carefully typed most of the tables and undertook much of the later word processing of the manuscript. Word processing was also conducted by Miss J L Williams and final processing was undertaken by Miss C C Wilson. Their patience and diligence are gratefully acknowledged.

Finally, I am extremely grateful for the support and encouragement of family, relatives and friends during the entire candidature. To you all, a very big "thank you".

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 : INTRODUCTION

Sheep's burnet (*Sanguisorba minor* ssp. *muricata* (Spach) Briq.) is a low growing, long-lived, palatable herb which is a native of southern, western and central Europe through to western Asia (Salmeron, 1966; Nordborg, 1967b; Proctor and Nordborg, 1968). In New Zealand, the plant has received intermittent attention for revegetating erosive, semi-arid country (Macpherson, 1910, 1911, 1912; Cockayne, 1920a, b, 1921, 1922a, b; Lunn, 1951; Sewell, 1952; Sievwright, 1956) and it has proved well adapted to drought-prone and semi-arid areas such as those of the eastern and central parts of the South Island. Since the early 1970's, modern selections of sheep's burnet have been evaluated in New Zealand for their dryland revegetation potential and to a lesser extent for their forage value (NWASCO, 1982; Sheppard and Wills, 1985, 1986; NWASCA, 1986; Wills *et al.*, 1987; Rys *et al.*, 1989).

Despite the rather extensive evaluations conducted on sheep's burnet and other *S. minor* subspecies, little is known about the optimum environmental conditions for their establishment and growth. The New Zealand and international literature on these agronomic aspects is based largely on qualitative assessments, although more detailed quantitative research has been forthcoming recently (Salmeron, 1966; Silvertown and Dickie, 1980; Gay *et al.*, 1982; Daly, 1984; Sydes and Grime, 1984; Sheppard and Wills, 1985; Wills *et al.*, 1987; Rys *et al.*, 1989).

Most of the New Zealand work has been conducted in the Mackenzie Basin, the Waitaki Valley, and in Central and North Otago. Establishment of sheep's burnet at these locations has varied from being a complete failure to being moderately successful from sowings in autumn or spring on shady or sunny faces (Macpherson, 1910-1912; Cockayne, 1920-1922; Ward, 1923; Tennent, 1935; Wills, 1983; Wills *et al.*, 1987). The most probable reasons for this variability are competition from resident vegetation, unfavourable temperatures and/or inadequate moisture principally because of sowing time and aspect, and variability in sowing depth and seed size (Ward, 1923; Wills, 1983; Sheppard and Wills, 1985).

Establishment of sheep's burnet is slow compared to other species such as lucerne (de Lacy, 1985; J S Sheppard, pers. comm.; B J Wills, pers. comm.), particularly under harsh environmental conditions such as those experienced in Central Otago. Accordingly, plants should receive only light or no grazing for the first 18 months following sowing (de Lacy, 1985; Sheppard and Wills, 1985, 1986; NWASCA, 1986), which is a relatively long time to have a forage excluded from stock. Apart from climatic influences, slow vegetative growth of sheep's burnet may be due to several factors including low photosynthetic efficiency, inherently high respiration, or favoured partitioning of photosynthates to the roots. In his non-technical article, de Lacy (1985) suggested that the slow establishment of the species was due to the development of a long tap root, which presumably acts as a major sink for photosynthate.

Regrowth of sheep's burnet originates from the top of the tap root and may depend primarily on carbohydrate reserves stored in the root, as with lucerne (Smith, 1962; Rapoport and Travis, 1984; Gabrielsen *et al.*, 1985). Young plants with developing root systems possibly have low root reserves which may explain their often unsatisfactory regrowth following intense defoliation (author; B J Wills, pers. comm.). A detailed examination of the foliar regrowth of young sheep's burnet swards, together with information on their physiological condition, may assist greatly in understanding regrowth and in formulating early defoliation strategies. Mature swards are much more tolerant of grazing (Sheppard and Wills, 1985).

Sheep's burnet has a growth habit, and perhaps forage yield and response to defoliation, similar to that of the more widely recognised dryland species of lucerne (*Medicago sativa* L.) (Douglas and Kinder, 1973; Musgrave *et al.*, 1975; Musgrave, 1976, 1982; Daly, 1984) and birdsfoot trefoil (*Lotus corniculatus* L.) (Duke, 1981; Scott and Charlton, 1983). Although sheep's burnet is potentially suitable for revegetating dryland areas, there have been few detailed trials conducted to compare its performance alongside and in combination with these "standard" dryland species. Such trials are highly desirable for determining whether sheep's burnet has worthwhile advantages as a pure sward over comparable species and/or would be suitable as a companion species.

It was therefore with two main themes in mind that a series of experiments was conducted between spring, 1985 and autumn, 1988. These themes were: 1) suitable establishment conditions; and 2) foliar regrowth responses of establishing plants. The overall objectives of the studies reported in this thesis were to determine:

1) some of the important agronomic requirements for satisfactory establishment of sheep's burnet;
2) the early growth and yielding ability of sheep's burnet, compared with that of standard dryland legumes; and
3) the foliar regrowth responses of establishing plants of sheep's burnet under limiting and non-limiting moisture conditions.

CHAPTER 2 : LITERATURE REVIEW

2.1 INTRODUCTION

This review is divided into three sections. The first section covers literature on sheep's burnet (*Sanguisorba minor* ssp. *muricata* (Spach) Briq.) and also that on closely related subspecies and/or species where there has been possible taxonomic confusion regarding which plant(s) were actually being described. Similarities and differences between members of the genus *Sanguisorba* have also been highlighted where appropriate to further characterise some of the plant's features. Section two deals with the influence of environmental and other factors on the seedling emergence and early vegetative growth of numerous other plant species. Literature on defoliation and nonstructural carbohydrate physiology is also reviewed. The final section is a brief review of multivariate analysis procedures.

The common and botanical names of the species and subspecies referenced in this thesis are listed in Tables 2.1a and b. Material is referred to subsequently by its common name.

2.2 SHEEP'S BURNET

2.2.1 TAXONOMY

Prior to the mid-1960's, sheep's burnet and other members of the tribe *Sanguisorbeae* were named by various binomials, or not at all (Cockayne, 1920a, b; Macpherson, 1920; Ward, 1923; McGillivray, 1929; McTaggart, 1935; Armstrong *et al.*, 1950, 1953; Sievwright, 1956; Anon, 1957, 1958; Raven and Thompson, 1961; Hafenrichter *et al.*, 1965; Salmeron, 1966) which caused considerable confusion in evaluative and descriptive work. Later in the same decade, a detailed revision of the classification system for the genus *Sanguisorba* was undertaken by Professor G Nordborg of Sweden (Nordborg, 1963, 1967a, b, 1968; Proctor and Nordborg, 1968). Her system has been adopted partially although there has still been some use of old

Table 2.1a Common and botanical names of grass species referenced.

COMMON NAME	BOTANICAL NAME
barley	*Hordeum vulgare* L.
blue gramagrass	*Bouteloua gracilis* (Kunth) Griffiths
branched wiregrass	*Aristida armata* Henr.
bromegrass	*Bromus* spp.
buffel grass	*Cenchrus ciliaris L.*
chewings fescue	*Festuca nigricans* Lam.
cocksfoot	*Dactylis glomerata* L.
crested wheatgrass	*Agropyron desertorum* (Fischer ex Link) Schultes
green panic	*Panicum maximum* var. *trichoglume* Robyns
kleingrass	*Panicum coloratum* L.
maize	*Zea mays* L.
mulga grass	*Thyridolepis mitchelliana* (Nees) S.T. Blake
paspalum	*Paspalum dilatatum* Poir.
perennial ryegrass	*Lolium perenne* L.
phalaris	*Phalaris aquatica* L.
prairie grass	*Bromus willdenowii* Kunth
rice	*Oryza sativa* L.
sheep's fescue	*Festuca ovina* L.
smooth bromegrass	*Bromus racemosus* L.
sorghum	*Sorghum bicolor* (L.) Moench.
spear grass	*Heteropogon contortus* (L.) Roem. and Schult.
summer grass	*Digitaria sanguinalis* (L.) Scop.
tall fescue	*Festuca arundinacea* Schreb.
timothy	*Phleum pratense* L.
weeping lovegrass	*Eragrostis curvula* (Schrad.) Nees
western wheatgrass	*Pascopyrum smithii* (Rydb.) A. Love
wheat	*Triticum aestivum* L.
Yorkshire fog	*Holcus lanatus* L.

Table 2.1b Common and botanical names of legume and non-grass species referenced.

COMMON NAME	BOTANICAL NAME
birdsfoot trefoil	*Lotus corniculatus* L.
chicory	*Chichorium intybus* L.
cicer milkvetch	*Astragalus cicer*
cotton	*Gossypium* spp.
fathen	*Chenopodium album* agg.
garden burnet	*Sanguisorba officinalis* L.
hairy canary clover	*Dorycnium hirsutum* (L.) Ser. in DC.
lotus	*Lotus pedunculatus* L.
lucerne	*Medicago sativa* L.
mouse-ear hawkweed	*Hieracium pilosella* L.
rape	*Brassica napus* L. ssp. *oleifera* DC.
red clover	*Trifolium pratense* L.
sainfoin	*Onobrychis viciifolia* Scop.
sheep's burnet	*Sanguisorba minor* ssp. *muricata* (Spach) Briq.
sheep's sorrel	*Rumex acetosella* L.
shepherd's purse	*Capsella bursa-pastoris* (L.) Med.
siratro	*Macroptilium atropurpureum* (D.C.) Urban
soybean	*Clycine max* (L.) Merr.
subterranean clover	*Trifolium subterraneum* L.
sugar beet	*Beta vulgaris* L. ssp. *vulgaris*
sunflower	*Helianthus annuus* L.
sweet clover	*Melilotus alba* Desr.
tobacco	*Nicotiana tabacum* L.
vetch	*Vicia lathyroides* L.
white clover	*Trifolium repens* L.
yarrow	*Achillea millefolium* L.

binomials and/or failure to specify a subspecies of *S. minor* (Nemati, 1978; Noor, 1978; Le Houerou, 1979; Gay *et al.*, 1982; Evert and Hartman, 1984), as well as occasional use of erroneous binomials for sheep's burnet (Campbell, 1979).

The system of Nordborg (1967b) has been adopted by researchers in New Zealand (Wills, 1983, 1984, 1986; Daly, 1984; Healy, 1984; Sheppard and Wills, 1985; Webb *et al.*, 1988), India (Purohit and Panigrahi, 1984) and the United States (Robertson, 1974). In view of its partial adoption, particularly locally, her classification is used in this thesis and hence sheep's burnet is referred to as *S. minor* ssp. *muricata* (Spach) Briq. Other binomials for sheep's burnet (Proctor and Nordborg, 1968; Healy, 1984; Sheppard and Wills, 1985), together with its taxonomic relationship with other members of the genus *Sanguisorba*, are shown in Table 2.2.

One of the earliest reports of burnet (probably sheep's burnet) in New Zealand was by Wilkin (1877). In early New Zealand investigations (Macpherson, 1910; Cockayne, 1920a, b, 1921, 1922; McGillivray, 1929; Sievwright, 1956), sheep's burnet was referred to incorrectly as *Poterium sanguisorba*, the salad burnet (Healy, 1984). Examination of naturalised plants near the early trial sites confirmed that sheep's burnet had indeed been grown (Sheppard and Wills, 1985) and this was also supported largely by the findings of surveys by Given (1982) and by Webb *et al.* (1988).

2.2.2 DISTRIBUTION AND ECOLOGY

Sheep's burnet is distributed naturally around the Mediterranean Basin (Sheppard and Wills, 1985) but is concentrated mainly in Central and Southern Europe (Nordborg, 1967b). The subspecies is also found sparsely in the northern part of North Africa, on the Canary Islands, and in Asia from Turkey to Afghanistan (Nordborg, 1967b). Introduced plants of the species complex have naturalised in North America, mainly around the Great Lakes and in Oregon and California (Nordborg, 1967b; Robertson, 1974; Sheppard and Wills, 1985). In New Zealand, sheep's burnet is now regarded as a member of the naturalised flora of Central Otago (Given, 1982).

Table 2.2 Taxonomy of some members of the genus *Sanguisorba*

FAMILY: ROSACEAE
TRIBE: SANGUISORBEAE
GENUS: *SANGUISORBA*
SUBGENERA: *Poterium, Sanguisorba*

1. Subgenus *Poterium* (L.) A. Braun and Bouche. Upper flowers of capitulum female, the middle and lower hermaphrodite. Stamens numerous. Stigmatic papillae long. Carpels 2.
   Sheep's burnet: *Sanguisorba minor* Scop. ssp. *muricata* (Spach) Briq.
   syn. *Poterium polygamum* Waldst. and Kit.
   Poterium muricatum Spach
   Sanguisorba muricata (Spach) Gremli
   Poterium sanguisorba L. ssp. *muricatum* (Spach) Rouy.
   Salad burnet: *Sanguisorba minor* Scop. ssp. *minor*
   syn. *Poterium sanguisorba* L.
   Sanguisorba dictyocarpa (Spach) Franchet

2. Subgenus *Sanguisorba*
   Flowers hermaphrodite. Stamens (2-) 4 (-15). Stigmatic papillae short. Carpel 1.
   Great burnet/garden burnet: *Sanguisorba officinalis* L.
   syn. *Sanguisorba polygama* F. Nyl.

The natural habitat of the *S. minor* complex is in dry grassland on rocky ground (Proctor and Nordborg, 1968) and it is restricted generally to alkaline or almost neutral soils (Nordborg, 1967b). Soil textures can vary from clay to gravel and stone and in 50 samples of soil collected from a diverse sample of sites supporting *S. minor* in Europe, Nordborg (1967b) found a pH range of 6.4 to 8.1. In Spain, Salmeron (1966) found sheep's burnet growing on slightly more acidic soils, with pH values as low as 5.6. The altitudinal range of sheep's burnet is frequently from sea level to about 1400 m (Salmeron, 1966; Nordborg, 1967b) but in Iran and Afghanistan, plants have been recorded at 1800-2000 m (Nordborg, 1967b).

Sheep's burnet is noted for its moderate to high drought tolerance in Australia (Scott, 1932; McTaggart, 1935; Anon, 1951), India (Thakur, 1957), Iran (Nemati, 1978; Campbell, 1979) and North Africa (Le Houerou, 1979). Swards of burnet in North Africa have persisted for over ten years on shallow soils with rainfall not exceeding 300 mm and production under a 300-400 mm rainfall is satisfactory, although not high (Le Houerou, 1979). In New Zealand, research conducted mainly in Central Otago and the Mackenzie Country (Sheppard and Wills, 1985) has also demonstrated the usefulness of sheep's burnet under dry conditions. The plant tolerates heavy winter frosts and high summer temperatures (Le Houerou, 1979; Sheppard and Wills, 1985, 1986).

2.2.3 MORPHOLOGY

Within the last three decades, there have been several morphological and anatomical descriptions of sheep's burnet and other members of the *S. minor* complex (Salmeron, 1966; Nordborg, 1967a, b, 1968; Proctor and Nordborg, 1968; Robertson, 1974; Purohit and Panigrahi, 1984; Sheppard and Wills, 1985), the most extensive being that by Nordborg (1967b). The following description is a summary extracted from these publications.

Sheep's burnet is a dicotyledonous perennial herb, 100-900 mm tall at flowering, glabrous or hairy, with a well developed basal rosette of pinnate leaves. The root is a long, tough and ligneous taproot which is branched, particularly in its lower parts.

Length may be up to 2 m but is usually about 1 m which has also been confirmed in Central Otago. The two cotyledons are petiolate and ovate with entire margins. The later developing basal leaves have 3-12 pairs of orbicular to elliptical leaflets, 10-15 x 15-22 mm, which are stalked frequently and have toothed or deeply dissected margins. Most leaflet pairs are approximately the same size as the apical leaflets with the exception of the basal pairs which are distinctly smaller and either frequently displaced to some degree, or single. Usually the lower side of the leaflets is lighter in colour than the upper and only the mid-vein is distinct.

Flowering stems, often 500-900 mm tall, are erect and commonly leafy with terminal globose or ovoid capitula, 7-15 x 7-24 mm. Upper flowers of the inflorescence are female while middle and lower flowers are hermaphrodite. The individual flowers have very short peduncles and true petals are absent. Members of the *S. minor* complex are all wind pollinated which is unusual among Rosaceae and there are several morphological changes that have resulted evidently from a shift from insect to wind pollination. These include the greenish inflorescences, numerous long stamens (up to 50), long stigmatic papillae, and the near absence of a nectar ring surrounding the mouth of the receptacle (floral tube). The number of pistils in *S. minor* is two, rarely one or three, and the ovaries develop into two (1-3) achenes, included in the receptacle. Receptacles are 4-angled with lateral, strongly compressed ridges (wings) on the angles, and have dimensions of 3-4 x 4-5 mm. They become dry and hard at the fruit stage and their surface is sculptured in different ways. The two true seeds within each receptacle may germinate and produce individual seedlings (Section 2.2.5). Such seedling pairs are referred to as "doubles" in this thesis.

The receptacle and its contents (hypanthium) are referred to correctly as fruits rather than seeds. Extraction of the seed is difficult but generally it is of little practical interest since germination of the fruits is quite satisfactory under suitable conditions (Salmeron, 1966). Because of its practical appeal and common usage, the individual units of sheep's burnet sown to establish swards are referred to as seeds rather than fruits. Seed weights (size) for sheep's burnet are highly variable and in Spain ranged from 128 to 171 seeds per gram (Salmeron, 1966). Nordborg (1967b) in her detailed

investigations of European material also found great variation in the length and breadth of receptacles.

The base chromosome number for members of the genus *Sanguisorba*, section *Poterium*, is seven and only tetraploids (2n=4x=28) and octoploids (2n=8x=56) occur naturally. The tetraploids have the greatest distribution while the octoploid races are restricted to the Mediterranean region. Natural hybridisation between subspecies of *S. minor* is possible but occurs rarely due to separation altitudinally, or ecological isolation.

2.2.3.1 SEED LINES

Almost all of the sheep's burnet seed used currently in New Zealand and elsewhere is common or unregistered germplasm. The only known cultivar is 'Delar' which was released by the Aberdeen Plant Materials Centre in about 1982 (J O Peterson, pers. comm., 1984 - USDA Corvallis Plant Materials Centre, Oregon), but there were seed shortages for several years. However, there was abundant unregistered material in Oregon which was practically identical to 'Delar' (B J Wills, pers. comm.) and large consignments from this source were purchased by at least one commercial company in New Zealand in the early 1980's. It is probable that all of the seed sold currently in this country, including that from local seed production, originates from Oregon (B J Wills, pers. comm.).

The results of extensive selection work for over a decade on several *S. minor* subspecies has resulted in the development of a line which is predominantly sheep's burnet and which is well adapted to New Zealand conditions, particularly those in the country's lower South Island (B J Wills, pers. comm.). Registration of the currently unnamed cultivar will be sought within the next few years.

2.2.4 AGRICULTURAL IMPORTANCE

Sheep's burnet is used for two main purposes, namely revegetation / soil conservation and as forage for grazing animals under dryland conditions. Both uses

are implied in most reports, if not mentioned specifically, and the plant has been evaluated for these uses in numerous parts of the world including Australia (Scott, 1932; McTaggart, 1935; Anon, 1951, 1958), India (Thakur, 1957), Iran (Nemati, 1978; Campbell 1979), New Zealand (Macpherson, 1912, 1920; Cockayne, 1920a, b; Ward, 1923; McGillivray, 1929; Lunn, 1951; Sewell, 1952; Sievwright, 1956; NWASCO, 1982; Sheppard and Wills, 1985, 1986; NWASCA, 1986; Rys *et al.*, 1989), North Africa (Le Houerou, 1979), Pakistan (Noor, 1978), Spain (Salmeron, 1966) and USA (Valassis *et al.*, 1957; Hafenrichter *et al.*, 1965). Attributes of sheep's burnet which are particularly advantageous for soil conservation and/or forage are:

- drought tolerance (McTaggart, 1935; Anon, 1951; Le Houerou, 1979), probably due mainly to the plant's well developed taproot;
- tolerance of heavy frosts (up to -12°C) and high summer temperatures (> 30°C) (NWASCA, 1986);
- a dense basal rosette (Nordborg, 1967b) which provides protective ground cover (Thakur, 1957; Sheppard and Wills, 1985), which is particularly efficacious in reducing erosion caused by rain (FAO, 1965);
- palatable foliage is grazed readily and established plants are tolerant of intense grazing (Wills, 1984; Sheppard and Wills, 1985);
- prolific seed production, reseeding capability, persistence and ability to spread (Thakur, 1957; Douglas, 1970; Le Houerou, 1979; Sheppard and Wills, 1985; Wills and Begg, 1986; Fisher *et al.*, 1987);
- satisfactory survival and production under low to moderate fertility conditions (Sewell, 1952; Scott *et al.*, 1985).

In New Zealand since the early 1970's, there has been renewed interest in lines of sheep's burnet and closely related subspecies predominantly for their erosion control potential (Sheppard and Wills, 1985). As regards ground cover, Wills (1983) reported interim results from autumn and spring oversowing trials on sunny and shady aspects at Otematata Station in the Mackenzie Basin in the South Island. Ground cover was superior on sunny compared with shady faces and cover on autumn sown faces in the first year after oversowing was about 10%. By the second

year, up to 27% of sunny faced plots were covered and cover on both faces was often equal to or better than that supplied by four legume species. Sheep's burnet has provided satisfactory and persistent ground cover at several sites in the South Island of New Zealand. These include shallow loessial soils on the Wither Hills in Marlborough and on some dry North Canterbury sites which are susceptible to wind erosion (de Lacy, 1985; NWASCA, 1986; J S Sheppard, pers. comm.).

In the lower North Island of New Zealand, several small, spaced plant evaluations of the dryland revegetation potential of sheep's burnet have been conducted (Douglas, 1985; Douglas and Foote, unpubl.; Foote, unpubl.). On west coast sand country, plant survival was satisfactory but on low organic matter coastal sites growth and ground cover were poor, while further inland on more developed soils, plants provided most satisfactory ground cover (Douglas and Foote, unpubl.). In Hawke's Bay, beneficial cover was provided by 12 and 16-month old sheep's burnet plants which were growing on drought-prone, gently sloping land facing the sea (Foote, unpubl.).

The perceived advantage of sheep's burnet producing forage highly palatable for a wide range of stock has resulted in descriptions of the plant's forage yield and seasonal distribution, and related characteristics, under New Zealand conditions (Daly, 1984; Sheppard and Wills, 1985; NWASCA, 1986; Wills *et al.*, 1987). Information on these characteristics has been based on observations of, and measurements from, existing field trials. Few trials have been conducted to examine these features of sheep's burnet specifically.

2.2.4.1 FORAGE AND SEED YIELDS

Herbage accumulation of sheep's burnet in an area of Tunisia receiving 300-400 mm rainfall annually was 4-6 t DM ha^{-1} yr^{-1} (Le Houerou, 1979). Under slightly heavier rainfalls (400-600 mm per year), herbage accumulation was 15-30 t DM ha^{-1} yr^{-1} (Le Houerou, 1974). Details on plant age, defoliation history and harvesting heights were not presented in either report. In Spain, Salmeron (1966) obtained up to 42 t ha^{-1} of fresh herbage per year under a six harvest cutting regime and at one harvest the dry

matter content was 22.7%. Assuming this to be approximately constant at other harvests, herbage accumulation was up to 9.5 t DM ha^{-1} yr^{-1}. Maximum growth occurred in the spring (74 kg DM ha^{-1} d^{-1}) while generally some herbage accumulation occurred during the rest of the year, except in winter.

Sheep's burnet (or possibly another subspecies of *S. minor*) has also received attention in the USSR. For example, in the Leningrad province, herbage accumulation over three years ranged from 19-40 t ha^{-1} yr^{-1} (Medvedev, 1969), but whether this represented fresh or dry herbage mass is unknown, although the high values suggest that fresh weight may have been reported. Nevertheless, the results showed that forage yields could be quite variable. In trials with *P. sanguisorba* on dry steppes in the Rostov province, herbage accumulation of burnet grown alone (3.5 t DM ha^{-1}) equalled that of lucerne (Kozov, 1965). The early spring growth of sheep's burnet was noted as well as its growth in late summer and early autumn. In Oregon, USA, the plant also produced well in late spring and summer and has been recommended as a component in mixtures to help provide maximum all-season forage on hill grazing lands with shallow soils (Valassis *et al.*, 1957). Rarely has sheep's burnet outyielded lucerne but this was reported on two experimental farms in New South Wales in 1957 (Anon, 1958). The ability of plants to grow actively in late summer and autumn was also highlighted at Canberra for some species of *Sanguisorba* and *Poterium* (Anon, 1951).

Seasonal patterns of herbage accumulation in New Zealand are similar to those reported overseas. Growth of sheep's burnet is maximal in the spring and may continue at a satisfactory level through the summer and autumn, depending mainly on moisture availability. In Central Otago in the lower South Island of New Zealand, there is frequently a distinct flush of growth in the autumn (Sheppard and Wills, 1985; NWASCA, 1986) and some growth occurs even under the harsh winters in that region (B J Wills, pers. comm.). During the spring, herbage accumulation under dryland conditions can amount to 3.0-4.5 t DM ha^{-1} with similar or slightly reduced accumulation occurring in the autumn (NWASCA, 1986). Under milder Manawatu nursery conditions, higher herbage accumulations of 11-12 t DM ha^{-1} yr^{-1} have been estimated from cutting trials (Foote, unpubl.). A significant advantage of sheep's

burnet is its ability to conserve and supply late winter - early spring feed before lucerne flushes (Wills, unpubl.). Herbage masses of sheep's burnet are within the range of those reported by Hoglund *et al.* (1979) for grass/white clover pastures at a range of New Zealand sites.

A few New Zealand studies have obtained data on the herbage accumulation of swards consisting of sheep's burnet and lucerne cv. 'Wairau' (Daly, 1984; Wills *et al.*, 1987). In a four year study at Tara Hills, sowing rates of each species used to establish mixed swards were 11 and 26 kg ha^{-1} for lucerne and sheep's burnet, respectively (Daly, 1984). Although sheep's burnet established rapidly and contributed about 25% of the herbage mass of the sown species by the second year, its contribution declined markedly thereafter. It was concluded that the species could enhance short-term production but was unsatisfactory as a long-term companion species with lucerne. The results of Wills *et al.* (1987) were obtained from a study over a shorter time but they also showed that under cutting, the contribution of sheep's burnet to the mixed sward herbage mass declined markedly at later harvests. This poor performance was attributed to low plant numbers and an intervening drought. Plant numbers of sheep's burnet declined from 44 plants m^{-2} to 7-8 plants m^{-2}.

Foliage of sheep's burnet has forage value similar to that of lucerne (Sheppard and Wills, 1985; NWASCA, 1986). However, mature herbage with well developed flower stalks is less valuable and has lower digestibility (Sheppard and Wills, 1985). Protein content is often lower than that of lucerne and in Spain has been estimated at 10.9% (Salmeron, 1966). The foliage is moderately to highly palatable to a range of livestock (McTaggart, 1935; Stapledon, 1948; Archer, 1971; Sheppard and Wills, 1985; Wills *et al.*, 1987) and there are no reports of bloat being caused by the plant (Sheppard and Wills, 1985).

Satisfactory seed production practices for sheep's burnet under New Zealand conditions have been outlined by Sheppard and Wills (1985, 1986). A major problem is the long period of seed ripening and hence uneveness and this disadvantage may be overcome partly by careful selection of genotypes with a shorter

duration of flowering. In first year crops, seed yields of up to 500 kg ha^{-1} have been achieved (Sheppard and Wills, 1986). This is slightly lower than those yields of 540-830 kg ha^{-1} obtained from mature plants in Oregon, USA, (Sheppard and Wills, 1986), but it is in partial agreement with average yields in the USSR of 300-400 kg ha^{-1} (Medvedev, 1969). The highest seed yields reported for sheep's (small) burnet were obtained in seed production trials in south-western Colorado, USA (Fisher *et al.*, 1987). Production there varied with year and row width but ranged from 400-1300 kg ha^{-1}.

2.2.5 SEED GERMINATION AND EMERGENCE

Both embryos of sheep's burnet 'seed' usually germinate and emerge (Nordborg, 1967b) with one seedling normally dominating (Sheppard and Wills, 1986). Under glasshouse conditions the first seedling frequently emerges 1-4 days earlier than the second member of the pair (personal observation), but the consequences of this for subsequent establishment are unknown. Anastomosis of the two seedlings has been observed in Central Otago, New Zealand and it has been suggested that regardless of the development of the seedling pairs, the outcome after a few months is a single plant equivalent (B J Wills, pers. comm.). Anastomosis, in the sense of combining vascular tissues, has not been reported elsewhere while members of a pair have been observed as separate, though close, entities by the author.

In Spain, germination of sheep's burnet of 60-85% in 30 days has been obtained in laboratory tests, while in the field most estimates of emergence have been at least 50% (Salmeron, 1966). The laboratory tests involved unspecified conditions and most of the germinating seed had germinated 9-10 days after conducive environmental conditions were imposed (Salmeron, 1966). Estimates of germination obtained by Nordborg (1967b) for hypanthia ranged from 8-76% but were usually between 30 and 60%. The interval from sowing to germination was about 10 days to 4-5 weeks. Sheep's burnet seed produced in New Zealand has given germinations under laboratory conditions of frequently at least 60% and sometimes 100% (Begg, unpubl.; Foote and Douglas, unpubl.).

The viability of sheep's burnet seed decreases with the number of years after harvest (Nordborg, 1967b) and is highly dependent on the storage humidity and to a lesser extent, the temperature (Hafenrichter *et al.*, 1965). In European material, the oldest seeds which germinated were eight years old and had a germination of 3% (Nordborg, 1967b) while seeds stored under cool, dry conditions in the United States had germination of 50-60% even after storage for 10-14 years (Hafenrichter *et al.*, 1965).

2.2.6 ESTABLISHMENT

Establishment of sheep's burnet has been successful in several countries but it has been relatively slow or sometimes a complete failure on certain sites. One of the earliest series of experiments century involving sheep's burnet this century was that commenced by the New Zealand Department of Agriculture in 1910, when seed was sown in enclosed areas in the Mackenzie Plains and at Earnscleugh in Central Otago, South Island (Macpherson, 1910). The land was well grubbed and harrowed, and rubbish was removed before sowing in October and November, 1910. Plots were harrowed and rolled after sowing (Macpherson, 1910). In late November, no seedlings had developed from the October sowing. However, some sheep's burnet sown the previous March had a patchy distribution at one of the Mackenzie Plains sites and this was perhaps due to the favourable intervening winter (Macpherson, 1910). By July/August, 1911 sheep's burnet at the Mackenzie Plains' sites had tolerated frosts satisfactorily and was generally "doing well". Spring sowings of the material were superior. However, at the Earnscleugh site the spring 1910 sowing was good but an April 1911 sowing was better (Macpherson, 1911).

A decade later Cockayne (1920a, b) reported on the Earnscleugh trials of Macpherson and found that sheep's burnet was abundant in isolated areas in the sown ground as well as in its original rows. The ability of the plant to re-establish itself under the harsh local conditions was shown by the establishment of some plants downhill from the original rows (Cockayne, 1920b). Cockayne later included sheep's burnet in fenced trials at Northburn Station on the western side of the Dunstan Range near Cromwell in 1920-21 (Cockayne, 1922a, b). Sheep's burnet seed with a germination

of 60% was sown at 2.7 kg ha^{-1} in March 1921. By May, 1922 most seed had germinated fairly freely, but plants were not established at the time of inspection (Cockayne, 1922b).

Periodic reports on the Northburn trials have continued until the present. Tennent (1935) noted excellent establishment of sheep's burnet on some parts of the sunny mid-altitude (600 m) faces but less satisfactory growth at lower altitudes (350-450 m). On densely grassed shady faces, sheep's burnet was generally poor or absent. Early in 1950, Lunn (1951) highlighted the growth and spread of the plant on sunny aspects and Douglas (1970) reported an increase in several palatable species, including sheep's burnet, mainly by reseeding. Plants also occasionally established short distances outside the fence but they were often overgrazed severely (Douglas, 1970). Recent observations have found well-established plants of sheep's burnet up to 10 m outside the fences of several plots and also in some other plots (Sheppard and Wills, 1985; Wills and Begg, 1986).

In another early New Zealand investigation, sheep's burnet was one of numerous species sown at several unfenced sites on Haldon Station in the Mackenzie district (Ward, 1923). Three aspects were sown in spring 1921 and 1922, and autumn 1922, namely: a) flat ground exposed to wind; b) sunny faces; and 3) shady faces. Sowings on the flats germinated but dried off while those on sunny faces were also very poor, with only weak strikes at best. Results were most satisfactory on the shady faces, however, and several species, including sheep's burnet, were placed second after Yorkshire fog, cocksfoot, ryegrass and yarrow as the most encouraging species. McGillivray (1929) later reported on these trials together with those initiated by Macpherson (1910) and noted that sheep's burnet was growing well, which indicates that its establishment was eventually satisfactory.

In addition to the most widely documented trials just mentioned, sheep's burnet has been evaluated elsewhere. At the Pisa experimental area in Central Otago, which is situated on bare exposed flats, sheep's burnet was sown at 2.4 kg ha^{-1} in a mixture with several other species (Lunn, 1951). The seed was sown by hand into cultivated and harrowed land in April, 1938 and then the site was chain harrowed and rolled.

The first summer after sowing was hot and dry but by early 1950, the growth and spread of sheep's burnet, particularly in stock excluded areas, was one of the outstanding features of the trial (Lunn, 1951). In another nearby trial, seed of sheep's burnet was hand broadcast on to areas of slightly better, fairly steep hill country, including southerly faces in March, 1949 (Lunn, 1951). Unfortunately, no comments on subsequent establishment were presented and it is suspected that establishment was a failure (J S Sheppard, pers. comm.).

At Hollbrook Station in the Mackenzie Basin, which receives approximately 450 mm rainfall annually, Sievwright (1956) sowed sheep's burnet in combination with several other species in 1948. When the plots were examined approximately 12 months later, sheep's burnet was not included in notes on the most outstanding features. Sievwright (1956) concluded that the plant established in bare, wind-blown areas, but made slight growth. At Craigieburn Station in the Castle Hill Basin, sheep's burnet was slow to establish but showed "promise" when spring sown on unfertilised land and where intercultivation between rows was practised until establishment (Sewell, 1952).

Preliminary evaluation of accessions of sheep's burnet for erosion control purposes commenced in 1973 in the South Island and there were very promising results at Bendigo, near Cromwell, and at Black Forest Station, in the Mackenzie Basin. Plants were vigorous and seeded freely (Sheppard, unpubl.; Sheppard and Wills, 1985) and at Black Forest Station some plants had established up to several metres from the parent plants (Sheppard and Wills, 1985), and similar re-establishment was also noted in some studies reviewed previously. These encouraging results prompted more extensive trials in the South Island and in 1978 three sites in the Mackenzie Basin and one site on the Wither Hills, Marlborough were each planted with 47 accessions, mostly of sheep's burnet (Sheppard and Wills, 1985). All sites were fenced and approximately three month old glasshouse prepared seedlings were used. Assessments were made for growth, survival and vigour and the top ten accessions using these criteria were all sheep's burnet (Sheppard and Wills, 1985).

At one site in the Mackenzie Basin, some accessions of sheep's burnet which were planted in May suffered heavy losses due to frost damage (Sheppard, unpubl.). This was due to plants being lifted completely from the ground or being partially heaved out only to die during summer. Establishment of material planted in August on the Wither Hills was very satisfactory while in the Mackenzie Basin it was poor. Competition from resident vegetation in the spring and early summer may have hindered plant establishment at the latter site (Sheppard, unpubl.; Sheppard and Wills, 1985).

In a recent trial at Tara Hills in the Mackenzie Basin, sheep's burnet (26 kg ha^{-1}) in combination with lucerne cv. 'Wairau' (11 kg ha^{-1}) established rapidly in the first season (Daly, 1984). However, an extremely dry summer during the second season resulted in higher than average plant mortality which was unexpected in view of the plant's recognised drought tolerance. Wills *et al.* (1987) reported on a trial at Hakataramea in Central Otago in which sheep's burnet established slowly when overdrilled at 10 kg ha^{-1} into a paddock of 18 year old 'Wairau' lucerne. Again, plant numbers of sheep's burnet were noticeably low due to drought and probably competition from established lucerne (B J Wills, pers. comm.). At Marlborough in the northern part of the South Island, sheep's burnet (70% germination) was sown in autumn, 1985 at 7.2 kg ha^{-1} (Sheppard, unpubl.). After one year, plant density averaged 28 plants m^{-2} and ranged from 14-52 plants m^{-2}.

Arising from early studies and more recent research, recommendations on establishment methods for sheep's burnet, including suitable sowing times and seeding rates, are now documented widely (NWASCO, 1982; Wills, 1983, 1984; Sheppard and Wills, 1985, 1986; NWASCA, 1986; Rys *et al.*, 1989). Seed can be sown in autumn or spring depending mainly on the growth limitations likely to be imposed soon after sowing by frosts or soil moisture deficits. Seed sown aerially should be applied at 10-20 kg ha^{-1} whereas rates for drilled seed can be lower (Sheppard and Wills, 1985; B J Wills, pers. comm.). An advantage of sheep's burnet highlighted recently is its ability to persist in mouse-ear hawkweed colonies without needing large fertiliser applications (NWASCO, 1982; NWASCA, 1986). Successful establishment of sheep's burnet in these colonies usually requires mechanical

scarification of the hawkweed plants or their complete destruction, and then sowing seed at a shallow depth by mechanical or stock-treading methods.

2.2.6.1 RESEARCH OVERSEAS

In early South Australian investigations, Scott (1932) conducted trials involving sheep's burnet at several sites. On slightly rocky, red loam soils burnet was one of thirteen species sown but it was not listed as being satisfactory. When sheep's burnet was sown with ryegrass, subterranean clover and chicory at a rate of 3.4 kg ha^{-1}, it established and provided useful forage after rain. On fertilised sandy soils overlying a good clay subsoil, sheep's burnet was sown at an unknown rate and established fairly well, but it did not produce as well as other species in the experiment. A trial conducted by McTaggart (1935) in the Canberra area also included sheep's burnet in various spring sown pasture mixes, at a rate of 1.1-2.2 kg ha^{-1}. Approximately thirteen months later, sheep's burnet had established satisfactorily and persisted, as shown by its moderately high average percentage ground cover immediately before three defoliation treatments were imposed. Responsiveness to increased rainfall was noted as an outstanding characteristic of the plant. In a sub-alpine area of Pakistan (elevation 2900 m) on a moderate slope of easterly aspect, autumn sown *Poterium sanguisorba* established equally as well as several grass and legume species (Noor, 1978), with an average of 32 plants m^{-2} five months later. However, ground cover of 20% was significantly less than that provided by red clover (57%) and vetch (37%). Iranian trials have also shown the ability of sheep's burnet to establish and persist on a diversity of depleted rangeland sites (Nemati, 1978; Campbell, 1979). For example, on four badly eroded (80-95% bare soil) areas in the Zagros Mountains of Iran, Campbell (1979) oversowed sheep's burnet and seven other species at two times, namely November 1975 when the soil was dry and nearly all resident vegetation was dead, and in February 1976 into the snow. Satisfactory establishment of all sown species from both sowings was achieved and averaged 32 plants m^{-2} after at least four months. Establishment was exceptionally good where there was no competition from resident species. Survival of sheep's burnet was noted 16-19 months after sowing on some sites of the more

favourable areas. Experience with sheep's burnet in Tunisia has found the seedlings to be aggressive, which facilitates establishment (Le Houerou, 1979).

Dryland trials conducted in south-western Colorado, USA, found that sheep's burnet was one of several species which established very well from a spring (April) sowing (Fisher *et al.*, 1987). Six weeks after sowing, assessments were made and burnet had approximately 75-100% of complete stand (regarded as 12 plants per m row). The species produced more seed than any other species (Fisher *et al.*, 1987), which indicated potential advantages for re-establishment.

2.2.7 RESPONSES TO DEFOLIATION

There is little detailed information on the regrowth responses of grazed or mown swards of sheep's burnet and other *S. minor* subspecies, particularly for establishing swards. Most reports are based on simple, brief observations and in only a few instances has there been any useful quantification of important variables such as defoliation intensity.

Early New Zealand trials involving sheep's burnet showed that the plant could tolerate infrequent defoliations of various intensities. Ten to fifteen month old plants in Central Otago in the South Island were strong and healthy despite being cut 2-3 times, and had seeded (Macpherson, 1912). Cockayne (1920a) reported that although sheep completely removed all leaf from established plants at Earnscleugh, subsequent regrowth was satisfactory. Exclusion of sheep from this trial site for approximately seven months resulted in a greatly increased amount of sheep's burnet. Plants were often 12.5-15.0 cm high, and leaves were from 7.5-12.5 cm long (Cockayne, 1921). Some plants were flowering and there were abundant seedlings. Virtually all regrowth occurred in September and October when there was only about 2.5 cm rainfall and examination of the detailed weather data presented by Cockayne (1921) showed that the abundant vegetative and reproductive growth of the established plants occurred under increasingly warm but very dry conditions.

Plants of sheep's burnet, which were less than 17 months old, were moderately tolerant of heavy stocking with sheep (and rabbits) at Haldon Station in the Mackenzie district, as shown by their intermediate ranking on a plant number basis (Ward, 1923). McGillivray (1929) reported on trials initiated by Macpherson (1910) and Ward (1923) at Haldon and noted that sheep's burnet was growing well despite occasional heavy grazing pressure. Rabbits were undoubtedly a problem in these early investigations but the extent of their influence on the results was uncertain.

The Northburn experiments (Cockayne, 1922a, b) provided some indication of the grazing tolerance of mature sheep's burnet. Stock were excluded from the plots for the first ten years, but from 1930-1956 the plots were opened to grazing, mainly during winter (Lunn, 1951; Douglas, 1970). Thereafter, stock were excluded again but there was some access to the plots by browsing animals due to deteriorating fencing (Douglas, 1970). After two and a half years' grazing (June, 1930 to December, 1932), Tennent (1935) noted that cocksfoot, Chewing's fescue, lucerne and yarrow had tolerated hard grazing particularly well, but there was no mention of sheep's burnet. However, in November 1946, sheep's burnet was one of six species regrowing fairly well, indicating that the plant had survived harder earlier grazing (Lunn, 1951). Lucerne and cocksfoot were less abundant. Sheep's burnet was again one of the prominent species four years later (Lunn, 1951). Douglas (1970) reported that following rabbit control and enclosure of the plots again, palatable species such as sheep's burnet, cocksfoot and lucerne had increased, mainly by reseeding. Sheep's burnet and cocksfoot were even found outside some plots, despite their severe overgrazing. Gow (cited by Stewart, 1979) noted that the recovery and regrowth of sheep's burnet in the plots after grazing by sheep was extremely poor but no further information was given. He would not recommend the plant as a grazing proposition. With continued exclusion of stock from the plots, a 1984/'85 assessment (Wills and Begg, 1986) found that sheep's burnet along with lucerne, cocksfoot, tall fescue and Chewing's fescue were the dominant species on sunny aspect plots. Sheep's burnet was less prominent on dark aspects. The results substantiated the benefits of allowing moderately long intervals between successive grazings to maintain satisfactory growth and persistence of sheep's burnet.

The ability of sheep's burnet to persist under intermittent unrestricted heavy grazing was also shown at other sites in the South Island of New Zealand. An accession evaluation at one Central Otago site in the 1970's was disrupted badly by the appearance of volunteer sheep's burnet plants following the exclusion of rabbits (J S Sheppard, pers. comm.). The site had been chosen in consultation with the runholder because of its apparent freedom from old trial sheep's burnet material. The old plants which appeared had well developed crowns at or just below the soil surface and it was estimated that these plants, or their seedlings, had persisted for 40-50 years (J S Sheppard, pers. comm.). On the Wither Hills in the northern part of the South Island, a small area of sheep's burnet has been grazed intensely during the winter and spelled during spring/summer. This management has been conducted annually for about ten years and the sheep's burnet is spreading (J S Sheppard, pers. comm.), despite periodic heavy grazing.

In early Australian research, three defoliation treatments were imposed on thirteen month old mixed swards in spring, 1932 (McTaggart, 1935). Three swards included sheep's burnet and the treatments were:

a) close mowing every six weeks to simulate frequent grazing;
b) close mowing every eight weeeks to simulate moderately-frequent grazing; and
c) close grazing by sheep when warranted.

Although the percentage ground cover of sheep's burnet under each treatment fluctuated considerably with rainfall, plants showed very satisfactory persistence. The results were affected markedly by grazing, with the sheep closely defoliating the palatable plants, while in the mown treatments the rosette-type plants were missed frequently by the mower knife. Details on the intensity of defoliation were omitted, which detracted from some of the value of the study.

The ability of sheep's burnet to persist under moderate to intensive grazing, and to recover satisfactorily when stock are excluded, was also shown in a study conducted in Oregon, USA (Valassis *et al.*, 1957). After ten years of variable grazing intensity, sheep's burnet was one of several species which had shown at least limited

persistence, and following two seasons' protection from grazing, plants made "remarkable recovery" (Valassis *et al.*, 1957). Under arid, high-country conditions in North Otago (Anon, 1957), sheep's burnet plants of unknown age yielded satisfactorily when mown during spring and summer.

The first known measurement of regrowth herbage mass of sheep's burnet over several harvests within a year was reported in Spain (Salmeron, 1966). Between 28 April 1965 and 25/30 April 1966, six harvests of herbage to an approximately constant 5 cm cutting height were made and accumulation was expressed on a fresh weight basis. However, on the assumption that herbage at all harvests had a dry matter content of about 22% (Section 2.2.4.1), the following findings are presented. No growth occurred in winter (June/August equivalent) while maximum herbage accumulation of 74 kg DM ha^{-1} d^{-1} occurred in late spring (November equivalent). At other times of the year, herbage accumulation ranged from 20-35 kg DM ha^{-1} d^{-1} and intervals between harvests varied from approximately five weeks in late spring/early summer to twelve weeks in autumn and early spring (Salmeron, 1966). Except during winter, when there was no growth, plants at harvest were 16-30 cm high and 20-26 cm wide.

Under very dry conditions, sheep's burnet plants become dormant after seed development and this was observed in spaced-plant evaluations of several *S. minor* subspecies on the Wither Hills in Marlborough in the South Island of New Zealand (Sheppard and Wills, 1985). Regrowth commenced with the onset of late autumn rains and developed from the top of the tap root (J S Sheppard, pers. comm.), as it does following grazing (Sheppard and Wills, 1985, 1986).

Recent New Zealand cutting trials have provided regrowth estimates for sheep's burnet. Under Manawatu nursery conditions in the lower North Island, approximately two-year old swards were cut five times per year for two years and each harvest left a 4-5 cm high stubble. Average herbage growth rate ranged from 13 kg DM ha^{-1} d^{-1} in autumn/winter to 100 kg DM ha^{-1} d^{-1} in late spring (Foote, unpubl.). Regrowth rates in a preliminary study in Central Otago, South Island (Wills, unpubl.) were much lower and varied from 2.5 kg DM ha^{-1} d^{-1} in winter to

5 kg DM ha^{-1} d^{-1} in late spring. In Hawke's Bay, North Island (Rys *et al.*, 1989), young sheep's burnet plants originating from an autumn sowing were first defoliated to leave a 3 cm high stubble at approximately five months after sowing. Cuttings were continued over two harvesting periods at frequencies ranging from 4-16 weeks. The two periods were 14/8/1986 to 30/4/1987 and 1/5/1987 to 15/5/1988 and there were no significant differences between defoliation frequencies. Total herbage mass (and herbage accumulation calculated by the author) averaged 14,700 kg DM ha^{-1} (57 kg DM ha^{-1} d^{-1}) and 9,200 kg DM ha^{-1} (24 kg DM ha^{-1} d^{-1}) over the two periods, respectively.

It is recommended currently that establishing swards of sheep's burnet, up to 18 months old in harsh semi-arid environments, should not be grazed or should not be given more than a short, light grazing (Sheppard and Wills, 1985, 1986; de Lacy, 1985; NWASCA, 1986). Thereafter, short periods of set stocking and relatively long intervals between grazings are suggested. Controlled rotational grazing is recommended for drought prone areas (Sheppard and Wills, 1986).

2.3 ESTABLISHMENT AND DEFOLIATION RESPONSES OF OTHER PLANT SPECIES

2.3.1 ESTABLISHMENT

Establishment is the most critical stage of a pasture's life (Culleton and McCarthy, 1983) since the result largely determines subsequent performance (Sears, 1961). In lowland pastures, cultivation followed by sowing is the most reliable and efficient method of establishment (Sears, 1961; Baker, 1970; Charlton and Thom, 1984), while in unploughable hill country, oversowing is the main method for the introduction of improved species (Suckling, 1966; Charlton, 1977; Charlton and Thom, 1984).

The subject of establishment may be divided conveniently into two broad developmental stages, namely 1) germination, and 2) emergence and early vegetative growth. The second stage is that examined usually in most agronomic research on establishment. Numerous studies on one or both of these aspects have been

In moist soil, the predominant environmental factor influencing the germination of seeds is temperature and for most species, the prevailing temperature determines both the proportion of seeds in a sample which germinate as well as their germination rate, and hence the duration of germination (Woods and MacDonald, 1971; McElgunn, 1973; Culleton and McCarthy, 1983; Hur and Nelson, 1985; Lafond and Baker, 1986a, b; Charlton *et al.*, 1986; Hampton *et al.*, 1987; Charlton, 1989; Jordan and Haferkamp, 1989). Germination percentage usually remains constant over a wide range of temperatures, occasionally 20°C or more, and decreases markedly on either side of this range (Thompson, 1970; Cooper, 1977).

Responses of a range of New Zealand herbage grasses and legumes to several constant, and one alternating, temperature regimes in the laboratory were investigated by Charlton *et al.* (1986) and Hampton *et al.* (1987), respectively. The grasses were examined at constant temperatures ranging from 5-30°C in five degree increments while the legumes were examined at the same temperatures with the exception of 25 and 30°C, which were excluded. A 5/10°C alternating temperature regime was common to both studies (Charlton *et al.*, 1986; Hampton *et al.*, 1987). Final percentage germination of ryegrass, white clover and lucerne were approximately constant over the respective ranges of temperature studied. Germination of 'Woogenellup' subterranean clover, 'Kahu' timothy and 'Maru' phalaris were reduced at 10°C or below while 'Raki' paspalum failed to germinate below 15°C. Higher temperatures also reduced the germination of some species such as 'Apanui' and 'Wana' cocksfoot and 'Matua' prairie grass at 30°C (Charlton *et al.*, 1986) and 'Mt Barker' subterranean clover at 20°C (Hampton *et al.*, 1987). As temperatures deviated more from the optimum, germination rates for all species declined, and the time to the commencement of germination increased (Charlton *et al.*, 1986; Hampton *et al.*, 1987). Similar studies over a range of temperatures have not been conducted for sheep's burnet or other *S. minor* subspecies.

In Ireland, two grasses (perennial ryegrass and prairie grass) and one legume (red clover) were evaluated for percentage and rate of germination at temperatures of 4, 8, 12, 16, 25 and 30°C (Culleton and McCarthy, 1983). Above 4°C, all species had greater than 80% germination after 50 days but after the same time at 4°C, the two

conducted and an indication of the vast knowledge accumulating in these areas is evidenced particularly by several recent reviews published in the "Advances in research and technology of seeds" series (Perry, 1976, 1980; Lovato, 1981; Kahre, 1983; Powell, 1988; Slaughter, 1988), and elsewhere (Black, 1959; Cooper, 1977).

2.3.1.1 GERMINATION

Germination is defined as "that consecutive number of steps which cause a quiescent seed, with a low water content, to show a rise in its general metabolic activity and to initiate the formation of a seedling from the embryo" (Mayer and Poljakoff-Mayber, 1982). Germination has also referred to "the appearance of the first aerial organ above the soil surface" but Black (1959) advocated that this should only be a definition for emergence, and this terminology is adopted presently. Favourable environmental conditions must exist for satisfactory germination (Mayer and Poljakoff-Mayber, 1982; Lovato, 1981) and generally the two main factors governing the event are moisture availability and temperature.

2.3.1.1.1 SOIL MOISTURE

The first step in germination is the uptake of moisture by the seed, termed imbibition (Lovato, 1981), with consequent swelling of the entire seed. In rape seed, Shaykewich (1973) found a linear relationship between water absorbed and seed volume, but for wheat and maize the increase in volume was more than the quantity of water absorbed. Imbibition of water in legume seed is much quicker than that of grasses and most of the water required for germination is imbibed during the first 4-8 hours (McWilliam *et al.*, 1970). Minimum seed moisture contents required for germination vary depending on species and examples are 30.5% (maize), 26.5% (rice), 50% (soybean) and 31% in sugar beet (Hunter and Erickson, 1952). Inadequate moisture in the seed bed is probably the main reason for failure of seeds to germinate (Lovato, 1981).

2.3.1.1.2 SOIL TEMPERATURE

grasses had less than 70% germination, while red clover still had greater than 80% germination. Germination rates for all species declined with lowering temperatures. McElgunn (1973) in Canada germinated lucerne (three cultivars), birdsfoot trefoil cv. 'Leo', sainfoin cv. 'Melrose' and sweet clover (two cultivars) at fixed temperatures of 7, 10, 13 and 21°C and 12-hour alternating temperatures of 2/13°C, 4/15°C, 7/18°C and 16/27°C. Total germination was similar under all temperature conditions except the 2/13°C regime, while over all temperatures, average germination rate was in the order sweet clover>lucerne and birdsfoot trefoil>sainfoin. McElgunn (1973) concluded that cold alternating temperatures reduced both germination rate and total germination while cold constant temperature reduced the germination rate but did not influence total germination. Birdsfoot trefoil germination rate was shown by Qualls and Cooper (1968) and Hur and Nelson (1985) to generally increase over the temperature ranges of 15.6-26.7°C and 6-30°C, respectively. In the later study, time to radicle emergence of the species decreased as temperature increased (Hur and Nelson, 1985).

Several studies have investigated the effects of both temperature and moisture level on germination, and two studies are reviewed presently. Woods and MacDonald (1971) used six constant temperatures of 10-35°C in five degree increments, and deionized water and various mannitol solutions of 0.25-0.8 MPa osmotic potential, to study germination of birdsfoot trefoil. Temperatures lower than 15°C delayed germination while at temperatures of 30°C or higher, germination was delayed or reduced. When averaged over all temperatures, there was no detectable effect of osmotic moisture stress on germination from stresses of -0.05 MPa or less. However, there was an almost linear reduction in germination with increasing stress in the range -0.1 to -0.8 MPa and the authors suggested that germination would cease at moisture stresses of -1.4 MPa or greater. Both factors interacted and delayed and reduced germination at high temperatures or osmotic moisture stresses. In a later study, nine spring wheat cultivars were germinated in distilled water at 5, 8, 12, 20, and 30°C and in polyethylene glycol solutions with osmotic potentials of 0.0, -0.4 and -0.8 MPa at 10 and 20°C (Lafond and Baker, 1986b). Final germination for all factor combinations exceeded 90% and rate of germination (reciprocal of median

germination time (t_{50})) was related linearly to temperature. Increasing osmotic moisture stress resulted in increases in t_{50} at 10 and 20°C.

2.3.1.1.3 SEED SIZE

The effect of seed size on germination was also examined in the two studies mentioned previously. Three seed sizes of wheat (Lafond and Baker, 1986b) ranging from small (2.8-3.2 mm) to large (3.6-3.9 mm) were investigated and in all instances small seeds germinated faster than large seeds. For example, the average median germination times (hours) for the three seed sizes of a 1981 seed lot over all temperatures, were 64.3 h (small), 66.6 h (medium) and 67.5 h (large). Final germination percentage was unaffected by seed size and there was also no difference in rate of water uptake (g kg^{-1} h^{-1}) by small and large seeds. Three seed sizes were also investigated in three cultivars of birdsfoot trefoil (Woods and MacDonald, 1971), but the findings were at variance with those found for wheat. While final germination of medium and large seeds was similar, small seeds had lower total germination which was partly attributable to their higher hard seed content. Apart from germination of small seeds being slightly less than larger seeds at 25°C or higher, temperature x seed size interaction was negligible. Small seeds also germinated more slowly than the larger seeds.

Working with 'Maku' lotus, Charlton (1989) found that large seed (0.78 g TSW (thousand seed weight)) had a significantly higher germination rate at 10°C than small seed (0.63 g TSW). There were also differences in final germination at three weeks, with large seeds having approximately 100% germination while small seeds achieved 70%. It was suggested that germination rate of 'Maku' lotus at low temperatures could be improved by selection for larger seeded material. Improved germination percentage and/or rate from using large seeds have also been found for tobacco (Kasperbauer and Sutton, 1977), sorghum (Maranville and Clegg, 1977) and several vegetable crops (Clarke, 1985). It was reviewed earlier that seed of sheep's burnet is highly variable in size/weight (Section 2.2.3). Such material may also vary correspondingly in germination characteristics and hence ultimate field performance.

Variability in seed size is inevitable as it depends on environmental conditions of mother plants (Bean, 1973). Factors which may influence seed size include nutrition of the mother plant, location on the inflorescence and stage of maturity at harvest (Perry, 1976). An important property of the seed is the proportion of seed which is embryo versus that which is endosperm (Perry, 1976), and how these proportions change with seed size. Both the amount of reserve material and the amount of meristematic tissue (embryo) generally increase linearly with seed size (Bremner *et al.*, 1963). At least in wheat (Bremner *et al.*, 1963), and probably several pasture grasses (Sangakkara *et al.*, 1985), the quantity of reserve material is more important than the size of the embryo in conferring any effects of seed size.

2.3.1.1.4 RESERVE POLYSACCHARIDES IN SEEDS

The main solid state reserve polysaccharide in seeds is starch and on a dry weight basis it may constitute 60-90% of the cereal seed and 30-40% of the legume seed (Mercier, 1985). Non-starchy polysaccharides which may occur in seeds, also referred to as cell wall storage polysaccharides (Meier and Reid, 1982), are those belonging to the mannan groups (including pure mannan, glucomannan and galactomannan), the xyloglucans and the galactans (Mercier, 1985). Only seeds of three families, namely Fabaceae (Leguminosae), Arecaceae (Palmae) and Liliaceae, were noted by Mercier (1985) as containing any of these polysaccharides. Mobilization of storage carbohydrates and other polymeric reserves in seeds have been reviewed by Davies and Slack (1981), Halmer (1985) and Slaughter (1988). The main reserve polysaccharide in seed of sheep's burnet is probably starch.

2.3.1.1.5 TIME OF SOWING

It should be apparent from previous sections (for example 2.3.1.1.2) that the most appropriate time for sowing is determined largely by the soil temperature and moisture requirements for satisfactory germination (and emergence) of the species to be sown. An equally important issue in harsh sites such as hill country and semi-arid lands is the environmental conditions likely to be encountered by the swards within the first few months of establishment. The two main concerns here are frosts

following autumn sowings and soil moisture deficits following sowings in the spring (Lancashire, 1984; Charlton and Thom, 1984; Lambert *et al.*, 1985; Scott *et al.*, 1985; Wills, 1986).

Most farmers sow pasture seeds in the autumn irrespective of the region (Sangakkara *et al.*, 1982) and under conditions in the South Island of New Zealand, White (1973) recommended that autumn sowings of pasture species should be completed by mid-March to ensure good establishment and growth before winter frosts begin. In the absence of irrigation, sowings on arable land in the summer-dry east coast, are restricted usually to autumn (Hume and Fraser, 1985). For summer-dry hill country, autumn oversowing is recommended and this should be conducted early where winters are severe (Lambert *et al.*, 1985). Oversowing summer-wet hill country is usually more successful in the spring than in autumn, particularly where winters are cold (Lambert *et al.*, 1985). Conditions for the germination of seeds and subsequent seedling establishment on the soil surface are considerably more severe than those experienced by buried seed (McWilliam and Dowling, 1970) and the usually poor establishment from oversowing (Charlton and Thom, 1984) is probably due largely to these factors.

2.3.1.2 EMERGENCE AND EARLY VEGETATIVE GROWTH

Following successful seed germination, seedlings emerge and become autotrophic, and foliar and root growth and differentiation continue. Several factors which may influence one or more stages of this sequence are temperature, moisture, sowing depth and seed size. Continued development of seedlings ultimately results in competition for scarce resources such as light and soil nutrients. This has important implications for sward production and botanical composition.

2.3.1.2.1 TEMPERATURE

The optimum temperature(s) for early seedling growth may be slightly different from that for germination. In sainfoin, Carleton *et al.* (1968) found that while seed germination was best at 15-23°C, seedling length during the first eight days of

germination increased most at slightly higher temperatures of 20-30°C. In newly germinated seeds, temperature may have a marked effect on the rate of transfer of stored reserves to the embryo axis (Derwyn *et al.*, 1966) and these reserves contribute substantially to seedling growth (Qualls and Cooper, 1968; Curtis and McKersie, 1984) and hence establishment.

The temperature optima for seedling emergence and growth vary with species (Namken *et al.*, 1974), and seedling growth often has a higher temperature optimum than that of older plants (Friend *et al.*, 1962). In reviewing the literature, McWilliam (1978) found that the optimum temperature for both dry matter accumulation and extension growth of numerous legumes and Festucoid grasses, was 20-25°C. Approximate minimum and maximum temperatures for these processes were 5 and 30-35°C, respectively. Some of these findings were supported by a study on seedling tall fescue which was grown at five temperatures (Robson, 1972) in five degree increments from 10 to 30°C. Leaf area ratio, involving leaf weight ratio and specific leaf area, increased with temperature and leaves at 25°C were twice as long and had twice the area of those at 10°C. This was due to the much higher growth rate at 25°C and the higher temperature was also close to the optimum for most aspects of leaf growth (Robson, 1972).

In a study of forage legumes, Smoliak *et al.* (1972) germinated and grew lucerne, cicer milkvetch, and sainfoin at soil temperatures of 7, 13, and 27°C for 28 days. At 7°C, lucerne and sainfoin emerged and grew but cicer milkvetch failed to emerge. However, the latter species emerged and grew slowly at 18°C. Best establishment of lucerne and cicer milkvetch occurred at 27°C while sainfoin grew equally well at 18 and 27°C. Results indicated that while warmer temperatures were suitable for establishment of cicer milkvetch, lucerne and particularly sainfoin were more adaptable to a wider range of soil temperatures.

Temperature also influences the rate of leaf extension (Williams and Biddiscombe, 1965). For example, in perennial ryegrass in the spring, Baker and Younger (1987) estimated an average increase in leaf extension rate of 0.4 mm d^{-1} for each 1°C increase in temperature over the range investigated. However, the relationship can

vary with season and this was observed for tall fescue (Wilhelm and Nelson, 1978) where leaf extension rates in the field averaged 8.54 and 4.15 mm d^{-1} during autumn and summer, respectively. It was suggested that the relatively low extension rates in the summer were due to high temperature and lowered plant water status (Wilhelm and Nelson, 1978). Studies on the effects of temperature on the growth of sheep's burnet are unknown.

2.3.1.2.2 MOISTURE

Seeds often germinate, but the seedlings may not survive because insufficient soil moisture has limited the development of a root system capable of supporting the plant through later periods of less favourable moisture conditions. For example, death of seedlings of blue grama on dry rangelands in the USA is attributed to poor root development and unsatisfactory extension into moist soil (Wilson and Briske, 1979). Distribution and quantity of water are also important determinants of seedling emergence and survival, and weeping lovegrass and kleingrass required two days of simulated rainfall to emerge at 24 and 30°C (Wester *et al.*, 1986). Emergence failed at 38°C, presumably because soil water evaporated before seeds could imbibe. Earliest emerged seedlings generally survived longer than seedlings which emerged later.

The emergence of aerially sown ryegrass seed in dry soils in eastern Australia was improved by the establishment of a temporary water table (Cornish, 1983). The water table maintained a surface water potential of more than -0.04 MPa for seven days resulting in an establishment of 12%. However, in the absence of a water table, emergence failed when the water potential of topsoil dropped below -0.04 MPa within one day, even if the soil had been wet initially to field capacity. On sand country trials in the Manawatu in the lower North Island of New Zealand, the numbers of lucerne seedlings from early and late autumn and spring sowings ranged from 130,000 to 260,000 ha^{-1} (Smith and Stiefel, 1978). This was interpreted as an indication of the dominant influence of soil moisture on germination and initial establishment.

Since the major review by Hsiao (1973) on plant responses to moisture stress, there have been numerous similar reviews (Boyer and McPherson, 1975; Turner and Begg, 1978; Ritchie, 1981; Hanson and Hitz, 1982; Tyree and Jarvis, 1982; Krieg, 1983; Morgan, 1984; Barker and Chu, 1985; Aspinall, 1986; Barlow, 1986; Kriedemann, 1986; McCree, 1986; Schulze, 1986; Turner, 1986). Literature reviewed by Turner and Begg (1978) suggested that morphological responses such as leaf area development, tillering and root growth were more sensitive to moisture deficits than physiological processes such as stomatal behaviour, photosynthesis, respiration and assimilate distribution. There is continuing support for this view from the few recent studies where both morphological and physiological attributes have been measured (Legg *et al.*, 1979; Brown and Tanner, 1983; McCree *et al.*, 1984; Cruz *et al.*, 1986). In a study on lucerne (Brown and Tanner, 1983), leaf and stem expansion, and stomatal responses, were related to various leaf water potentials. Shoot growth was negligible at approximately -1.0 MPa while stomatal conductance, transpiration, and probably photosynthesis remained high until leaf water potential was about -1.5 MPa, and then decreased steadily (Brown and Tanner, 1983).

The sensitivity of leaf expansion (Dale, 1988) and other morphological traits to moisture deficits has important practical implications, particularly for forage plants where the foliage constitutes most of the economic yield. Both the intensity and duration of moisture deficits are important determinants of losses in herbage mass (Barker and Chu, 1985). Recent estimates of the reduction in leaf extension of forage species due to moisture deficits are rare, there being apparently more interest in investigating other plant attributes in the presence of moisture deficits (Ludlow *et al.*, 1985; Sambo and Aston, 1985). In the mid-1970's, Ludlow and Ng (1976) reported an 80% reduction in leaf extension rate of green panic when the leaf water potential dropped from -0.4 to -0.7 MPa, and elongation ceased at -1.0 MPa. Later studies on prairie grass indicated that the leaf extension rate of stressed plants declined rapidly when the reductions in leaf water potential became apparent (Chu and McPherson, 1977). Data presented indicated that leaf extension rate was approximately 30 mm d^{-1} under well watered conditions and declined to about 5 mm d^{-1} after a 9-10 day drying treatment. The corresponding change in leaf water potential was from -0.5 MPa (moist soil) to -1.5 MPa. Following rewatering, plants

exhibited some compensatory growth with leaf extension rates exceeding initial "pre-drought" rates for several days (Kerr and McPherson, 1978; Chu *et al.*, 1979).

There is increasing evidence of osmotic adjustment (Tyree and Jarvis, 1982; Morgan, 1984; Barlow, 1986; Turner, 1986) in a range of species including barley (Blum, 1989), cotton (Ackerson, 1985), maize (Westgate and Boyer, 1985), sorghum (Turner *et al.*, 1978), sunflower (Turner *et al.*, 1978; Conroy *et al.*, 1988) and wheat (Johnson *et al.*, 1984). Two recent studies have indicated that osmotic adjustment also occurs in the temperate pasture species phalaris (Sambo and Aston, 1985), in the tropical grasses green panic, buffel grass and spear grass (Ludlow *et al.*, 1985), and to a slight extent in the legume Siratro (Ludlow *et al.*, 1985). Adjustment results in the maintenance of turgor pressure to a lower water potential than possible in unadjusted plants and therefore potentially benefits turgor dependent processes such as cell elongation, and hence leaf expansion, and daytime stomatal opening. The occurrence and extent of osmotic adjustment depends upon three factors (Turner and Jones, 1980): a) the rate of drying; b) degree or level of water deficit; and c) species and genotype. Solutes which may contribute to osmotic adjustment include sugars, amino acids, organic acids, prolinebetaine and potassium (Turner and Begg, 1978; Aspinall, 1986).

Evapotranspiration (ET) from any well developed pasture or crop is controlled primarily by the weather (Penman, 1956; Kerr and McPherson, 1978), although in practice ET is influenced usually by soil and plant factors such as species, stage of growth, area of transpiring leaf and soil moisture availability (Ritchie and Burnett, 1971; Ritchie, 1974; Kerr and McPherson, 1978; Barker and Chu, 1985). Working with crops, Ritchie and Burnett (1971) found that ET increased with leaf area index (LAI) up to a value of about 3, and thereafter ET was unrelated to LAI. Hence, apart from choice of species and young swards, these findings suggested that reductions in leaf area by defoliation may offer some control of ET and consequent conservation of soil water. Results from simulated grazing studies on a tropical grass (Toft *et al.*, 1987) supported this view, as did some of the literature reviewed by Barker and Chu (1985).

2.3.1.2.3 SEED SIZE AND SOWING DEPTH

The effects of seed size on emergence rate and/or early plant growth have been studied for a range of forage species including birdsfoot trefoil (Carleton and Cooper, 1972; McKersie *et al.*, 1981; Curtis and McKersie, 1984), cicer milkvetch (Townsend and Wilson, 1981), crested wheatgrass (Rogler, 1954), lucerne (Beveridge and Wilsie, 1959; Carleton and Cooper, 1972), red clover (Evans, 1973), ryegrass (Arnott, 1969; Evans, 1973; Hayes, 1975; Brown, 1977), sainfoin (Carleton and Cooper, 1972; Fransen and Cooper, 1976), subterranean clover (Black, 1956, 1957b, c; 1958), sweetclover (Haskins and Gorz, 1975), tall fescue (Hayes, 1975; Lewis and Garcia, 1979), white clover (Mytton, 1973), and yorkshire fog (Hayes, 1975). In most of these studies, seedlings from large seeds have emerged earlier and produced more vegetative growth than those seedlings arising from small seeds. A notable exception to this trend was emergence of lucerne (Beveridge and Wilsie, 1959) where days for maximum emergence were the same for the three seed sizes investigated. Greater plant weights per seedling, however, originated from larger seed.

Working with sainfoin seedlings from four seed sizes of ten genotypes, Fransen and Cooper (1976) found that seedlings from large seed emerged earlier and developed more rapidly than seedlings from small seed. In sweetclover, three seed sizes of two cultivars were investigated (Haskins and Gorz, 1975). Seedlings from spring sown medium and large seeds emerged significantly earlier than those from small seeds and had higher estimates of stand count, plant height and herbage mass of shoots and roots. Only stand count and herbage masses were estimated in an autumn sowing, but the same pattern occurred. Studies on perennial ryegrass (Lam and Ridout, 1985) showed that normal (2.10 g TSW) seeds had a higher percentage emergence than small (1.25 g TSW) seeds, but by eight weeks there was no difference in seedling mass (g/pot). An examination of two seed weight groups of the same species (Arnott, 1969) found that at similar sowing depths, heavy seeds produced heavier seedlings which developed more leaves and tillers than those from light seed. Red clover was one of several species investigated by Evans (1973) and medium- and high-weight seeds produced seedlings which had heavier shoot and root masses and

which had longer root lengths and quicker root elongation rates than seedlings from low-weight seeds.

The effects of seed size are confined largely to early establishment because of later interplant competition, and this results frequently in similar forage yields being achieved in first and later seasons, regardless of initial seed size (Black, 1959; Perry, 1980). However, the contribution which large seeds may make to improving establishment is highly desirable, particularly where the species is being sown for rapid ground cover/soil protection (FAO, 1965; van Kraayenoord, 1986).

Sowing depth also has an important influence on emergence and establishment and has been discussed in several reviews (Herriott, 1958; Black, 1959; Perry, 1976; Cooper, 1977). Interactions between sowing depth and seed size have been reported for emergence and other characters (Beveridge and Wilsie, 1959; Arnott, 1969; Haskins and Gorz, 1975). Average emergence of birdsfoot trefoil from sowing depths of 1.0, 2.5 and 3.8 cm was 53, 25 and 18%, respectively (Stickler and Wassom, 1963). In sweetclover, sowing depth had the greatest effect on stand count and it interacted with seed size (Haskins and Gorz, 1975). Stand counts from medium and large seeds were higher than those from small seeds, particularly at 3.8 and 5.7 mm sowing depths. Emergence from deeper sowings was also much slower than from shallow sowings. In ryegrass (Arnott, 1969), shoot emergence was dependent on seed weight and sowing depth. Most shoots of seedlings which developed from heavy seeds were able to emerge from greater depths than those from light seeds. An examination of seed sizes of lucerne and their sowing depths (Erickson, 1946) showed that the most favourable sowing depth for large seed (1.9 cm) was approximately three times deeper than the best depth for small seed (0.6 cm). For sheep's burnet it is recommended currently that seed be sown at a depth of approximately 1 cm (B J Wills, pers. comm.) but there is no information on how this should be adjusted for various seed sizes.

The practical implications of the above findings are numerous. Large seeds can be sown at greater depth than small seeds and this could have benefit where topsoils dry rapidly. However, deeper sowings may also result in weakened seedlings and

increase the opportunity for disease. Ultimately, a compromise must be made between deeper sowings into moister soil and those depths where emergence and subsequent vegetative growth are acceptable. Additional factors which may be important in emergence are seed orientation (Perry, 1976; Lovato, 1981) and mechanical impedance such as soil crusting (Perry, 1976; Clarke and Moore, 1986).

2.3.1.2.4 COMPETITION

Competition arises "when each of two or more organisms seeks the measure it requires of any particular factor and when the immediate supply of the particular factor is below the combined demand of the organism" (Donald, 1963). Competition among plants often occurs for water, nutrients and light (Donald, 1963; Rhodes, 1970; Hall, 1978; Rhodes and Stern, 1978; Haynes, 1980), although competition for other resources may also occur. Intraspecific competition possibly is more intense than interspecific competition since plants of the same species often have more similar requirements (Haynes, 1980).

Numerous literature reviews and studies on competition in forage species have been conducted (Donald, 1963; Harper, 1967; Hill and Shimamoto, 1973; Harris and Sedcole, 1974; Pineiro and Harris, 1978a, b; Rhodes and Stern, 1978; Haynes, 1980; Scott and Lowther, 1980; Berendse, 1981; Snaydon and Satorre, 1989). Of most interest has been the effect of competition on shoot and/or root mass of the components in a mixture. The identification of which specie(s) are relatively aggressive, that is, increase their yield in the presence of other species, under the particular environmental conditions, is often an important part of the research. In a literature review, Rhodes (1970) concluded that in establishing swards, root competition usually precedes shoot competition for nutrients and water while in older swards, competition for light becomes important.

Some researchers have explained their findings in terms of limiting resources. For example, in a study of shoot and root competition between 'Huia' white clover and 'Maku' lotus (Scott and Lowther, 1980), yield data showed that competition in the mixture was for soil resources and not for light, with 'Maku' the aggressor. It was

concluded that a major factor accounting for Huia's relatively low yield was its inability to absorb phosphate. The effect of competition on herbage mass is by far the most widely investigated but studies involving plant attributes such as tiller production (Norrington-Davies, 1968), leaf size and appearance rate (Rhodes, 1968), and total nitrogen (Hall, 1974a), have also been conducted.

2.3.1.2.4.1 STUDY METHODS AND ANALYSES

Competition in mixed stands has been studied using two contrasting experimental designs: additive (for example, Donald (1958)) and replacement series (for example, de Wit (1960)), with the latter being used most widely (Hall, 1974a, 1978; Trenbath, 1978). In replacement series, a constant total density of plants is used and the planting density of one species is proportionately decreased as the planting density of the second species is increased. In additive series, various densities of a second species supplement a constant density of an indicator species and hence there is always a change in total plant density. A disadvantage of additive designs compared with replacement designs is the scarcity of adequate mathematical models to quantify competition effects and to make predictions on various competitive situations (Spitters and van den Bergh, 1982).

Numerous methods of experimentation and statistical analysis have been devised which attempt to evaluate competition in terms of biologically meaningful parameters (de Wit, 1960; Hill and Shimamoto, 1973; Hall, 1974a, b, 1978; Trenbath, 1978; Gleeson and McGilchrist, 1980; Sinclair and Gleeson, 1984; Snaydon and Satorre, 1989).

Two types of experiment which have been widely used are the mixture diallel (derived from genetic terminology where diallell means two alleles (Mather and Jinks, 1971) and the replacement series. In mixture diallels, several genotypes are grown in all possible pair combinations, together with monocultures of the genotypes (Hill and Shimamoto, 1973; Trenbath, 1978; Gleeson and McGilchrist, 1980). In the mixture plots, the two genotypes are grown in 50:50 proportions at the same total density as in monoculture plots. Diallel competition experiments are closely related

to genetic diallel cross experiments and indeed similar methods of analysis are often conducted (for example, Norrington-Davies (1967) and Trenbath (1978)).

When seeking to investigate how two genotypes compete when grown in different proportions, a replacement series is frequently used (de Wit, 1960; Trenbath, 1978). Any two monocultures and their 50:50 mixture from a diallel experiment might be viewed as a special case of a replacement series, and analysed as such. One of the foremost methods of analysing repalcement series experiments is that developed in the Netherlands by de Wit and his colleagues (de Wit, 1960; de Wit and van den Bergh, 1965; Baeumer and de Wit, 1968) and both experimental and simulated data have shown the importance of the de Wit model in providing a sound basis for analysing replacement series experiments (Hall, 1978; Trenbath, 1978). Single experiments involving both mixture diallels and replacement series have been conducted (Hill and Shimamoto, 1973; Sinclair and Gleeson, 1984).

Another method of analysing competition between pairs of genotypes, when both monocultures and mixtures have been grown, is to plot the yield per unit area of one genotype against that of the other. These plots, termed bivariate diagrams, are applicable for additive and replacement designs, but they have not been widely accepted, mainly because of a lack of ecological understanding of the diagrams until very recently (Snaydon and Satorre, 1989). Routine use of the method by research workers is yet to be achieved. Although analyses for pairs of genotypes are widely available, there has been very little development of analysis techniques for more than two species mixtures, as occur in real vegetation.

Most statistical analyses of competition experiments such as those of mixture diallels and replacement series, are based on models of either the "additive" or "proportional" type (Trenbath, 1978). In additive models, the expectation of the gain per-plant yield (Y) by the aggressor (for example, genotype i) in the ij-th mixture over that in its monoculture, is equal to a corresponding loss by the subordinate (genotype j) in the same mixture compared with its own monoculture. The proportional model is based on the expectation that the proportional gain per-plant mixture yield in the aggressor

is equal to a corresponding proportional decrease in the subordinate compared in each case with per-plant yields in the genotype's own monoculture.
The models are expressed as:-

a) additive: $$Y_{ij}-Y_{ii} = Y_{jj}-Y_{ji}$$

b) proportional: $$\frac{Y_{ij}-Y_{ii}}{Y_{ii}} = \frac{Y_{jj}-Y_{ji}}{Y_{jj}}$$

Both models are equivalent when monoculture yields of all genotypes are the same (Trenbath, 1978), since in the proportional model, the denominators on both sides of the equation are equal.

Due to the use of a replacement series experiment in the field trials (Chapter 4) and the appropriateness of analysing such data using the de Wit (1960) competition analysis (Hall, 1978; Trenbath, 1978), a more detailed section on this analysis follows.

2.3.1.2.4.2 THE DE WIT MODEL

This model was originally developed by de Wit (1960) and his colleagues to obtain a quantitative description and assessment of competition in experiments involving pure and mixed stands of barley and oats. The model has since been applied to the analysis of competition between a diverse range of species (Hill and Shimamoto, 1973; Hall, 1974a, b; Berendse, 1981; Sinclair and Gleeson, 1984).

De Wit (1960) derived his model using an analogy with diffusion processes in gases and the full analysis usually consists of a graphical analysis and a more mathematical approach (de Wit, 1960; de Wit and van den Bergh, 1965). In the graphical analysis, dry matter yield data are presented in replacement diagrams where the yields of each genotype are plotted against the relative frequency of one genotype. Original data may be plotted alone but usually a fitted curve, as described below, is presented as well or by itself. It is possible to interpret from the replacement diagrams such information as whether competition between the two genotypes is occurring, which

is the more aggressive/competitive genotype, and whether there are advantages or disadvantages in combining the two genotypes. Replacement diagrams and their interpretation have been discussed fully elsewhere (for example de Wit (1960), Hill and Shimamoto (1973) and Hall (1978)).

The mathematical basis for the de Wit (1960) model and the estimation and interpretation of some biologically important indices have received much attention (Thomas, 1970; Hall, 1974a, b, 1978; Machin and Sanderson, 1977; Trenbath, 1978; Berendse, 1981; Sinclair and Gleeson, 1984). The main prediction of the model is that the yield of a binary mixture varies non-linearly with the proportion of one (or the other) component. The formulae describing this non-linear relationship for the observed herbage masses per unit area of genotype *i* grown with genotype *j* (Y_{ij}), and the reciprocal mixture (Z_{ji}), are respectively

$$Y_{ij}=\frac{Mk_{ij}x}{(k_{ij}-1)x+1};\ and\ Z_{ji}=\frac{Nk_{ji}z}{(1-k_{ji})x+k_{ji}}$$

where M and N are the yields of the monocultures of genotypes *i* and *j*, respectively; x and z are the proportions of genotypes *i* and *j*, respectively (x+z=1); and k_{ij} and k_{ji} are measures of the competitive power or ability of one genotype over the other and are usually termed relative crowding coefficients (de Wit, 1960; Sinclair and Gleeson, 1984).

Relative crowding coefficients are unitless constants which can assume any positive value and they are of fundamental importance in the de Wit (1960) model for several reasons. The k estimates determine the shape of the fitted curve of mixture yield against the proportion of genotype in the mixture. Values greater than unity define curves for mixture components which have higher yields between proportions of 0 and 100% than those expected when yield is related linearly to proportion. When k estimates are less than unity, the reverse is true, that is the components at proportions intermediate between 0 and 100% yield less than those expected from a linear relationship. In the special case where k=1, the response is linear for the corresponding species, that is Y_{ij}=Mx and Z_{ji}=Nz using the previous symbols. Hence, when k=1, yield of a component in a binary mixture is dependent on its monoculture yield and proportion in the mixture.

The product of the relative crowding coefficients, that is $k_{ij}k_{ji}$, is used to describe the level and type of interference occurring between the two genotypes. The model states (de Wit, 1960) that when $k_{ij}k_{ji}=1$, the two genotypes are competing for the same 'biological space' (a composite of all growth factors and resources), that is, they are mutually exclusive. If $k_{ij}k_{ji}>1$, the genotypes may be competing partly for the same space and partly for different space. Hence, non-competitive interference is perhaps occurring in addition to competitive interference. That two genotypes can be regarded as competing for the same space when $k_{ij}k_{ji}=1$ may be shown as follows. Since $k_{ij}k_{ji}=1$ then $k_{ij}=1/k_{ji}$ and substituting in the formula

$$Y_{ij}=\frac{Mk_{ij}x}{(k_{ij}-1)x+1}$$

and simplifying gives

$$Y_{ij}=\frac{Mx}{(1-k_{ji})x+k_{ji}}$$

The denominator in the above equation is the same as in the equation for Z_{ji} mentioned previously and therefore both earlier equations may be simplified when $k_{ij}k_{ji}=1$ to

$$Y_{ij}=Mx \quad \text{and} \quad Z_{ji}=Nk_{ji}z$$

These equations describe straight lines which may have equal slope, that is $M=Nk_{ji}$, although this is not necessary. Hence, the yield of one component in the binary mixture is dependent on the proportion of the other component and in this regard the genotypes are mutually exclusive.

Competitive performance may also be examined by estimating relative yields, that is the productivity of a genotype in a mixture relative to its monocultural yield at similar densities. The relative yields of genotype i and j are Y_{ij}/M and Z_{ji}/N respectively and their sum is termed relative yield total (RYT) (de Wit and van den Bergh, 1965). Hence,

$$(RYT)_{ij}=Y_{ij}/M+Z_{ji}/N$$

The relative yield of a genotype in a mixture indicates the genotype's ability to obtain environmental resources for growth. Hence, it is analagous to the relative crowding coefficient (k) and interpretations of RYT are similar to those obtained from the product of the relative crowding coefficients. This is shown by the formula derived by van den Bergh (1968) which relates maximum RYT to k-product, namely:

$$RYT_{max} = \frac{2[k_{ij}k_{ji} - (k_{ij}k_{ji})^{0.5}]}{k_{ij}k_{ji} - 1}$$

It can be seen that as $k_{ij}k_{ji}$ approaches infinity, RYT approaches 2; as $k_{ij}k_{ji}$ approaches zero, RYT approaches zero; and when the two genotypes are competing for the same space, $k_{ij}k_{ji}$=RYT=1. In an ecological context, as RYT increases from one (mutual exclusion/competitive interference) and approaches two, the relative importance of non-competitive interference increases and there is a greater potential for coexistence between the genotypes (Hall, 1978).

2.3.2 DEFOLIATION RESPONSES

Defoliation is defined as the partial or complete removal of the above-ground parts of plants (Hodgson, 1979; Thomas, 1980) and is usually achieved by grazing or mowing (Thomas, 1980). However, the process may also be by numerous other means including fire, cultivation, herbicides and extreme environmental conditions (Harris, 1978). Three terms are necessary to define defoliation accurately, namely: a) frequency - the period between successive defoliations; b) intensity - the degree of defoliation which may be defined by terms including severity, height and residual leaf area; and c) timing - in relation to plant growth stage and season (Harris, 1978).

2.3.2.1 EFFECTS OF DEFOLIATION

Investigations and reviews on numerous temperate and tropical forage species have shown generally that increasingly frequent and intense defoliation results in reduction of herbage mass (Weinmann, 1948; Troughton, 1957; Humphreys, 1966; Whiteman, 1969; Colman and Lazenby, 1970; Evans, 1971, 1973; Harris, 1978; McLean and

Wikeem, 1985; Stroud *et al.*, 1985; Brown, 1987), and several examples follow. It should be noted that while grasses are generally adapted well to grazing/defoliation (Langer, 1973), other species such as sheep's burnet and many legumes are probably less well adapted. However, literature on grass responses to defoliation has been reviewed as some of the basic principles may apply to sheep's burnet.

The effects of defoliation on root growth of perennial ryegrass were investigated by Evans (1971) using three defoliation intensities which left stubble heights of 2.5, 5.0 and 7.5 cm. Single defoliations caused a rapid fall in root elongation, and the 2.5 cm treatment had the greatest effect, with some roots dying. Herbage masses of shoot and root were not significantly different between the treatments but were less than the respective undefoliated controls. Following further defoliation, the 2.5 cm treatment experienced considerable root death, but associated herbage masses were not presented. A later study on three pasture grasses and two legumes (Evans, 1973) involved defoliation intensities which left 2.5, 5.0 and 10.0 cm stubble heights. Again, the most intense defoliation had the greatest effect and for most species caused complete or nearly complete cessation of root elongation.

Studies on two arid zone grasses, branched wiregrass and mulga grass, showed that young plants (7 weeks old) were more affected by clipping than 10 week and older plants (Brown, 1987). Two defoliation intensities, namely 50% residual leaf area (rLA) and 20% rLA, were investigated and the laxer defoliation had no significant effect on root growth in either species. Of the 20% rLA plants, root masses of young clipped plants were about half those of undefoliated plants but the reductions in root mass from the clipping of older plants were frequently negligible. Root death was not observed in the study and Brown (1987) noted the lack of agreement with some American findings on grasses where root growth ceased following 40-50% of foliar removal and root death occurred at higher defoliation intensities.

Cessation of root growth and possible death following defoliation may have important practical implications for water and nutrient uptake and this was shown by Oswalt *et al.* (1959). Plants of cocksfoot and bromegrass were defoliated and when radioactive phosphorus was placed into the existing root zone (15 cm depth) two

days after clipping, it took at least 19 days before the phosphorus was reached by new roots. Harris (1978) also reviewed evidence on the reduction in nutrient uptake due to the decreased transpiration of the remaining leaf area following defoliation.

Tillering of grasses may be partially maintained or enhanced by defoliation (Schwass and Jacques, 1956; Reid, 1959; Lambert, 1982) or affected adversely, as under severe defoliation regimes or pot conditions (Mitchell, 1953, 1954; Mitchell and Coles, 1955; Wright, 1962; Stroud *et al.*, 1985; Brown, 1987). In a study mentioned previously (Brown, 1987), severe defoliation (20% rLA) suppressed tillering significantly and increased tiller death compared with lighter defoliation (50% rLA). Western wheatgrass was evaluated by Stroud *et al.* (1985) under cutting and the most severe defoliation treatment imposed was clipping plots four times during the summer to leave a 2.5 cm stubble height. Tiller numbers in this treatment decreased by approximately half over a 16 month study period and several months later were about 30% of those for other treatments. Harris (1978) suggested that the literature on tillering and defoliation is full of contradiction and that the apparent confusion would be resolved if the experimental conditions and precise nature of the defoliation treatments were considered.

Changes of botanical composition of mixed swards arising from defoliation are documented widely and depend on numerous factors including species and growth habit, frequency and intensity of defoliation and their relationship with the growth stages of the sward components, method of defoliation, and relative competitive abilities (Humphreys, 1966; Harris, 1978; Watkin and Clements, 1978; Haynes, 1980). For example, a major advantage of ryegrass is that its yield is not reduced nearly as much as most grasses by continuous stocking compared to periodic grazing or cutting (Smetham, 1973), and the species tillers more profusely under heavy stocking (Suckling, 1975). Growth habit of legumes is also a major determinant of their suitability for grazing (Haynes, 1980). There is, for instance, a diversity of growth habits within the birdsfoot trefoil cultivars (Haynes, 1980; Scott and Charlton, 1983), ranging from prostrate to erect types, with the latter material being generally less persistent in swards, and best suited to periodic defoliation, as with lucerne (White, 1982; Scott and Charlton, 1983). Recently, replacement series and other

arrangements have been used to investigate compositional changes in mixed swards which have been defoliated (Harris, 1971, 1973, 1978; Harris and Thomas, 1973; Pineiro and Harris, 1978a, b), and these methods should continue to provide valuable information.

The method of defoliation, particularly mowing versus grazing, may have a major influence on the characteristics of the resulting sward (Cuykendall and Marten, 1968; Watkin and Clements, 1978; Christiansen and Svejcar, 1987) and the danger of extrapolating from mowing studies to grazing practice is well known (Humphreys, 1966). Factors which may account for differences between the results of mowing and grazing studies have been reviewed extensively by Watkin and Clements (1978) and include grazing selectivity, treading damage and animal excretion. Animal factors were also investigated in a recent study by Broom and Arnold (1986). Nevertheless, similar results have been obtained between the two methods of defoliation (Cuykendall and Marten, 1968) and the results of cutting (simulated grazing) studies continue to be used to suggest likely grazing management and/or to assist in interpreting existing grazing practices (Sheaffer, 1983; Stroud *et al.*, 1985; Atkinson, 1986; Brown, 1987).

2.3.2.2 MANNER OF REGROWTH

Regrowth herbage mass of a sward following defoliation may be described adequately by a sigmoid growth function (Walton, 1983) and is explained frequently by changes in leaf area and hence light interception characteristics (Humphreys, 1966; Brown and Blaser, 1968; Harris, 1978; Walton, 1983). The work of Brougham (1955, 1956, 1958) and others (Davidson and Donald, 1958) has enhanced considerably the understanding of leaf area and light relationships in defoliated swards. Following defoliation which is complete, or nearly so, initial regrowth is exponential in form until the approximate attainment of critical leaf area index (critical LAI) (Brougham, 1956), where the leaf area intercepts 95% of the incident light (Thomas, 1980). At this stage, growth rate attains a maximum and a period of growth at this rate, the linear phase, generally follows (Brougham, 1955; Leafe *et al.*, 1974; Noy-Meir, 1975). As herbage accumulation continues, LAI increases to a

ceiling where leaf senescence lower in the canopy balances new leaf production (Harris, 1978). For maximum herbage accumulation, defoliation intensities which remove herbage to the point where maximum growth rate is first attained and intervals between defoliations which permit regrowth until maximum growth rate declines, seem most appropriate (Harris, 1978; Walton, 1983).

Apart from environmental factors influencing the rate and extent of regrowth, other factors which may also be important include the arrangement and photosynthetic efficiency of the residual leaves or stubble, stage of development (particularly vegetative versus reproductive), and number of meristematic tissues and their ability to develop and contribute to regrowth (Brown *et al.*, 1966; Harris, 1978; Richards and Caldwell, 1985). Experiments with white clover and lucerne (Brown *et al.*, 1966) showed that in white clover, the age of residual leaves influenced their photosynthetic efficiency, with young leaves generally having higher net assimilation rates than leaves 2-3 weeks older. Young leaves also had superior herbage accumulation rates. In lucerne, leaves were removed from the top, middle and bottom of plants and those at higher positions (youngest) had the highest net assimilation rates (Brown *et al.*, 1966). It was suggested that the older leaves towards the base of the canopy may be more of a hardship than advantage during regrowth and that perhaps these should be removed in a mowing or rotational grazing system. Higher rates of photosynthesis in younger leaves have also been reported for sheep's fescue (Atkinson, 1986). Reproductive swards are more efficient photosynthetically than vegetative swards due to the elevation of leaves higher in the swards as a result of stem extension (Leafe *et al.*, 1974). The role of nonstructural carbohydrate reserves in regrowth is dealt with in Section 2.3.2.3.

The sites of regrowth vary considerably between different pasture and forage species and this dictates the most suitable type of defoliation management. The grasses are extremely well adapted to defoliation (Langer, 1973) and regrowth arises from initiation of new leaves from axillary buds which lie close to the soil surface. These are often well below practical defoliation intensities and therefore the growing points and axillary buds escape damage. In legumes, growing points are at the tip of an elongated stem which is frequently elevated (Haynes, 1980; Walton, 1983). During

defoliation, the growing points of most legumes are removed and growth of the elongating stems terminated. Regrowth in lucerne originates from previously dormant buds near the crown (Leach, 1978; Haynes, 1980; Walton, 1983), while for species such as birdsfoot trefoil and cicer milkvetch, regrowth from axillary buds or shoots on remaining stubble is important (Townsend *et al.*, 1978; Scott and Charlton,1983).

2.3.2.3 NONSTRUCTURAL CARBOHYDRATES

The energy for regrowth following defoliation may originate from two main sources, namely a) current photosynthate from residual leaf area and/or b) stored reserves (Booysen and Nelson, 1975; Richards and Caldwell, 1985; Volenec, 1986; Christiansen and Svejcar, 1987). Evaluations of the contribution and importance of one or both of these energy sources to regrowth, under a variety of environmental conditions, have been conducted for a wide range of herbage species including birdsfoot trefoil (Smith, 1962), cicer milkvetch (Gabrielsen *et al.*, 1985), cocksfoot (Ward and Blaser, 1961; Brown and Blaser, 1965; Davidson and Milthorpe, 1965), lotus (Sheath, 1978), lucerne (Reynolds and Smith, 1962: Smith, 1962; Cooper and Watson, 1968; Chatterton *et al.*, 1974; Rapoport and Travis, 1984; Gabrielsen *et al.*, 1985; Barta, 1988a, b), red clover (Smith, 1950, 1962), ryegrass (Davies, 1965; Alberda, 1966; Davies *et al.*, 1989), sainfoin (Cooper and Watson, 1968), tall fescue (Brown and Blaser, 1965; Booysen and Nelson, 1975; Volenec, 1986; Schnyder and Nelson, 1987) and timothy (Reynolds and Smith, 1962). Where investigated, most of these studies have indicated an increasing contribution of stored carbohydrate reserves to regrowth following more intense defoliation.

Carbohydrate reserves usually decline just after defoliation (Smith, 1950; Reynolds and Smith, 1962; Brown and Blaser, 1965; Humphreys, 1966; Gabrielsen *et al.*, 1985). As new leaves develop, current photosynthate increasingly satisfies the respiratory demands of existing tissue and the growth of new tissue (White, 1973), and there is usually an accompanying replenishment of carbohydrate reserves to about their pre-defoliation level (Smith, 1962; Davies, 1965; Davidson and Milthorpe, 1965; Alberda, 1966; Gabrielsen *et al.*, 1985). Defoliating once or more before

replenishment has occurred lowers the reserve carbohydrate content (Walton, 1983), reduces plant vigour (Humphreys, 1966, Booysen and Nelson, 1975) and may lead to plant death (Alberda, 1966).

The general patterns outlined may be modified or masked by numerous factors. Environmental conditions, particularly temperature and light, influence the amount of photosynthesis and respiration of plant tissues (Ludlow, 1978; McWilliam, 1978) and hence the extent of net photosynthesis and potential carbohydrate availability for storage. Working with ryegrass, Davies (1965) found that carbohydrate levels of light pretreated plants fell and were replenished more quickly at 20°C than at 10°C. In discussing their findings on the accumulation of carbohydrate reserves in cocksfoot and tall fescue, Brown and Blaser (1965) suggested that reductions in growth rate by low temperatures, soil moisture or nitrogen could result in rapid accumulation of reserves, even at low LAI estimates. With increasing temperature, the concentration of total nonstructural carbohydrates declines in lucerne and several other forage species. These and other factors have been discussed by White (1973), Harris (1978) and Walton (1983).

There are several main types of nonstructural carbohydrates in plants (Percival, 1952; Weinmann, 1961; Ojima and Isawa, 1968; Meier and Reid, 1982; Walton, 1983). The most important sugar, quantitatively, is sucrose and estimates reviewed by Percival (1952) were 5-6% of dry weight. Most of the non-reducing sugar component in a range of grass and legume species was sucrose and ranged from 1-12% of dry weight (Ojima and Isawa, 1968). Starch is formed in all higher plants in which it has been investigated (Meier and Reid, 1982) and Ojima and Isawa (1968) reported levels of starch mostly in the range of 0-4% of dry weight. Nonstarch reserve polysaccharides of vegetative tissues are mainly of the fructan and of the mannan type and although reserve mannans probably occur only in vegetative tissues of monocotyledons, fructans are present in both mono- and dicotyledons (Meier and Reid, 1982). In their review of fructans in dicotyledonous species, Meier and Reid (1982) listed numerous families of plants known to contain fructans in their vegetative tissues. A notable omission was the family Rosaceae and it is therefore probable that sheep's burnet has starch as its major reserve polysaccharide. The

family Fabaceae (Leguminosae) was also absent and this was in agreement with the findings of Ojima and Isawa (1968).

Nonstructural carbohydrates may be stored temporarily in all plant tissues (Harris, 1978; Walton, 1983) although lower parts of the foliage and upper areas of roots are the major sites of storage. In forage grasses, the primary area of storage is the stem base or stubble (Okajima and Smith, 1964; Volenec, 1986) but stolons, corms and rhizomes may also be important (Walton, 1983). Roots are major storage areas in legumes (Walton, 1983) such as lucerne and red clover (Smith, 1962).

The role of other reserve substances in regrowth has been indicated, particularly under conditions of low carbohydrate reserve status (Turner, 1949). For example, an experiment on cocksfoot found that the contribution to new growth and to respiration from protein and other fractions was four times that from carbohydrate reserves (Davidson and Milthorpe, 1965). However, such findings are rare and further studies in this area would be informative.

2.4 MULTIVARIATE ANALYSIS

2.4.1 INTRODUCTION

Multivariate analysis is the simultaneous analysis of two or more random variables (Lindeman *et al.*, 1980) and an assortment of descriptive and inferential techniques have been developed for the analysis of such data (Hope, 1968; Cooley and Lohnes, 1971; Overall and Klett, 1972; Press, 1972; Lindeman *et al.*, 1980; Harris, 1985). A key advantage of the techniques is that they incorporate all the information in the data, including the covariances or correlations among all variables.

In his introductory comments, Harris (1985) noted that multivariate statistical techniques can achieve two main tasks for the researcher. Firstly, they combine the variables in an optimum way according to a set of solving rules, which vary depending on the technique. In this regard, the techniques summarise the data and they frequently facilitate interpretation. Relative importance of the variables may

also be ascertained. A second broad task achieved by the multivariate techniques is that they provide a way of explicitly controlling the experiment-wise error rate and hence provide a solution to the problem of multiple comparisons (Harris, 1985).

Although the theory behind many of the multivariate techniques was developed early in this century (Hotelling (1936) and other early investigators reported by Press (1972) and Harris (1985)), it was not until the late 1960's / early 1970's that numerous books, such as those cited earlier (Hope, 1968; Cooley and Lohnes, 1971; Press, 1972), appeared. Computer programmes such as BMDP, SAS and SPSSX have also become available recently (Harris, 1985) and these offer a range of multivariate analyses. Hence, large problems can be handled with ease and this should result in wider use of the procedures. Several examples of the use of multivariate analyses in plant research have been reported by Glenday and Feijer (1956), Hussaini *et al.* (1977), Stroup and Stubbendieck (1983), Castonguay and Dube (1985) and Jolliffe and Hoddinott (1988).

2.4.2 UNDERLYING PRINCIPLES

2.4.2.1 DISPERSION MATRIX

Central to most multivariate techniques is the assemblage of the data into a square matrix **D** of size p x p, where p is the number of variables or characters (Cooley and Lohnes, 1971; Overall and Klett, 1972). Matrix **D**, called the dispersion or variance-covariance matrix, has sample variances (s^2) as diagonal elements and sample covariances (cov) as off-diagonal elements:

$$\mathbf{D} = \begin{bmatrix} s_1^2 & cov_{12} & \cdots & cov_{1P} \\ cov_{21} & s_2^2 & \cdots & cov_{2P} \\ cov_{P1} & cov_{P2} & \cdots & s_P^2 \end{bmatrix}$$

Associated with this matrix, or any square matrix, is a single number called a determinant which is a sum of terms involving the elements of the matrix. It is represented symbolically as **D** and the number of terms in the sum is equal to the factorial of the order, n, of the determinant. Methods for estimation of the determinant are documented widely (Searle, 1966; Cooley and Lohnes, 1971; Overall and Klett, 1972; Press, 1972). For the present matrix, $|\mathbf{D}|$ represents a generalised variance (Cooley and Lohnes, 1971). If $|\mathbf{D}|$ is greater than zero, as it usually is (Overall and Klett, 1972), the matrix is termed nonsingular and it has an inverse, which enables an operation analagous to division in univariate statistics to be undertaken. Methods of treating singular matrices ($|\mathbf{D}| = 0$) are also available (Overall and Klett, 1972).

Scale types of the variables (Siegel, 1956) are important issues in the application of most multivariate techniques (Harris, 1985). It is assumed for statistical inference that the underlying multivariate populations are well established and are often multivariate normal, with homogeneity of variance-covariance matrices being frequently a prime assumption (Press, 1972; Lindeman *et al.*, 1980). This of course, is an extension of the familiar univariate assumption of independently and normally distributed random variables. The difficulties of nominal and ordinal scales have been discussed by Press (1972) and Anderberg (1973). While qualitative (nominal scale) data are often treated nonparametrically, ordinal data are modified frequently by replacing the data points with their ranks (Press, 1972). Scales of all variables are often equalised by normalisation (mean of zero and unit variance) to aid interpretation (Overall and Klett, 1972; Press, 1972; Harris, 1985), but this is not essential. With normalised data, the variance estimates in **D** are replaced with 1's while correlations substitute for the covariances.

All square matrices have associated with them a characteristic equation (Cooley and Lohnes, 1971; Overall and Klett, 1972; Lindeman *et al.*, 1980). This is formed by subtracting some scalar value λ (variously termed eigenvalue, characteristic root, latent root, proper value) from each of the diagonal elements of the matrix, where λ is chosen so that the determinant of the resulting matrix is zero. Hence, the characteristic equation of a second order matrix **A** may be written:

$$| A-\lambda I | = \begin{vmatrix} a_{11}-\lambda & a_{12} \\ a_{21} & a_{22}-\lambda \end{vmatrix} = 0$$

For a matrix of order p, there may be as many as p different values of λ which will satisfys the equation. A feature of the eigenvalues is that their sum is equal to the trace of **A**, which the sum of its diagonal elements. Furthermore, the product of the eigenvalues is equal to the determinant of **A**. For each eigenvalue, there is a corresponding vector, **v**, called the eigenvector (also termed characteristic vector and latent vector), which is selected to satisfy the equations

$$\mathbf{Av}=\lambda\mathbf{v} \quad \text{or} \quad (\mathbf{A}-\lambda\mathbf{I})\mathbf{v} = 0$$

I is an identity matrix containing 1's on the diagonal and 0's on the off-diagonal positions. The complete eigenstructure of **A** is defined by the relation

$$\mathbf{AV}=\mathbf{VL}$$

where **L** is a diagonal matrix of the complete set of eigenvalues of **A**, and **V** is the entire set of associated eigenvectors arranged as columns. An important property of the eigenvectors is that they are orthogonal (uncorrelated or independent) (Lindeman *et al.*, 1980). Any set of linear equations with constraints always leads to the above algebra and hence to solutions via eigenstructures (Harris, 1985).

2.4.2.2 OPTIMAL DISPERSION FUNCTIONS

A feature of most multivariate procedures is the estimation of a new variable, which is a linear (weighted) combination of the original variables, defined according to a set of solving rules (Overall and Klett, 1972; Harris, 1985). This may be represented as

$$u_{jk} = v_{1j}x_{1k} + v_{2j}x_{2k} + ... + v_{pj}x_{pk}$$

where

u_{jk} is the new variable (aggregate score) estimated for the j-th solution (j=1...m, where m is the number of solutions for a set of solving rules) and the k-th entity (k=1...n, where n is the number of entities or experimental units),

x_{pk} is the p-th attribute or character (p=1...o, where o is the number of attributes) measured on the k-th entity, and

v_{pj} is the coefficient or weight for the p-th attribute and the j-th solution.

The coefficients (v_{pj}'s) of the attributes are estimated using specific solving rules which involve dispersion (variance-covariance) matrices mentioned in the previous section. If all j solutions are combined, we can write in matrix notation

$$\mathbf{u=V'x}$$

where **u** and **x** are column vectors and **V'** is a matrix of the complete set of corresponding **v**'s arranged as rows. Where normalised data (z-scores) are used, the relation may be rewritten as

$$\mathbf{u=V'z}$$

and the two **V'** would be different. Dispersion matrices of the resulting two new variables may be stated as

$$\mathbf{D_u=V'D_zV} \quad \text{(for normalised data) and} \quad \mathbf{D_u=V'D_xV}$$

being the usual quadratic form definition of the variance of a linear function (Lindeman *et al.*, 1980).

2.4.3 TYPES OF ANALYSES

There is a large and often bewildering range of multivariate analysis techniques including principal component analysis, rotated and unrotated factor analyses, multivariate analysis of variance, canonical correlation analysis, and discriminant analysis (including multiple discriminant, Fisher's discriminant, classificatory, canonical and stepwise analyses) (Hope, 1968; Cooley and Lohnes, 1971; Overall and Klett, 1972; Press, 1972; Lindeman *et al.*, 1980; Harris, 1985). There are also the techniques of cluster analysis (a non-parametric approach) and other classification analyses as well as the more familiar analysis of covariance and multiple regression and correlation analyses (Le Clerg *et al.*, 1962; Overall and Klett, 1972; Lindeman *et al.*, 1980; Steel and Torrie, 1980; Harris, 1985). Only some of the multivariate techniques used less frequently in agronomic research, including those used in this thesis, are described further.

Principal component analysis is a method to derive a smaller set of statistically independent linear combinations (principal components) of a set of variables that retain as much of the information in the original variables as possible (Overall and Klett, 1972; Harris, 1985). The technique is used appropriately for analysis of data collected for one group, treatment or population (Harris, 1985). The solving rule is that linear function of the variables which best discriminates amongst the entities in the single population. Factor analysis, also suitable for only a single set of variables, aims to explain the relationships (correlations or covariances) among a set of variables in terms of a relatively few unobservable, latent variables (Overall and Klett, 1972; Lindeman *et al.*, 1980). Generally, the latent variables are not

computable as linear combinations of the original variables. The overall effect of the analysis is a regrouping of the data into patterns which can usually be interpreted meaningfully. Rotation of principal components or factors may be conducted to aid interpretation (Press, 1972; Lindeman *et al.*, 1980).

The simultaneous analysis of two sets of variables is termed canonical correlation analysis. A linear combination of the variables in each set is found, called a canonical variable (variate), such that the correlation (canonical R) between the two canonical variables is maximised. The maximum number of canonical correlations is equal to the number of variables in the smaller set (Lindeman *et al.*, 1980; Harris, 1985). The techniques of multivariate analysis of variance and discriminant analysis have been detailed (Hope, 1968; Cooley and Lohnes, 1971; Overall and Klett, 1972; Press, 1972; Lindeman *et al.*, 1980; Harris, 1985) and a summary of relevant information is presented henceforth due to the wide use of these techniques in all experimental work described in this study.

2.4.3.1 MULTIVARIATE ANALYSIS OF VARIANCE

This technique tests the differences among the multivariate means of several treatments or populations and may be viewed as an extension of univariate analysis of variance to the case in which the dependent variable is a vector rather than a scalar. The multivariate generalisation of analysis of variance involves partitioning the matrix of total sums of squares and cross products, usually referred to as T (for Total), in a manner that is identical to the partitioning of the sums of squares in univariate analysis of variance. The matrices for hypothesis and error are identified usually as **H** and **E**, respectively. It is noteworthy that the diagonal elements of **T**, **H** and **E** are the sums of squares for the corresponding partitions of the univariate model. In univariate analysis of variance, the optimal testing criterion is generally agreed to be an F-test of the sample variance ratio. The analagous procedure in multivariate analysis is to develop test statistics using the ratio of **H** to **E**. As matrices cannot be divided, an equivalent operation is the inverse of **E** multiplied by H, that is $\mathbf{E}^{-1}\mathbf{H}$. Four test statistics based on this matrix have been developed and all are related to its characteristic roots and vectors (Section 2.4.2.1). The most

widely recommended statistic is Wilks' likelihood ratio criterion (Press, 1972; Lindeman *et al.*, 1980) which is

$$\frac{|\mathbf{E}|}{|\mathbf{T}|} \quad \text{or} \quad \frac{|\mathbf{E}|}{|\mathbf{H}+\mathbf{E}|}$$

The determinants of **E** and **T** (**H** + **E**) are scalar indices of multivariate generalised variance (Section 2.4.2.1) and hence the ratio is one of within-groups error variance to total variance. For the univariate case, the F value and Wilks' criterion are related inversely (Lindeman *et al.*, 1980) and in the multivariate situation, the larger the true treatment differences, the larger the denominator will be and hence the smaller the value of Wilks' criterion.

In the univariate case, Wilks' criterion has an exact F distribution. This is also true for many practical research situations involving multivariate analysis of variance where the number of variables or the number of treatments (groups) is low. However, as both the numbers of variables and treatments increase, the exact sampling distribution of Wilks' criterion does not conform to any well-known model such as F or chi-square (Lindeman *et al.*, 1980). When an exact test cannot be conducted, approximate tests developed by Bartlett (1947) and Rao (1952) may be used. Rao's test yields a slightly better approximation to the exact significance probability of Wilks' criterion and is distributed approximately as F.

2.4.3.2 DISCRIMINANT FUNCTIONS

Where there are overall significant differences between treatments according to Wilks' criterion (and the other test statistics), it is most useful to determine the linear combination of the original variables which is accounting for maximum discrimination between the treatments. One type of discriminant function (variously called canonical discriminant function, multiple discriminant function, and canonical

analysis of discriminance) is appropriate to achieve this objective (Overall and Klett, 1972; Lindeman *et al.*, 1980; Harris, 1985).

The number of discriminant functions for a given problem is equal to the smaller of p, the number of variables and g-1, where g is the number of groups or treatments. To determine these, the eigenvalues of $\mathbf{E}^{-1}\mathbf{H}$ first need to be calculated, together with their associated eigenvectors (Section 2.4.2.1) which are the coefficients or weights given to each variable in the linear function(s) (Section 2.4.2.2). The prime solving rule for estimating the discriminant functions is the maximisation of the ratio of amongst-groups to the within-groups sums of squares and cross-products matrices. The ratio is termed the discriminant criterion, λ, and may be represented as

$$\lambda_j = \frac{\mathbf{v}_j{}'\mathbf{H}\mathbf{v}_j}{\mathbf{v}_j{}'\mathbf{E}\mathbf{v}_j}$$

where λ_j is the discriminant criterion for the j-th (j=1...m, where m is the number of solutions) discriminant function, **H** and **E** are the hypothesis (amongst-groups) and error (within-groups) sums of squares and cross-products matrices, respectively, and $\mathbf{v}_j$ and $\mathbf{v}_j'$ are the eigenvector and its transpose for the j-th function. In order to achieve a finite number of solutions to the problem, constraints are imposed on the eigenvectors such that for a given discriminant function, j, premultiplying the eigenvector $\mathbf{v}_j$ by its transpose equals unity, that is $\mathbf{v}_j'\mathbf{v}_j=1$. Another restriction is that the eigenvectors for different functions are orthogonal/uncorrelated, that is $\mathbf{v}_j'\mathbf{v}_{j'}= 0$ ($_j \neq_{j'}$).

The solving function (θ_j) may be written as

$$\theta_j=\mathbf{v}_j'(\mathbf{E}^{-1}\mathbf{H})\mathbf{v}_j - \lambda_j(\mathbf{v}_j'\mathbf{v}_j - 1) - \kappa_j\mathbf{v}_j'(\mathbf{E}^{-1}\mathbf{H})\mathbf{v}_{j-1}$$

where all terms are as defined previously and λ_j and κ_j are Lagrange multipliers for the j-th solution. Differentiation of θ_j with respect to $\mathbf{v}_j$ and maximisation of the

resulting derivative (set equal to zero) yields for the first (and subsequent) solutions an equation of the general form

$$(\mathbf{E}^{-1}\mathbf{H}-\lambda\mathbf{I})\mathbf{v}=0$$

That is, the outcome is an eigenstructure and further information on this relation and its solutions was presented in Section 2.4.2.1. The largest eigenvalue (λ) and its associated eigenvector define the linear function that gives the maximum F value for the hypothesis defined by a given **H** and **E** and hence is the best discriminator amongst the treatments. The second largest eigenvalue and its associated eigenvector define a second function providing the second largest F value for the hypothesis, and so forth. Hence, the analysis produces functions which are ordered from greatest to least with regard to the extent to which they discriminate between the treatments (groups). In most situations, it is hoped that much of the dispersion in the data is accounted for by one or a few discriminant functions as this facilitates interpretation. That is, functions with high discriminatory ability are generally desired.

The discriminant power of each function assists in identifying those functions with high discriminatory ability and is equal to λ. The sum of all λ's gives the total discriminant power and hence the proportion of discriminant power due to a specific function can be determined (Cooley and Lohnes, 1971). A satisfactory parsimonious set of discriminant functions for most practical purposes is that set of one or more functions which accounts for 70-80% of the data dispersion. Significance tests of all λ's corresponding to each discriminant function may also be conducted (Cooley and Lohnes, 1971; Lindeman *et al.*, 1980) to identify those which are significantly different from zero. When this is so for the first (largest) λ, it may be removed from consideration and the test repeated for the second lower-order amalgamation of solutions, and so forth.

A description of what a discriminant function measures must be based on the relative magnitudes of each variable in the function. To aid interpretation, differences in scaling and/or variability among the set of variables are overcome by standardising the elements of the eigenvector for the function(s) of interest. This is achieved by

dividing the elements of the eigenvector by the standard error of the corresponding function. The resulting net score then has unit within-treatment variance when the function is applied to the original variables. Also, the original variables can be normalised (*z*-scores) so that both the original variables and the net scores are scale free and have an equal variance of unity. This information may be used to determine the relative importance of the variables in contributing to discrimination between the treatments and is analagous to the use of standardised partial regression coefficients (Steel and Torrie, 1980). Another useful type of information is contained within structure vectors or matrices (Lindeman *et al.*, 1980). These are the pooled within-group correlations between the variables (on original or normalised scales) in a function and the net scores on the same function, and are analagous to partial correlations. Hence, judgements of relative importance can again be made but from a different perspective. Both importance criteria are useful in attempting to name a discriminant function on the basis of its contents.

The treatment (group) means may be estimated in two ways. Firstly, the unadjusted net scores (components) can be calculated from the linear function with coefficients and raw variables unadjusted. Alternatively and more frequently, adjusted net scores, usually called factors, are used and these are calculated from the linear function with coefficients standardised, or coefficients standardised and original variables normalised. However, both methods give similar or identical ordinations of the treatment means. Some examples of mean estimation and interpretation are presented by Hope (1968), Overall and Klett (1972), and Lindeman *et al.* (1980). Differences between pairs of treatment means can be tested using Hotelling's (1931) T^2 statistic which is a multivariate generalisation of the familiar univariate t test (Steel and Torrie, 1980). Other methods for making comparisons between treatment mean vectors have also been generalised from the univariate case (Lindeman *et al.*, 1980; Harris, 1985).

CHAPTER 3 : MATERIALS AND METHODS

3.1 INTRODUCTION

The materials and methods described are those common to mostly two or more experiments. Sections are ordered chronologically, seed lines and herbage handling methods being followed by statistical analyses.

3.2 SEED LINES

Seed of sheep's burnet which originated from Oregon, USA (Section 2.2.3.1), was used in all experiments. It was probably from a 1982 harvest (B J Wills, pers. comm.). In experiments described in Chapters 6 and 7, sheep's burnet seed collected from the early Cockayne plots (Section 2.2.6) by Dr B J Wills in 1985, was also included. 'Rere' lucerne which was obtained from a commercial outlet in autumn 1985, was included as a dryland standard in the experiments described in Chapters 4, 6 and 7.

3.3 PLANT MORPHOLOGY

Plant material harvested ranged from seedlings a few days after emergence to well differentiated material aged several months. Main plant characteristics of sheep's burnet, including those of the hypanthium ("seed"), are illustrated in Figures 3.1a and b.

For young seedlings, the following plant parts were defined:

1. Foliage was the above ground component, normally demarcated from the roots by a colour change from light green to white (the latter corresponding to healthy root) at the soil surface or up to 2-3 mm below the surface. Roots were the remaining below ground component. Excision of roots and foliage was conducted at this approximate zone of colour change;

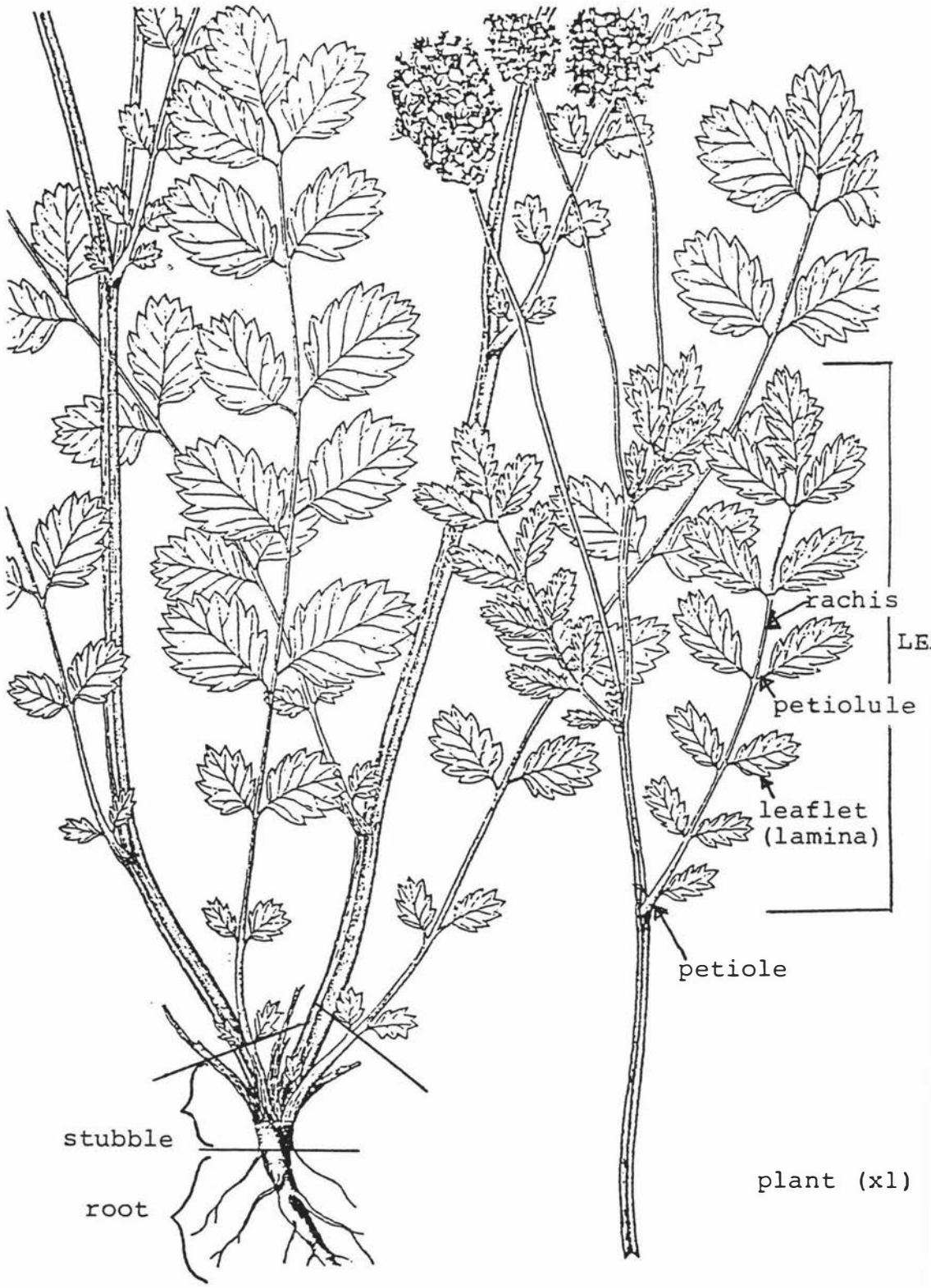
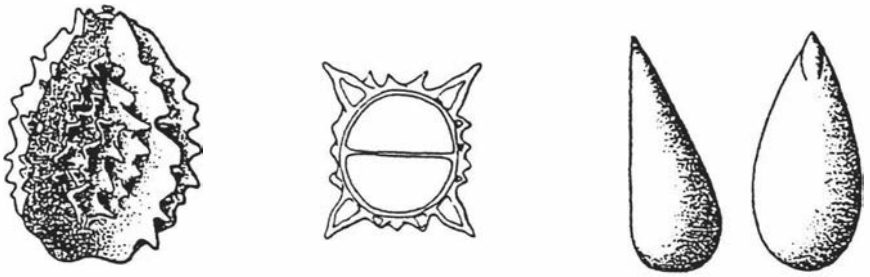


Figure 3.1a Morphology of sheep's burnet (adapted from Salmeron (1966)).

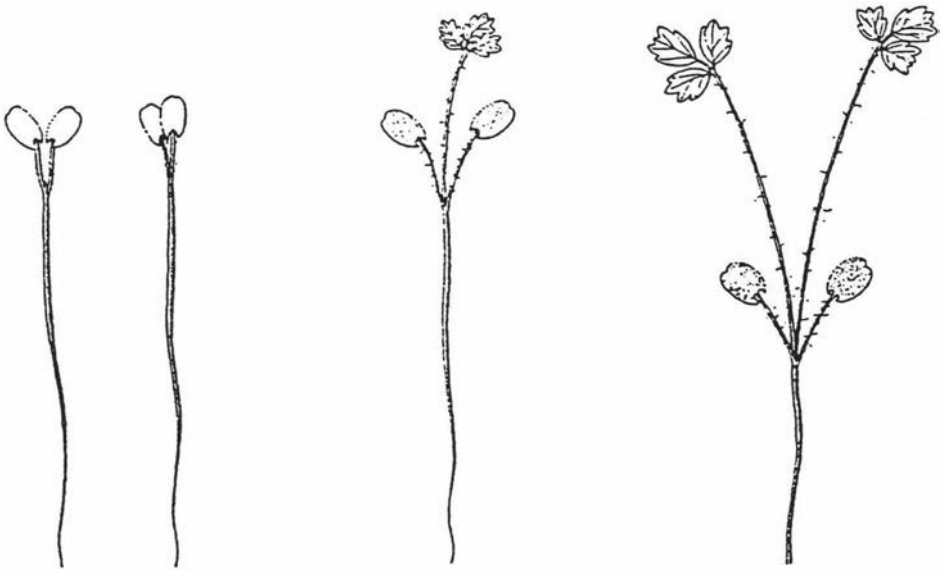


hypanthium ("seed")

cross-section of hypanthium

true seed

(x6)



seedlings (x1)

Figure 3.1b Hypanthium and seedling morphology of sheep's burnet (adapted from Salmeron (1966)).

2. Petiole was that part of the foliage which supported the leaflets (leaf laminae).

In older and more differentiated plants, the following plant components were defined:

1. Complete leaves were intact compound structures (Figure 3.1a) which consisted of a single terminal leaflet, various numbers of leaflet pairs and supporting petioles and petiolules;

2. Incomplete leaves lacked a terminal leaflet and often one or more other leaflets plus accompanying supporting structures;

3. Stubble was the plant material extending from immediately below the lowest leaflet pair of each leaf to immediately above the first branch from the roots. It comprised mainly petioles, growth zone material where new shoots originated, and small (< 10 mm long) complete leaves whose removal seemed unjustified in view of their expected low contribution to any leaf dry weight determinations;

4. Root was all plant material immediately below the highest branch of the below ground structure.

3.4 LEAF AREA AND HERBAGE MASS DETERMINATIONS

Leaf area of seedlings was determined by removing all leaf laminae from their supporting structures and then estimating lamina area using LI-COR 1600 or 3100 leaf area meters. For more developed plants with numerous complete leaves and many leaflets, leaf area was determined for the entire structure, that is petiole, rachis, laminae and petiolules. An exception was the tagged leaves remaining after defoliation in the regrowth experiments reported in Chapters 8 and 9. There, leaf laminae were measured for area due to the low number of leaflets involved.

Plant components were dried initially at 100°C for one hour to reduce enzyme activity quickly, and thereafter at 70°C for 24 hours. Similar drying regimes are used commonly, particularly when preparing material for analysis of nonstructural carbohydrates (Reynolds and Smith, 1962; Ojima and Isawa, 1968; Smith, 1973; Gabrielsen *et al.*, 1985; Herberer *et al.*, 1985). Any samples destined for nonstructural carbohydrate determinations were immediately ground finely and stored for up to two months in a freezer with a silica-gel desiccant. Storage of tissues under such low temperatures and moisture levels, for short periods, minimises the interconversion of carbohydrates (Nelson and Smith, 1972).

3.5 ANALYSIS OF TOTAL NONSTRUCTURAL CARBOHYDRATES

Numerous extraction techniques have been used to determine the levels of total nonstructural carbohydrates (TNC) (Dekker and Richards, 1971; Smith, 1971; Haissig and Dickson, 1979) and each method provides different estimates (Smith, 1981). The main nonstructural carbohydrates in sheep's burnet are soluble sugars (mainly sucrose) and probably starch (Section 2.3.2.3), and the method chosen to determine the concentrations of these fractions was that of Haslemore and Roughan (1976). Apart from the relative simplicity of the technique and the ability to process a large number of samples quickly, an important advantage was the familiarity with the technique by some members of the department in which the current studies were conducted. The main steps involved in the technique and the slight modifications were as follows:

1. Soluble sugars

Plant material (approximately 100 mg) was extracted with 10 cm^3, 62.5% (v/v) methanol at 55°C for 30 minutes. A 4 cm^3 aliquot of this extract was collected, from which non-carbohydrate, interfering materials (phenols, pigments) were precipitated by the addition of 0.1 cm^3 of a 0.5 M solution of lead acetate. Chlorophyll pigments, and therefore galactolipids, were removed by shaking with 5 cm^3 chloroform. Soluble sugars were retained within an upper aqueous phase and the amount present in a 50 μl aliquot was determined using the phenol-sulphuric acid procedure of

Dubois *et al.* (1956). Following cooling to room temperature, absorbances of the orange coloured samples at 490 nm were read. Standards equivalent to 0, 4, 8, 12 and 20% soluble sugar (stock solution of 2 mg sucrose cm^{-3} in 62.5% methanol) were also processed similarly.

2. Starch

The residual plant material from above was treated with 4 cm^3 100% methanol at 100°C for five minutes and then this procedure was repeated. Distilled water (4 cm^3) was added and the aqueous suspension was boiled subsequently for 60 minutes to gelatinise the starch. After cooling, 0.1 cm^3 of an amyloglucosidase preparation (10 mg cm^{-3} amyloglucosidase (Sigma Chemical Company product from *Rhizopus* mould) and 1 mg cm^{-3} alpha-amylase in 25 mM sodium citrate of pH 6.0) was added to hydrolyse the starch. Free glucose in the diluted hydrolysate was then determined using glucose oxidase reagent. A water blank and a range of glucose standards were treated similarly. A magenta colour was produced by adding 5 cm^3 of 5 M hydrochloric acid and absorbances at 540 nm were read. Two starch standards (4 and 8 mg starch) were processed in the same way for each analytical run and the average recovery of glucose from the starch standards was in the range 75% to 90%. Starch levels of plant tissues were expressed on the basis of 100% recovery of glucose by multiplying the estimate by the appropriate conversion factor.

3.6 FUNCTION ANALYSIS OF SEEDLING EMERGENCE AND REGROWTH

Growth functions (Richards, 1959; Landsberg, 1977; Moore, 1979; Ratkowsky, 1983) were used, where possible, to describe seedling emergence and foliar regrowth (expressed in various forms such as leaf number, extension, area and dry weight) in one or more experiments. Two important advantages in the use of growth functions were, firstly, their ability to describe the overall growth processes in relatively simple mathematical terms, and secondly, they estimated parameters which could be interpreted biologically.

The logistic and monomolecular functions (Richards, 1959; Ratkowsky, 1983) were employed. Their parameterisations were:

1. Logistic

$$y=\frac{\beta_0}{1+e^{(\beta_1-\beta_2 t)}}$$

2. Monomolecular

$$y=\beta_0(1-\beta_1 e^{-\beta_2 t})$$

where

	y	=	dependent variable estimate;
	t	=	time from start of the measurement period;
	β_0	=	estimate of ultimate limiting value or upper asymptote;
	β_1	=	a parameter which is usually unimportant biologically (Richards, 1959); and
	β_2	=	estimate of 'rate constant' which determines the spread of the curve along the time axis.

Parameterisations for each function were presented by Richards (1959) and that for the monomolecular function was adopted here. However, for the logistic function an alternative parameterisation was used following the findings of Ratkowsky (1983). Six parameterisations of the logistic function were examined (Ratkowsky, 1983) to identify those whose behaviour most closely approached that of a linear model. Such parameterisations are advantageous since they enable estimation of parameters which are more unbiased, more normally distributed and which more closely approach minimum possible variance (Ratkowsky, 1983). Another important benefit is the relative ease in obtaining least squares estimates of the parameters. The parameterisation selected was one of two identified by Ratkowsky (1983) as being most suitable for practical use.

3.6.1 SEEDLING EMERGENCE

Time zero was regarded as one day before the first observance of emergence and seedlings were considered emerged when both cotyledons were fully exposed and approximately horizontal. Measurements ceased when seedling numbers were unchanged after two consecutive recordings. Comparisons between the emergence characteristics of "seed" of sheep's burnet, which produces up to two seedlings per hypanthium (Section 2.2.3), and those of lucerne and/or birdsfoot trefoil seed which produce one seedling per seed, were conducted. The number of emerged sheep's burnet seedlings was expressed as though only one seedling was produced per hypanthium. This was achieved by subtracting the number of close seedling pairs ("doubles"), derived from the same hypanthium, from the total number of seedlings emerged. This estimate was referred to as the adjusted seedling number. The proportion of doubles was calculated as the number of doubles divided by adjusted seedling number and it was expressed as a percentage.

Emergence was expressed in two ways, firstly as a percentage of seeds sown and secondly, as a percentage of the final number of seedlings emerged. Both methods of expression have been employed in previous germination and/or emergence studies (Schimpf *et al.*, 1977; Hsu *et al.*, 1984; Scott *et al.*, 1984; Lafond and Baker, 1986a, b; Bahler *et al.*, 1989; Carberry and Campbell, 1989; Jordan and Haferkamp, 1989). Logistic functions were fitted to each datum type. The first method was preferable, where a logistic function could be fitted satisfactorily to the data for each treatment x block combination, since all estimates of $ß_0$, $ß_1$ and $ß_2$ could then be analysed simultaneously in a multivariate analysis of variance (Section 3.7). An important biological advantage of this approach was the ability to analyse jointly the rate of germination ($ß_2$) and the extent of germination ($ß_0$) in the one analysis, and hence determine their relative importance in treatment discrimination. Unfortunately this methodology was only possible for the glasshouse experiment reported in Chapter 6. Difficulties in fitting the logistic function satisfactorily to the data for some of the treatment x block combinations in the experiments described in Chapters 4 and 7, precluded its use there.

In the experiments reported in these chapters, final emergence (%) was estimated independently of rate of emergence as the ratio of the highest seedling number

recorded during the measurement period to the number of seeds sown. In Chapter 7, emergence was also expressed as a percentage of final seedlings emerged. Fitting the function to these data (pooled over blocks) enabled an examination of the effects of treatments on the rate of seedling emergence. In this situation, $ß_0$ was approximately 100% in all cases. For the field trials reported in Chapter 4, the logistic function did not provide a satisfactory fit for all treatment x block sets, nor did other expressions, such as weighted and unweighted linear regressions (Steel and Torrie, 1980), quadratic logistic, and Gompertz and monomolecular functions (Richards, 1959; Schimpf *et al.*, 1977; Ratkowsky, 1983; Scott *et al.*, 1984) provide a satisfactory fit.

An additional approach to examining the rate of emergence was undertaken involving times to reach arbitrary stages of emergence which were of biological interest. These were a low level of emergence (10%=t_{10}), intermediate emergence (50%=t_{50}) and high emergence (90%=t_{90}) and were estimated from the logistic functions fitted, as described in Chapters 6 and 7. For the cumulative emergence data in Chapter 4, freehand curves of unknown function were drawn for each location x species x block combination and the three stages of emergence were estimated from these curves. The practical interpretation and relevance of the estimates of t_{10}, t_{50} and t_{90} were improved by their addition to the number of days after sowing when time zero for the curves was chosen.

3.6.2 FOLIAR REGROWTH

Logistic functions (Richards, 1959; Ratkowsky, 1983) were used to describe the regrowth patterns of young swards of sheep's burnet in Chapter 5. The number of complete leaves (m^{-2}), leaf area of complete leaves (cm^2 m^{-2}), proportion of leaves comprising lamina (%), and herbage mass (kg DM ha^{-1}) were all described by logistic equations. The time of harvest for herbage mass was used as time zero for all functions and further details are presented in Chapter 5. Times to reach 10, 50 and 90% of the final levels (asymptotes) for each character were also estimated, as described previously for seedling emergence data.

Leaf extension measurements were used as indicators of foliar regrowth in the glasshouse defoliation experiments described in Chapters 8 and 9. Although leaf extension may follow a sigmoidal growth pattern (Moore, 1979; Brown and Tanner, 1983; Barlow, 1986), which can probably be described suitably by a logistic function, extension data in the present experiments were fitted with monomolecular functions (Richards, 1959). This was because the initial leaf length measurements were conducted some time after leaf extension first commenced. Thus measurements in the very early stages of extension were unavailable. Furthermore, a true origin where leaf extension was zero could not be ascertained. Measurements were made when it first became practical to measure leaf length with a ruler.

3.6.3 FITTING THE FUNCTIONS

Both logistic and monomolecular functions were fitted by the nonlinear least squares procedure in SAS (1982), called NLIN. The program required that the first derivatives of the equations be specified with respect to each of the three parameters (β_0, β_1 and β_2). The derivatives were determined using standard differentiation methods and were:

1. Logistic

$$\frac{dy}{d\beta_0} = \frac{1}{1+e^{(\beta_1-\beta_2 t)}}$$

$$\frac{dy}{d\beta_1} = \frac{-\beta_0 e^{(\beta_1-\beta_2 t)}}{[1+e^{(\beta_1-\beta_2 t)}]^2}$$

$$\frac{dy}{d\beta_2} = \frac{\beta_0 e^{(\beta_1-\beta_2 t)} t}{[1+e^{(\beta_1-\beta_2 t)}]^2}$$

2. Monomolecular

$$\frac{dy}{d\beta_0} = 1 - \beta_1 e^{-\beta_2 t}$$

$$\frac{dy}{d\beta_1} = -\beta_0 e^{-\beta_2 t}$$

$$\frac{dy}{d\beta_2} = \beta_0 \beta_1 t e^{-\beta_2 t}$$

All symbols were defined near the start of Section 3.6. The Gauss-Newton method of iteration was used to meet the convergence criterion, 10^{-8}, at which the smallest residual was met (Ratkowsky, 1983). Useful features of the printout included an analysis of variance, estimates of the parameters and their asymptotic standard errors, and an asymptotic correlation matrix of the parameters.

3.7 ANALYSES OF VARIANCE

An important part of the statistical analyses in most chapters was multivariate analysis of variance and the estimation of multiple discriminant functions (Section 2.4). These techniques were adopted because of the likelihood that two or more of the characters examined in any experiment were correlated, and this should therefore be accounted for in a worthwhile analysis. Also, the data-set was more the focus than the individual characters. In the highly unlikely situation where all correlations (or covariances) between a set of characters are zero, the outcome and interpretation of the multivariate analysis would be identical to those of the separate univariate analyses of variance.

Most experiments involved the measurement/estimation of a dozen or more characters and the multivariate approach provided a means of: 1) conducting a simultaneous,

overall analysis of variance of these characters; 2) reducing the mass of information into a relatively small number of linear combinations of the original characters; and 3) identifying characters which provided superior discrimination between the various treatments. These matters and others were discussed in Section 2.4 and more detailed information is available in Hope (1968), Cooley and Lohnes (1971), Overall and Klett (1972), Press (1972), Lindeman *et al.* (1980) and Harris (1985).

Multivariate analysis of variance was conducted using the MANOVA option in the ANOVA and GLM procedures of SAS (1982). The treatments in all experiments were regarded preferably as samples of larger populations. That is, it was desired to assume a random effects model (Steel and Torrie, 1980; Lindeman *et al.*, 1980) for all significance tests. However, this was not always possible due to insufficient error degrees of freedom to conduct the multivariate tests. Other models (mainly mixed and fixed effects) were therefore assumed and these are presented in the appropriate chapters.

Each MANOVA produced four different statistics for testing the various hypotheses, including Wilks' likelihood ratio criterion (Section 2.4.3.1), which is recommended the most widely (Press, 1972; Lindeman *et al.*, 1980). Output also included all non-zero characteristic roots (eigenvalues) and associated eigenvectors (Section 2.4.3.1). The elements of each eigenvector were the coefficients of a linear function of the original variables and defined a new variable termed a canonical variable (multiple discriminant function). The proportion of total dispersion in the variance-covariance matrix accounted for by each variable (discriminant power as described in Section 2.4.3.2) was also presented.

The multiple discriminant functions so calculated were difficult to interpret further due to the frequently mixed scales of the original characters plus the variability in the data. This was overcome by requesting a canonical analysis of the hypothesis and error matrices (SAS, 1982), and two "adjusted" types of coefficients for characters in the linear function were calculated. These were:

1. standardised canonical coefficients which were normalised to give canonical variables with unit within-class variance when applied to the standardised variables; and

2. raw (unstandardised) canonical coefficients which were normalised to give canonical variables with unit within-class variance when applied to the original unstandardised characters.

The standardised canonical coefficients (type 1) were utilised to estimate multiple discriminant function mean scores for the different treatments. They had an advantage for interpretative purposes in that they indicated the relative abilities of the standardised characters to discriminate between the treatments. The treatment means of all characters were standardised and used as inputs into the equations to estimate the multiple discriminant function mean scores. The canonical analysis also provided structure matrices (Section 2.4.3.2), containing the correlations between the original variables and the multiple discriminant functions.

The more traditional and still widely adopted approach of examining the characters one by one with univariate analysis of variance (Le Clerg *et al.*, 1962; Steel and Torrie, 1980), was also used but was regarded generally as of secondary importance in discriminating between the various treatments. The models assumed for significance testing (random, mixed and fixed effects) (Steel and Torrie, 1980) were the same as those assumed in the equivalent multivariate analyses. Treatment means and their standard errors were estimated for all characters.

3.8 SIGNIFICANCE LEVELS

The following classification was used for all significance tests:

NS	=	$P > 0.10$;
(NS)	=	$0.10 \geq P > 0.05$;
*	=	$0.05 \geq P > 0.01$;
**	=	$0.01 \geq P > 0.001$;
***	=	$P \leq 0.001$.

CHAPTER 4 : FIELD ESTABLISHMENT AND GROWTH OF SHEEP'S BURNET IN THE LOWER NORTH ISLAND OF NEW ZEALAND

4.1 INTRODUCTION

Evaluations of sheep's burnet in New Zealand have been conducted mainly in Central Otago (eg. Cockayne, 1920a, b; 1921; 1922a, b; Sheppard and Wills, 1985, 1986) and elsewhere in the South Island (NWASCO, 1982; NWASCA, 1986; J S Sheppard, pers. comm.). There have been several recent field evaluations in the lower North Island, including coastal sand country on the east and west coasts, and at a few sites in Hawke's Bay (Douglas, 1985; Foote, unpubl.). Most of these evaluations were conducted with transplanted glasshouse prepared seedlings and in several instances sheep's burnet survived satisfactorily and provided dense low-growing, protective ground cover and adequate herbage production.

These results were sufficiently encouraging to indicate that sheep's burnet may be useful in the lower North Island, and it was therefore desirable to obtain more detailed information on the establishment and vegetative growth of the plant, as well as on its responses to defoliation. Detailed quantitative information on the plant under South Island conditions is also scarce. Hence, the main objective of the present research was to quantify the previously mentioned features of sheep's burnet at potentially suitable lower North Island sites. Two such sites were chosen mainly because of their relatively high summer temperatures, and hence likelihood of drought, and their cold winters and low to moderate soil fertility. Reasonable closeness to Massey University to enable regular monitoring of important growth processes was also a selection criterion.

A further objective was to compare sheep's burnet alongside and in combination with some widely recognised dryland species to appraise, realistically, the possible value of the species. The two legumes, birdsfoot trefoil and particularly lucerne, are used widely in dryland revegetation and have been compared, often qualitatively, with each other and to a lesser extent with sheep's burnet (Ward, 1923; Lunn, 1951; Scott and Charlton, 1983; Daly, 1984; de Lacy, 1985; Rys *et al.*, 1989). In view of their

moderately similar growth habits, regrowth patterns and likely defoliation managements, it was decided to compare sheep's burnet with these species. It was also of interest to evaluate mixtures of the species, where sheep's burnet may derive a yield advantage from the legume association, as reported for various legume - grass mixtures (Haynes, 1980).

4.2 MATERIALS AND METHODS

4.2.1 THE SITES

Trials were located at Flock House, approximately 15 km south-west of Bulls (grid reference; NZMS 1, Tangimoana N148 / 798481) and at the Massey University farm Riverside, about 15 km north of Masterton (grid reference; NZMS 1, Masterton N158 / 111758). The site at Flock House was a yellow brown sand (Himatangi series) while that at Riverside was a shallow stony silt loam to fine sandy loam. Soil fertility levels at both sites were low to moderate (Appendix) and it is under such conditions that sheep's burnet may be similar to, or superior to, higher fertility demanding species such as lucerne. Fertiliser was not applied for two years before the trial started, or during its execution.

The existing vegetation at Flock House, which consisted almost exclusively of weeds such as summer grass, fathen and sheep's sorrel, was sprayed with glyphosate (1.8 kg ha^{-1}) on 24 July, 1985. At Riverside, a perennial ryegrass/white clover pasture predominated and was sprayed on 8 August, 1985 with glyphosate (1.4 kg ha^{-1}) and 0.8 kg ha^{-1} 2,4-D plus 0.4 kg ha^{-1} dicamba.

4.2.2 TREATMENTS AND EXPERIMENTAL DESIGN

There were nine treatments, comprising three monocultures (sheep's burnet, lucerne cv. 'Rere' and birdsfoot trefoil cv. 'Granger') and six binary mixtures involving various arbitrary proportions of sheep's burnet with either legume species, namely:

100% sheep's burnet (S); 75%S:25%L; 50%S:50%L; 25%S:75%L

100% birdsfoot trefoil (T); 75%T:25%S; 50%T:50%S; 25%T:75%S
cv. 'Granger'
100% lucerne cv. 'Rere' (L).

All proportions were based on a viable seed weight basis (Section 4.2.3). Treatments were arranged in three randomised complete blocks and plots measured 3 x 12 m. There were 0.5 m buffers between plots within blocks and 2 m buffers between blocks.

4.2.3 STAND ESTABLISHMENT

In September, some four weeks after spraying, the sites were rotary-hoed, harrowed and Cambridge rolled. Seed was broadcast by hand and each plot then raked lightly to cover most of the seed. Sowings were conducted on 3 September (Flock House) and 17 September (Riverside), 1985. Seed of birdsfoot trefoil was obtained commercially in autumn, 1985 and the sources of the sheep's burnet and lucerne seed were described in Section 3.2. The legume seeds were inoculated with the appropriate *Rhizobium* strains, namely NZP 2238 for birdsfoot trefoil, and NZP 4010 for lucerne. This was conducted within 48 hours of sowing (Cooper, 1977) to ensure that a relatively high number of viable bacterial cells were available for subsequent seedling infection.

Sheep's burnet was sown at 12 kg ha^{-1} (B J Wills, pers. comm.; J S Sheppard, pers. comm.) which gave 141 viable seeds m^{-2}. Sowing rates for lucerne and birdsfoot trefoil to provide similar viable seed numbers m^{-2} were 4.5 kg ha^{-1} and 2.4 kg ha^{-1}, respectively. The rate for lucerne was adequate to establish pure swards in some districts under favourable conditions (Wyn-Williams, 1982) while that for birdsfoot trefoil was lower than recommended (Scott and Charlton, 1983).

At Flock House, emergence of seedlings of sheep's burnet was very poor. Close inspection of superficial and buried seed revealed that in many instances most of the internal parts of the seed had been destroyed completely. Sand dune weevils (*Cecyropa discors* Broun.) were suspected as being the cause of the damage (W

Stiefel, pers. comm.) although this was never verified. Following a spraying of all emerged seedlings with paraquat, the trial was resown on 20 September. Pellets of lindane (a.i.=20%) were applied immediately prior to sowing at 11 kg ha^{-1} and the site was irrigated subsequently. Applications of oxamyl (0.4 kg ha^{-1}) were also made. Resulting seedling emergence was very satisfactory and it was assumed that the contribution of the first sowing to present seed numbers, and hence rate of sowing, was negligible. Hard seed contents of the legume species were also low (Appendix). Emergence at Riverside following the first sowing was satisfactory and therefore no resowing was required.

4.2.4 MEASUREMENTS

Hourly recordings of soil temperature were conducted at each location during the germination and seedling emergence phases at the surface, one, five and ten cm depths. Gravimetric soil moisture contents were determined approximately every second day at depths of 0-5, 5-10 and 10-20 cm using New Zealand Standards (1980). Soil water contents were determined thereafter at Riverside at approximately 1-3 weekly intervals until May, 1986. Air temperature and relative humidity were recorded at Riverside from 21 October, 1985 to 12 February, 1986 usually for 4-5 days every 1-2 weeks.

Seedling counts were made every second day on two randomly sited fixed quadrats (0.125 m^2) per monocultural plot. An emerged seedling had both cotyledon leaves horizontal. Counts were conducted from days 7-23 and days 7-21 after sowing at Riverside and at Flock House, respectively. For the last three recording periods at each site, seedling counts of each species in all mixed plots were also conducted. The number of seedling pairs per quadrat, assumed to be mainly from seed (fruits or hypanthia) producing two seedlings (Sections 2.2.3 and 2.2.5), was also recorded.

No seedling measurements were made at Flock House beyond 21 days after sowing because of the growth of weeds, principally summer grass, fathen and shepherd's purse. Weed growth had likely adverse effects (for example, shading) on the sown species which may have distorted their growth patterns to an unknown extent.

Furthermore, the effects of weeds on seedling growth was not an objective of the studies. Effective control of the weeds was generally unsatisfactory and was hindered by the restricted range of herbicides which could be used safely in the presence of both leguminous and non-leguminous species.

At Riverside, the vegetative growth of each species in monocultural plots was determined by removing four seedlings within two 0.125 m^2 randomly selected quadrats at 38, 45, 52 and 59 days after sowing. At these times, sheep's burnet seedlings averaged respectively at least 2, 3, 5 and 6 fully expanded leaves. For each harvest, seedlings were washed thoroughly in water and then measured for longest foliar and root lengths. At day 59, leaf area per seedling was also determined (Section 3.4). Foliage and roots were separated (Section 3.3) and their dry weights determined (Section 3.4).

The vegetative cover of all plots was determined twice before the first harvest for herbage mass on 28/11/1985 and 9/12/1985 and immediately prior to every harvest in the first season on 17/12/1985, 5/2/1986 and 15/4/1986. The method involved analysing 100 points along a fixed transect (Levy and Madden, 1933). One hit per needle was recorded at all times except on 9/12/1985 and 17/12/1985 where up to 3 hits per needle were recorded to obtain more detailed information on canopy structure within the different swards.

Herbage mass of the swards was determined three times on 17/12/1985, 5/2/1986 and 23/4/1986 by mowing a randomly selected strip in each plot to a height of approximately 5-7 cm. Harvests were conducted when lucerne swards reached 10-30% flowering. A lenient cutting height was adopted for all species since more intense defoliation could well have seriously reduced plant vigour, crown development and regrowth (Musgrave, 1982; Wynn-Williams, 1982; Scott and Charlton, 1983; B J Wills, pers. comm.). Prior to each harvest, two 0.125 m^2 quadrats were cut to a similar height as above to determine the botanical composition of the swards on a dry weight basis.

4.2.5 STATISTICAL ANALYSES

4.2.5.1 ENVIRONMENTAL PARAMETERS

Mean daily soil temperatures at each location and depth, and air temperatures at Riverside were calculated by averaging the minimum and maximum daily values recorded (New Zealand Meteorological Service, 1983). The mean soil temperature data were then regarded as replicates for a given depth and used in a two-tailed t-test (Steel and Torrie, 1980) to compare locations. Gravimetric soil moisture content (%) data during the arbitrarily defined germination and emergence phases at Riverside (17/9-22/10/1985) and Flock House (20/9-22/10/1985), were analysed using univariate split plot analyses of variance over sampling times (Steel and Torrie, 1980). A similar analysis was also conducted for the complete moisture content data (September, 1985-May, 1986) at Riverside.

4.2.5.2 SEEDLING EMERGENCE

All seedling emergence data for sheep's burnet were expressed on the basis of one seedling per seed to facilitate comparisons with lucerne and birdsfoot trefoil (Section 3.6.1). Plot means for all species were calculated subsequently and expressed as a percentage of the 141 viable seeds sown per square metre (Section 4.2.3). Final emergence per plot was taken as the highest count throughout the measurement period.

Variances of the means for all treatments (pure and mixed swards) were quite heterogeneous and transformations to logarithmic, square root and arcsine scales (Steel and Torrie, 1980) failed to markedly reduce this problem. Therefore, a desirable pooled analysis over locations for the emergence data (Cochran and Cox, 1957; Le Clerg *et al.*, 1962) was not conducted. Instead, means and their standard errors were estimated for all treatments and the main treatments of interest, namely the pure swards within and between locations, were compared using pair wise t-tests (Steel and Torrie, 1980).

For pure swards of sheep's burnet, the proportion of "doubles" was estimated (Section 3.6.1) for individual quadrats and plot means at each recording time were

then calculated. Analyses showed that these estimates were not significantly ($P<0.05$) different between recording times within each location and therefore times were used as additional replication in a two-tailed t-test between locations (Steel and Torrie, 1980).

Two analyses were conducted on the final seedling emergence data of mixed swards. Firstly, the percentage of sheep's burnet in each mixture was calculated to determine if there were distinct proportions of the species in the trial. Data were subjected to a pooled analysis of variance over locations (Le Clerg *et al.*, 1962) and a random effects model was assumed for all tests of significance (Steel and Torrie, 1980). The ratios of estimated mean squares were tested (F-test) for significance in the usual manner (Crump, 1951; Le Clerg *et al.*, 1962). For source of variation due to locations, a complex F-ratio (F') was estimated using linear combinations of mean squares (Crump, 1951). Degrees of freedom for the test were estimated using the formula of Satterthwaite (1946). In the second analysis, the final seedling emergences of each species in a mixture were subjected to a chi-square test (Steel and Torrie, 1980) to ascertain the accuracy of the planned arbitrary seedling ratios such as 75%:25% and 50%:50%. Seedling counts were summed over blocks before conducting the tests and a correction for continuity as proposed by Yates (Steel and Torrie, 1980) was used throughout.

Times to reach 10%, 50% and 90% of final emergence were estimated from freehand curves of cumulative emergence for each location x species x block combination (Section 3.6.1). The resulting three characters (t_{10}, t_{50} and t_{90}) were then analysed simultaneously using a multivariate analysis of variance pooled over locations (Section 3.7), which was an extension of the familiar pooled univariate analysis (Le Clerg *et al.*, 1962). There were insufficient error degrees of freedom for multivariate tests (Section 3.7) for species, when using the species x location interaction matrix as the error matrix and therefore the residual matrix was used. For source of variation due to locations, the location(block) matrix was used as the error matrix. This is a crude test, since locations also includes a component due to the species x location interaction, although the test is obviously more valid when the interaction is unimportant.

4.2.5.3 VEGETATIVE GROWTH AND HERBAGE MASS

Seedling foliar length and dry weight, and root dry weight data, were transformed to logarithms to approximately stabilise the variances. Root length data were satisfactory on the original measurement scale. All data were then subjected to a split-plot multivariate analysis of variance (Section 3.7) over the four harvests. Species were regarded as main plots and harvests as sub-plots. A significance test for species used the species x block interaction matrix as the error matrix while all other sources of variation were tested assuming a random effects model (Section 3.7). Leaf area data for monocultural plots were subjected to an analysis of variance on the original measurement scale.

Totals of first hits of sown species per plot were calculated to give percentage sown species and data were analysed using a split-plot analysis of variance, with time as a sub-plot (Steel and Torrie, 1980). A random effects model was assumed for all tests of significance (Section 3.7). For the additional point analysis information recorded on 9/12/1985 and 17/12/1985, the total hits of sown species were expressed as a percentage of total hits of all vegetation (sown species, grasses and weeds) (Analysis D of Levy and Madden, 1933). Data were then subjected to an analysis of variance as described above.

Total herbage mass per plot was expressed as t DM ha^{-1} and the proportion of weeds (%) was calculated using the botanical composition data. Herbage mass and weed content at the three harvests were analysed simultaneously using a multivariate analysis of variance (Section 3.7). Data for each harvest were regarded as separate characters to allow for any covariances between the harvests. A random effects model was assumed for all tests of significance (Steel and Torrie, 1980). A univariate analysis of variance of herbage mass totalled over the three harvests was also conducted, as well as analyses for herbage mass and weed content at each harvest.

4.2.5.3.1 COMPETITION ANALYSES

Herbage mass data for the two replacement series involving sheep's burnet and birdsfoot trefoil, and sheep's burnet and lucerne, were analysed for each harvest and over all harvests using the de Wit (1960) competition model. Mean absolute and relative masses of each species were plotted against their mean proportions in the mixed swards, and relative yield totals (RYT's) and relative crowding coefficients (*k*'s) (Section 2.3.1.2.4) were estimated.

The equations (Section 2.3.1.2.4) describing the non-linear curves were fitted using the NLIN procedure in SAS (1982). The programme required the specification of the first derivative for all parameters in the model. Hence, for species *i* growing in combination with species *j*, herbage mass (Y_{ij}) is described by

$$Y_{ij} = \frac{Mk_{ij}x}{(k_{ij}-1)x+1}$$

where all parameters were defined in Section 2.3.1.2.4, and the first derivatives are

$$\frac{dY_{ij}}{dM} = \frac{k_{ij}x}{(k_{ij}-1)x+1}$$

and

$$\frac{dY_{ij}}{dk_{ij}} = \frac{Mx}{(k_{ij}-1)x+1} - \frac{Mkx^2}{[(k_{ij}-1)x+1]^2}$$

$$= \frac{Mx(1-x)}{[(k_{ij}-1)x+1]^2}$$

Similar derivatives were obtained for the equation (Section 2.3.1.2.4) describing the herbage mass of species *j* growing with species *i* (Z_{ji}). The seedling proportions obtained in the field were used as the points on the horizontal axis.

The NLIN procedure was advantageous in providing asymptotic standard errors for the parameter estimates and of particular interest were those for the k estimates. The k-product was of biological interest since according to the de Wit (1960) model, if it is equal to unity, the species are regarded as exhibiting competitive interference and being mutually exclusive (Section 2.3.1.2.4.2). Alternatively, if the product is >1,

the species are showing non-competitive interference in addition to competitive interference (Section 2.3.1.2.4.2). To enable statistical tests (t-tests) of the k-product being equal to or >1, the standard error of the product of the k's was calculated from the approximation of the variance of a product (Kendall and Stuart,1958), namely,

$$\sigma_{xy}^2 = x^2\sigma_y^2 + y^2\sigma_x^2 - \sigma_x^2\sigma_y^2 + cov(x^2y^2) - cov^2(xy) - 2xycov(xy)$$

Since the curves for each species were fitted independently, all covariances were assumed zero.

In order to determine if there were differences in competitive power/ability between harvests for each species, pairwise two-tailed t-tests (Steel and Torrie, 1980) were conducted. Similar t-tests between the k estimates of the two species, that is sheep's burnet and lucerne, and sheep's burnet and birdsfoot trefoil, were also conducted at each harvest and in the pooled analysis to identify any differences in competitive ability between the mixture components. The k-products at each harvest and in the pooled analysis were tested for departure from unity using two-tailed t-tests, and where the result was significant ($P<0.05$), a one-tailed t-test was used to test if the k-product was greater than one.

Total herbage masses of all pure and mixed swards in the two replacement series were also subjected to analysis of variance.

There was practical interest in how the interpretations about competition between sheep's burnet and the legumes would change if the originally planned ratios (Section 4.2.2) had been used in the analyses. Hence, the above analyses were repeated using the original ratios with attention focused again on the k estimates and their product.

4.3 RESULTS

4.3.1 ENVIRONMENTAL PARAMETERS

Data on the air temperatures and mean relative humidity recorded at Riverside between 21 October, 1985 and 12 February, 1986 are presented in Table 4.1. Mean air temperature ranged from 10.4°C in October to 18.2°C in January. The highest relative humidity was recorded in October and the lowest in November.

Soil temperatures at each depth were higher at Flock House than at Riverside, usually by about 4°C (Table 4.2), and temperatures decreased as depth increased. Contents of soil moisture at both locations were relatively low at 10-20 cm depth compared with those at shallow depths (Table 4.2). There was also variation in mean moisture content at different sampling times at Flock House which ranged from 12.2-20.0%. In the analysis of all moisture content data at Riverside, highly significant ($P<0.001$) effects were found for soil depth and sampling time, and their interaction. Interaction means were mostly in the range of 25-45% moisture content and the overall mean was 34.2% (SE=0.5%).

4.3.2 SEEDLING EMERGENCE

Overall final seedling emergences (%) for all swards were similar at the two locations (Table 4.3) and averaged 60.9% at Flock House and 50.5% at Riverside. There were no significant differences between pure swards within each location but the level of emergence of sheep's burnet at Flock House (66.2%) was superior to that at Riverside (26.5%) (Table 4.3). Both pure legume swards had similar emergences at the two locations. An establishment objective to achieve approximately uniform levels of emergence of all swards was mostly successful and emergence averaged over all swards and locations was about 56%.

The mean proportions of doubles (%) in pure swards of sheep's burnet were 31.1% (SE=3.2%) at Flock House and 25.1% (SE=6.5%) at Riverside. Seedling pairs were

TABLE 4.1 Air temperature (°C) and relative humidity (%) at Riverside from October 1985-February 1986.

Parameter	>21 October	November	December 1985	January 1986	<12 February
No. of days recorded	6	19	22	20	10
Highest recorded (°C)	17	24	26	29	28
Average daily maximum (°C)	13.7	18.6	18.9	23.1	23.4
Mean (°C)	10.4	13.9	15.3	18.2	17.8
Average daily range (°C)	6.7	9.4	7.3	9.9	11.3
Average daily minimum (°C)	7.0	9.2	11.6	13.2	12.1
Lowest recorded (°C)	4	2	8	5	7
Relative humidity at 9 am (%)	78	64	69	68	76

TABLE 4.2 Mean soil temperature (°C) and moisture (%) at various depths during the germination and emergence phases at Flock House and Riverside in spring, 1985 (standard errors in parentheses).

	Soil temperature (°C)	
Depth (cm)	Flock House	Riverside
0	19.4 (0.6)	15.2 (0.4)
1-2	17.4 (0.4)	13.2 (0.3)
5	16.5 (0.3)	12.0 (0.2)
10	16.0 (0.3)	11.7 (0.2)
	Soil moisture (%)	
0-5	17.1 (0.4)	43.9 (0.8)
5-10	17.6 (0.6)	35.1 (0.6)
10-20	12.1 (0.5)	29.8 (0.3)

TABLE 4.3 Mean final seedling emergence (%) for all swards at Flock House and Riverside in spring, 1985 (standard errors in parentheses).

Sward	Flock House (%)	Riverside (%)	Sward mean
100% sheep's burnet	66.2 (2.5)	26.5 (5.0)	46.3 (9.2)
100% lucerne	63.4 (13.3)	86.1 (28.0)	74.7 (14.8)
100% birdsfoot trefoil	45.4 (13.4)	33.1 (3.4)	39.2 (6.8)
75% sheep's burnet: 25% lucerne	64.3 (20.6)	54.8 (18.4)	59.6 (12.5)
50% sheep's burnet: 50% lucerne	71.9 (0.9)	65.2 (5.7)	68.6 (3.0)
25% sheep's burnet: 75% lucerne	43.5 (10.7)	37.8 (3.8)	40.7 (5.2)
75% sheep's burnet: 25% birdsfoot trefoil	76.6 (24.7)	48.2 (8.2)	62.4 (13.3)
50% sheep's burnet: 50% birdsfoot trefoil	57.7 (7.7)	58.6 (18.1)	58.2 (8.8)
25% sheep's burnet: 75% birdsfoot trefoil	59.6 (11.5)	44.4 (6.6)	52.0 (6.8)
Location mean	60.9 (4.3)	50.5 (5.0)	55.7

TABLE 4.4 Mean percentage of sheep's burnet seedlings in all binary mixtures with birdsfoot trefoil and lucerne at Flock House and Riverside in spring, 1985

Sward	Flock House (%)	Riverside (%)	Sward mean
25% sheep's burnet: 75% lucerne	35.7	13.9	24.8 c
25% sheep's burnet: 75% birdsfoot trefoil	54.4	13.9	34.1 c
50% sheep's burnet: 50% lucerne	65.8	37.6	51.7 b
50% sheep's burnet: 50% birdsfoot trefoil	63.1	32.6	47.8 b
75% sheep's burnet: 25% lucerne	82.4	70.7	76.6 a
75% sheep's burnet: 25% birdsfoot trefoil	86.8	62.5	74.6 a
Location mean	64.7 a +	38.5 b	

+ Figures scored by different letters differ at the 5% significance level

first observed at Flock House thirteen days after sowing, and at Riverside six days later.

The mean percentage of sheep's burnet seedlings in all mixtures with the two legumes at each location, are presented in Table 4.4. There were significant ($P<0.01$) differences between locations and swards, with performance at Flock House (64.7%) superior to that at Riverside (38.5%). Regardless of the companion legume, sward means accurately reflected the intended proportions of sheep's burnet. Swards had significantly decreasing means in the order 75% > 50% > 25% sheep's burnet (Table 4.4). The results of chi-square tests provided additional interpretations on the component ratios within the mixed swards (Table 4.5). Rather than comparisons between swards, these tests examined the seedling ratios within each sward. The planned ratio of 75% sheep's burnet to 25% lucerne was achieved in the field at Riverside. However, all other ratios were not attained accurately, as indicated by the significant chi-square tests (Table 4.5).

There were significant differences between locations and species in the multivariate analysis of times to reach various stages of emergence (Table 4.6a). The validity of using the location(block) matrix as the error matrix for testing locations was strengthened because of the absence of a species x location interaction. The most important character in the single discriminant function for locations (Table 4.6a) was time to reach 50% final emergence (t_{50}). It had a positive loading and was approximately four times more important than t_{90} (Table 4.6b). Location means for these characters (Table 4.6c) showed that emergence at Flock House was 3-4 days earlier than at Riverside.

The first of two discriminant functions for species accounted for 97% of total dispersion in the data (Table 4.7a) and in this function t_{10} and t_{50} were approximately equal in importance and had positive loadings (Table 4.7b). The results showed that sheep's burnet was slower to emerge than birdsfoot trefoil and lucerne, and that lucerne was the quickest emerging species. Univariate means indicated a similar pattern (Table 4.7c).

TABLE 4.5 Total seedling numbers (per m^2) of binary mixtures and their components together with the results of a chi-square test to ascertain the accuracy of planned seedling ratios in the field.

	Flock House					Riverside				
Sward	total seedlings (m^{-2})	sheep's burnet (m^{-2})	birdsfoot trefoil (m^{-2})	lucerne (m^{-2})	chi-square +	total seedlings (m^{-2})	sheep's burnet (m^{-2})	birdsfoot trefoil (m^{-2})	lucerne (m^{-2})	chi-square
25% sheep's burnet: 75% lucerne	184	60	-	124	4.36*	160	24	-	136	8.01**
50% sheep's burnet: 50% lucerne	304	200	-	104	29.69***	276	104	-	172	16.26***
75% sheep's burnet: 25% lucerne	272	220	-	52	4.71*	232	164	-	68	2.07
75% sheep's burnet: 25% birdsfoot trefoil	324	280	44	-	21.93***	204	132	72	-	10.99***
50% sheep's burnet: 50% birdsfoot trefoil	244	152	92	-	14.27***	248	100	148	-	8.91**
25% sheep's burnet: 75% birdsfoot trefoil	252	136	116	-	111.24***	188	24	164	-	14.36***

+ with 1 degree of freedom

TABLE 4.6a Important statistics for the discriminant function for locations involving times to reach various stages of emergence.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significar
1	100.00	56.22	3	2	*

Table 4.6b Mean scores for the single discriminant function for locations.

Location	Mean score
Flock House	-4.33
Riverside	4.33

two largest standardised coefficients:

1. t_{50} (12.48)
2. t_{90} (-3.26)

Table 4.6c Location means for t_{50} and t_{90} (standard errors in parentheses).

Location	t_{50} (days)	t_{90} (days)
Flock House	10.4 (0.7)	13.6 (0.7)
Riverside	13.3 (1.1)	18.1 (0.6)

TABLE 4.7a Important statistics for the discriminant functions for species involving times to reach various stages of emergence.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	97.12	10.92	6	12	***
2	2.88	2.45	2	7	NS

Table 4.7b Mean scores for the first discriminant function for species.

Species	Mean score
birdsfoot trefoil	0.15
lucerne	-4.04
sheep's burnet	3.89

two largest standardised coefficients:

1. t_{10} (1.95)
2. t_{50} (1.79)

Table 4.7c Species means for t_{10} and t_{50} (standard errors in parentheses).

Species	t_{10} (days)	t_{50} (days)
birdsfoot trefoil	8.5 (0.7)	12.1 (0.8)
lucerne	6.7 (0.4)	8.8 (0.4)
sheep's burnet	11.4 (0.9)	14.6 (1.0)

4.3.3 VEGETATIVE GROWTH AND HERBAGE MASS

There were highly significant differences ($P<0.001$) between the four harvests according to the multivariate analysis of variance. Other sources of variation were nonsignificant. For species however, the result for Wilks' criterion was not consistent with that of another multivariate test, namely Pillai's trace ($F=10.91$ with 8,4 degrees of freedom), which was significant at the 5% level of probability. Because of this discrepancy, it was decided to estimate the discriminant functions for species (Table 4.8a). The first function accounted for most of the total variability (87%) and therefore further results for this function are presented henceforth. Foliar dry weight, with a standardised coefficient of 6.8, was the most important character and was about four times more important than the second most influential character, namely root dry weight (Table 4.8b). Species rankings on the function were sheep's burnet > lucerne > birdsfoot trefoil (Table 4.8b) and the inferiority of birdsfoot trefoil was also shown by species' means for foliar and root dry weights (Table 4.8c). Estimates of leaf area at 59 days after sowing also partly supported these patterns with sheep's burnet being similar to lucerne but significantly greater than birdsfoot trefoil (Table 4.8d).

The first of three discriminant functions for harvests accounted for about 87% of the dispersion (Table 4.9a) and again foliar dry weight was the most important character (Table 4.9b). Foliar length was approximately half as important and there was an overall increase in mean score with increasing days after sowing (Table 4.9b), which was also shown univariately (Table 4.9c).

Results of the point analysis of first hits of sown species are presented in Table 4.10. The only significant effect was that due to time and there was an increase in mean hits over the first four recording times (Table 4.10). Despite some notable specific differences in ground cover, such as between pure swards of sheep's burnet and the two legume species (Table 4.10), the overall F-test for swards was not significant when assuming a random effects model. In the more detailed point analysis at two recording times (Table 4.11), pure swards of sheep's burnet were superior to those of both lucerne and birdsfoot trefoil. As well, all mixed swards involving sheep's

TABLE 4.8a Important statistics for the two discriminant functions for species involving vegetative characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	86.58	7.92	8	2	NS
2	13.42	8.20	3	2	NS

Table 4.8b Mean scores for the first discriminant function for species

Species	Mean score
birdsfoot trefoil	-3.99
lucerne	0.89
sheep's burnet	3.10

two largest standardised coefficients:

1. foliar dry weight (6.84)
2. root dry weight (-1.68)

Table 4.8c Species means for foliar and root dry weight (standard errors in parentheses).

Species	foliar dry weight (log)	root dry weight (log)
birdsfoot trefoil	1.96 (0.18)	1.58 (0.08)
lucerne	3.05 (0.21)	2.41 (0.08)
sheep's burnet	3.23 (0.23)	2.23 (0.10)

Table 4.8d Leaf area (cm^2) per seedling at 59 days after sowing at Riverside (standard errors in parentheses).

Species	Mean (cm^2)
birdsfoot trefoil	4.10 (0.77)
lucerne	9.66 (1.35)
sheep's burnet	13.90 (1.80)

TABLE 4.9a Important statistics for the three discriminant functions for harvests involving vegetative characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significa
1	86.79	17.00	12	8	***
2	11.86	7.77	6	8	**
3	1.34	4.46	2	5	(NS)

Table 4.9b Mean scores for the first discriminant function for harvests.

Harvest (days after sowing)	Mean score
38	-4.84
45	-3.09
52	1.48
59	6.46

two largest standardised coefficients:

1. foliar dry weight (4.25)
2. foliar length (2.10)

Table 4.9c Harvest means for foliar dry weight and length (standard errors in parentheses).

Harvest (days after sowing)	foliar dry weight (log)	foliar length (log)
38	2.02 (0.17)	3.17 (0.08)
45	2.30 (0.18)	3.18 (0.07)
52	2.91 (0.24)	3.56 (0.09)
59	3.74 (0.24)	3.94 (0.09)

TABLE 4.10 Mean percentage of first hits of sown species in pure and mixed swards at Riverside in 1985/1986 (standard errors in parentheses).

	Recording time					
	1985			1986		
Sward.	28/11	9/12	17/12	5/2	15/4	Sward mean
100% sheep's burnet	37.7	59.3	70.0	79.0	72.3	63.7 (4.6)
100% lucerne	13.7	16.0	19.0	35.7	40.7	25.0 (3.6)
100% birdsfoot trefoil	7.3	10.3	29.3	51.0	40.3	27.7 (5.1)
75% sheep's burnet: 25% lucerne	36.0	51.3	75.0	81.3	74.0	63.5 (5.4)
50% sheep's burnet: 50% lucerne	34.0	53.0	68.3	81.3	73.3	62.0 (4.6)
25% sheep's burnet: 75% lucerne	33.7	44.3	49.3	65.7	65.7	51.7 (4.4)
75% sheep's burnet: 25% birdsfoot trefoil	36.3	62.7	77.3	85.7	78.7	68.1 (5.2)
50% sheep's burnet: 50% birdsfoot trefoil	29.3	41.3	63.7	73.3	74.0	56.3 (5.5)
25% sheep's burnet: 75% birdsfoot trefoil	19.7	31.0	47.0	66.0	63.7	45.5 (6.0)
Time mean	27.5 d+	41.0 c	55.4 b	68.8 a	64.7 a	

+ Figures scored by different letters differ at the 5% significance level.

TABLE 4.11 Means of total hits of sown species as a percentage of total hits of all vegetation in pure and mixed swards at Riverside in 1985.

Sward	Recording time 9/12 1985	17/12 1985	Sward mean
100% sheep's burnet	77.3	86.4	81.9 ab
100% lucerne	41.4	29.7	35.5 d
100% birdsfoot trefoil	30.9	47.3	39.1 d
75% sheep's burnet: 25% lucerne	78.0	88.3	83.1 ab
50% sheep's burnet: 50% lucerne	87.4	86.7	87.0 a
25% sheep's burnet: 75% lucerne	69.0	63.0	66.0 bc
75% sheep's burnet: 25% birdsfoot trefoil	84.7	88.8	86.7 a
50% sheep's burnet: 50% birdsfoot trefoil	74.9	75.5	75.2 abc
25% sheep's burnet: 75% birdsfoot trefoil	60.5	67.5	64.0 c
Time mean	67.1	70.3	

+ Figures scored by different letters differ at the 5% significance level.

burnet also had significantly higher means than the pure legume swards. There was no overall difference between the two time means (Table 4.11).

Herbage masses and weed contents of all swards were similar according to the results of the multivariate analysis of variance (Section 4.2.5.3) and the character means and their standard errors for all swards at each harvest are presented in Table 4.12. Only differences between swards were detected for herbage mass at the first harvest and these were due mainly to the frequent superiority of sheep's burnet and lucerne over birdsfoot trefoil. For all swards, herbage masses totalled over the three harvests were similar and averaged 6.3 t DM ha^{-1} (Table 4.12). Estimates of mean herbage mass (regrowth) at harvests two (3.5 t DM ha^{-1}) and three (2.0 t DM ha^{-1}) were similar (Table 4.12) and averaged approximately 2.8 t DM ha^{-1}. Mean herbage accumulation between harvests one and two was 70 kg DM ha^{-1} d^{-1} which was about three times greater than that between harvests two and three, namely 26 kg DM ha^{-1} d^{-1}.

4.3.3.1 COMPETITION ANALYSES

4.3.3.1.1 SHEEP'S BURNET / LUCERNE SWARDS

Total herbage masses for all pure and mixed swards of the sheep's burnet/lucerne replacement series were similar within each harvest and means are presented in Table 4.12. The similarity in total absolute yields (Figure 4.1) also meant that relative yields were alike and that their totals (RYT) were approximately unity at each harvest. Relative crowding coefficient (k) estimates are presented in Table 4.13 and they were all similar between harvests for each species at the 5% level of significance. However, the harvest one estimate for lucerne was significantly higher than estimates at the other two harvests at $P<0.10$. The k estimates for sheep's burnet appeared larger than those for lucerne at each harvest and in the pooled analysis (Table 4.13), but due to sometimes considerable variability in the estimates, there were no significant ($P<0.05$) differences between the estimates. An exception was at harvest two where sheep's burnet was greater than lucerne ($P<0.01$). Hence, in most

TABLE 4.12 Mean herbage masses (t DM/ha) and weed contents (%) of pure and mixed swards at Riverside in the establishment year (standard errors in parentheses).

Sward	Harvest time: 17/12/1985 Herbage mass (t DM/ha)	17/12/1985 Weed (%)	5/2/1986 Herbage mass (t DM/ha)	5/2/1986 Weed (%)	23/4/1986 Herbage mass (t DM/ha)	23/4/1986 Weed (%)	Total mass (t DM/ha)
100% sheep's burnet	0.94 (0.07) ab+	12.7 (0.6)	3.33 (0.70)	12.2 (2.0)	1.76 (0.81)	13.2 (8.1)	6.03 (1.44)
100% lucerne	0.75 (0.03) bcd	6.7 (2.5)	3.11 (0.30)	10.0 (7.0)	2.48 (0.20)	15.9 (7.3)	6.34 (0.46)
100% birdsfoot trefoil	0.44 (0.08) e	12.4 (1.3)	3.99 (0.88)	29.9 (7.7)	1.85 (0.41)	31.1 (6.0)	6.27 (0.91)
75% sheep's burnet:							
25% lucerne	0.84 (0.10) abc	13.5 (4.0)	3.98 (0.34)	11.4 (6.3)	1.49 (0.44)	16.2 (5.6)	6.32 (0.24)
50% sheep's burnet:							
50% lucerne	0.88 (0.10) abc	5.0 (1.3)	3.53 (0.30)	11.0 (3.5)	2.11 (0.49)	14.6 (3.7)	6.52 (0.63)
25% sheep's burnet:							
75% lucerne	1.04 (0.07) a	7.3 (1.3)	3.41 (0.32)	16.9 (6.0)	2.59 (0.65)	12.9 (4.7)	7.05 (0.84)
75% sheep's burnet:							
25% birdsfoot trefoil	0.68 (0.17) cde	2.9 (0.7)	3.40 (0.10)	11.9 (1.7)	2.01 (0.63)	7.1 (3.3)	6.08 (0.57)
50% sheep's burnet:							
50% birdsfoot trefoil	0.86 (0.13) abc	9.2 (3.6)	2.92 (0.18)	17.2 (3.4)	1.68 (0.18)	13.3 (3.4)	5.46 (0.35)
25% sheep's burnet:							
75% birdsfoot trefoil	0.59 (0.04) de	7.7 (3.8)	3.58 (0.09)	11.2 (2.7)	2.13 (0.34)	24.6 (5.1)	6.30 (0.28)
Time mean	0.78 (0.14)	8.6 (4.2)	3.47 (0.80)	14.6 (9.0)	2.01 (0.89)	16.5 (9.8)	6.26 (1.33)

+ Figures scored by different letters differ at the 5% significance level.

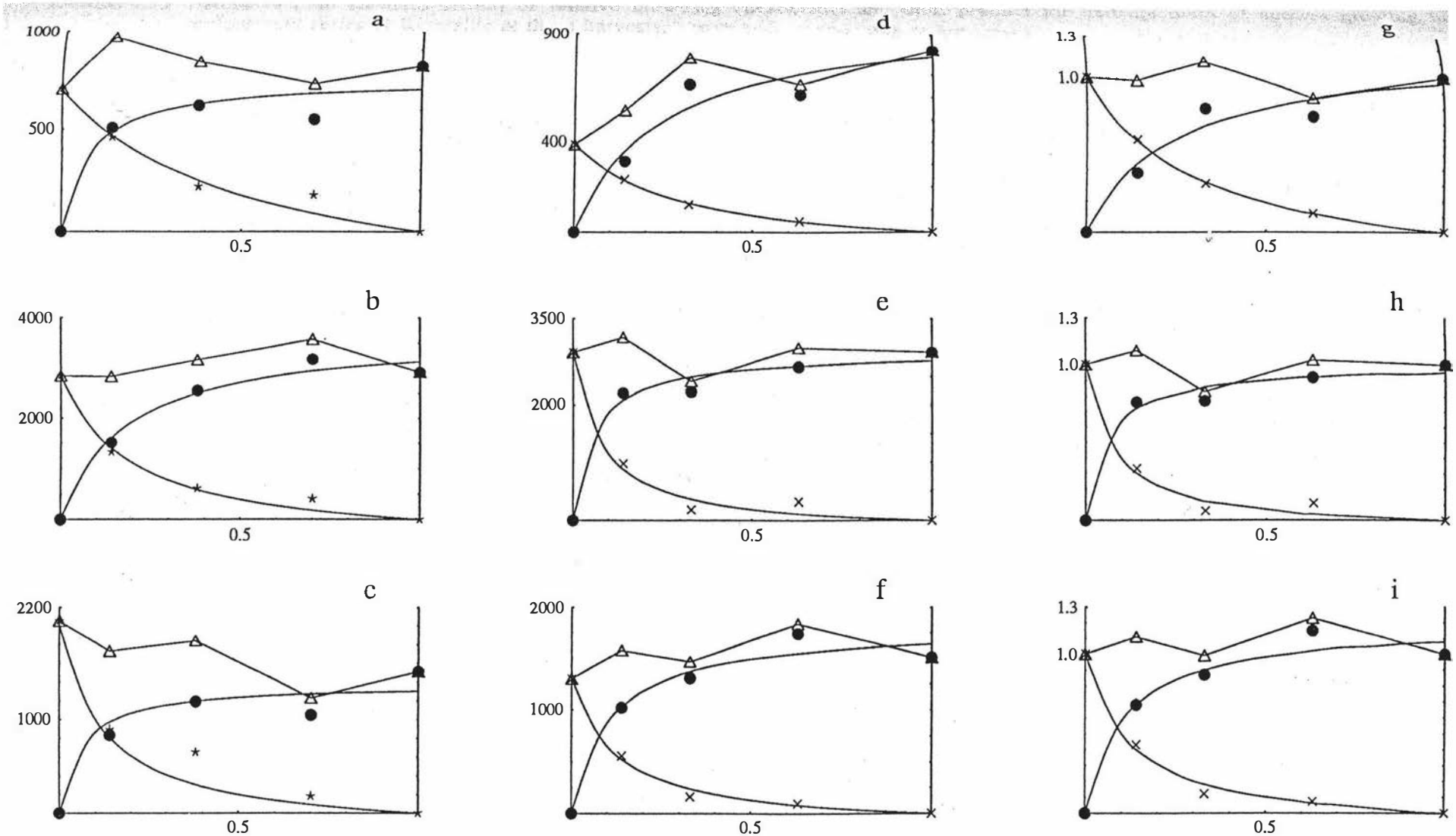


Figure 4.1 Replacement diagrams for sheep's burnet/legume mixtures at Riverside. Graphs a-c are herbage masses (kg DM/ha on the vertical axis) of sheep's burnet (•) and lucerne (⋆) against seedling proportion at harvests 1-3, respectively. Graphs d-f are herbage masses of sheep's burnet (•) and birdsfoot trefoil (×) against seedling proportion at harvests 1-3, respectively and graphs g-i involve the corresponding relative yields. The proportion of sheep's burnet increases from left to right on all graphs and yield totals are represented by △ .

TABLE 4.13 Estimates (and standard errors) of relative crowding coefficients and their product for herbage mass of species growing in two replacement series at Riverside at three harvests.

Mixture	Seedling proportions used	Species	Harvest time 17/12/1985		Harvest time 05/02/1986		Harvest time 23/04/1986		Pooled analysis	
sheep's burnet/ lucerne	realised	{ sheep's burnet	14.02	(11.11)	6.44	(1.67)	18.04	(11.86)	9.23	(11.38)
		{ lucerne	0.34	(0.09)	0.16	(0.03)	0.11	(0.05)	0.16	(0.09)
		{ product (k_{sl} x k_{ls})	4.77	(3.86)	1.03	(0.32)	1.98	(1.47)	1.48	(1.72)
	planned	{ sheep's burnet	6.12	(4.27)	3.39	(1.13)	4.51	(2.44)	3.84	(4.51)
		{ lucerne	0.60	(0.13)	0.30	(0.03)	0.32	(0.06)	0.34	(0.17)
		{ product (k_{sl} x k_{ls})	3.67	(2.62)	1.02	(0.35)	1.44	(0.81)	1.31	(1.48)
sheep's burnet/ birdsfoot trefoil	realised	{ sheep's burnet	5.11	(2.13)	18.69	(8.04)	10.36	(4.15)	11.89	(15.21)
		{ birdsfoot trefoil	0.23	(0.01)	0.07	(0.02)	0.11	(0.02)	0.09	(0.07)
		{ product (k_{sb} x k_{bs})	1.18	(0.49)	1.31	(0.66)	1.14	(0.49)	1.07	(1.20)
	planned	{ sheep's burnet	2.43	(0.95)	9.06	(3.92)	5.12	(2.16)	5.78	(7.42)
		{ birdsfoot trefoil	0.46	(0.02)	0.15	(0.04)	0.22	(0.03)	0.19	(0.15)
		{ product (k_{sb} x k_{bs})	1.12	(0.44)	1.36	(0.67)	1.13	(0.50)	1.10	(1.22)

TABLE 4.13 Estimates (and standard errors) of relative crowding coefficients and their product for herbage mass of species growing in two replacement series at Riverside at three harvests.

Mixture	Seedling proportions used	Species	Harvest time						Pooled analysis	
			17/12/1985		05/02/1986		23/04/1986			
sheep's burnet/ lucerne	realised	{ sheep's burnet	14.02	(11.11)	6.44	(1.67)	18.04	(11.86)	9.23	(11.38)
		{ lucerne	0.34	(0.09)	0.16	(0.03)	0.11	(0.05)	0.16	(0.09)
		{ product (k_{sl} x k_{ls})	4.77	(3.86)	1.03	(0.32)	1.98	(1.47)	1.48	(1.72)
	planned	{ sheep's burnet	6.12	(4.27)	3.39	(1.13)	4.51	(2.44)	3.84	(4.51)
		{ lucerne	0.60	(0.13)	0.30	(0.03)	0.32	(0.06)	0.34	(0.17)
		{ product (k_{sl} x k_{ls})	3.67	(2.62)	1.02	(0.35)	1.44	(0.81)	1.31	(1.48)
sheep's burnet/ birdsfoot trefoil	realised	{ sheep's burnet	5.11	(2.13)	18.69	(8.04)	10.36	(4.15)	11.89	(15.21)
		{ birdsfoot trefoil	0.23	(0.01)	0.07	(0.02)	0.11	(0.02)	0.09	(0.07)
		{ product (k_{sb} x k_{bs})	1.18	(0.49)	1.31	(0.66)	1.14	(0.49)	1.07	(1.20)
	planned	{ sheep's burnet	2.43	(0.95)	9.06	(3.92)	5.12	(2.16)	5.78	(7.42)
		{ birdsfoot trefoil	0.46	(0.02)	0.15	(0.04)	0.22	(0.03)	0.19	(0.15)
		{ product (k_{sb} x k_{bs})	1.12	(0.44)	1.36	(0.67)	1.13	(0.50)	1.10	(1.22)

cases the analyses showed that the competitive power or ability of the two species was equal.

The k-products at all harvests and in the pooled analysis were not significantly ($P<0.05$) different from unity which indicated that sheep's burnet and lucerne were mutually exclusive/competing for the same environmental resources (competitive interference).

4.3.3.1.2 SHEEP'S BURNET / BIRDSFOOT TREFOIL SWARDS

The total herbage masses of members of the sheep's burnet/birdsfoot trefoil replacement series (Table 4.12) were similar within each harvest. Swards at harvest one were an exception where sheep's burnet yielded significantly ($P<0.05$) more than birdsfoot trefoil and 25% sheep's burnet : 75% birdsfoot trefoil. The graphs of relative yield at each harvest (Figure 4.1) showed that RYT was approximately one. All relative crowding coefficients (k) for sheep's burnet were similar (5% level) at each harvest while the k estimate for birdsfoot trefoil at harvest one was greater ($P<0.01$) than those of later harvests (Table 4.13). The estimates for harvests two and three were not significantly different at the 10% level. Sheep's burnet had a higher k estimate than birdsfoot trefoil at harvests one and two ($P<0.10$), and at harvest three ($P<0.05$), but estimates were not significantly different in the pooled analysis over the three harvests. Hence, most of the results indicated that sheep's burnet was more competitive than birdsfoot trefoil in mixtures.

Sheep's burnet and birdsfoot trefoil were competing for the same environmental resources as indicated by the k-products for each harvest and the pooled analysis not being significantly different from unity. These findings were similar to those found in sheep's burnet and lucerne mixtures (Section 4.3.3.1).

4.3.3.1.3 PLANNED SEEDLING PROPORTIONS

In the sheep's burnet/lucerne replacement series, the k estimates for sheep's burnet were not significantly ($P<0.05$) different between harvests, while the estimate for

lucerne at harvest one was greater ($P<0.10$) than at the other harvests (Table 4.13). Comparisons between species at each harvest and in the pooled analysis showed that all k estimates were similar except at harvest two where sheep's burnet was significantly ($P<0.05$) greater than lucerne. All k-products were not significantly different from unity.

Tests for the sheep's burnet/birdsfoot trefoil replacement series found that sheep's burnet had similar k estimates at all harvests, but birdsfoot trefoil was more competitive at harvest one than at later harvests. Sheep's burnet had a significantly ($P<0.10$) higher k estimate than birdsfoot trefoil at all harvests. No k-product was significantly ($P<0.05$) different from unity.

From the results presented above for planned seedling proportions and those found for seedling proportions realised in the field (Sections 4.3.3.1.1. and 4.3.3.1.2), it can be seen that interpretations about relative competitive power/ability and the occurrence of competitive interference (mutual exclusion), were very similar.

4.4 DISCUSSION

Establishing plants at Riverside experienced air temperatures and relative humidities (Table 4.1) which were very similar to the long term (at least 38 years) averages recorded at the Waingawa meteorological station near Masterton (New Zealand Meteorological Service, 1983). The trial was therefore conducted during a typical season. Due to the early termination of the trial at Flock House (Section 4.2.4), similar comparisons with long-term averages at that location were unwarranted. Mean temperatures of the sandy soil at Flock House were consistently higher than for the silt loam at Riverside by about 4°C (Section 4.3.1). For the 10 cm depth, long-term averages show a similar trend but the difference is smaller (New Zealand Meteorological Service, 1983). At Flock House in September, for example, the average soil temperature is 10.0°C whereas at Waingawa the corresponding mean is 8.5°C.

Soil moisture levels during germination and emergence at the two locations, and during the later establishment and regrowth phases at Riverside (Section 4.3.1), were generally very satisfactory for plant survival and growth. At Flock House, all mean soil moisture contents were greater than the permanent wilting point (5%) and generally less than the moisture content at field capacity (20-35%), for the soil at the trial site (Stiefel, unpubl). A similar situation occurred for the silt loam at Riverside with all soil moisture contents greatly exceeding the permanent wilting point (4-6%), as determined by a pressure plate apparatus (Todd, unpubl.).

Although most seedling emergences of the various swards within and between locations were similar (Section 4.3.2), a notable exception was the superior emergence of sheep's burnet at Flock House compared with at Riverside. This was probably due to the higher soil temperatures experienced at the sandy site, which were in the range of 16.0-19.4°C (Table 4.2). These findings were in agreement with those from studies on other species where temperature frequently had a major influence on the proportion of seeds germinating and/or emerging (Woods and MacDonald, 1971; McElgunn, 1973; Hur and Nelson, 1985; Charlton *et al.*, 1986; Hampton *et al.*, 1987). The differences in emergence of sheep's burnet between the two locations suggested that this species was more sensitive to variations in temperature than lucerne and birdsfoot trefoil and this may lead to possible differences in subsequent establishment, ground cover and forage yield. However, the hypothesis could not be tested presently due to the early termination of the trial at Flock House. Emergence of pure swards of sheep's burnet at Flock House (66%) and at Riverside (26.5%) were in partial agreement with findings overseas. In Sweden, emergence is usually between 30 and 60% (Nordborg, 1967b) while in Spain, field emergence is frequently more than 50% (Salmeron, 1966).

The quicker emergence rate of all species at Flock House compared with at Riverside (Section 4.3.2) was probably due to the relatively high temperatures at the former site. Times to reach various stages of emergence at Flock House were about 3-4 days shorter than those at Riverside. The response of emergence rate of sheep's burnet to temperature was undocumented previously but the relationship is well known for lucerne (Hampton *et al.*, 1987) and birdsfoot trefoil (Woods and

MacDonald, 1971; Hur and Nelson, 1985). The results suggested that sowing into warm soils, by selection of appropriate locations and/or sowing times, could hasten sward establishment and associated ground cover. The two locations differed mainly in time to reach 50% emergence (Table 4.6b) and estimation of this character alone in future studies should provide satisfactory discrimination between locations.

Sheep's burnet had a slower overall emergence rate than birdsfoot trefoil and lucerne (Tables 4.7b and c). Lucerne is renowned for its rapid emergence (Cooper, 1977) while birdsfoot trefoil is slow and non-competitive during establishment (Scott and Charlton, 1983; Curtis and McKersie, 1984; Hur and Nelson, 1985) and the results supported these findings. They suggested that sheep's burnet might not be overly competitive at an early stage of development and therefore the species should only be sown alone or in mixtures with other slowly emerging species. Thorough weed control practices should also be adopted. Similar recommendations are made currently (Sheppard and Wills, 1985, 1986; NWASCA, 1986) and the present findings provided strong, quantitative support for them.

The proportion of 'seeds' of sheep's burnet producing two seedlings was approximately 30% at each location (Section 4.3.2). In European studies, it was found that the two achenes of seed of sheep's burnet, as well as seed of other members of the genus *Sanguisorba*, subgenus *Poterium*, may each produce new plants (Nordborg, 1967b). However, the extent of this development and the environmental conditions under which it occurs were not reported. Results of the present study have contributed in this regard. The early appearance of seedling pairs at Flock House was probably due to the relatively high soil temperatures at that location. Under conditions in the South Island of New Zealand, one of the two seedlings usually dominates (Sheppard and Wills, 1986) and anastomosis of the two seedlings has also been observed (Section 2.2.5). The result after a few months is frequently a single plant equivalent (B J Wills, pers. comm.) and therefore there may be no forage yield advantage from the production of extra seedlings. However, the production of doubles could be advantageous in achieving rapid ground cover, but this remains to be determined.

The superior emergence of sheep's burnet at Flock House resulted in the species occupying a greater proportion of the mixed swards there (Table 4.4) than at Riverside. This provided further evidence of the likely pronounced effect of temperature on emergence of sheep's burnet and the results suggested that the relative sowing rates of the species should be adjusted to achieve similar seedling proportions at different soil temperatures.

Target seedling ratios in the mixed swards were difficult to attain in the field at Flock House and at Riverside on the basis of viable seed numbers per unit area (Table 4.5). Most studies involving the development of mixed swards for detailed study have eliminated this problem by using specified numbers of seedlings of each species in a rigid planting pattern (Hall, 1974; Harris and Sedcole, 1974; Scott and Lowther, 1980). Such procedures, however, have generally been utilised in pot studies in controlled environments and not in larger scale exploratory field trials. In several field investigations involving mixtures, swards were established in the manner adopted presently (Pineiro and Harris, 1978a, b) although the accuracy of the resulting seedling ratios has rarely been evaluated. Despite their frequent inaccuracies (Table 4.5), the mixed swards were at least distinct with respect to their proportions of sheep's burnet (Table 4.4). Therefore, subsequent comparisons of ground cover (point analysis) and herbage mass of the various swards (Section 4.2.5.3) were warranted.

The consequences of not attaining planned seedling proportions in the field (Section 4.3.2) were not of major importance, at least as far as the interpretations of the competition analyses (Section 4.3.3.1) were concerned. However, the disparity between the planned and realised seedling proportions raises an interesting philosophical question of how the realised proportions might be adjusted to give those seedling proportions which were planned. The use of the planned proportions of seedlings as a covariate is inappropriate because the concomitant data would not be independent of treatments. Independence of the covariate and treatments is a necessary assumption for the valid use of covariance adjustment (Steel and Torrie, 1980). Furthermore, there would also be little practical interest in expressing seedling number on the basis of a common mean proportion of seedlings. Another

character may be suitable as a covariate but an appropriate one is not immediately apparent.

The initial difficulty in obtaining satisfactory seedling emergence of sheep's burnet at Flock House (Section 4.2.3) highlighted the potential practical problems of evaluating a species which is relatively new to a region. For up to two years before the trial was commenced, local personnel conducted preliminary small-scale sowings of sheep's burnet. Results were generally unsatisfactory with very patchy seedling distributions developing at best, and consequently comments and unpublished reports were made stating that sheep's burnet was unsuitable for sand country revegetation. This study suggested that these earlier criticisms were premature and unjustified and that satisfactory seedling emergence can be achieved following the use of an appropriate insecticide. This practice could be a general requirement for satisfactory emergence of the species on similar sand country. In this regard, sheep's burnet is disadvantaged compared with the two legume species, which were undamaged by the pest(s). The potential of sheep's burnet as a revegetation plant and forage source on sand country remains to be determined.

Seedling emergence (Section 4.2.4) was a net measurement since the counts were equal to the number of seedlings emerged minus those seedlings which disappeared, perhaps due to death and/or removal. Disappearance of seedlings was not observed although it may be significant in some unfenced, harsh environments. Under these circumstances, its measurement should therefore be conducted.

Despite the slow emergence rate of sheep's burnet at both locations (Table 4.7b), it recovered quickly at Riverside with early vegetative growth per seedling equalling or slightly surpassing that of lucerne. Growth of birdsfoot trefoil was inferior to both species (Section 4.3.3). Foliar dry weight and leaf area were useful characters in distinguishing between the species and the canopy development of sheep's burnet was most satisfactory, particularly compared with birdsfoot trefoil. The relatively high leaf areas of sheep's burnet and lucerne (Table 4.8d) indicated that these species provided more early ground cover than birdsfoot trefoil. The leaf area of the sheep's burnet seedlings was also more dispersed horizontally than that of lucerne which may

be advantageous for soil protection. The findings for seedlings suggested that sheep's burnet and lucerne are more competitive than birdsfoot trefoil and that the latter species may be disadvantaged in mixed swards. Slow establishment of birdsfoot trefoil has also been noted by Scott and Charlton (1983). The early vegetative growth results were at variance with those for seedling emergence rate where sheep's burnet was relatively poor (Section 4.3.2). Genetic manipulation and/or use of large seed may improve emergence rate which would make sheep's burnet a more attractive option for rangeland seeding, particularly when coupled with its satisfactory early vegetative growth, as found presently.

A valuable find in distinguishing between the vegetative growth of sheep's burnet and the two legume species was that the most important characters for this purpose were foliar and root dry weights, rather than the simpler length measurements (Section 4.3.3). This has important practical usefulness in deciding on the number and types of characters to be measured in future similar research.

Sheep's burnet provided superior ground cover, as evidenced by its point analysis performance as a monoculture and in mixtures with lucerne and birdsfoot trefoil (Table 4.11), although not always (Table 4.10). The findings supplied further evidence of the suitability of the species for soil conservation (McTaggart, 1935; Thakur, 1957; Campbell, 1979; Sheppard and Wills, 1985). In South Island studies on very depleted faces (Wills, 1983), autumn sown sheep's burnet provided about 10% ground cover in the first year and up to 27% cover in the second year. Under the more favourable conditions of the present study, pure swards of the species had an average cover of approximately 80% (Table 4.11).

Caution should be exercised in interpreting the results of the point analysis since the growth habit of sheep's burnet probably favoured increased point contact compared with lucerne and birdsfoot trefoil. Sheep's burnet has a pronounced basal rosette of pinnately compound leaves (Nordborg, 1967b; Sheppard and Wills, 1985), many of which are horizontal to semi-erect in orientation. In contrast, the birdsfoot trefoil and lucerne cultivars used in the present study are typified by a more upright growth habit (Haynes, 1980; Scott and Charlton, 1983). Seedling doubles (Section 2.2.5)

may have also contributed to the results but the effect was probably short-lived due to likely single plant equivalents existing several months after sowing (B J Wills, pers. comm.).

Overall herbage masses and weed contents of swards of sheep's burnet and mixtures involving the species, were similar to pure swards of lucerne and birdsfoot trefoil (Section 4.3.3). This suggested that sheep's burnet was relatively tolerant of cutting and that it may make a worthwhile contribution to the supply of palatable forage in the first year. A similar conclusion was reached from a four year cutting trial conducted in the South Island of New Zealand (Daly, 1984) but it was noted that the contribution of sheep's burnet in subsequent seasons declined markedly. Some support for this was also found in a continuation of the present trial under periodic grazing for about the next three years (Foote, unpubl.), with the content of sheep's burnet in early 1989 being negligible.

The mean herbage mass totalled over spring/summer (three harvests) of 6.3 t DM ha^{-1} was superior to the spring production of sheep's burnet on yellow-grey earth soils in Central Otago (Sheppard and Wills, 1985). In another study, Wills (unpubl.) conducted monthly dry matter harvests on two mature stands of sheep's burnet, near Alexandra in Central Otago. Herbage masses from November to April were 2.5 and 3.2 t DM ha^{-1}, respectively. Rigorous comparisons between these results and those of the present study have doubtful value since apart from the differences in stand age and cutting frequencies, there were distinct environmental variations as suggested by climate records (New Zealand Meteorological Service, 1983). However, it is likely that much of the difference between these South Island results and those obtained presently was attributable to the frequently drier conditions experienced at the South Island sites. Similarities in the yielding ability of swards of sheep's burnet and lucerne found in the present Wairarapa study have also been obtained in studies in the USSR (Kozov, 1965). There was no indication presently that sheep's burnet outyielded lucerne, as reported in New South Wales, Australia (Anon, 1958).

The similarities in herbage mass showed that sheep's burnet was not slow to establish, as suggested under generally harsher South Island conditions (de Lacy,

1985). Furthermore, the species could be defoliated at the same time as lucerne and birdsfoot trefoil, without loss of productivity. Therefore, the results suggested that under relatively mild conditions, there was no need to delay defoliation because of possible slow establishment. Similar studies conducted under grazing would provide information complementary to that obtained currently.

The Riverside trial was conducted under low to moderate fertility (Section 4.2.1) conditions and the results indicated that sheep's burnet may be a useful species under such circumstances. The reductions in growth and yield of the two legume species, which are more suited to moderate to high fertility situations (Leach, 1978; Scott and Charlton, 1983), was unknown. Sheep's burnet would not be considered under high fertility (and high moisture) conditions since there are almost invariably many higher yielding alternative species (Scott and Charlton, 1983; Scott *et al.*, 1985; J S Sheppard, pers. comm.; B J Wills, pers. comm.). Furthermore, these other species are also usually more competitive under fertile conditions and therefore sheep's burnet may not perform as well with them in mixtures (N C Lambrechtsen, pers. comm.), as it did in the mixtures at Riverside (Section 4.3.3.1). This may partly account for the relatively unsatisfactory performance of sheep's burnet in combination with lucerne under moderately fertile conditions in Otago, South Island (Wills *et al.*, 1987). It is suggested that the site characteristics, such as soil fertility levels, should be ascertained routinely before comparing sheep's burnet with other species.

The similarity between herbage masses of pure swards of sheep's burnet and those of mixed swards of sheep's burnet and each legume (Table 4.12), indicated that there was no total yield advantage from the herb-legume association. This finding was at variance with those of grass-legume associations, where a greater total forage yield may be obtained by growing a grass and a legume in association, rather than as monocultures, in the absence of fertiliser nitrogen (Haynes, 1980). However within each mixture, sheep's burnet produced similar or higher herbage masses than the legumes, and this may have been partly due to the symbiotically fixed nitrogen from the legumes. At Tara Hills in the South Island of New Zealand, application of nitrogen to a pure sward of sheep's burnet was advised (N C Lambrechtsen, pers.

comm.), but the basis for this recommendation was unknown. It was assumed presently that the often well nodulated legume plants fixed nitrogen which was potentially available to the sheep's burnet plants and detailed research in this area may assist in explaining the observations. Although there were no total yield advantages from the herb-legume association, other potential benefits of including a legume with sheep's burnet are increased protein content and digestibility of the herbage, providing herbage with superior well-balanced mineral content, and increasing the duration of availability of green forage (Marten, 1985; Minson, 1985).

The competition analyses introduced a refinement on most previous research since standard errors were calculated for all relative crowding coefficients and their products (Table 4.13). These enabled valid statistical tests of all differences between the various estimates rather than incomplete tests or judgement on the basis of the relative magnitudes of the estimates (for example, Harris (1972), Hall (1974a, b), Ivens and Lowe (1980), and Martin (1984)). The present results could have been interpreted completely erroneously if it was not for the t-tests, as the numerical differences between the two species were apparently very great (Table 4.13). Standard error estimation and appropriate statistical tests should be routine practice in this type of research. Interpretations of the relative crowding coefficients and their products in the competition analyses involving planned and achieved seedling proportions were very similar (Section 4.3.3.1.3). The results of this study therefore suggested that collecting seedling count data for the sole purpose of using them to determine seedling proportions for inclusion in competition analyses, was unnecessary. However, omitting seedling counts should not be taken as a general recommendation in competition analyses until further comparisons between analyses based on planned and realised seedling proportions are undertaken.

Despite the relatively moderate emergence rate and poor early vegetative growth of birdsfoot trefoil (Section 4.3), its total herbage mass over summer and autumn was similar to that of sheep's burnet and lucerne (Table 4.12). Photosynthate partitioning favouring the foliage over the root may have partly accounted for this observation. Conversely, sheep's burnet and lucerne have well developed tap roots (Heinrichs, 1963; Nordborg, 1967b), which in the case of lucerne and probably sheep's burnet,

are relatively large stores of photosynthetic assimilate (Rapoport and Travis, 1984). In these species, partitioning favouring the foliage is presumably less pronounced.

At the first harvest (Table 4.12), which represented uninterrupted growth from sowing, birdsfoot trefoil had inferior herbage mass compared with pure swards of sheep's burnet and lucerne, and several mixtures. As well, the trefoil swards had relatively patchy ground cover. However, despite these disadvantages, the weed content of the trefoil swards was similar to that of all other sward types (Table 4.12). It is suggested that in more weed-prone sites, ingress of weeds into birdsfoot trefoil swards could be a greater problem than in the other swards examined presently. The similar herbage accumulations between all swards (Section 4.3.3) suggested that they may be useful for providing palatable forage over summer. Pasture accumulation on the Riverside farm during this period is quite variable and since 1986/87 it has ranged from about 10-30 kg DM ha^{-1} d^{-1} in December to negligible levels in January and February (Parker *et al.*, 1989; W J Parker, pers. comm.). The estimates for the present sward types of 26 and 70 kg DM ha^{-1} d^{-1} (Section 4.3.3) compared very favourably with the values for pasture, although they were obtained in the 1985/86 season. Comparisons between the experimental swards and resident pasture in the same season(s) would clarify this issue. The experiment was also conducted under cutting and hence extrapolation of the current findings to the grazing situation should be conducted cautiously (Humphreys, 1966; Watkin and Clements, 1978). Similar future research involving grazing animals would complement the present study.

CHAPTER 5 : FOLIAR REGROWTH OF ESTABLISHED SWARDS OF SHEEP'S BURNET

5.1 INTRODUCTION

Foliage of sheep's burnet is palatable at all stages of vegetative growth and the species may provide worthwhile forage yields in some localities (Cockayne, 1920a, b; 1921; Stewart, 1979; Sheppard and Wills, 1985). Despite its suitability as a forage, there is little detailed information on the manner and rate of regrowth which is necessary to develop optimum grazing management systems (Gabrielsen *et al.*, 1985).

An early report on sheep's burnet (Macpherson, 1912) indicated that its regrowth following two or three cuttings was very satisfactory and plants in some instances were described as "splendid". Similar indications were obtained from several other studies (Cockayne, 1921; Ward, 1923; McGillivray, 1929; Anon, 1957). Plants regrow from the basal crown and the upper part of the root stock (Sheppard and Wills, 1985; Sheppard, unpubl.) and in a preliminary Central Otago study, regrowth ranged from 2.5 kg DM ha^{-1} d^{-1} in winter to 5 kg DM ha^{-1} d^{-1} in late spring (Wills, unpubl.). Under milder Manawatu conditions, higher regrowth rates (13 kg DM ha^{-1} d^{-1} in autumn/winter and 100 kg DM ha^{-1} d^{-1} in late spring (Foote, unpubl.)) have been recorded.

The objectives of the present study were to determine for sheep's burnet the duration and rate of foliar regrowth of established plants under field conditions, morphological changes associated with foliar regrowth, and to estimate likely maximum values for herbage mass. A further aim was to use this information to suggest appropriate defoliation strategies.

5.2 MATERIALS AND METHODS

5.2.1 THE SITE

Pure swards of sheep's burnet at Riverside (grid reference; NZMS 1, Masterton N158 /111758), as described in Chapter 4, were used for the present investigation. The swards had been defoliated previously three times (17 December, 1985 and 5 February and 23 April, 1986) to leave a residual height of approximately 5-7 cm at each cutting.

5.2.2 MEASUREMENTS

All plants within one 0.125 m^2 randomly selected quadrat were removed from each of the three plots on 7 May, 1986, two weeks after the last defoliation. Material was washed thoroughly and the foliage was severed from the roots, immediately above the top branching root. This point of demarcation was characterised frequently by a colour change from light green to brown. All weeds and severed roots were discarded. For each quadrat, foliage was separated into the following categories: complete leaves, which represented regrowth (Section 3.3); incomplete leaves and stubble (Section 3.3); and remainder. The latter category consisted of relatively lignified growth zone material from which new leaf shoots developed, and senescing and dead tissues. The number of complete leaves was recorded and approximately 10-20% of them were used to estimate leaf area and leaf lamina and rachis/petiole dry weights. Dry weights of remaining complete leaves, incomplete leaves and stubble, and remainder were also determined. The above procedure was repeated six times at weekly intervals on 14, 21 and 28 May, and 4, 11 and 18 June to monitor the regrowth patterns.

At the last two harvests on 11 and 18 June, the number of primary shoots, defined as those shoots developing from the main central foliar/root axis, and the number of higher order shoots which developed from the primary shoots, were recorded. The lengths of the foliar/root axes where primary and higher order shoots developed (growth zones), were also measured. For each shoot order, complete leaves,

incomplete leaves, and stubble components were separated. Three categories of complete leaves were employed based mainly on arbitrary leaf lengths, namely small (frequently curled and with short rachis distances between adjacent leaflets, < 70 mm long), medium (70-130 mm) and large (fully expanded leaves with relatively large rachis distances between adjacent leaflets, > 130 mm long). The separation of incomplete leaves and stubble enabled determination of the relative importance of these components which was impossible previously due to their bulking. Numbers and dry weights of complete leaves (three categories), incomplete leaves and stubble were determined for primary and higher order shoots.

5.2.3 STATISTICAL ANALYSES

5.2.3.1 ALL HARVESTS

Four characters were calculated for complete leaves over all seven harvests (times). These were: 1) herbage mass expressed as kg DM ha^{-1} after including the weight contribution from the subsamples for leaf area determinations; 2) leaf number (units) expressed on a per m^2 basis; 3) leaf area (cm^2) expressed on a per m^2 basis; and 4) the proportion (%) of leaf lamina, using the leaf lamina and rachis/petiole dry weight data. Plots of each of the four characters against time, involving three points (from three blocks) per time, indicated that sigmoid curves (Richards, 1959; Landsberg, 1977) were suitable model functions to describe the patterns of development of these characters.

In this regard, the three parameter logistic model (Ratkowsky, 1983) of the form (Section 3.6)

$$y = \frac{\beta_0}{1 + e^{(\beta_1 - \beta_2 t)}}$$

was fitted to all data sets utilising a nonlinear least squares procedure (Section 3.6.3). Time zero in all cases was the third harvest for dry matter yield on 23 April (Chapter 4) and all characters were assumed zero at this time because there were negligible

complete leaves. Estimates of time to reach a low level of regrowth, arbitrarily defined as 10% of the asymptotic value for each character (t_{10}), 50% regrowth (t_{50}) and almost complete regrowth (90% regrowth=t_{90}) were estimated from each curve (Section 3.6.2). The maximum regrowth rate per day was obtained by calculating the value of the first derivative of the logistic model function at t_{50} for each character. The equation for the first derivative (dy/dt) was

$$\frac{d\,y}{d\,t} = \frac{\beta_0\beta_2 e^{(\beta_1-\beta_2 t)}}{[1+e^{(\beta_1-\beta_2 t)}]^2}$$

where the parameters ($ß_0$, $ß_1$ and $ß_2$) and t were defined previously (Section 3.6).

Herbage masses of complete leaves at each harvest were also combined with the corresponding masses for incomplete leaves and stubble, and remainder, in a multivariate analysis of variance (Section 3.7) involving time. The analysis enabled an assessment of the relative importance of each character (Section 3.7) in accounting for the changes between harvests. The three herbage masses were also expressed as a proportion (%) of their sum (total herbage mass) at each harvest and all six characters (absolute and proportional masses) were analysed using univariate analyses of variance.

5.2.3.2 FINAL TWO HARVESTS

The numbers of primary and higher order (other) shoots were used as dependent variables in separate linear regression analyses (Steel and Torrie, 1980) against growth zone length to determine if there was any functional relationship between these biologically important characters. A similar analysis was also conducted to determine if there was a relationship between the number of primary shoots and the number of other shoots. This was based on the assumption that subsequent production of other shoots may have occurred after a certain number of primary order shoots were produced. For each shoot order, mean percentages over the two sampling times of small, medium and large complete leaves were estimated on a number and dry weight basis. Average numbers of incomplete leaves and stubble

sections per m^2 were also calculated. Additional dry weight data were used to estimate the mean proportions of all components as a percentage of total green dry weight for each shoot order.

5.3 RESULTS

5.3.1 ALL HARVESTS

Plots of the logistic functions describing leaf number (m^{-2}), leaf area (cm^2 m^{-2}), leaf lamina proportion (%) and herbage mass (kg DM ha^{-1}) of complete leaves, are shown in Figure 5.1. Upper asymptotic values for each character together with maximum daily regrowth rates and times to reach various stages of regrowth are presented in Table 5.1. All characters attained close to maximum value (t_{90}) about four weeks after defoliation and maximum daily regrowth rates occurred two to three weeks after defoliation (Table 5.1). There was a relatively slow initial increase (t_{10}=2.3 weeks) in leaf area but by t_{90}, apparent differences between leaf area and the other three characters had disappeared (Table 5.1). Maximum average herbage mass of sheep's burnet was 880 (SE=52) kg DM ha^{-1} and regrowth rate over the harvests peaked at 46 kg DM ha^{-1} d^{-1} (Table 5.1). Although leaf regrowth was dominated initially by increases in rachis/petiole dry weight, dry weights of lamina and rachis/petiole were approximately equal four weeks after defoliation (Figure 5.1), with the proportion of leaf lamina of complete leaves being about 55%.

There were highly significant ($P<0.001$) differences between harvests in the multivariate analysis of variance of the herbage mass data for complete leaves, incomplete leaves and stubble, and remainder (Table 5.2a). The first of three discriminant functions for harvests (Table 5.2a) accounted for 82% of the total dispersion in the data. The most important character on this function was dry weight of incomplete leaves and stubble which was only slightly more important than that for complete leaves (Table 5.2b). The different loadings on these dry weight characters (Table 5.2b) indicated that complete leaves (regrowth) increased with time after cutting while the reverse trend occurred for incomplete leaves and stubble.

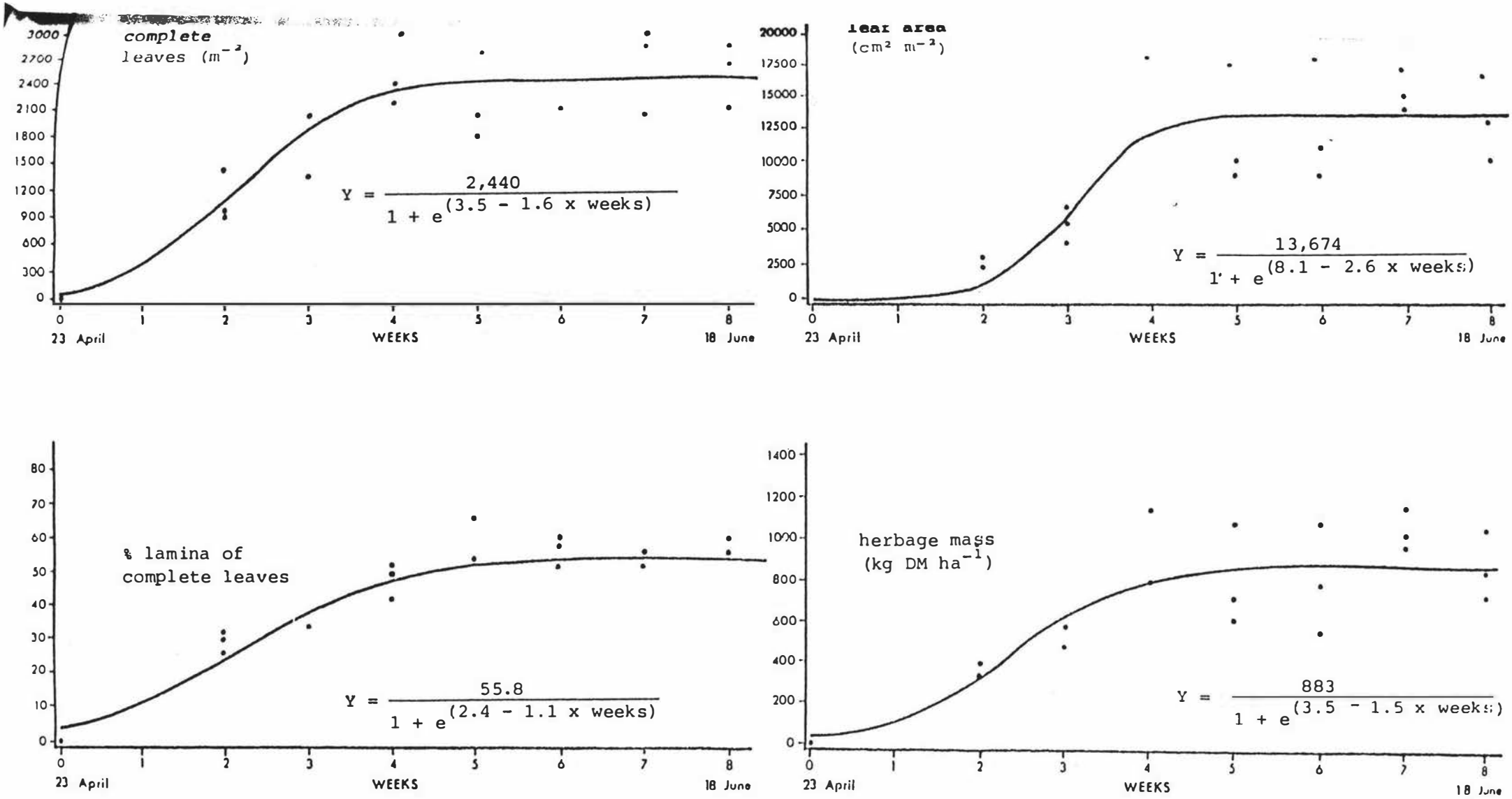


Figure 5.1 Logistic growth functions describing the number, area, percentage lamina and herbage mass of complete leaves (regrowth) after defoliation on 23 April, 1986.

TABLE 5.1 Times to reach various stages of regrowth together with maximum regrowth rates and final levels (asymptotes) achieved, following defoliation on 23 April, 1986 (standard errors in parentheses).

Character	t_{10} (weeks)	t_{50} (weeks)	t_{90} (weeks)	asymptote	maximum regrowth rate (/day)
number of complete leaves (/m²)	0.8	2.2	3.6	2440 (110)	138
leaf area (cm²/m²)	2.3	3.1	3.9	13670 (850)	1280
leaf lamina content (%)	0.2	2.2	4.3	56 (1)	2
herbage mass (kg DM/ha)	0.9	2.4	3.9	880 (52)	46

TABLE 5.2a Important statistics for the three discriminant functions for harvests involving herbage masses of complete leaves, incomplete leaves and stubble, and remainder.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	82.11	4.44	18	29	***
2	16.15	2.05	10	22	(NS)
3	1.74	0.66	4	12	NS

Table 5.2b Mean scores for the first discriminant function for harvests at Riverside from 7 May-18 June, 1986.

Harvest date	Mean score
7 May	4.09
14 May	1.91
21 May	1.48
28 May	-0.02
4 June	-1.83
11 June	-2.47
18 June	-3.15

two largest standardised coefficients:

1. dry weight of incomplete leaves and stubble (1.94)
2. dry weight of complete leaves (-1.47)

Table 5.2c Harvest date means for herbage masses of incomplete leaves and stubble, and complete leaves (standard errors in parentheses).

Harvest date	Incomplete leaves and stubble (t DM/ha)	complete leaves (t DM/ha)
7 May	0.63 (0.06)	0.35 (0.02)
14 May	0.53 (0.08)	0.57 (0.05)
21 May	0.70 (0.14)	0.91 (0.11)
28 May	0.46 (0.04)	0.79 (0.14)
4 June	0.27 (0.04)	0.81 (0.13)
11 June	0.34 (0.01)	1.03 (0.05)
18 June	0.14 (0.03)	0.85 (0.10)

This pattern was also shown by the respective univariate means (Table 5.2c) and on a proportional dry weight basis (Figure 5.2). Mass of the remainder component was unimportant in the discriminant analysis as it had a standardised coefficient of approximately zero. This was also supported on a proportional basis where its content of total herbage mass was similar at all harvests (Figure 5.2) and averaged 39% (SE=5%).

5.3.2 FINAL TWO HARVESTS

The total number of primary and other shoots averaged 28.6 (SE=2.0) shoots per m^2 and primary shoots were approximately 80% of the total. The mean length of the growth zone was 12.1 mm (SE=0.4 mm). Results of the regression analyses of the number of primary shoots and other shoots regressed individually against growth zone length, and an analysis of other shoot numbers against the number of primary shoots, are presented in Table 5.3. All regressions were highly significant ($P<0.001$) but the variability accounted for by each analysis was low (R-squares 0.20-0.40). Length parameters ($ß_1$) were not significantly different from each other and were greater than zero. In the regression of primary shoot numbers against the length of growth zone, $ß_0$ (intercept) was not significantly different from zero. This indicated that on average, a 1 mm increase in growth zone length resulted in 1.6 primary shoots per m^2 being produced (Table 5.3). Before other shoots were produced, however, the growth zone was at least 5.5 mm long. The analysis of numbers of other shoots against primary shoot numbers showed that the number of other shoots was dependent partly on the number of existing primary shoots. From the appropriate regression equation (Table 5.3), it was estimated that at least five primary shoots were required before higher order shoots were produced.

About 73% of the 2600 complete leaves per m^2 were produced by primary shoots and regardless of shoot order, approximately 80% of the leaves were small (Table 5.4). Primary shoots contributed 75% and 80% of the total numbers of incomplete leaves and of stubble sections, respectively. The importance of primary shoots over other shoots was also reflected in the dry weights for each component (Table 5.5a), and for each shoot order, complete leaves (regrowth) accounted for about 80% of the

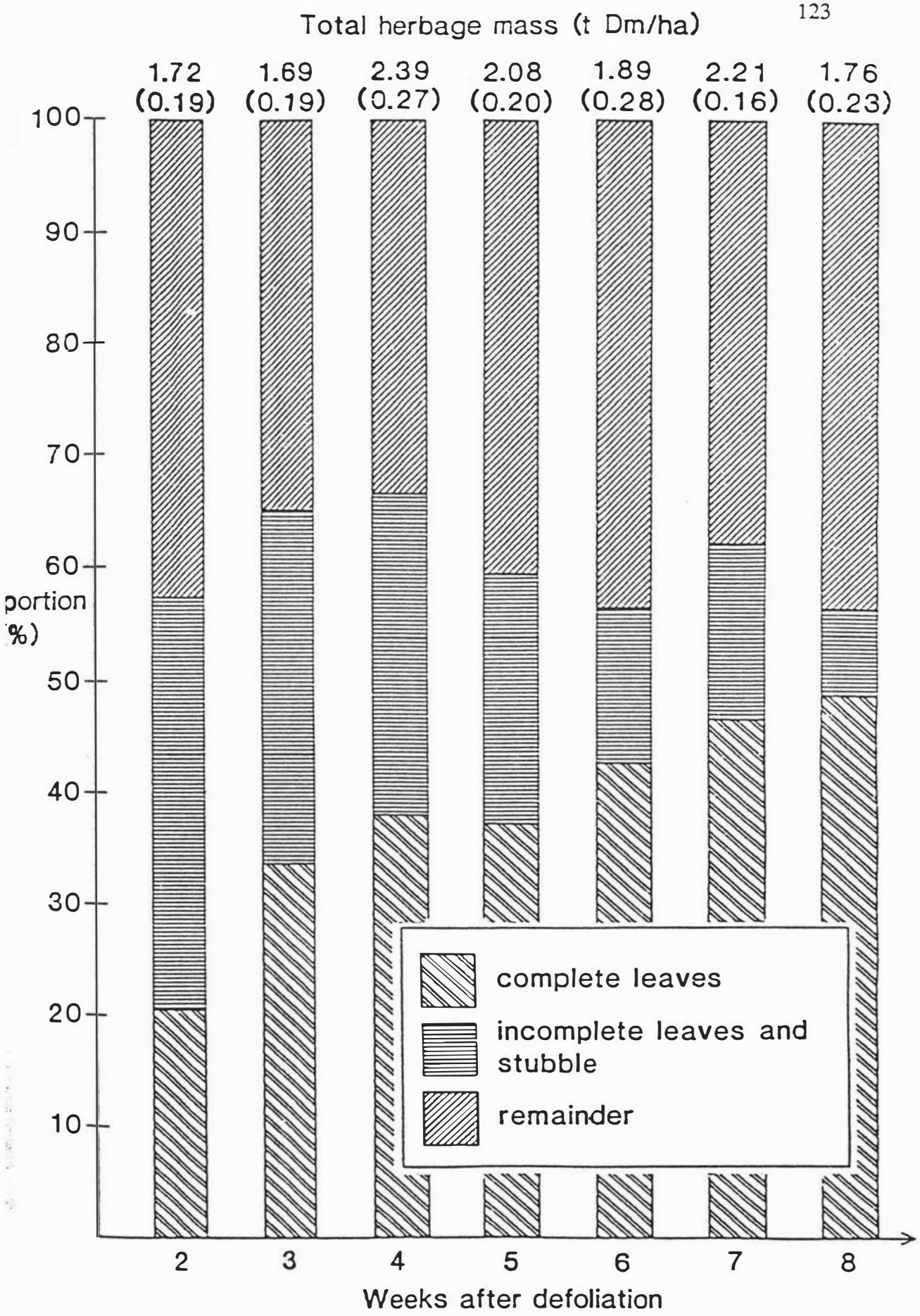


Figure 5.2 Patterns of change in the relative proportions of foliar herbage components at Riverside following defoliation on 23 April, 1986 (standard errors in parentheses).

TABLE 5.3 Various statistics for linear regressions involving primary shoots, other shoots and length of growth zone.

Character	Regression equation	$\hat{SE}(Y)$	F-test	R-square	$\hat{SE}(\beta_0)$	$\hat{SE}(\beta_1)$
Primary shoots	Y= -0.48 + 1.64 x length+	9.44	47.33***	0.31	3.03	0.24
Other shoots	Y= -7.46 + 1.36 x length	10.53	26.23***	0.20	3.39	0.27
Other shoots	Y= -3.63 + 0.65 x primary shoots	9.12	70.82***	0.40	1.75	0.08

+ length = growth zone length (mm); SE= standard error.

TABLE 5.4 Some characteristics of shoots averaged over harvests six and seven at Riverside (standard errors in parentheses).

Character	Proportion of different sized complete leaves (%) small	medium	large	number of complete leaves (/m²)	number of incomplete leaves (/m²)	number of stubble sections (/m²)
Primary shoots	80.8 (3.5)	15.4 (2.5)	3.8 (1.3)	1895 (136)	652 (107)	220 (50)
Other shoots	85.8 (3.9)	13.2 (3.3)	1.0 (0.8)	697 (122)	217 (49)	56 (24)
Total shoots	-	-	-	2595 (168)	869 (134)	276 (74)
Proportion of primary shoots (%)	-	-	-	73	75	80

TABLE 5.5a Mean herbage masses (kg DM/ha) over harvests six and seven for several foliar herbage components of primary and other shoots (standard errors in parentheses).

Character	Complete leaves small (kg DM/ha)	Complete leaves medium (kg DM/ha)	Complete leaves large (kg DM/ha)	Incomplete leaves (kg DM/ha)	Stubble (kg DM/ha)	Total herbage mass (kg DM/ha)
Primary shoots	447.2 (55.9)	213.3 (44.1)	72.3 (27.3)	168.3 (31.9)	28.8 (9.5)	929.9 (80.3)
Other shoots	145.7 (27.7)	54.7 (18.3)	4.0 (3.0)	37.3 (9.9)	5.7 (3.0)	247.5 (46.8)

Table 5.5b Mean proportions (%) of total herbage mass over harvests six and seven for several foliar herbage components of primary and other shoots (standard errors in parentheses).

Character	Complete leaves small (%)	Complete leaves medium (%)	Complete leaves large (%)	Total complete leaves (%)	Incomplete leaves (%)	Stubble (%)	Total herbage mass (kg DM/ha)
Primary shoots	50.0 (7.7)	22.3 (3.4)	7.3 (2.6)	79.6 (2.6)	17.3 (2.0)	3.1 (1.0)	929.9 (80.3)
Other shoots	62.5 (6.7)	20.1 (4.2)	1.3 (0.9)	83.9 (3.0)	14.4 (2.7)	1.7 (0.9)	247.5 (46.8)

total green dry weight which consisted of complete leaves, incomplete leaves and stubble (Table 5.5b). Small complete leaves had the highest dry weight proportion of any component while the contribution of stubble was negligible.

5.4 DISCUSSION

The regrowth of complete leaves of sheep's burnet was characterised initially by slow regrowth followed by a period of rapid increase and finally a levelling off (Figure 5.1). Such sigmoidal growth patterns occur in a wide range of plant species (Moore, 1979; Bircham and Korte, 1984). The initial slow phase of regrowth may have been because of relatively low nonstructural carbohydrate levels in the form of reserves and/or reduced current photosynthate supply due to inadequate residual leaf area. Relatively low shoot and cell numbers may also have been contributing factors. Swards attained a stable leaf area index (Watson, 1947; Donald, 1963) four weeks after defoliation of 0.4 which coincided with the onset of maximum herbage mass. The figure provided a rough estimate of critical leaf area index (Brougham, 1955) for the species and it was considerably lower than those found previously for lucerne (4.6) and white clover (3.5) (Walton, 1983), which also have nearly horizontal, broad leaves.

Plant densities were not measured but it may be assumed that they were reasonably typical of what could be expected under favourable environmental conditions following recommended sowing rates (Section 4.2.3). It is suggested that the regrowth responses found, including estimates of maximum herbage mass and leaf area index, could be generally applicable in similar seasons, but there would be undoubtedly some variations due to factors such as temperature and light fluctuations (Ludlow, 1978; McWilliam, 1978). Estimates of the duration and efficiency of the leaf area of sheep's burnet would assist in appraising the likely production of photosynthate and hence herbage accumulation, and rates of leaf tissue turnover would also be useful.

Although the maximum regrowth rate was 46 kg DM ha^{-1} d^{-1} (Table 5.1), the average over four weeks was approximately 31 kg DM ha^{-1} d^{-1} which was at least twice the

rate found by Foote (unpubl.) under favourable Manawatu nursery conditions. One of the main reasons for this discrepancy was a likely lower plant density in the Manawatu study as suggested by a greater visual proportion of bare ground following cutting. There was also a two month interval between cuttings and the results of the present study suggested that an interval half as long may have been more appropriate, since after this time there were no further increases in new herbage mass (Figure 5.1). Other factors accounting for differences between the studies could have been leaf area duration and the extent of tissue senescence and death. The latter factor may have been particularly relevant in the Manawatu study because of the longer cutting frequency. Regrowth estimates in the Riverside investigation were considerably higher than those recorded under harsher Central Otago conditions where even in spring, when maximum production occurs (Sheppard and Wills, 1985), regrowth was only 5 kg DM ha^{-1} d^{-1} (Wills, unpubl.).

In the establishment stages of the Riverside trial, the highest average regrowth rate was 70 kg DM ha^{-1} d^{-1} between 17 December, 1985 and 5 February, 1986 (Chapter 4). As detailed samplings of regrowth at frequent intervals were not undertaken during this summer period, the time when new herbage mass started to peak and level off, could not be determined. The generally higher temperatures and light intensities experienced during the summer probably accounted for the superior regrowth rates and ceiling herbage masses compared with those found in the present autumn study (Ludlow, 1978; McWilliam, 1978; Lancashire, 1984).

The ceiling regrowth yield and/or regrowth rate of sheep's burnet at Riverside was superior or inferior to that found for several other species suitable for dryland conditions. In Canterbury in the South Island of New Zealand, herbage accumulation of lucerne during April to June ranges typically from approximately zero to 12 kg DM ha^{-1} d^{-1} (White, 1982) which is lower than the estimates obtained for sheep's burnet in the current study. One of the advantages of sheep's burnet over lucerne may be its ability to provide superior quantities of herbage in mid to late autumn and this has been suggested by observations in the South Island (J S Sheppard, pers. comm.). Also in Canterbury, swards of perennial ryegrass cv. 'Nui' were subjected to several grazing managements over summer and pasture regrowth in the absence

of stocking was measured after 6-7 weeks (Vartha and Hoglund, 1983). Herbage mass in the autumn ranged from 600 to 1300 kg DM ha^{-1} depending on the year of measurement and results in the present study (Section 5.3.1) were approximately intermediate within this range.

In the establishment year, regrowth rates under mowing of four autumn sown dryland grass cultivars were reported by Lancashire and Brock (1983). However, the autumn/winter figures presented were for the first few months of growth only and therefore their comparison with results of the present investigation was inappropriate. A more meaningful comparison was between growth rates in the summer, with that of sheep's burnet being 70 kg DM ha^{-1} d^{-1} as discussed above while growth rates for the grasses ranged from 42 kg DM ha^{-1} d^{-1} for perennial ryegrass cv. 'Nui' to 65 kg DM ha^{-1} d^{-1} for tall fescue cv. 'Roa' (Lancashire and Brock, 1983). The comparisons indicated that sheep's burnet had a relatively favourable growth rate and future studies initiated at the same sowing time would clarify this issue. The swards should also be established using similar viable seed numbers per unit area to enable valid comparisons of establishment and regrowth. Recent estimates of pasture herbage accumulation at Riverside farm from April to June have ranged from 4-37 kg DM ha^{-1} d^{-1} (Parker *et al.*, 1989) and the current average figure for sheep's burnet of 31 kg DM ha^{-1} d^{-1} was at the upper end of this range. The results suggested that the species could be a viable option in similar environments.

About eight month old swards of sheep's burnet attained nearly stable ceiling regrowth yields after approximately four weeks (Figure 5.1). This suggested that monthly defoliation frequencies during autumn are appropriate where each defoliation leaves a stubble height of about 5-7 cm, as practised in this study. However, this could be difficult to manage consistently in a grazing situation. In most cases, an increased defoliation intensity would delay the commencement of the exponential regrowth phase (Walton, 1983), increase the most suitable grazing frequency, and hence result in reduced herbage dry matter yield (Harris, 1978). Such results however would depend very much on weather factors such as radiation inputs and therefore recommendations for various regions could differ. In Spain, Salmeron (1966) recommended a defoliation interval in autumn/winter of 40-72 days but

appropriate defoliation intensities were undefined. Under Central Otago conditions, Wills (unpubl.) used defoliation intensities comparable to those in the current investigation and advocated a grazing interval in March/May of 20-25 days which was only slightly less than that suggested presently.

Swards in the Riverside study were mown uniformly and there are obvious difficulties in extrapolating current results to grazed situations because of problems of accounting exactly for animal grazing characteristics (Watkin and Clements, 1978). Factors such as animal selectivity and treading damage may be quite important in the grazing situation but they have received little attention in studies on sheep's burnet. They should therefore be examined in future investigations. Leafy, actively growing herbage of sheep's burnet is quite palatable to livestock (Sheppard and Wills, 1985; Wills *et al.*, 1987) and is sought eagerly during grazing. From personal observations on lightly grazed swards, residual vegetation generally comprises various proportions of complete leaves, incomplete leaves and stubble, which are at variance with the findings of the present investigation where mowing resulted in negligible complete leaves. Under heavy grazing pressures however, few complete leaves also remain. From a herbage regrowth viewpoint, at least during establishment, it seems desirable to initially have numerous complete leaves in the early stages of unfolding and extension, but whether this can be achieved in practical grazing situations remains to be determined.

Despite the consistent total herbage masses (t DM ha^{-1}) across all harvests, prominent changes over time occurred in the proportion of green sward components due to the increasing number and hence weight of complete leaves (Figure 5.2). However, it is noteworthy that at six to eight weeks after defoliation, complete leaves represented only about half of the total mass and incomplete leaves and stubble an additional 10-15%. The combined proportion of these two components (60% of total mass) was consistent over all samplings and in a grazing context they represented desirable herbage on offer. It is likely that if stock, particularly sheep, were introduced to the regrowing swards they would have grazed complete leaves initially in preference to incomplete leaves and stubble. This was because the former tissues were younger and generally more accessible and similar observations have been reported for pasture

species (Watkin and Clements, 1978; Rattray and Clark, 1984). Continued removal of complete leaves would presumably lower the forage quality of the swards and result in a reduced preference by livestock as well as a decline in sward vigour.

The remaining non-green portion of total herbage mass, approximately 40% of the total mass for all harvests, consisted of dead matter and relatively heavily lignified growth zone material. Dead matter is rejected by stock because of low preference and limited accessibility in the canopy base and at least in the case of pasture, it has a very low digestibility (40%) compared with green material (80%) (Rattray and Clark, 1984). Similar comments probably apply to the growth zone material. Although the proportion of dead matter in the current study was not ascertained, the fact that it was at least less than 40% of total herbage mass suggested (Rattray and Clark, 1984) that high digestibilities of the sheep's burnet herbage should be achieved easily in practice. This is supported partly by *in vitro* digestibility estimates determined for leafy, actively growing tissues of the species of 69.4% for dry matter digestibility and 68.8% for organic matter digestibility (Sheppard and Wills, 1985). Mature herbage with a large proportion of stalks has lower digestibility (Sheppard and Wills, 1985).

A major proportion of the regrowth herbage developed from the primary shoots with higher order shoots (secondary and others) being relatively unimportant (Section 5.3.2). This was the situation at harvests six and seven where ceiling levels for complete leaf number, area and herbage mass were achieved (Figure 5.1). The production of shoot meristems and their development as young shoots after this time may have continued, albeit slowly. However, it is doubtful whether these new shoots and their associated complete leaves would have contributed significantly to regrowth herbage mass. This is because their development would probably have been curtailed severely in the low light intensities existing within the dense canopy of complete leaves (Ludlow, 1978). A corollary of this, of course, is that timely defoliation to remove much of the existing canopy could have encouraged a proliferation of new shoots. This has implications for grazing management and the scheduling of grazing on a growth stage basis should receive increased attention.

The signals for initiation and development of higher order shoots were equivocal. The results indicated that a minimum growth zone length of 5.5 mm per plant was required before higher order shoots were produced. Furthermore, at least five primary shoots were produced before subsequent higher order shoots appeared (Section 5.3.2). The factors controlling this sequence were unknown but it is suggested that the levels of total nonstructural carbohydrates and their distribution could be influential, as well as the extent of development of vascular tissues. Hormonal effects may also be important. Possible manipulation of growth zone length by varying the sowing depth could increase the potential number of primary shoots produced following defoliation. This would have an advantage in increasing herbage accumulation.

The rate of regrowth of sheep's burnet immediately after cutting (or grazing) may be accounted for by at least two factors, namely assimilate energy from current photosynthate and secondly reutilisation of reserve carbohydrates stored in stubble and/or roots. Related factors which may be important include the age and photosynthetic efficiency of any residual leaf area, the rate of assimilate transport to regrowth sites, and the numbers of meristems available for regrowth together with their physiological activity (Section 2.3.2). The relative importance of the two energy sources is unknown for sheep's burnet but it has been investigated for a wide range of other forages (Ward and Blaser, 1961; Harris, 1978; Walton, 1983; Gabrielsen *et al.*, 1985). Generally, intense defoliation results in immediate reductions in the levels of carbohydrate reserves, and depletion may continue until photosynthesis is established at a level that meets plant requirements for respiration and growth (Harris, 1978). From a review of numerous papers, estimates of the duration of dependence on reserves for regrowth ranged from 2-7 days for grasses to 21 days for lucerne (Harris, 1978). Sheep's burnet has a well developed, large taproot (Nordborg, 1967b; Sheppard and Wills, 1985) and it is likely that this is a major storage site of reserve carbohydrates, as has been shown for lucerne (Cooper and Watson, 1968; Rapoport and Travis, 1984). Some evidence for this view was provided by the accidental severence of mature nursery grown plants of sheep's burnet immediately below the existing growth zone (Douglas, unpubl). In most

cases, regrowth occurred from the severed roots despite an extremely harsh treatment and the absence of a current photosynthate source.

CHAPTER 6 : IMPROVED EARLY VEGETATIVE GROWTH OF SHEEP'S BURNET FROM USING NEW SELECTIONS AND LARGE SEEDS

6.1 INTRODUCTION

The size of commercially available seed of sheep's burnet is quite variable and seed weights reported overseas have ranged from 128 to 171 seeds per gram (Salmeron, 1966). In European material, Nordborg (1967b) found great variation in the length and breadth of receptacles of the species. However, no investigations were conducted to determine whether this morphological variability was associated with rates of germination, seedling emergence, or early vegetative growth. Seed size in numerous grass and legume species is frequently a major determinant of the rate of emergence and seedling development (Black, 1959; Stickler and Wassom, 1963; Thomas, 1966; Cooper, 1977; Dalianio, 1980; Scott and Hampton, 1985; Charlton, 1989).

Most available selections of sheep's burnet originated from Europe, or reached New Zealand via western North America, and newer accessions are generally superior to naturalised material from the old Macpherson and Cockayne trials in Central Otago, with respect to growth, survival and vigour (Sheppard and Wills, 1985). The advantages of the newer selections would be enhanced if they were also superior at the seedling emergence and early vegetative growth stages.

The main objectives of this study, therefore, were to compare a range of seed sizes of sheep's burnet and current and previously used selections of the species with respect to their seedling emergence and early vegetative development.

6.2 MATERIALS AND METHODS

6.2.1 LOCATION

The study was conducted in a glasshouse at the Plant Growth Unit, Massey University. Temperature settings were 25°C (day) and 15°C (night) on a 12 hour cycle. Occasional monitoring of temperatures showed that on some fine days, the glasshouse temperature exceeded 25°C by several degrees. During the night, temperatures did not fall below 10°C. Relative humidity at 0900 hours was 50-70%.

6.2.2 PLANT ESTABLISHMENT AND TREATMENTS

Seed of a commercial selection of sheep's burnet (originally from Oregon, USA) and a selection from the Cockayne plots of Central Otago, New Zealand (Section 3.2), was passed through two sieves (2.0 and 2.8 mm each) to obtain samples of three different sized seeds. These were labelled as small (< 2.0 mm), medium (2.0-2.8 mm) and large (> 2.8 mm). Unsieved lucerne cv. 'Rere' seed (Section 3.2) of approximately uniform size was included as a dryland "standard".

All seed was sown on 24 March, 1986 at 7.5 mm depth into a 1 part soil : 10 parts sand mix which was contained within PB10 planter bags with flattened dimensions of 12 x 46 cm. The mix was used to facilitate later root removal and 3-4 month slow-release fertiliser (N:P:K=15:5.2:12.5) was distributed uniformly in the medium at a rate of 5 $g\ell^{-1}$ to ensure that media nutrient levels did not limit seedling growth. Sheep's burnet and lucerne were sown at a rate of six seeds per bag. Each plot (experimental unit) consisted of five bags. The seven treatments, namely two seed lines x three seed sizes of sheep's burnet, and 'Rere' lucerne, were arranged in four randomised complete blocks. All bags were supported on trolleys and the media were kept moist by ensuring that bag weights did not fall below 200 g of the bag weights at field capacity (11.3 kg). The moisture content at field capacity was 6.6% which represented about 740 g water.

6.2.3 MEASUREMENTS

Weights were determined for 4 x 50 seed samples of the three seed sizes of each selection of sheep's burnet together with their proportions by weight in the original seed batches. Weights were determined on the seed as supplied (ISTA, 1985). Seed

moisture content was ascertained after drying at 105°C for 17 hours (ISTA, 1985) and averaged 8.73% (SE=0.04%) across all seed sizes. The levels of total nonstructural carbohydrates were estimated for each seed size of the Oregon selection using the rapid chemical analysis method of Haslemore and Roughan (1976), which is detailed in Section 3.5. The seed was dried at 40°C for 24 hours (Sangakkara *et al.*, 1985) and ground finely. Five samples of each seed size were analysed.

Seedling emergence counts per plot were recorded daily from three days after sowing, when emergence first occurred, until thirteen days after sowing when seedling numbers in each plot had been equal for at least the last two days. A seedling was deemed emerged when both cotyledons were exposed fully and approximately horizontal. The number of "doubles" (Chapter 3) of sheep's burnet was recorded thirteen days after sowing and the smaller of any seedling pair was removed so that all remaining seedlings developed on a comparable basis.

All seedlings within one randomly selected bag per plot were harvested seventeen days after sowing on 10 April. At this time, most lucerne seedlings had one fully expanded trifoliate leaf. Seedlings were measured for longest shoot and root lengths, and leaf area (Chapter 3). Dry weight determinations were made for leaf, stem and root components (Chapter 3). Four further harvests were conducted weekly on 17 and 24 April and 1 and 8 May.

6.2.4 STATISTICAL ANALYSES

6.2.4.1 SEEDLING EMERGENCE

Seed weights were converted to thousand seed weights (TSW) and seeds per gram since one or both parameters are quoted frequently (Salmeron, 1966; Nordborg, 1967b; ISTA, 1985; Sheppard and Wills, 1985). Data for TSW were analysed for each seed line using an analysis of variance and a random effects model was assumed for all significance tests (Steel and Torrie, 1980). Carbohydrate data for the Oregon line of sheep's burnet were expressed as % glucose, % starch and % total nonstructural carbohydrates (%TNC=% glucose + % starch). Absolute masses of

starch were also calculated. All carbohydrate data were analysed using one-way analysis of variance (Steel and Torrie, 1980).

Cumulative seedling emergence expressed as a percentage of seeds sown was described for each treatment x block combination by a logistic function (Section 3.6). Details on the biological significance of the function and the method of fit were presented in Sections 3.6.1 and 3.6.3. The three parameters (β_0, β_1 and β_2) defining the function for each treatment and block had one or more covariances between them and therefore the data were subjected to a multivariate analysis of variance (Section 3.6.1). The procedure was repeated for the sheep's burnet treatments only and sources of variation included seed line and seed size and their interaction. This enabled a more detailed examination of the potential sources of variation in the experiment. Although it was desired to assume a random effects model (Steel and Torrie, 1980) for all significance tests, there were insufficient error degrees of freedom for multivariate tests for seed line and seed size, when using the seed line x seed size interaction matrix as the error matrix. A fixed effects model was therefore adopted with the residual matrix being used as the denominator in all significance tests. Univariate analyses of variance were also conducted.

The proportion of doubles for each sheep's burnet treatment (Section 3.6.1) was estimated as a percentage. Data were analysed subsequently using a factorial analysis of variance including sources of variation due to seed line and seed size and their interaction.

Times to reach various stages of emergence (t_{10}, t_{50} and t_{90}) for all treatments were estimated from the logistic functions (Section 3.6.1). Data were then analysed jointly using a multivariate analysis of variance (Section 3.7). For the sheep's burnet treatments only, an additional analysis was conducted which was similar to that used for the logistic function parameters above. Again the residual matrix was used as the denominator in all significance tests and univariate analyses of variance were also conducted.

6.2.4.2 VEGETATIVE GROWTH

Variable numbers of seedlings per plot were harvested. Adjustment of treatment means for each character using seedling number as a covariate was not conducted since all R-square estimates were low and generally less than 0.20. Instead, arithmetic means per seedling were calculated for all characters. To approximately stabilise the variances, all characters were transformed to logarithms (Steel and Torrie, 1980). Since there were likely covariances between at least some of the six characters, data were analysed simultaneously using a split plot multivariate analysis of variance over harvests (Section 3.7). A random effects model was assumed for all tests of significance (Steel and Torrie, 1980) except for source of variation due to treatment, where the block x treatment interaction matrix was used as the denominator in the F-test. Additional multivariate analyses were conducted on the sheep's burnet data alone and included sources of variation due to seed line, seed size and their interaction. In these latter analyses, the residual matrix was used as the denominator in all significance tests for the same reasons as those mentioned in Section 6.2.4.1. Univariate analyses of variance were also conducted for all characters.

6.3 RESULTS

6.3.1 SEED SIZE AND COMPOSITION

Seed line x seed size means are presented for several physical and chemical characters of seed of sheep's burnet in Table 6.1. Thousand seed weights for each line increased significantly ($P<0.05$) with increasing seed size but the mean seed weights for them were similar. There were 124 and 133 seeds per gram for the Oregon and Cockayne seed lines, respectively (Table 6.1). The contents of nonstructural carbohydrates (glucose and starch) were the same for all seed sizes of the Oregon line, but as suggested by the weight differences between the three seed sizes, heavier seeds had greater absolute quantities of glucose and starch. Starch for example, increased by approximately 5mg / 1000 seeds from small to medium to large seeds (Table 6.1). The arbitrarily chosen sieve sizes enabled the batches of

TABLE 6.1 Some physical and chemical attributes of seed of sheep's burnet.

Seed line	Seed size	1,000 seed weight (g)	Seeds per gram[x]	Proportion by weight (%)	glucose content (%)	starch content (%)	TNC content (%)	starch /1000 seeds (mg)
Oregon	small	4.32 c+	231.6	28.9	1.29	0.19	1.48	8.21 c
Oregon	medium	8.20 b	122.3	39.2	1.07	0.16	1.23	13.12 b
Oregon	large	11.63 a	86.0	31.9	1.13	0.17	1.30	19.77 a
Mean		8.05	124.2		1.16	0.17	1.34	13.70
Cockayne	small	4.04 c	247.8	29.7	-	-	-	-
Cockayne	medium	7.15 b	140.1	39.2	-	-	-	-
Cockayne	large	11.30 a	88.6	31.1	-	-	-	-
Mean		7.50	133.3					

+ figures scored by different letters differ at the 5% level of significance

x calculated from 1,000 seed weight data

seed of each selection to be separated into three seed sizes of about equal proportion by weight.

6.3.2 SEEDLING EMERGENCE

There were significant differences between lucerne and some of the sheep's burnet treatments according to the results of the multivariate analysis of logistic function parameters. The first of three discriminant functions for treatments accounted for 90% of the data dispersion (Table 6.2a) and mean scores on this function are presented in Table 6.2b. Univariate means for $ß_0$ and $ß_2$, together with the proportions of doubles for the sheep's burnet treatments, appear in Table 6.2c. The parameters $ß_0$ and $ß_2$ were of greatest interest because they represented final emergence and emergence rate, respectively (Section 3.6.1). The most important of these in the first function was $ß_2$ which had a positive loading (Table 6.2b). This indicated that increasing emergence rate resulted in more positive mean scores and lucerne was superior to several sheep's burnet treatments in this respect (Tables 6.2b and c). Final emergence ($ß_0$) was about half as important as $ß_2$ and it had a negative loading (Table 6.2b).

All sheep's burnet treatments and lucerne had similar final seedling emergences ($ß_0$ from logistic function) (Table 6.2c), with an overall average of 51.4%. The proportion of doubles (%) was similar for both lines of sheep's burnet and their respective seed sizes, although the seed line x seed size interaction was significant at the 10% level. An examination of the treatment means (Table 6.2c) showed that for the sheep's burnet from Oregon, an increase in seed size resulted in a higher proportion of doubles, whereas for the Cockayne material this trend was relatively unimportant. The mean proportion of doubles was 32.9%.

Amongst the sheep's burnet treatments, there was an inconsistent relationship between seed line and seed size in the analysis of the logistic function parameters. On the first discriminant function for the interaction, which accounted for 87% of total data dispersion (Table 6.3a), $ß_2$ (emergence rate) was again the most important character and it was approximately twice as important as $ß_0$ (Table 6.3b). An

TABLE 6.2a Important statistics for the three discriminant functions for treatments from the multivariate analysis of logistic function parameters for sheep's burnet and lucerne.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	90.16	2.84	18	46	**
2	9.40	0.77	10	34	NS
3	0.44	0.10	4	18	NS

Table 6.2b Mean scores for the first discriminant function for treatments

Seed line	Seed size	Mean score
Lucerne	-	3.67
Oregon	small	0.66
Oregon	medium	-0.19
Oregon	large	0.66
Cockayne	small	-2.34
Cockayne	medium	-0.55
Cockayne	large	-0.57

standardised coefficients:

1. β_2 (1.98)
2. β_1 (-1.16)
3. β_0 (-1.13)

Table 6.2c Treatment means for β_0 and β_2 and proportion of doubles (standard errors in parentheses).

Seed line	Seed size	β_0	β_2	Proportion of doubles (%)
lucerne	-	34.6 (6.8)	2.4 (0.6)	-
Oregon	small	47.0 (4.7)	1.7 (0.2)	10.9 (7.4)
Oregon	medium	46.9 (5.9)	1.1 (0.1)	31.3 (4.8)
Oregon	large	57.4 (13.6)	1.9 (0.3)	57.8 (14.1)
Cockayne	small	68.0 (5.9)	1.4 (0.5)	23.9 (6.9)
Cockayne	medium	53.0 (7.7)	1.3 (0.2)	35.6 (10.9)
Cockayne	large	52.8 (8.6)	1.2 (0.1)	37.7 (7.8)

TABLE 6.3a Important statistics for the two discriminant functions for seed line x seed size interaction from the multivariate analysis of logistic function parameters for sheep's burnet.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	86.69	2.91	6	26	*
2	13.31	1.42	2	14	NS

Table 6.3b Mean scores for the first discriminant function for sheep's burnet treatments.

Seed line	Seed size	Mean score
Oregon	small	1.75
Oregon	medium	0.44
Oregon	large	-0.03
Cockayne	small	-2.26
Cockayne	medium	0.06
Cockayne	large	0.02

standardised coefficients:

1. β_2 (2.27)
2. β_1 (-1.81)
3. β_0 (-1.20)

examination of the treatment means (Table 6.2c) indicated that while $ß_2$ estimates were similar for the Cockayne seed sizes, medium sized sheep's burnet seed from Oregon emerged more slowly than small and large seed. This probably accounted for most of the interaction and the results indicated that selection for seed size in Oregon sourced material could be worthwhile.

The multivariate analysis of times to reach 10, 50 and 90% emergence for all treatments (sheep's burnet and lucerne) was significant for treatments. The first discriminant function for this source of variation accounted for 82% of data dispersion (Table 6.4a) and mean scores on the function together with the two largest standardised coefficients are presented in Table 6.4b. The most important character was t_{50} with a negative loading and it was almost twice as important as t_{90}. Lucerne had a much more negative mean score than all sheep's burnet treatments (Table 6.4b) which indicated that it exhibited superior emergence rate. This was also supported by the univariate means (Table 6.4c) with lucerne being relatively early in reaching specific stages of emergence. A separate multivariate analysis of the sheep's burnet treatments alone found that they were all similar in their emergence rate characteristics.

6.3.3 VEGETATIVE GROWTH

There was a significant harvest x treatment interaction in the multivariate analysis of all characters for lucerne and the sheep's burnet treatments. The first three discriminant functions accounted for a total of 78% of data dispersion (Table 6.5a). On the first function (42% of dispersion), leaf area was the most important character and it was about four times more important than root length (Table 6.5b). Mean scores indicated that lucerne and Oregon sheep's burnet were similar over several harvests. The Cockayne seed line was relatively inferior, although responses did vary with seed size (Table 6.5b). Univariate means for leaf area (Table 6.6a) and root length (Table 6.6b) partly supported these findings.

The second discriminant function for the interaction accounted for 21% of data dispersion and the most important character was stem dry weight but it was only

TABLE 6.4a Important statistics for the three discriminant functions for treatments from the multivariate analysis of times to reach various stages of emergence for sheep's burnet and lucerne

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	81.65	5.65	18	46	***
2	14.27	2.68	10	34	*
3	4.08	1.70	4	18	NS

Table 6.4b Mean scores for the first discriminant function for treatments.

Seed line	Seed size	Mean score
Lucerne	-	-5.23
Oregon	small	0.67
Oregon	medium	0.60
Oregon	large	0.71
Cockayne	small	1.83
Cockayne	medium	1.02
Cockayne	large	0.40

two largest standardised coefficients:

1. t_{50} (-1515.5)
2. t_{90} (946.4)

Table 6.4c Treatment means for t_{50} and t_{90} (standard errors in parentheses).

Seed line	Seed size	t_{50} (days)	t_{90} (days)
lucerne	-	3.4 (0.2)	4.4 (0.4)
Oregon	small	7.6 (0.4)	8.9 (0.4)
Oregon	medium	7.5 (0.2)	9.4 (0.3)
Oregon	large	7.2 (0.8)	8.5 (0.9)
Cockayne	small	8.5 (0.5)	10.5 (1.0)
Cockayne	medium	7.9 (0.4)	9.8 (0.8)
Cockayne	large	7.5 (0.3)	9.3 (0.3)

TABLE 6.5a Important statistics for the six discriminant functions for the harvest x treatment interaction from the multivariate analysis of vegetative characters for sheep's burnet and lucerne.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	41.64	1.29	144	470	*
2	20.81	0.99	115	397	NS
3	14.84	0.85	88	323	NS
4	11.87	0.73	63	246	NS
5	6.02	0.56	40	166	NS
6	4.82	0.53	19	84	NS

Table 6.5b Mean scores for the first discriminant function for harvest x treatment interaction.

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	-1.70	0.27	0.47	0.94	1.18
Oregon	small	-2.27	-0.77	-0.48	2.22	3.44
Oregon	medium	-1.13	-0.61	0.07	1.12	3.63
Oregon	large	-0.57	-0.56	0.68	3.02	4.52
Cockayne	small	-0.81	-2.34	-2.52	-0.46	0.35
Cockayne	medium	-1.70	-2.38	-1.50	-1.15	-0.14
Cockayne	large	0.21	-1.92	-0.83	-0.39	2.07

two largest standardised coefficients:

1. leaf area (6.18)
2. root length (-2.70)

Table 6.5c Mean scores for the second discriminant function for harvest x treatment interaction.

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	-1.66	-0.40	2.26	3.91	5.88
Oregon	small	-4.74	-1.65	1.43	2.04	3.90
Oregon	medium	-3.87	-0.20	1.39	2.33	3.64
Oregon	large	-4.02	-0.89	1.98	3.83	4.28
Cockayne	small	-5.97	-2.76	-1.26	0.71	0.92
Cockayne	medium	-4.62	-2.19	-0.70	0.01	1.05
Cockayne	large	-6.74	-2.08	0.24	1.22	2.72

two largest standardised coefficients:

1. stem dry weight (1.60)
2. shoot length (1.38)

Table 6.5d Mean scores for the third discriminant function for harvest x treatment interaction.

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	-3.13	-2.02	-2.18	-1.58	-2.40
Oregon	small	0.23	-0.55	0.93	0.20	1.60
Oregon	medium	-0.98	-0.61	-0.09	0.24	0.90
Oregon	large	-1.28	-0.02	-0.31	0.61	1.99
Cockayne	small	-0.37	-0.90	0.41	1.92	1.45
Cockayne	medium	-0.45	-0.28	-0.07	1.03	1.50
Cockayne	large	-0.76	-0.23	0.98	1.86	2.37

two largest standardised coefficients:

1. shoot length (-5.45)
2. leaf area (4.88)

TABLE 6.6a Treatment means for leaf area (log scale) for the harvest x treatment interaction (standard errors in parentheses).

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	1.04 (0.15)	1.72 (0.30)	2.91 (0.21)	3.79 (0.14)	4.63 (0.08)
Oregon	small	-0.24 (0.65)	1.06 (0.41)	2.63 (0.12)	3.40 (0.58)	4.49 (0.28)
Oregon	medium	0.36 (0.18)	1.77 (0.22)	2.63 (0.50)	3.27 (0.12)	4.36 (0.27)
Oregon	large	0.28 (0.30)	1.51 (0.51)	3.02 (0.24)	4.33 (0.08)	4.74 (0.25)
Cockayne	small	-0.53 (0.25)	0.27 (0.12)	0.99 (0.35)	2.33 (0.08)	2.60 (0.25)
Cockayne	medium	-0.29 (0.25)	0.58 (0.21)	1.39 (0.42)	1.82 (0.30)	2.66 (0.53)
Cockayne	large	-0.45 (0.30)	0.67 (0.24)	1.99 (0.26)	2.59 (0.29)	3.77 (0.16)

Table 6.6b Treatment means for root length (log scale) for the harvest x treatment interaction (standard errors in parentheses).

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	3.9 (0.1)	4.1 (0.1)	4.9 (0.1)	5.4 (0.1)	5.7 (0.1)
Oregon	small	3.5 (0.2)	3.9 (0.2)	4.9 (0.1)	5.0 (0.1)	5.4 (0.2)
Oregon	medium	3.7 (0.1)	4.4 (0.1)	4.9 (0.2)	5.1 (0.1)	5.3 (0.1)
Oregon	large	3.6 (0.1)	4.3 (0.2)	4.9 (0.1)	5.4 (0.1)	5.4 (0.2)
Cockayne	small	3.3 (0.1)	3.8 (0.1)	4.5 (0.2)	5.0 (0.1)	4.9 (0.4)
Cockayne	medium	3.4 (0.1)	4.0 (0.1)	4.4 (0.2)	4.9 (0.2)	5.2 (0.2)
Cockayne	large	3.4 (0.2)	4.0 (0.1)	4.8 (0.1)	5.2 (0.1)	5.5 (0.1)

slightly more important than shoot length (Table 6.5c). Both characters had positive loadings which indicated that increasing values resulted in more positive mean scores. Univariate means are presented in Tables 6.6c and d. On the third function (15% of dispersion), shoot length and leaf area were the most important characters (Table 6.5d). Lucerne frequently had shoot lengths superior to those of the sheep's burnet treatments (Table 6.6c), particularly that material from the Cockayne plots.

The multivariate analysis of the sheep's burnet data alone found significant sources of variation for seed size and harvest x seed line interaction. The first of two discriminant functions for seed size accounted for 82% of data dispersion (Table 6.7a) and mean scores on the function, together with its two largest standardised coefficients, are presented in Table 6.7b. Leaf area had a positive loading and it was about 2.5 times more important than shoot length which was loaded negatively. Larger values for both characters were desirable agronomically and their combination gave an overall positive mean score. Hence, the results indicated that large seed gave superior vegetative growth and this was also supported by the univariate means for leaf area and shoot length (Table 6.7c).

For the harvest x seed line interaction, there were four discriminant functions but the first one accounted for most (78%) of the data dispersion (Table 6.8a). Leaf area was the most important character in the function and it had a positive loading (Table 6.8b). This indicated that increasing leaf area resulted in more positive mean scores. Root length was about half as important as leaf area. The results indicated that sheep's burnet from Oregon was frequently superior to that from the Cockayne plots and this was supported by the univariate means (Tables 6.8c and d). The interaction was due mainly to the relatively similar performance of the two selections at the first harvest and different performances in subsequent harvests, particularly for leaf area (Table 6.8d).

6.4 DISCUSSION

Rapid emergence and early seedling growth are of paramount importance in the efficient establishment of pasture and crop species (Black, 1959; Beveridge and

Table 6.6c Treatment means for shoot length (log scale) for the harvest x treatment interaction (standard errors in parentheses).

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	4.0 (0.1)	4.1 (0.2)	4.7 (0.1)	5.1 (0.1)	5.6 (0.1)
Oregon	small	3.0 (0.4)	3.6 (0.2)	4.4 (0.1)	4.6 (0.2)	4.9 (0.1)
Oregon	medium	3.4 (0.1)	4.0 (0.1)	4.3 (0.2)	4.6 (0.1)	4.9 (0.2)
Oregon	large	3.3 (0.2)	3.8 (0.3)	4.5 (0.1)	5.0 (0.1)	5.0 (0.1)
Cockayne	small	2.8 (0.1)	3.3 (0.1)	3.6 (0.1)	4.0 (0.1)	4.1 (0.1)
Cockayne	medium	3.0 (0.2)	3.4 (0.1)	3.8 (0.2)	3.9 (0.1)	4.2 (0.2)
Cockayne	large	2.8 (0.1)	3.4 (0.2)	3.9 (0.1)	4.1 (0.2)	4.5 (0.1)

Table 6.6d Treatment means for stem dry weight (log scale) for the harvest x treatment interaction (standard errors in parentheses).

Seed line	Seed size	1986 10/4	17/4	24/4	1/5	8/5
lucerne	-	-1.11 (1.24)	1.47 (0.48)	3.17 (0.25)	3.86 (0.56)	5.60 (0.11)
Oregon	small	-3.63 (1.01)	0.89 (0.22)	0.57 (0.96)	2.74 (1.15)	4.48 (0.31)
Oregon	medium	-3.16 (1.11)	1.55 (0.18)	2.59 (0.25)	3.58 (0.48)	4.32 (0.30)
Oregon	large	-1.86 (1.29)	1.25 (0.47)	2.91 (0.27)	3.83 (0.51)	4.84 (0.23)
Cockayne	small	-3.68 (0.98)	-0.04 (0.32)	0.89 (0.41)	2.49 (0.11)	2.81 (0.18)
Cockayne	medium	-1.74 (0.79)	0.25 (0.51)	1.41 (0.43)	2.42 (0.58)	2.67 (0.66)
Cockayne	large	-4.76 (0.99)	0.69 (0.23)	2.10 (0.20)	2.71 (0.54)	3.79 (0.18)

TABLE 6.7a Important statistics for the two discriminant functions for seed size from the multivariate analysis of vegetative characters for the sheep's burnet treatments.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	82.34	4.20	12	150	***
2	17.66	1.91	5	76	NS

Table 6.7b Mean scores for the first discriminant function for seed size.

Seed size	Mean score
small	-0.64
medium	-0.21
large	0.84

two largest standardised coefficients:

1. leaf area (4.85)
2. shoot length (-1.89)

Table 6.7c Treatment means for leaf area and shoot length (standard errors in parentheses).

Seed size	leaf area (log)	shoot length (log)
small	1.70 (0.27)	3.8 (0.1)
medium	1.85 (0.23)	3.9 (0.1)
large	2.25 (0.28)	4.0 (0.1)

TABLE 6.8a Important statistics for the four discriminant functions for harvest x seed line interaction from the multivariate analysis of vegetative characters for the sheep's burnet treatments.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	78.38	2.24	24	263	**
2	13.93	0.86	15	210	NS
3	5.23	0.59	8	154	NS
4	2.46	0.50	3	78	NS

Table 6.8b Mean scores for the first discriminant function for harvest x seed line interaction.

Seed line	1986 10/4	17/4	24/4	1/5	8/5
Oregon	-1.70	-0.48	0.45	2.50	4.20
Cockayne	-1.47	-2.16	-1.53	-0.64	0.83

two largest standardised coefficients:

1. leaf area (5.29)
2. root length (-2.74)

Table 6.8c Harvest x seed line means for leaf area (log scale) (standard errors in parentheses).

Seed line	1986 10/4	17/4	24/4	1/5	8/5
Oregon	0.13 (0.24)	1.45 (0.23)	2.76 (0.18)	3.67 (0.23)	4.53 (0.15)
Cockayne	-0.42 (0.14)	0.51 (0.11)	1.46 (0.22)	2.25 (0.16)	3.01 (0.24)

Table 6.8d Harvest x seed line means for root length (log scale) (standard errors in parentheses).

Seed line	1986 10/4	17/4	24/4	1/5	8/5
Oregon	3.6 (0.1)	4.2 (0.1)	4.9 (0.1	5.2 (0.1)	5.4 (0.1)
Cockayne	3.4 (0.1)	3.9 (0.1)	4.6 (0.1)	5.0 (0.1)	5.2 (0.1)

Wilsie, 1959; Sears, 1961; Perry, 1980; Charlton and Thom, 1984; Lafond and Baker, 1986a, b). Seed line and seed size, as examined in the current study, are two of the relatively easily manipulated factors which may influence these parameters.

The arbitrarily obtained seed sizes of sheep's burnet covered a large range of thousand seed weights (Table 6.1) and provided a basis for examining the effect of different seed sizes on early establishment. The average weights for the Oregon and Cockayne seed lines were lower than those reported by Lindenbein (1956) (cited by Nordborg, 1967b) for commercial seed (12.52 g) and seed collected from nursery plants (10.05 g), but the latter estimate was slightly less than those for large seed in the present study. Differences in average seed weight (size) between the two studies could be due to numerous factors including variations in nutrition of the mother plant, position of the inflorescence and stage of maturity at harvest (Perry, 1976, 1980). The averages of 124 and 133 seeds per gram for Oregon and Cockayne selections, respectively, were approximately at the lower end of the range reported for Spanish material (Salmeron, 1966).

Under the controlled environmental conditions of the present study, final emergence (%) for all sheep's burnet treatments and lucerne was similar at approximately 50% (Section 6.3.1). Field emergence of sheep's burnet in Spain (Salmeron, 1966) has surpassed 50% in the majority of cases and these findings partly supported those reported here. In Sweden, emergence is usually between 30 and 60% (Nordborg, 1967b). For lucerne, the estimate was in agreement with normal levels of final field emergence (Palmer and Wynn-Williams, 1982; Wynn-Williams, 1982). In contrast with the usual situation where lucerne has a relatively high hard seed content (for example, Scott and Hampton (1985)), 'Rere' lucerne in this investigation exhibited a low content of 1%. Hence, further germination after emergence counts ceased was unlikely.

Similar levels of final emergence for the three seed sizes of sheep's burnet were at variance with most of the findings from studies on other species, where higher emergences resulted frequently from using large seeds (Black, 1959; Powell, 1988). Results from this study suggested that satisfactory emergence of sheep's burnet can

be achieved regardless of the size distribution of the seed sample, but the situation in field sowings remains to be determined. Apart from potentially large field variability in factors such as soil moisture and temperature, soil physical attributes have also received recent research interest (Clarke and Moore, 1986; Powell, 1988), and these may be relevant in field sowings of sheep's burnet. For example, it is possible that the extent of emergence of large seeds of the species may be lower than small seeds in soils prone to crusting due to the presumably greater mechanical impedance of their relatively large cotyledons.

All seeds were placed carefully at constant depth and therefore there was no opportunity to determine suitable planting depths for satisfactory emergence of each seed line and seed size of sheep's burnet. For a given seed size, a compromise in sowing depth must be made between the desirability of having soil depths where the seed is exposed to levels of soil moisture conducive to satisfactory germination, and those depths where emergence and subsequent vegetative growth are acceptable. Large seeds for numerous species have often germinated and produced satisfactory early vegetative growth from greater depths than small seeds (Rogler, 1954; Black, 1959; Arnott, 1969), and this may be true for sheep's burnet. Inevitably, however, emergence is reduced when planting depth exceeds a critical value (Beveridge and Wilsie, 1959; Arnott, 1969). In some species, orientation of the seed may influence the rate of seedling emergence (Perry, 1976; Lovato, 1981), but this is probably only of theoretical interest in the case of sheep's burnet since its practical manipulation in the field would be extremely difficult.

Estimates of the proportion of doubles from different seed sizes were undocumented previously and averaged 33% (Section 6.3.1). In European material, the two achenes per seed of sheep's burnet may each produce new plants (Nordborg, 1967b), although the extent of this development and the environmental conditions under which it occurs were not reported. While the proportion of doubles of the older Cockayne selection was not related to seed size, increasing numbers of doubles with heavier seed were suggested for the Oregon material (Table 6.2c). These ranged from 11% to 58% for small and large seeds, respectively. The advantages and disadvantages of a high proportion of doubles remain to be determined. It would be particularly

important to find the effect of the proportion of doubles on the overall vigour of a stand. Due to the very close physical (and perhaps chemical) association of doubles, the second emerged member of a pair may frequently experience competition, particularly for light, earlier than seedlings with a non-doubles history. However, the extent of this competition and its ultimate effect on seedling growth and survival is largely unknown. Varying the occurrence of doubles under South Island field conditions probably has little practical relevance since the seedling pairs either anastomose or one seedling dominates (Wills, 1984; Sheppard and Wills, 1986), with the result that after a few months a single plant equivalent exists (B J Wills, pers. comm.).

The similar rates of seedling emergence of the three seed sizes of Cockayne sheep's burnet (Section 6.3.1) suggested that there would be no advantage in using different sized seeds of this naturalised material in field sowings. Conversely, small and large seed of the Oregon selection had slightly superior emergence rates compared with the medium seed size (Table 6.2c), which suggested that some benefit may result from using these seed sizes. There was no apparent explanation for the slight inferiority of medium sized seed. Various seed sizes could be obtained by physical separation in the seed dressing plant or by possible improvement via genetic means. The size of genetic advances leading to increased yields of small or large seed in the Oregon seed line would depend very much on the heritability of seed size and the selection intensities adopted in any practical breeding programme (Allard, 1960; Poehlman, 1979).

The slow emergence of all sheep's burnet treatments compared with lucerne suggested that mixtures involving these two species should consist of relatively low seeding rates of lucerne to reduce competition, particularly for light, with the later emerging seedlings of sheep's burnet. Because of similar growth forms and the competitiveness of lucerne, mixtures of these species are not recommended under many conditions in the South Island of New Zealand (B J Wills, pers. comm.). Shading or low light intensity affects the distribution of dry matter into foliage and roots in numerous species and with decreasing light intensity, less dry matter is partitioned into seedling roots (Black, 1958; Cooper, 1977; Ludlow, 1978; Barta,

1988a, b). This has important practical implications under dry conditions where shaded seedlings, with their restricted root development, may be more susceptible to drought (Cooper, 1977). Due to the slow to moderate emergence and early vegetative growth of sheep's burnet, it is recommended currently that the species should only be sown into ground with a depleted vegetative cover (Wills, 1984; Sheppard and Wills, 1984, 1985), and results from the present investigation support this recommendation.

The proportions of embryo (excluding cotyledons) and cotyledons in the different seed sizes were not determined in this experiment. In many species, large seeds usually possess larger embryos than small seeds (Bremner *et al.*, 1963; Perry, 1980), but the situation for sheep's burnet is unknown. However, cotyledons from large seeds in this study were generally larger than those from small seeds and this was pronounced for the Oregon seed line. This suggested that the major increase in seed size was accounted for by cotyledon size and hence nutrient reserves. The composition of these reserves was equivocal since the seed content of starch, which is usually a significant proportion of seed dry weight in many species (Halmer, 1985; Mercier, 1985; Sangakkara *et al.*, 1985), was low in the present investigation. More important energy sources could be proteins (albumins and globulins) (Lovato, 1981), lipids, polyphenols and nucleic acids (Slaughter, 1988).

One of the two achenes in a double is usually larger than the other (B J Wills, pers. comm.). This may account for the frequent observation, at least in sheep's burnet from Oregon, that one of the seedlings is often larger than its partner. In small seed of this material in the current investigation, a low proportion of doubles (11%) was produced (Table 6.2c). This may have resulted from frequently inadequate development of the embryo in the smaller of the two achenes.

Despite the probably greater quantities of storage reserves in larger seeds of each seed line of sheep's burnet, emergence rates almost without exception, were generally similar to those of small seeds (Section 6.3.1.). This suggested that other factors may be limiting the rate of emergence and these could include genetic limits on the rates of various biochemical processes associated with germination, and on the elongation

rate of the radicle/hypocotyl (Black, 1959). Limits on the rate of transfer of reserves to the embryo axis may also be an important factor.

An advantage of the multivariate analysis of the logistic function parameters used in the current investigation was that it enabled a simultaneous analysis of final emergence level ($ß_0$) and emergence rate ($ß_2$). This provided valuable information on the relative importance of these two parameters in discriminating between the various treatments and therefore gave a more fundamental understanding of the underlying biological principles. In all instances, emergence rate ($ß_2$) was the most important character in distinguishing between the treatments and the results indicated that it may be unnecessary to measure $ß_0$ in other similar studies.

The frequently superior early vegetative growth of sheep's burnet from Oregon compared with that from the Cockayne plots (Section 6.3.2) highlighted the merits of the relatively recent material compared with germplasm used in early New Zealand trials. Seedlings of the Oregon seed line had generally greater leaf area, stem dry weight and shoot and root lengths (Section 6.3.2), which may confer several advantages in practical field sowings. For example, longer shoot lengths and hence taller seedlings, enable seedlings to exhibit a greater competitive advantage for light, thereby minimising or delaying the deleterious effects of reduced light intensities mentioned previously. A larger leaf area may result in greater absolute dry weight increases during the early part of the vegetative phase and also provide earlier protective ground cover. The often slightly longer roots of the Oregon selection suggested that it may reach deeper and moister layers of the soil more rapidly than sheep's burnet from the Cockayne plots. This feature is highly advantageous in dry environments where it is often essential to rapidly develop a deep and efficient root system (Evans, 1973; Wilson, 1984).

The superiority of the Oregon seed line was in agreement with the findings from several field evaluations of a range of glasshouse prepared seedlings of sheep's burnet in the South Island of New Zealand (Sheppard and Wills, 1985). Apart from the locational differences, seedlings in the field studies were older than those studied presently and measurements were simpler (J S Sheppard, pers. comm.). A

continuation of the current study may have provided further evidence of the superiority of the Oregon material.

The improved vegetative growth of both seed lines of sheep's burnet from using relatively large seed was consistent with the findings for many other species, where seedlings developed from large seeds are frequently superior for one or more of leaf area, leaf number, root and leaf dry weights, root length and root elongation rate (Black, 1959; Arnott, 1969; Evans, 1973; McKersie *et al.*, 1981; Powell, 1988). However, the present study was also a considerable refinement on previous research since it identified leaf area from several commonly measured seedling features as being the character most useful for discriminating between the various seed sizes. Its measurement alone should suffice in future similar investigations. The results suggested that seedlings from large seed could provide superior early ground cover which is an important benefit for soil conservation plantings. However, it is doubtful whether the advantages in the early vegetative stages would have also been reflected in ultimate forage yields, due to the gradual onset of inter-plant competition (Black, 1959; Perry, 1980).

Soil moisture and various nutrients were adequately supplied in this investigation and therefore results represented the upper potential of those which could be realised in the field. These conditions would probably have favoured the growth of lucerne (Scott and Charlton, 1983; Scott *et al.*, 1985). Differences between lucerne and the seed lines of sheep's burnet might have been smaller under low fertility conditions.

The present study provided guidelines on the most appropriate vegetative characters which should be measured in future similar investigations. Useful discrimination between the means for lucerne and all sheep's burnet treatments was provided by the foliar characters of leaf area, stem dry weight and shoot length, with leaf area being particularly useful. Shoot length was also the easiest vegetative character to measure, thereby making it a desirable character for practical seedling evaluations. Furthermore, the results suggested that destructive samplings for root length and dry weight determinations were unnecessary for satisfactory treatment discrimination. This finding has several advantages in practical evaluation programmes, foremost

being considerable savings on relatively labour intensive operations.

CHAPTER 7 : INTERACTIONS BETWEEN SEVERAL FACTORS IN THE SUCCESSFUL EARLY ESTABLISHMENT OF SHEEP'S BURNET UNDER CONTROLLED CONDITIONS

7.1 INTRODUCTION

Numerous environmental and seed factors may influence successful early establishment of sheep's burnet and two of these factors, namely seed line and seed size, were examined in the previous study (Chapter 6). Variability in seed size and seed line contributed to differences in early vegetative growth but did not affect emergence.

Two other factors which may influence seedling establishment are temperature and sowing depth. Apart from soil moisture, temperature is the dominant environmental factor governing the seedling establishment of many species (Woods and MacDonald, 1971; Perry, 1976; Cooper, 1977; Lovato, 1981; Garcia-Huidobro *et al.*, 1982; Hur and Nelson, 1985; Muendel, 1986; Charlton, 1989). Major sub-optimal effects of temperature increases include higher germination (and hence emergence) rates, shorter duration of emergence, increased absorption of some nutrients and raised root/foliage ratios. The effects of temperature on seedling emergence and early establishment of sheep's burnet are poorly documented.

The standard recommendation is that seed of sheep's burnet should be sown not deeper than about 1 cm (B J Wills, pers. comm.). More precise information on sowing depth is unavailable and in view of the importance of this agronomic feature (Beveridge and Wilsie, 1959; Black, 1959; Perry, 1976; Cooper, 1977; Tischler and Voigt, 1983), particularly for seed which has highly variable size (weight), there is a need for specific recommendations.

This group of experiments examined the effects of temperature and sowing depth, in addition to seed line and seed size, on the emergence and early vegetative growth of sheep's burnet. Associated with this was the aim of identifying the presence or

absence of any interactions among these factors which could be applied to practical sowings.

7.2 MATERIALS AND METHODS

7.2.1 LOCATION

The investigation was conducted in a controlled environment room at the Climate Laboratory, Plant Physiology Division, DSIR, Palmerston North. Four experiments were conducted at constant air temperatures of 10, 15, 20 and 25°C during the period from 24 June to 7 November, 1986 (Appendix). Daylength was 12 hours, relative humidity was approximately 70%, and photosynthetic irradiance was 140-150 Wm^{-2}.

Light was supplied by 4 x 1000W Sylvania "Metalarc" high pressure discharge lamps and 4 x 1000W tungsten halogen lamps. Further details on the system are available elsewhere (Warrington *et al.*, 1978). The carbon dioxide level was monitored and remained within 331-431 ppm. Air flow down through the plants was 0.3-0.5 ms^{-1} as measured with an Alnor Instruments thermoanemometer.

7.2.2 PLANT ESTABLISHMENT AND TREATMENTS

Seeds of three different sizes of the Oregon and Cockayne accessions were used, as described in Section 6.2.2. Due to a shortage of large Cockayne seed, unsieved lucerne cv. 'Rere' seed of approximately uniform size was used instead in the 15 and 25°C experiments.

Sowing times at each temperature were: 10°C (22 July); 15°C (16 September); 20°C (24 June) and 25°C (21 October). Seeds of each line and size were sown 5 and 10 mm deep into a 1 part soil : 10 parts sand mix contained in 1.2 *l* plastic pots. Sand was used to facilitate later root removal and no fertiliser was added to the potting medium. The twelve treatments, namely two seed lines x three seed sizes x two sowing depths (including lucerne where appropriate), were arranged in each of three randomised complete blocks and each plot (experimental unit) consisted of four pots,

giving a total pot number per experiment of 144. Four seeds were sown per pot. All pots were supported on trolleys and the media were watered uniformly by hand at a rate of 70-130 cm^3 d^{-1} depending on temperature.

7.2.3 MEASUREMENTS

The number of seedlings emerged was counted daily until the number of seedlings in any treatment had been equal for at least two days. A seedling was deemed emerged when both cotyledons were exposed fully and approximately horizontal. The number of doubles (Chapter 3) of sheep's burnet was recorded at the end of the emergence count period. At all temperatures, counts were conducted for three of the four pots per plot because the seedlings in the remaining pot were harvested during the counting periods, as detailed henceforth.

One pot per plot was harveted on each of four occasions to determine the early vegetative growth of the seedlings in each treatment. The initial harvest at each temperature was conducted for all treatments when the first seedling appeared in any treatment. Subsequent harvests were conducted on a growth stage basis. These were: harvest 2 - cotyledons fully expanded and first leaf starting to emerge; harvest 3 - first leaf fully expanded and second leaf starting to emerge; and harvest 4 - second leaf fully expanded and third leaf starting to emerge. At each harvest, shoot and root lengths were measured for individual seedlings while dry weights were determined for the bulked seedlings per pot at harvest one (Chapter 3). For harvests two to four, seedlings were dissected into foliage and roots and bulked as such for dry weight determinations (Chapter 3). At each harvest, seedlings which were not at the appropriate growth stages were discarded.

7.2.4 STATISTICAL ANALYSES

7.2.4.1 SEEDLING EMERGENCE

The stage where emerged seedling numbers had stabilised for at least two consecutive days was regarded as final emergence (Section 3.6.1). Data were

expressed subsequently as a percentage of seed sown per plot and the proportion of doubles was estimated for individual plots (Section 3.6.1).

To compare the relative rates of seedling emergence of each treatment, cumulative emergence at each time was totalled over blocks and expressed as a percentage of final emergence (Section 3.6.1). This was conducted for the Oregon seed line only since this was frequently the superior material in the previous study (Chapter 6). The rate constants (β_2) (Section 3.6) were compared using pairwise t-tests (Steel and Torrie, 1980) between those estimates for depths within each temperature x seed size combination, between seed sizes within each depth x temperature combination and between temperatures within each seed size x depth combination.

Times to reach 10, 50 and 90% emergence (t_{10}, t_{50} and t_{90}, respectively) were also estimated from the fitted functions and then added to the number of days after sowing when time zero for each curve occurred (Section 3.6.1). The inverses of the estimates for t_{50} were then regressed linearly against temperature for each depth x seed size combination to determine the threshold temperature required for emergence (Kanemasu *et al.*, 1975; Angus *et al.*, 1981). There were no significant differences between the parameter estimates for each equation and therefore all data were bulked and the regression analysis repeated. An estimate of threshold temperature was obtained by backward solution of the regression function and an estimate of its standard error was calculated using the formula of Gordon *et al.* (1979).

7.2.4.2 VEGETATIVE GROWTH

At each harvest, a variable number of seedlings per plot was harvested. R-square estimates of total dry weight against seedling number at each temperature and harvest ranged from low to high and therefore since the estimates were not consistently high, no analysis of covariance and adjustment of treatment means were conducted. Instead, arithmetic means per seedling were calculated for all dry weight data and this approach was also adopted for the shoot and root length data.

7.2.4.3 ANALYSES OF VARIANCE

Three different analyses were conducted with emphasis on multivariate analysis of variance (Section 3.7). Univariate analyses of variance were also conducted for all characters. All analyses of variance were pooled over temperatures (Le Clerg *et al.*, 1962) and where possible, a random effects model was assumed for tests of significance (Steel and Torrie, 1980). Characters measured over several harvests were regarded as separate characters at each harvest to include possible covariances between successive harvests in the analyses.

The first analysis was for the Oregon seed line and involved three seed sizes, two depths and four temperatures. Earlier research (Chapter 6) showed that sheep's burnet from Oregon was superior for a variety of features and therefore the present analysis was of major interest. Eighteen characters were used in the multivariate analysis of variance and they were:

emergence (%)
proportion of doubles (%)
time of final emergence (days)
shoot length at harvest 1 (H1) (mm)
shoot length at H2 (mm)
shoot length at H3 (mm)
shoot length at H4 (mm)
root length at H1 (mm)
root length at H2 (mm)
root length at H3 (mm)
root length at H4 (mm)
total seedling dry weight at H1 (mg)
shoot dry weight at H2 (mg)
shoot dry weight at H3 (mg)
shoot dry weight at H4 (mg)
root dry weight at H2 (mg)
root dry weight at H3 (mg)
root dry weight at H4 (mg)

All sources of variation were tested against the residual matrix because there were insufficient error degrees of freedom for multivariate tests when any other matrix was used as the denominator. In addition to the equivalent univariate analyses of variance which tested all sources of variation against the residual mean square, temperature was tested against temperature(block) while all first order interactions were tested against the temperature x depth x seed size interaction. Depth and seed size were tested against appropriate first order interactions, thereby more closely resembling ratio choices when assuming a random effects model.

In the second analysis, three seed sizes and two depths were investigated for both Oregon and Cockayne seed lines when grown at two temperatures (10 and 20°C). All eighteen characters listed above were analysed simultaneously and again the residual matrix was used as the denominator to test all sources of variation. The third order interaction was omitted due to likely interpretation difficulties. Analagous univariate analyses were conducted and in addition temperature was tested against temperature(block).

Five sheep's burnet treatments and lucerne were investigated at two depths and at two temperatures (15 and 25°C) in the final analysis. All characters listed previously were analysed in the multivariate analysis of variance with the exception of the proportion of doubles, which lucerne does not produce. Sources of variation were tested against the residual matrix for the reason mentioned previously. In addition to the equivalent univariate analyses, temperature was tested against temperature(block) while all first order interactions were tested against the temperature x depth x treatment interaction. Pseudo-random effects ratios for treatment and depth were constructed by testing these sources of variation against appropriate first order interactions.

7.3 RESULTS

7.3.1 SEEDLING EMERGENCE

Final seedling emergence and the proportion of doubles were influenced significantly by seed size (Table 7.1a) with there being approximately a 10% increase in emergence with larger seed. Small seed produced a much lower proportion of doubles than medium and large seed. Emergence levels and the proportion of doubles across all temperatures were similar (Table 7.1b) and averaged 70.4% and 43.1%, respectively.

Seedling emergence rates were frequently unaffected by seed size as shown by the generally similar rate constant (β_2) estimates for all seed sizes at each temperature x depth combination (Table 7.2a). Two exceptions occurred at low temperatures (10

TABLE 7.1a Seed size means for emergence (%) and proportion of doubles (%) for Oregon sheep's burnet (standard errors in parentheses).

Seed size	emergence (%)	proportion of doubles (%)
small	61.5 (2.8)	8.8 (1.9)
medium	69.3 (2.9)	51.3 (2.0)
large	80.6 (2.7)	69.3 (3.4)

Table 7.1b Temperature means for emergence (%) and proportion of doubles (%) for Oregon sheep's burnet (standard errors in parentheses).

Temperature (°C)	emergence (%)	proportion of doubles (%)
10	69.9 (3.6)	42.1 (6.2)
15	69.9 (3.6)	47.0 (6.9)
20	67.4 (4.2)	37.4 (6.6)
25	74.5 (3.5)	46.0 (7.3)

TABLE 7.2a Rate constant (β_2) estimates from the logistic functions describing cumulative emergence (totalled over blocks) over time for Oregon sheep's burnet - comparing seed sizes.

	Depth (mm)							
	5				10			
Seed size	temperature (°C)				temperature (°C)			
	10	15	20	25	10	15	20	25
small	0.79 ab+	1.84	2.26	3.16	1.37	0.87 b	1.24	2.59
medium	0.74 b	1.43	2.36	4.06	1.05	1.99 ab	1.77	3.00
large	1.23 a	2.46	1.88	4.27	1.38	2.51 a	2.18	2.56

+ figures underscored differ at the 5% level of significance.

Table 7.2b Rate constant (β_2) estimates from the logistic functions describing cumulative emergence (totalled over blocks) over time for Oregon sheep's burnet - comparing temperatures.

	Depth (mm)					
	5			10		
Temperature	Seed size			Seed size		
(°C)	small	medium.	large	small	medium	large
10	0.79 b+	0.74	1.23	1.37 b	1.05 c	1.38 c
15	1.84 a	1.43	2.46	0.87 b	1.99 b	2.51 ab
20	2.26 a	2.36	1.88	1.24 b	1.77 abc	2.18 abc
25	3.16 a	4.06	4.27	2.59 a	3.00 a	2.56 a

+ figures underscored differ at the 5% level of significance.

and 15°C at 5 and 10 mm depths, respectively), where large seed sometimes had significantly higher emergence rates than medium or small seed. The most important factor influencing emergence rate was temperature, as evidenced by often superior rates at high temperatures (Table 7.2b). Significant temperature effects featured prominently at the deeper sowing depth for all seed sizes. The often increased emergence rates at high temperatures were reflected in a slight general decrease in the duration of emergence with increasing temperature from about 5-7 days at 10°C to 2-4 days at 25°C. Emergence rates of small seed differed between sowing depths at 10 and 15°C, with deeper sowing having the highest rate at 10°C while the reverse trend occurred at 15°C (Table 7.2a). No other depth effects were significant ($P<0.05$).

Times to reach each stage of emergence decreased with increasing temperature while the effects of seed size and sowing depth on t_{10}, t_{50} and t_{90} were less apparent (Table 7.3). Temperature influenced both the timing of emergence and its duration. For example, at 10°C it took approximately 13 days to reach 10% emergence and a further three days to attain 90% emergence (Table 7.3). Conversely, 10% emergence was reached in about four days at 25°C and most emergence (t_{90}) occurred within the next 1-2 days.

The regression analysis of the inverse of time to reach 50% emergence ($1/t_{50}$), against temperature, was highly significant and approximately 92% of the variability in the dependent variable was accounted for by temperature. Estimates of the parameters were: $ß_0$=-0.05 (SE=0.01) and $ß_1$=0.01 (SE=0.00). The threshold temperature required for emergence was 4.85°C (SE=1.86°C). Parameter estimates and their standard errors for the regression equations involving the same variables for each Oregon sheep's burnet treatment, are presented in the Appendix.

7.3.2 VEGETATIVE GROWTH

Temperature had a pronounced effect on harvest time, with higher temperatures shortening the time to attain specific stages of growth. For example, time of harvest one ranged from 12 days after sowing at 10°C to 4 days at 25°C. Furthermore, as

TABLE 7.3 Days to reach 10, 50 and 90% of final seedling emergence for variously sized Oregon sheep's burnet seed sown at 5 and 10 mm depths at 10, 15, 20 and 25°C.

Temperature (°C)	Seed size	Depth (mm)	t_{10}	t_{50}	t_{90}
10	small	5	13.0	15.8	18.6
10	medium	5	12.9	15.9	18.8
10	large	5	12.0	13.8	15.6
10	small	10	15.6	17.2	18.8
10	medium	10	14.5	15.6	17.7
10	large	10	13.1	14.7	16.3
15	small	5	8.7	9.9	11.1
15	medium	5	6.8	8.4	9.9
15	large	5	7.5	8.4	9.3
15	small	10	9.3	11.8	14.3
15	medium	10	9.1	10.2	11.3
15	large	10	9.1	10.0	10.9
20	small	5	5.7	6.6	7.6
20	medium	5	4.4	5.3	6.2
20	large	5	4.3	5.4	6.6
20	small	10	5.0	6.8	8.6
20	medium	10	4.2	5.4	6.7
20	large	10	5.7	6.7	7.7
25	small	5	3.4	4.1	4.8
25	medium	5	3.4	4.0	4.5
25	large	5	3.3	3.9	4.4
25	small	10	4.5	5.3	6.2
25	medium	10	4.0	4.7	5.4
25	large	10	3.6	4.5	5.4

temperature increased, harvest times for seedlings at the same growth stage in different treatments became aligned more closely. Most seedling numbers were less than five and generally in the range of 2-3 seedlings/harvest.

7.3.3 ANALYSES OF VARIANCE

7.3.3.1 OREGON SEED LINE AT FOUR TEMPERATURES

All sources of variation in the multivariate analysis of variance were significant ($P<0.05$) except the temperature x depth interaction. A particularly important feature of the results was the significance of most of the interactions, namely temperature x seed size, depth x seed size and temperature x depth x seed size, which showed that there was a complex interplay between the factors when all characters were analysed simultaneously. This contrasted with the results of most univariate analyses where all interactions were generally non-significant. There was frequent agreement between the significance of the main effects in the multivariate and univariate analyses. Only interactions are discussed further due to their significance.

Approximately 70% of the dispersion in the temperature x seed size interaction data was accounted for by the first two discriminant functions (Table 7.4) and mean scores on these two functions are presented in Table 7.5a together with the four largest standardised coefficients for each function. On the first function, time of final emergence had the highest coefficient which indicated that it was the most important character in the function. Total seedling dry weight at harvest one was slightly less important. Three characters had similar importance in the second function, namely root dry weight at harvest two and harvest one root and shoot lengths. The latter two characters were related inversely. The general patterns accompanying an increase in seed size on the first function were later final emergence, and increases in total seedling dry weight, shoot length at harvest two and the proportion of doubles. These trends were supported frequently by the univariate means (Tables 7.5b to h). Apart from delayed final emergence, increasing values of these characters were desirable. An anomaly occurred at 20°C for small and medium seed with the latter seed size having the lower mean score and this probably accounted for most of the

TABLE 7.4 Important statistics for the discriminant functions for three sources of variation from the multivariate pooled analysis of variance of numerous vegetative characters for Oregon sheep's bumet at 10, 15, 20 and 25°C.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
Temperature x seed size					
1	42.22	1.85	108	139	***
2	25.69	1.48	85	120	*
3	15.15	1.18	64	100	NS
4	8.89	0.94	45	78	NS
5	4.87	0.76	28	54	NS
6	3.17	0.67	13	28	NS
Depth x seed size					
1	65.61	2.15	36	46	**
2	34.39	1.69	17	24	NS
Temperature x depth x seed size					
1	44.29	1.35	108	139	*
2	30.82	1.04	85	120	NS
3	11.52	0.71	64	100	NS
4	6.32	0.57	45	78	NS
5	3.82	0.50	28	54	NS
6	3.22	0.51	13	28	NS

TABLE 7.5a Mean scores for the first and second discriminant functions (DF) for temperature x seed size interaction for Oregon sheep's burnet.

Temperature (°C)	DF	Seed size small	medium	large
10	1	0.02	7.84	8.98
10	2	- 0.72	-1.67	-1.01
15	1	- 2.45	2.15	5.01
15	2	- 4.83	-4.16	-1.75
20	1	- 1.40	-3.69	2.11
20	2	2.33	2.98	4.28
25	1	-10.35	-6.07	-2.16
25	2	- 2.42	1.97	5.02

four largest standardised coefficients:

DF1	DF2
1. time of final emergence (3.35)	1. H2 root dry weight (1.71)
2. H1 total seedling dry weight (2.46)	2. H1 root length (-1.64)
3. H2 shoot length (1.79)	3. H1 shoot length (1.62)
4. proportion of doubles (1.34)	4. H1 total seedling dry weight (1.22)

Table 7.5b Temperature x seed size means for time of final emergence (%) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	17.5 (0.3)	18.0 (0.9)	17.2 (0.3)
15	13.0 (0.5)	12.0 (0.5)	12.5 (0.5)
20	9.0 (0.7)	7.7 (0.2)	7.7 (0.2)
25	6.0 (0.3)	5.8 (0.2)	6.2 (0.4)

Table 7.5c Temperature x seed size means for total seedling dry weight at harvest one (mg) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	0.88 (0.24)	2.12 (0.26)	2.72 (0.16)
15	0.82 (0.27)	1.57 (0.14)	2.03 (0.23)
20	3.71 (0.97)	2.64 (0.42)	4.27 (0.40)
25	0.61 (0.23)	1.70 (0.10)	2.12 (0.16)

Table 7.5d Temperature x seed size means for shoot length at harvest two (mm) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	19.4 (1.1)	9.3 (1.2)	19.9 (1.0)
15	18.1 (1.4)	20.5 (1.3)	20.2 (1.1)
20	19.2 (1.2)	17.5 (0.7)	19.4 (1.2)
25	18.1 (0.8)	18.8 (0.6)	22.4 (1.1)

Table 7.5e Temperature x seed size means for the proportion of doubles (%) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	9.5 (3.0)	52.4 (3.8)	64.5 (5.7)
15	12.4 (4.7)	51.2 (2.4)	77.5 (5.4)
20	5.7 (3.7)	44.4 (2.8)	62.1 (9.5)
25	7.7 (4.1)	57.2 (5.1)	73.3 (5.4)

Table 7.5f Temperature x seed size means for root dry weight at harvest two (mg) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	1.90 (0.29)	1.14 (0.16)	1.20 (0.14)
15	0.96 (0.21)	0.76 (0.17)	0.93 (0.13)
20	0.83 (0.25)	0.87 (0.18)	0.91 (0.08)
25	0.35 (0.13)	0.50 (0.11)	0.52 (0.11)

Table 7.5g Temperature x seed size means for root length at harvest one (mm) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	8.7 (1.6)	19.0 (2.2)	25.2 (3.6)
15	14.8 (2.0)	20.6 (1.4)	22.0 (2.2)
20	16.6 (5.0)	24.3 (3.2)	18.8 (2.5)
25	20.8 (3.0)	29.6 (2.6)	24.4 (2.0)

Table 7.5h Temperature x seed size means for shoot length at harvest one (mm) (standard errors in parentheses).

Temperature (°C)	Seed size small	medium	large
10	4.3 (0.8)	7.4 (1.1)	8.0 (0.6)
15	4.4 (0.4)	6.2 (0.9)	6.2 (0.7)
20	6.6 (2.0)	9.4 (1.1)	5.8 (0.8)
25	8.0 (1.4)	10.4 (1.2)	10.7 (1.3)

significance of the interaction. Similar increases on the second function occurred with larger seed but there was an irregularity at 10°C, also between small and medium seed (Table 7.5a).

The first discriminant function for depth x seed size interaction accounted for 66% of total dispersion (Table 7.4) and mean scores on this function are shown in Table 7.6a along with the four largest standardised coefficients. The four coefficients were of similarly high importance but differed in their loadings. Increases in seedling dry weight at harvest one and root dry weight at harvest two resulted in larger mean discriminant scores while decreases in scores resulted from increases in harvest four shoot length and root length at harvest three. Agronomically, increases in all these characters were desirable but because of the loading differences, the most appropriate mean score was close to zero. Small seed sown at 5 mm depth satisfied this criterion. Score means at 10 mm depth were greater than those at 5 mm depth for small and large seeds but were less for medium seed and this probably accounted for the significance of the interaction. Univariate means of the four most important characters are presented in Tables 7.6b, c, d and e.

Dispersion in the temperature x depth x seed size interaction matrix was accounted for adequately (75%) by two discriminant functions (Table 7.4). Score means and the four largest standardised coefficients for each function are presented in Table 7.7a. Total seedling dry weight at harvest one was again a prominent character and on the first function it was approximately two to three times more important than the three length characters. Differences in importance between characters were less pronounced on the second function. The proportion of doubles was the most important character while length and dry weight of roots at harvests two and four, respectively, were slightly less important. The two highest coefficients in the first function were positive (Table 7.7a) indicating that increasing values of the respective characters resulted in increasing mean scores. Although there was an overall pattern at each temperature and depth of increases (function one) and decreases (function two) in mean score with larger seed sizes, there were several exceptions to these trends. The most noteworthy anomalies were medium seed at 10°C and 5 mm depth,

TABLE 7.6a Mean scores for the first discriminant function for depth x seed size interaction for Oregon sheep's burnet.

Depth (mm)	Seed size small	medium	large
5	0.24	0.74	-1.98
10	1.71	-1.65	0.94

four largest standardised coefficients:

1. H1 total seedling dry weight (1.85)
2. H4 shoot length (-1.81)
3. H2 root dry weight (1.76)
4. H3 root length (-1.51)

Table 7.6b Depth x seed size means for total seedling dry weight at harvest one (mg) (standard errors in parentheses).

Depth (mm)	Seed size small	medium	large
5	0.89 (0.19)	1.97 (0.24)	2.66 (0.25)
10	2.11 (0.67)	2.04 (0.19)	2.91 (0.37)

Table 7.6c Depth x seed size means for shoot length at harvest four (mm) (standard errors in parentheses).

Depth (mm)	Seed size small	medium	large
5	24.9 (1.4)	27.4 (1.6)	30.8 (2.0)
10	29.3 (1.4)	31.2 (1.5)	32.2 (1.7)

Table 7.6d Depth x seed size means for dry weight at harvest two (mg) (standard errors in parentheses).

Depth (mm)	Seed size small	medium	large
5	1.10 (0.21)	0.93 (0.12)	0.92 (0.13)
10	0.92 (0.19)	0.70 (0.13)	0.86 (0.08)

Table 7.6e Depth x seed size means for root length at harvest three (mm) (standard errors in parentheses).

Depth (mm)	Seed size small	medium	large
5	71.4 (3.2)	66.5 (2.3)	77.0 (3.3)
10	63.4 (2.5)	71.6 (2.7)	65.0 (3.1)

ABLE 7.7a Mean scores for the first and second discriminant functions (DF) for temperature x depth x seed size interaction for Oregon sheep's burnet.

mperature (°C)	Depth (mm)	DF	Seed size small	medium	large
10	5	1	-0.08	5.70	5.09
		2	4.87	5.87	2.72
	10	1	2.71	3.05	4.94
		2	7.57	1.65	0.24
15	5	1	-3.84	-1.69	-0.54
		2	3.04	0.67	-3.33
	10	1	-1.95	-1.65	0.45
		2	4.72	-0.75	-2.97
20	5	1	-0.83	2.39	5.45
		2	5.02	1.69	-4.97
	10	1	9.29	0.76	6.40
		2	-1.11	-1.03	-3.99
25	5	1	-6.92	-6.53	-5.63
		2	2.70	-4.50	-7.18
	10	1	-9.68	-4.74	-2.15
		2	-0.72	-3.78	-6.44

ur largest standardised coefficients:

DF1	DF2
. H1 total seedling dry weight (3.02)	1. proportion doubles (-2.07)
. H3 shoot length (1.68)	2. H2 root length (1.46)
. H1 root length (-1.21)	3. H4 root dry weight (1.44)
. H2 root length (1.21)	4. H4 shoot dry weight (-1.07)

ınd small seed at 20°C and 10 mm depth. Univariate means for the relatively mportant characters in each function are presented in Tables 7.7b and c.

The results indicated that there was a complex interaction between all three factors of seed size, depth and temperature with the latter factor probably being of greatest importance. Interaction between seed size and the other factors occurred predominantly for the small and medium seed sizes. Large seeds had frequently high mean scores on the first discriminant function (Table 7.7a) and there was a slight improvement in seedling dry weight from deeper sowings at higher temperatures.

7.3.3.2 OREGON AND COCKAYNE SEED LINES AT 10 AND 20°C

All main effects and several interactions, namely temperature x seed line, temperature x depth, temperature x seed size and depth x seed size x seed line, were significant in the multivariate analysis of variance (Table 7.8). The results indicated that the relationships between the factors were more complex than that conveyed by the univariate analyses where very few first or higher order interactions were important.

Mean scores for the single discriminant function for the temperature x seed line interaction, together with the four largest standardised coefficients, are presented in Table 7.9a. The largest coefficient was for root dry weight at harvest two and it was almost double that of total seedling dry weight at harvest one. Of less importance were harvest one root length and shoot dry weight at harvest two, and both characters had negative loadings. At 10°C the Oregon material was inferior to that from the Cockayne plots while at 20°C the reverse was true due to Oregon's superior root dry weight at harvest two and greater seedling dry weight (H1). These trends were supported partly by the univariate means (Table 7.9b).

For the temperature x depth interaction, root and shoot dry weights at harvest two were the most important characters in the discriminant function, but shoot length at harvest four and time of final emergence were also important (Table 7.10a). It is noteworthy that harvest two root dry weight was also the most important character

Table 7.7b Temperature x depth x seed size means for three characters of Oregon sheep's burnet (standard errors in parentheses).

Temperature (°C)	Depth (mm)	total seedling dry weight at harvest one (mg)			shoot length at harvest three (mm)			root length at harvest one (mm)		
		Seed size			Seed size			Seed size		
		small	medium	large	small	medium	large	small	medium	large
10	5	0.40 (0.20)	2.04 (0.30)	2.59 (0.20)	17.6 (0.7)	20.1 (0.1)	21.9 (1.0)	6.7 (2.3)	16.5 (3.7)	26.3 (7.9)
10	10	1.35 (0.14)	2.20 (0.49)	2.85 (0.26)	20.2 (1.0)	23.5 (1.0)	24.0 (1.0)	10.7 (1.7)	21.5 (2.0)	24.1 (2.0)
15	5	0.87 (0.44)	1.37 (0.14)	2.30 (0.30)	19.2 (2.7)	20.2 (0.2)	23.2 (0.6)	15.7 (4.2)	18.4 (0.9)	24.3 (2.1)
15	10	0.77 (0.39)	1.77 (0.21)	1.76 (0.31)	24.7 (0.2)	26.2 (0.8)	26.0 (2.0)	14.0 (1.2)	22.7 (2.1)	19.6 (3.8)
20	5	1.57 (0.28)	2.61 (0.84)	3.81 (0.44)	29.9 (2.4)	30.4 (2.0)	35.5 (2.1)	24.3 (6.0)	20.1 (4.5)	16.5 (3.4)
20	10	5.87 (0.22)	2.66 (0.40)	4.73 (0.64)	28.9 (1.8)	34.3 (0.8)	35.0 (2.3)	8.9 (5.6)	28.4 (3.7)	21.2 (3.8)
25	5	0.74 (0.38)	1.86 (0.14)	1.94 (0.13)	27.5 (2.5)	29.0 (1.9)	27.8 (1.3)	20.7 (4.5)	33.0 (3.1)	24.0 (2.3)
25	10	0.47 (0.32)	1.54 (0.07)	2.29 (0.09)	27.7 (0.4)	32.8 (2.5)	32.4 (1.5)	21.0 (5.1)	26.2 (3.5)	24.9 (3.7)

Table 7.7c Temperature x depth x seed size means for four characters of Oregon sheep's burnet (standard errors in parentheses).

Temperature (°C)	Depth (mm)	root length at harvest two (mm) Seed size small	medium	large	proportion of doubles (%) Seed size small	medium	large
10	5	37.0 (13.4)	64.3 (6.4)	56.5 (7.1)	14.3 (7.1)	53.0 (8.3)	62.2 (12.1)
10	10	53.7 (2.2)	40.1 (4.8)	49.8 (3.0)	4.8 (4.8)	51.9 (1.9)	66.7 (3.3)
15	5	40.4 (1.6)	42.4 (2.2)	48.3 (5.3)	20.6 (5.1)	52.3 (5.3)	82.8 (9.6)
15	10	41.2 (3.8)	36.2 (1.5)	42.7 (6.9)	4.2 (4.2)	50.0 (3.1)	72.1 (4.7)
20	5	57.0 (5.9)	61.8 (2.9)	57.2 (8.3)	4.8 (4.8)	42.1 (4.8)	68.7 (12.1)
20	10	47.8 (3.8)	59.5 (7.8)	62.0 (8.1)	6.7 (6.7)	46.7 (3.3)	55.6 (9.5)
25	5	44.7 (5.1)	46.1 (3.8)	35.9 (5.4)	3.7 (3.7)	60.8 (0.8)	75.7 (9.1)
25	10	33.2 (3.1)	39.8 (4.0)	41.2 (3.6)	11.7 (7.3)	53.5 (4.7)	70.8 (7.7)

Table 7.7c continued.

root dry weight at harvest four (mg) Seed size small	medium	large	shoot dry weight at harvest four (mg) Seed size small	medium	large
4.10 (0.96)	7.06 (0.99)	6.30 (1.09)	9.43 (0.89)	12.82 (1.72)	13.27 (1.81)
4.39 (0.94)	6.15 (0.39)	5.83 (0.70)	8.13 (1.75)	13.82 (0.97)	12.90 (1.03)
4.16 (0.58)	5.87 (0.84)	6.49 (0.17)	12.25 (0.83)	17.72 (1.18)	21.89 (0.73)
4.83 (0.65)	4.78 (0.52)	6.63 (0.66)	13.53 (1.27)	15.99 (1.81)	20.41 (0.72)
2.66 (0.42)	3.98 (1.13)	3.87 (0.52)	9.03 (1.50)	13.36 (1.73)	15.85 (1.56)
2.88 (0.40)	2.96 (0.23)	4.96 (1.29)	13.20 (1.04)	14.56 (2.36)	19.49 (2.16)
2.74 (0.33)	2.61 (0.32)	2.74 (0.13)	14.34 (1.57)	16.58 (1.81)	15.61 (1.76)
2.37 (0.18)	2.33 (0.12)	2.66 (0.15)	18.03 (1.70)	14.94 (0.44)	16.88 (0.91)

TABLE 7.8 Important statistics for the discriminant function for four sources of variation from the multivariate pooled analysis of variance of numerous characters for Oregon and Cockayne sheep's burnet at 10 and 20°C.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
Temperature x seed line					
1	100.00	7.03	18	29	***
Temperature x depth					
1	100.00	2.60	18	29	*
Temperature x seed size					
1	82.70	1.66	36	58	*
2	17.30	0.72	17	30	NS
Depth x seed size x seed line					
1	77.01	1.64	36	58	*
2	22.99	0.90	17	30	NS

TABLE 7.9a *Mean scores for the first discriminant function for temperature x seed line interaction for Oregon and Cockayne sheep's burnet.*

Temperature (°C)	Seed Line: Oregon	Seed Line: Cockayne
10	-1.77	2.06
20	1.28	-1.57

four largest standardised coefficients:

1. H2 root dry weight (2.86)
2. H1 total seedling dry weight (1.63)
3. H1 root length (-1.24)
4. H2 shoot dry weight (-1.15)

Table 7.9b Temperature x seed line means for four characters of Oregon and Cockayne sheep's burnet at 10 and 20°C (standard errors in parentheses).

	root dry weight at harvest two (mg)		total seedling dry weight at harvest one (mg)		root length at harvest one (mm)		shoot dry weight at harvest two (mg)	
	Seed line		Seed line		Seed line		Seed line	
Temperature (°C)	Oregon	Cockayne	Oregon	Cockayne	Oregon	Cockayne	Oregon	Cockayne
10	1.41 (0.14)	2.69 (0.18)	1.91 (0.22)	1.88 (0.16)	17.6 (2.2)	14.1 (1.1)	3.92 (0.20)	4.92 (0.33)
20	0.87 (0.10)	0.66 (0.09)	3.54 (0.39)	2.35 (0.23)	19.9 (2.2)	25.0 (2.7)	2.94 (0.17)	3.01 (0.25)

TABLE 7.10a *Mean scores for the first discriminant function for temperature x depth interaction for Oregon and Cockayne sheep's burnet.*

	Depth (mm)	
Temperature (°C)	5	10
10	2.58	-1.59
20	-0.45	-0.55

four largest standardised coefficients:

1. H2 root dry weight (2.59)
2. H2 shoot dry weight (-2.44)
3. H4 shoot length (-2.20)
4. time of final emergence (-1.88)

Table 7.10b Temperature x depth means for four characters of sheep's burnet at 5 and 10mm depth and at 10 and 20°C (standard errors in parentheses).

	root dry weight at harvest two (mg)		shoot dry weight at harvest two (mg)		shoot length at harvest four (mm)		time to final emergence (days)	
	Depth (mm)		Depth (mm)		Depth (mm)		Depth (mm)	
Temperature (°C)	5	10	5	10	5	10	5	10
10	2.16 (0.23)	1.93 (0.21)	4.33 (0.30)	4.51 (0.30)	19.0 (0.5)	22.1 (0.5)	17.7 (0.3)	18.1 (0.3)
20	0.82 (0.11)	0.71 (0.10)	3.14 (0.24)	2.81 (0.17)	29.2 (1.0)	31.8 (1.1)	7.7 (0.3)	8.2 (0.2)

in the temperature x seed line interaction. Within the 5 mm sowing depth, an increase in temperature resulted in increases in harvest two shoot dry weight, shoot length at harvest four and a longer time to final emergence. However, the temperature increase was also accompanied by a decrease in harvest two root dry weight. The results also showed that at 10°C, seedlings at greater depth (10 mm) had relatively low root dry weight (H2) and delayed time of final emergence, but increased harvest two shoot dry weight and harvest four shoot length. Seedling performance at 5 and 10 mm depths was similar at 20°C. Univariate means are presented in Table 7.10b.

An increase in seed size at 10°C resulted in a corresponding increase in mean score whereas at 20°C there was a decrease in mean score with increasing seed size (Table 7.11a). Shoot length at harvest four had a negative loading and was the most important character, while three dry weight characters were moderately important and had positive loadings. Interaction means for shoot length at harvest four were similar at 10°C but increased with seed size at 20°C (Table 7.11b).

About 77% of the dispersion in the depth x seed size x seed line interaction data was accounted for by the first discriminant function (Table 7.8) and mean scores and the four largest standardised coefficients for this function are presented in Table 7.12a. Total seedling dry weight (H1) and root dry weight (H2) were most important and increases in these characters resulted in more positive scores. Mean scores decreased with increasing values of shoot dry weight at harvest two and time of final emergence. For the Oregon and Cockayne sheep's burnet seed lines at 5 and 10 mm depths, respectively, there was an increase in mean score with increasing seed size. However, the patterns for Oregon sheep's burnet at 10 mm depth and Cockayne sheep's burnet at 5 mm depth were equivocal. Mean scores either increased or decreased with sowing depth depending on the seed line x seed size combination. For example, the small Oregon and large Cockayne seeds of sheep's burnet were the only treatments which had higher mean scores with increased depth (Table 7.12a). Generally higher mean scores for large seed of both seed lines at each depth indicated superior total seedling dry weight at harvest one and root dry weight at harvest two compared with that from small and medium seed under similar

TABLE 7.11a Mean scores for the first discriminant function for temperature x seed size interaction for Oregon and Cockayne sheep's burnet.

Temperature (°C)	Seed size small	medium	large
10	2.03	4.23	4.70
20	-2.30	-3.65	-5.00

four largest standardised coefficients:

1. H4 shoot length (-2.84)
2. H1 total seedling dry weight (1.38)
3. H3 root dry weight (1.01)
4. H4 root dry weight (0.93)

Table 7.11b Temperature x seed size means for four characters of sheep's burnet of three seed sizes at 10 and 20°C (standard errors in parentheses).

	Temperature (°C)					
	10			20		
	Seed size			Seed size		
Character	small	medium	large	small	medium	large
shoot length at harvest four (mm)	19.3(0.8)	20.7(0.6)	21.7(0.8)	27.3(1.1)	30.1(1.0)	34.0(1.1)
total seedling dry weight at harvest one (mg)	1.04(0.17)	2.12(0.15)	2.52(0.13)	2.80(0.56)	2.61(0.25)	3.42(0.40)
root dry weight at harvest three (mg)	2.85(0.25)	3.63(0.23)	4.35(0.32)	1.56(0.14)	1.69(0.15)	1.62(0.14)
root dry weight at harvest four (mg)	3.70(0.41)	5.02(0.70)	5.45(0.43)	2.38(0.19)	2.90(0.37)	3.52(0.43)

TABLE 7.12a **Mean scores for the first discriminant function for depth x seed size x seed line interaction** for Oregon and Cockayne sheep's burnet.

Seed line	Depth (mm)	Seed size		
		small	medium	large
Oregon	5	-3.08	1.46	1.54
Oregon	10	0.98	-2.08	1.06
Cockayne	5	-0.35	0.94	0.59
Cockayne	10	-2.81	0.60	1.15

four largest standardised coefficients:

1. H1 total seedling dry weight (2.51)
2. H2 root dry weight (1.99)
3. H2 shoot dry weight (-1.97)
4. time of final emergence (-1.68)

Table 7.12b Depth x seed line means for four characters of Oregon and Cockayne sheep's burnet of three seed sizes at 5 and 10mm depth (standard errors in parentheses).

Seed line	Depth (mm)	total seedling dry weight at harvest one (mg)			root dry weight at harvest two (mg)			shoot dry weight at harvest two (mg)			time to final emergence (days)		
		Seed size			Seed size			Seed size			Seed size		
		small	medium	large	small	medium	large	small	medium	large	small	medium	large
Oregon	5	0.98 (0.30)	2.33 (0.42)	3.20 (0.35)	1.52 (0.43)	1.13 (0.18)	1.13 (0.15)	3.44 (0.33)	3.23 (0.42)	3.84 (0.33)	12.8 (1.9)	12.8 (2.4)	12.3 (2.1)
Oregon	10	3.61 (1.00)	2.43 (0.30)	3.79 (0.52)	1.21 (0.25)	0.88 (0.16)	0.98 (0.10)	2.98 (0.42)	3.50 (0.49)	3.62 (0.32)	13.7 (2.0)	12.8 (2.4)	12.5 (2.2)
Cockayne	5	1.73 (0.33)	2.04 (0.20)	2.32 (0.41)	1.29 (0.37)	1.86 (0.64)	2.03 (0.53)	2.77 (0.26)	3.96 (0.66)	5.19 (0.59)	13.2 (2.3)	12.7 (2.6)	12.5 (2.4)
Cockayne	10	1.36 (0.31)	2.66 (0.30)	2.56 (0.36)	1.29 (0.44)	1.42 (0.40)	2.16 (0.56)	2.92 (0.46)	3.84 (0.36)	5.10 (0.80)	14.2 (2.6)	12.7 (2.2)	13.0 (2.1)

conditions. Time of final emergence was also earlier. Univariate means for the relatively important characters in the function (Table 7.12b) partly supported these trends.

7.3.3.3 SHEEP'S BURNET AND LUCERNE AT 15 AND 25°C

All main effects and one of the four interactions, namely temperature x treatment, were significant ($P<0.05$) in the multivariate analysis of variance, which indicated that the factors were mostly independent of each other. The results were in agreement with those of many of the univariate analyses. Statistics for the discriminant functions for depth and the temperature x treatment interaction are shown in Table 7.13a.

Mean scores for the single discriminant function for depth along with the four largest standardised coefficients are presented in Table 7.13b. The three largest coefficients were of about equal magnitude and hence importance, and had positive loadings, while shoot length at harvest one was approximately half as important. Seedlings arising from deeper sowings emerged later and had longer shoots at the first three harvests and this was also shown by the individual character means (Table 7.13c).

Approximately 85% of the dispersion in the temperature x treatment interaction data was accounted for by the first two discriminant functions (Table 7.13a) and mean scores on these two functions are presented in Table 7.13d together with the four largest standardised coefficients for each function. Univariate means feature in Table 7.13e. Shoot lengths were important in both functions and in the first function, time of final emergence was the most important character. Positive loadings on this character and shoot lengths at harvests two and three, indicated that increasing values of these characters gave larger scores while the reverse was true for root length at harvest one. Lucerne had a distinctly lower mean score than all sheep's burnet treatments at 15°C while at 25°C lucerne and some other treatments had similar scores. The results indicated that there were large differences in performance of all sheep's burnet treatments at the two temperatures, whereas lucerne's performance was relatively stable. That is, at 25°C sheep's burnet had earlier emergence and

TABLE 7.13a Important statistics for the discriminant functions for two sources of variation from the multivariate pooled analysis of variance of numerous vegetative characters for sheep's burnet and lucerne at 15 and 25°C.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
Depth					
1	100.00	17.25	17	28	***
Temperature x treatment					
1	66.21	2.66	85	140	***
2	18.45	1.60	64	116	*
3	9.04	1.12	45	90	NS
4	5.14	0.78	28	62	NS
5	1.16	0.34	13	32	NS

Table 7.13b Mean scores for the first discriminant function for depth for sheep's burnet and lucerne.

Depth (mm)	Mean score
5	-2.53
10	2.53

four largest standardised coefficients:

1. time of final emergence (2.30)
2. H3 shoot length (2.28)
3. H2 shoot length (2.14)
4. H1 shoot length (1.13)

Table 7.13c Depth means for four characters of sheep's burnet and lucerne (standard errors in parentheses).

Depth (mm)	time to final emergence (days)	shoot length at harvest one (mm)	shoot length at harvest two (mm)	shoot length at harvest three (mm)
5	8.5 (0.6)	7.2 (0.5)	16.2 (0.4)	24.4 (1.0)
10	9.0 (0.6)	9.1 (0.7)	19.9 (0.5)	28.7 (1.0)

Table 7.13d Mean scores for the first and second discriminant functions (DF) for temperature x treatment interaction for sheep's burnet and lucerne.

Temperature (°C)	DF	Treatment + 0-small	0-medium	0-large	C-small	C-medium	lucern
15	1	5.60	5.62	6.50	6.88	4.24	-7.21
15	2	-3.29	-3.54	-2.46	-0.59	-1.03	4.24
25	1	-2.85	-3.68	-1.05	-4.89	-4.02	-5.14
25	2	-0.50	2.03	1.69	-2.18	0.24	5.40

four largest standardised coefficients:

DF1	DF2
1. time of final emergence (3.37)	1. H3 shoot length (2.07)
2. H2 shoot length (2.24)	2. H4 shoot dry weight (-0.92)
3. H1 root length (-1.28)	3. H1 shoot length (0.90)
4. H3 shoot length (1.09)	4. H4 shoot length (0.88)

longer roots at harvest one than at 15°C, but at harvests two and three it sometimes also had reduced shoot lengths.

On the second function, which accounted for a further 18% of total dispersion (Table 7.13a), shoot length at harvest three was at least twice as important as any other character. It had a positive loading along with shoot lengths at harvests one and four (Table 7.13d). At 15 and 25°C, lucerne had more positive mean scores on the second function than any sheep's burnet treatments and these trends were also shown for the relatively important shoot length characters (Table 7.13e).

7.4 DISCUSSION

Establishment is the most critical stage of a pasture's life (Culleton and McCarthy, 1983) since the result largely determines subsequent performance (Sears, 1961). Seedling growth is determined by the properties of the seed from which it originates, such as seed line and seed size, and by the interaction of the seedling with its environment which includes the factors of temperature and sowing depth. Results from this investigation showed that all four factors may influence emergence and early vegetative growth of sheep's burnet and that commonly they do not act independently. Interactions between some of these factors have also been found for other species (Arnott, 1969; Tischler and Voigt, 1983; Lafond and Baker, 1986a, b) which complicates the decisions which must be made at sowing.

The dominant influence of temperature on all phases of seedling growth was shown clearly in the present study. Effects of increases in temperature ranged from enhancement of seedling emergence rates and hence reduced duration of emergence, to reducing the times for seedlings to attain specific growth stages. Similar findings demonstrating the importance of temperature have been reported for a diversity of species (Woods and MacDonald, 1971; Perry, 1976; Cooper, 1977; Hur and Nelson, 1985; Charlton *et al.*, 1986; Muendel, 1986; Hampton *et al.*, 1987; Charlton, 1989).

The present findings have important implications for field establishment of sheep's burnet, particularly with regard to sowing time. Most farmers sow pasture seeds in

TABLE 7.13e Temperature x treatment means for characters of sheep's burnet and lucerne (standard errors in parentheses).

Character	Temperature (°C)	Treatment+ O-small	O-medium	O-large	C-small	C-medium	C-large
time to final emergence (days)	15	13.0 (0.5)	12.0 (0.5)	12.5 (0.5)	14.3 (0.4)	12.0 (0.4)	5.7 (0.3)
	25	6.0 (0.3)	5.8 (0.2)	6.2 (0.4)	6.8 (0.4)	7.3 (0.3)	3.5 (0.2)
shoot length at harvest two (mm)	15	18.1 (1.4)	20.5 (1.3)	20.2 (1.1)	15.4 (1.0)	16.3 (0.9)	14.5 (0.9)
	25	18.1 (0.8)	18.8 (0.6)	22.4 (1.1)	15.6 (1.1)	16.8 (1.1)	19.7 (1.0)
root length at harvest three (mm)	15	14.8 (2.0)	20.6 (1.4)	22.0 (2.2)	13.3 (0.4)	21.1 (1.5)	49.4 (3.7)
	25	20.8 (3.0)	29.6 (2.5)	24.4 (2.0)	17.1 (2.3)	22.8 (3.2)	38.7 (1.0)
shoot length at harvest three (mm)	15	22.0 (1.7)	23.2 (1.4)	24.6 (1.1)	21.0 (0.6)	22.0 (1.1)	32.5 (1.4)
	25	27.6 (1.1)	30.9 (1.6)	30.1 (1.4)	19.0 (1.3)	26.5 (1.7)	39.0 (1.5)
shoot dry weight at harvest four (mg)	15	12.89 (0.74)	16.85 (1.04)	21.15 (0.57)	10.00 (0.64)	13.83 (2.75)	14.29 (2.12)
	25	16.19 (1.32)	15.76 (0.91)	16.24 (0.93)	8.41 (0.87)	10.13 (0.83)	12.86 (0.86)
shoot length at harvest one (mm)	15	4.4 (0.3)	6.2 (0.9)	6.2 (0.7)	4.7 (0.5)	6.8 (0.6)	13.8 (1.1)
	25	8.0 (1.4)	10.4 (1.2)	10.7 (1.3)	6.7 (1.5)	6.9 (1.0)	12.5 (1.0)
shoot length at harvest four (mm)	15	25.4 (1.1)	29.5 (1.1)	29.3 (0.7)	21.0 (0.8)	24.9 (1.1)	41.3 (1.7)
	25	32.5 (1.2)	33.9 (1.1)	36.6 (2.0)	23.8 (0.9)	27.2 (1.3)	46.7 (2.2)

+ 0 = Oregon sheep's burnet; C = Cockayne sheep's burnet

the autumn irrespective of the region (Sangakkara *et al.*, 1982) and under South Island conditions, White (1973) recommended that autumn sowings of pasture species should be completed by mid-March to ensure good establishment and growth before winter frosts begin. The minimum temperature for satisfactory emergence of Oregon sheep's burnet found presently was 4.9°C which, when compared with long term averages of grass minimum and 10 cm depth soil temperatures at several Central Otago sites (New Zealand Meteorological Service, 1983), also suggested that March and perhaps April could be suitable autumn times for sowing. Practical experience with the species in this region has found that autumn sowing gives more satisfactory establishment on most country (Wills, 1983; Sheppard and Wills, 1985, 1986) than spring sowings and these sowings may also be less prone to bird predation. However, late autumn sowings should be avoided since there are problems with frost heave prior to the seedling becoming properly established (B J Wills, pers. comm.). Soil temperatures in the spring are conducive to satisfactory establishment of sheep's burnet but there is a risk of soil moisture limitations which may severely reduce seedling emergence, plant number and early vegetative growth (Wills *et al.*, 1987), even to the extent of complete establishment failure (Sheppard and Wills, 1985, 1986). Under milder conditions in both the North and South Islands, and/or where irrigation is available, there should be no major difficulties in establishing sheep's burnet in autumn or spring, provided soil temperatures are adequate.

The superiority of the Oregon seed line of sheep's burnet compared with that from the Cockayne plots was highly temperature dependent (Section 7.3.3.2) and was expressed only under warm (20°C) temperatures. This suggested that the two seed lines were adapted to different environments and therefore further comparative evaluations of them should be undertaken to identify their most suitable environments. The comparative field trials of Sheppard and Wills (1985) in the Mackenzie Basin and on the Wither Hills in Marlborough, circumvented the important emergence and early vegetative growth stages examined here by evaluating glasshouse prepared seedlings. They found that Oregon material and others out-performed naturalised material obtained from the Cockayne plots, but in view of the routine practice of establishing sheep's burnet from seed, a reappraisal of both lines from seed seems appropriate.

Temperature interactions with sowing depth and seed size (Section 7.3.3.2) indicated that adjustments to depth and seed size should be made when sowing into soils of different temperatures. An important advantage of a shallow (5 mm) sowing at 10°C was superior root dry weight at harvest two which suggested that survival of these seedlings may be at least equal to those from deeper sowings, particularly under dry conditions, due to their probably greater ability to extract moisture (and nutrients) (Evans, 1973; Wilson, 1984). The findings for root and shoot dry weight at harvest two (Table 7.10b) partly supported the view that deeper sowing results frequently in weakened seedlings (Beveridge and Wilsie, 1959; Cooper, 1977; Tischler and Voigt, 1983). A possible explanation is that seedlings from the 10 mm depth used more of their food reserves for emergence and therefore had less nourishment available for the initial development of photosynthetic tissue. It is likely that such seedlings would also be disadvantaged in the early post-emergence stage compared with seedlings from shallower sowings.

Reasons for the sometimes similar performance of material arising from 5 and 10 mm depths at 20°C (Table 7.10a) were equivocal but it is suggested that one or more physiological processes could be limiting at 20°C and that these limited the rate of development of seedlings from each depth. Further studies at this temperature are required to identify sowing depth limits for the establishment of satisfactory seedlings.

The frequent dry weight advantages from using larger seed were in agreement with the findings for many species including perennial ryegrass, prairie grass and cocksfoot (Arnott, 1969; Evans, 1973; Sangakkara *et al.*, 1985), birdsfoot trefoil (Curtis and McKersie, 1984), wheat (Lafond and Baker, 1986a, b), and cicer milkvetch (Townsend and Wilson, 1981). With increasing seed size at 10 and 20°C, there was a corresponding increase in shoot length (H4) and several other characters (Table 7.11b), and similar findings have been found for other species (Haskins and Gorz, 1975; Scott and Hampton, 1985). Larger seed of sheep's burnet could be obtained by physical separation and possibly by a suitable plant breeding programme (Allard, 1960; Poehlman, 1979).

The much larger differences in overall performance between sheep's burnet and lucerne at 15°C compared with at 25°C (Table 7.13d) suggested that sheep's burnet may be a more suitable alternative to 'Rere' lucerne in warm to hot environments than cool ones. This is probably partly a consequence of sheep's burnet's Mediterranean origin. Of particular agronomic interest was the indication that root lengths (H1) were more similar between the two species at 25°C than at 15°C, which suggested that both species may have similar abilities to reach moisture under higher temperatures. This has important implications for the revegetation of semi-arid lands where rapid development of a deep root system is frequently vital for successful seedling establishment (Evans, 1973; Cooper, 1977).

The arbitrary moisture regimes adopted at each temperature were sufficient for satisfactory early seedling growth as there was no visible wilting at any stage during the experiments. The effect of temperature on the loss of soil water by evaporation (Namken *et al.*, 1974) was minimised by increasing the water supply at higher temperatures and it was therefore possible to study the effects of temperature on seedling growth, unencumbered by those of moisture. It would now be appropriate for the inclusion of soil moisture as a factor in further investigations on the early establishment of sheep's burnet.

Worthwhile characters for mean discrimination were identified which should prove most useful for future similar studies. Probably the most consistently important were the shoot and root dry weight characters. It is noteworthy that total seedling dry weight at harvest one featured prominently and it was a relatively simple character to measure. Shoot length was particularly useful for comparing sheep's burnet and lucerne and the character is highly advantageous because of its simplicity and rapidity of measurement.

CHAPTER 8 : IMPROVED FOLIAR REGROWTH FROM PARTIAL COMPARED WITH COMPLETE DEFOLIATION OF YOUNG SHEEP'S BURNET

8.1 INTRODUCTION

Following successful emergence and early vegetative growth, swards of sheep's burnet become suitable for defoliation, either by grazing or mowing. Although recommendations on the management of mature swards are documented (Sheppard and Wills, 1985) and mature plants can withstand very intense grazing (Sheppard and Wills, 1986), little detailed information is available on the management of younger swards.

Under moderately harsh conditions in the South Island of New Zealand where establishment is slow (de Lacy, 1985), sheep's burnet should not be grazed, or at best only very leniently, for the first 18 months after sowing (Wills, 1983; Sheppard and Wills, 1985, 1986). Earlier grazing of swards may be practised under milder conditions but again defoliation intensity should be lenient to permit satisfactory plant survival and regrowth (J S Sheppard, pers. comm.). The rates and amount of regrowth from partially and completely defoliated young plants are largely unknown and any information on these agronomic aspects would assist in formulating defoliation strategies for young swards. Regrowth characteristics of these swards may also change with plant age and defoliation history.

A factor which might account for differences in regrowth between variously defoliated young plants is the source of the energy for regrowth. Nonstructural carbohydrates (NC), as current photosynthate from residual leaf area and/or stored reserves, may determine much of the early regrowth potential of numerous grass and legume species (Davies, 1965; Humphreys, 1966; Harris, 1978; Volenec, 1986). In lucerne for example, NC reserves stored mainly in the upper part of the taproot are a major source of energy for regrowth in many situations (Reynolds and Smith, 1962; Smith, 1962; Rapoport and Travis, 1984; Gabrielsen *et al.*, 1985; Barta, 1988a, b) and they may also be important in the regrowth of young sheep's burnet plants. By

determining the relative contributions of current photosynthate and stored reserves to regrowth, a physiological justification for the lenient defoliation of young plants may be provided.

The main objective of this investigation was to study the early regrowth responses of variously defoliated young sheep's burnet plants which differed in their management history. A further aim was to determine the contribution of nonstructural carbohydrates to regrowth processes.

8.2 MATERIALS AND METHODS

Two similar experiments were conducted, henceforth referred to as experiments one and two, which varied mainly in the age and defoliation history of the young plants used. The similarities and differences between the two experiments are highlighted in the following sections.

8.2.1 LOCATION

Both studies were conducted in the same glasshouse at the Plant Growth Unit, Massey University. Temperature settings were 25°C (day) and 15°C (night) on a 12 hr cycle. Periodic monitoring of temperature and relative humidity from sowing time until completion of the experiments showed that temperatures rarely fell below 15°C. In the summer of 1986/'87, daily maxima reached occasionally 31-33°C but most mean daily temperatures were 20-25°C. The relative humidity at 0900 hours was consistently in the range, 50-70%.

8.2.2 PLANT ESTABLISHMENT AND TREATMENTS

Six medium sized seeds of the Oregon seed line (Section 3.2) were sown at 5 mm depth into sand contained in PB10 planter bags with flattened dimensions of 12 x 46 cm. Only sand was used to facilitate later removal of roots. A slow-release fertiliser (N:P:K= 15:5.2:12.5) was distributed uniformly in the medium at a rate of $5gl^{-1}$. Experiment one was sown on 3 September, 1986 while the second experiment was

sown on 17 June, 1986. Bags were placed on the glasshouse concrete floor due to the physical difficulty of supporting their weight on trolleys or tables. Seedlings were established under well watered conditions which continued until the completion of each experiment. Thinning of seedlings to one healthy seedling per bag was conducted before shading from neighbouring seedlings occurred.

All plots (experimental units) in each experiment consisted of two bagged plants and were arranged in four randomised complete blocks. As seedlings developed in experiment one, the first eight leaves which had at least three leaflet pairs, were tagged sequentially. This allowed for possible differences in the ability of the variously aged leaves to supply nonstructural carbohydrates for regrowth. In experiment two, plants were permitted initially to reach 10-50% flowering and then defoliated completely. This procedure was repeated twice and after the third defoliation on 18 February 1987, leaf tagging was conducted as described above. Following tagging in experiment one, almost half of all plants were covered with boxes for 72 hours on 7 December, 1986 to reduce the levels of nonstructural carbohydrate reserves (Ward and Blaser, 1961; Davies, 1965). On removal of these, leaves of some plants were slightly chlorotic.

Treatments were imposed on 10 December, 1986 and 5 March, 1987 for experiments one and two, respectively, when the last (youngest) of the eight tagged leaves of all plants in each experiment was fully expanded. All foliage produced after tagging ceased, plus various proportions of the tagged leaves, were removed to create the treatments which were:

Unshaded (uncovered)

1. 100% residual leaf area (rLA) - no tagged leaves removed but all untagged foliage removed.

2. 75% rLA - removal of the two oldest tagged leaves per plant plus all untagged foliage.

3. 50% rLA - removal of the four oldest tagged leaves per plant plus all untagged foliage.

4. 25% rLA - removal of the six oldest tagged leaves per plant plus all untagged foliage.

5. 0% rLA - removal of all foliage (tagged and untagged).

Shaded (covered previously)

6. 100% rLA - same as 1.
7. 50% rLA - same as 3.
8. 0% rLA - same as 5.

Only the unshaded treatments in the range 0-75% rLA were examined in the second experiment. In all instances, leaves were defoliated immediately below the lowest leaflet pair.

8.2.3 MEASUREMENTS

8.2.3.1 LEAF EXTENSION

Basic information on the rate and magnitude of early regrowth of new leaves was obtained by measuring leaf extension of the first three leaves appearing after defoliation, every second day. These leaves were distinguished by coloured tags and all measurements were conducted at similar times of day to minimise possible diurnal variability in leaf extension rate (Dale, 1988) and hence length. Measurements were continued until there was no further increase in leaf length over two consecutive recordings.

8.2.3.2 VEGETATIVE FEATURES AND TOTAL NONSTRUCTURAL CARBOHYDRATES

Two harvests were conducted on plants from each treatment to determine some of the morphological and physiological changes occurring over the foliar regrowth period. The first harvests were made one day after the defoliation treatments were imposed on 11 December, 1986 (experiment one) and 6 March, 1987 (experiment two). Characters measured in both experiments were:

- number of tagged leaves
- leaf area of tagged leaves (cm^2)
- lamina and petiolule/rachis dry weights of tagged leaves (g)
- stubble and root dry weights (g)
- soluble sugar and starch levels of tagged leaves (%)
- soluble sugar and starch levels of stubble and root (%)

Definitions of the plant parts measured as well as the procedures for determining leaf area, dry weights, and levels of nonstructural carbohydrates were presented in Chapter 3.

The second harvests in each experiment were conducted when the three tagged leaves used for leaf extension measurements in all treatments were fully expanded. Harvest dates were 6 January and 8 April, 1987 for experiments one and two, respectively. The stubble and root characters measured at harvest one were determined similarly as well as dry weights and levels of nonstructural carbohydrates of the leaves. An additional character measured in experiment one was the number of leaves at harvest two.

8.2.4 STATISTICAL ANALYSES

8.2.4.1 LEAF EXTENSION

Mean times of first measurement of each leaf after defoliation were calculated for each treatment x experiment combination. Analyses of variance were not conducted on these data due to the frequently non-normal data distributions which could not be rectified by transformation.

Plots of length against time for each leaf per treatment and experiment were conducted for individual blocks to suggest likely types of curves to describe the leaf extension/regrowth events over time. In all instances, the plots indicated that an asymptotic regression model was suitable. In this regard, the three parameter monomolecular function (Richards, 1959; Landsberg, 1977) of the form

$$y = \beta_0 (1 - \beta_1 e^{-\beta_2 t})$$

was fitted to all data sets utilising a nonlinear least squares procedure (Section 3.6). Time zero was regarded as the time when measurement on any leaf first occurred and methods of fitting the function were presented in Section 3.6.3.

Correlations, and hence covariances, existed between some or all of the estimates of the monomolecular function parameters (β_0, β_1, and β_2) and therefore treatments were analysed simultaneously using multivariate analysis of variance (Section 3.7). Due to the structure of the data, including the presence of two experiments, three different analyses were conducted. Firstly, all parameter estimates for each leaf were analysed jointly for the unshaded, variously defoliated plants in experiment one and a random effects model was assumed for all significance tests (Lindeman *et al.*, 1980; Steel and Torrie, 1980). In the second analysis, all parameter estimates for the previously shaded and unshaded 0, 50 and 100% rLA defoliation intensities in experiment one were analysed. Although it was desired to assume a random effects model for all significance tests, multivariate tests of shade and intensity using the intensity x shade

interaction matrix as the residual were not possible due to insufficient error degrees of freedom. A fixed effects model was therefore assumed for all significance tests. In the final analysis, data for the previously unshaded 0, 25, 50 and 75% rLA treatments common to both experiments were analysed as a multivariate analysis of variance pooled over experiments. Sources of variation were tested against the residual matrix for the same reason mentioned above.

All parameters were also analysed univariately. In the second set of analyses, significance tests appropriate for fixed and random effects models were conducted. For the latter, shade and intensity were tested against the intensity x shade interaction. In the pooled analyses over experiments, fixed effects models were assumed. In addition, intensity was tested against the experiment x intensity interaction and a pseudo-random effects ratio for experiments was constructed by testing it against block(experiment).

8.2.4.2 VEGETATIVE FEATURES AND TOTAL NONSTRUCTURAL CARBOHYDRATES

The effects of the treatments on foliar regrowth were determined by calculating the differences between harvests two and one as

$$\text{change} = (\text{harvest two} - \text{harvest one})$$

for all characters. Harvest one data represented the condition of the treatments while harvest two data were influenced by the treatments themselves. The change scores were important biologically since in the case of the dry weight characters they represented net herbage accumulation (Thomas, 1980). Herbage losses due to senescence and decomposition were negligible so that the change scores were approximate measurements of the growth rate of new herbage.

The three different multivariate analyses of variance described for the leaf parameters in the previous section were performed on the change scores. Supporting univariate analyses of variance were also conducted. For the two multivariate analyses of

variance of material in experiment one, change scores for the levels of stubble and root starch were transformed using square roots to approximately stabilise the variances. In the pooled analysis over experiments, stubble and root starch data could not be transformed satisfactorily and were therefore omitted. This was justified further for the stubble starch data because the 0 and 25% rLA treatments in experiment two had no change in the levels of starch. That is, mean changes were zero and variances were also zero. Changes in root and leaf dry weights were transformed using arcsine and logarithms, respectively.

8.3 RESULTS

8.3.1 LEAF EXTENSION

First measurements of initial leaf production after defoliation for each treatment were conducted three to four days later. Measurements on the next two leaves were commenced at approximately one to three day intervals.

Defoliation intensity had a significant ($P<0.05$) effect in the multivariate analysis of the monomolecular function parameters for each leaf. Approximately 70% of the dispersion in the data was accounted for by the first discriminant function (Table 8.1a) and mean scores on this function are presented in Table 8.1b together with the three largest standardised coefficients. The final length ($ß_0$) of the first leaf produced after defoliation was the most important character in the first discriminant function and had a positive loading. It was approximately twice as important as final length of the second leaf which had a negative loading (Table 8.1b). There were relatively small differences between the leaf regrowth rates ($ß_2$) of each treatment since these characters were relatively unimportant in the function. The results showed that partial defoliation, with more positive mean scores on the function, was desirable agronomically and this was also supported by the univariate means (Table 8.1c). Of the partially defoliated plants, the 50% rLA material had relatively long first and second leaves.

TABLE 8.1a Important statistics for the four discriminant functions for defoliation intensity from the multivariate analysis of previously unshaded plants in experiment one - leaf parameters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	69.45	2.60	36	17	*
2	17.96	1.84	24	15	NS
3	8.78	1.57	14	12	NS
4	3.81	1.37	6	7	NS

Table 8.1b Mean scores for the first discriminant function for defoliation intensity in experiment one.

Defoliation intensity (% rLA)	Mean score
100	0.50
75	-0.05
50	4.31
25	1.72
0	-6.49

three largest standardised coefficients:

β_0 - leaf 1 (5.02)
β_0 - leaf 2 (-2.77)
β_1 - leaf 1 (2.33)

Table 8.1c Defoliation intensity means for some leaf parameters (standard errors in parentheses).

Defoliation intensity (% rLA)	β_0 - leaf 1	β_0 - leaf 2	β_1 - leaf 1
100	232.7 (13.7)	222.4 (15.9)	0.9 (0.0)
75	253.4 (11.0)	248.9 (23.2)	0.9 (0.1)
50	278.7 (15.6)	241.7 (16.4)	0.9 (0.0)
25	215.6 (16.3)	226.0 (17.1)	0.9 (0.0)
0	143.6 (12.0)	161.6 (11.2)	0.8 (0.0)

In the analysis of previously unshaded and shaded plants in the first experiment, defoliation intensity was also the only significant source of variation. The first discriminant function accounted for most (96%) of the data dispersion (Table 8.2a) and mean scores on this function together with the three largest standardised coefficients are presented in Table 8.2b. The most important character was final length of the first leaf after defoliation, as found previously. Completely defoliated plants (0% rLA) again had significantly shorter first leaves than the partially defoliated treatments (Table 8.2c). Although $ß_1$ estimates for the second and third leaves, particularly the former, also had notable discriminatory ability (Table 8.2b), no biological importance could be gleaned from these findings (Section 3.6).

Sources of variation due to experiment and defoliation intensity were significant in the multivariate analysis pooled over experiments (Table 8.3a). As there were only two experiments, one discriminant function for experiment accounted for the total data dispersion. The first of three discriminant functions for defoliation intensity accounted for about 70% of data dispersion. Mean scores on these functions for experiment and defoliation intensity, as well as the three largest standardised coefficients, are presented in Tables 8.3b and 8.3d, respectively. Corresponding univariate means appear in Tables 8.3c and e. For experiment, final length ($ß_0$) and growth rate ($ß_2$) of the third leaf were equally important in the discriminant function and approximately three times greater than $ß_1$ (leaf 3). The mean scores showed that the third leaves of plants in experiment one had superior growth rates and were longer than those in experiment two. Means for the respective characters showed similar trends (Table 8.3c). The first two leaves after defoliation were relatively unimportant in discriminating between the experiment means.

On the first discriminant function for defoliation intensity (Table 8.3d), final length of leaf two was the most important character but was followed closely by growth rate and final length of the third leaf. Since moderate to high values of all three characters were desirable and loadings on the three coefficients differed, an agronomically desirable mean score was approximately -2.5 (from addition of the three coefficients). This was close to the mean score for 0% rLA which indicated that this treatment had the best combination of the three most important characters.

TABLE 8.2a Important statistics for the two discriminant functions for defoliation intensity from the multivariate analysis of previously shaded and unshaded plants in experiment one - leaf parameters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	95.98	2.95	18	14	*
2	4.02	0.57	8	8	NS

Table 8.2b Mean scores for the first discriminant function for defoliation intensity in experiment one.

Defoliation intensity (% rLA)	Mean score
100	1.46
50	2.60
0	-4.06

three largest standardised coefficients:

β_0 - leaf 1 (3.10)
β_1 - leaf 2 (-2.00)
β_1 - leaf 3 (-0.98)

Table 8.2c Defoliation intensity means for some leaf parameters (standard errors in parentheses).

Defoliation intensity (% rLA)	β_0 - leaf 1	β_1 - leaf 2	β_1 - leaf 3
100	235.2 (8.8)	0.8 (0.0)	0.8 (0.0)
50	252.8 (13.0)	0.8 (0.0)	0.8 (0.0)
0	151.3 (8.2)	0.7 (0.0)	0.9 (0.0)

3a Important statistics for the discriminant functions for experiment and defoliation intensity from the multivariate analysis pooled over experiments - leaf parameters.

nt	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
	100.00	9.38	9	10	***
on intensity					
	72.30	2.01	27	30	*
	23.48	1.16	16	22	NS
	4.22	0.50	7	12	NS

Table 8.3b Mean scores for the discriminant function for experiment in the pooled analysis.

Experiment	Mean score
1	2.18
2	-2.18

three largest standardised coefficients:

β_2 - leaf 3 (1.57)
β_0 - leaf 3 (1.55)
β_1 - leaf 3 (0.51)

Table 8.3c Experiment means for some leaf parameters (standard errors in parentheses).

Experiment	β_2 - leaf 3	β_0 - leaf 3	β_1 - leaf 3
1	0.3 (0.0)	229.0 (12.9)	0.9 (0.0)
2	0.2 (0.0)	130.7 (9.5)	0.9 (0.0)

Table 8.3d Mean scores for the first discriminant function for defoliation intensity in the pooled analysis.

Defoliation intensity (% rLA)	Mean score
75	0.80
50	0.52
25	1.53
0	-2.84

three largest standardised coefficients:

β_0 - leaf 2 (2.99)
β_0 - leaf 3 (-2.76)
β_2 - leaf 3 (-2.75)

Table 8.3e Defoliation intensity means for some leaf parameters (standard errors in parentheses).

Defoliation intensity (% rLA)	β_0 - leaf 2	β_0 - leaf 3	β_2 - leaf 3
75	194.4 (24.0)	198.8 (22.7)	0.2 (0.0)
50	188.4 (22.0)	206.3 (28.6)	0.2 (0.0)
25	182.0 (19.5)	164.2 (21.5)	0.2 (0.0)
0	130.1 (14.3)	150.2 (19.2)	0.3 (0.0)

If only the final lengths of leaves two and three were of interest, then the best treatments were 50 and 75% rLA while the 0% rLA treatment was inferior.

8.3.2 VEGETATIVE FEATURES AND TOTAL NONSTRUCTURAL CARBOHYDRATES

The condition of the variously defoliated, previously unshaded plants in experiment one at harvest one are shown in Table 8.4. Differences in leaf characters between some of the defoliation intensities were created while all root and stubble characters were similar for each treatment. Starch in these young plants was absent in the stubble and root.

The multivariate analysis of change scores for this material found that there were significant ($P<0.05$) differences between the defoliation intensities. The first discriminant function for defoliation intensity accounted for most of the dispersion in the data (Table 8.5a) and mean scores on this function together with the four largest standardised coefficients, are presented in Table 8.5b. The most important character was root dry weight and it had a negative loading which indicated that increasing changes in root dry weight between the two harvests resulted in increasingly negative mean scores. Changes in the levels of leaf soluble sugars were slightly less important and this character also had a negative loading. Dry weight changes in leaves and stubble were approximately half as important as those of the root. Apart from soluble sugar levels of the leaves, changes in other nonstructural carbohydrate fractions were relatively unimportant in the discriminant function. Positive changes in the dry weights of leaf, stubble and root occurred for all treatments (Table 8.5c) and larger changes in this direction, which resulted in more negative mean scores on the function, were preferred. The situation for changes in leaf soluble sugars was less clear. When treatments were imposed (harvest one), sugar levels were approximately equal in all partially defoliated material and a similar situation occurred at harvest two. Only the 0% rLA treatment had an often greater change in the level of sugars (Table 8.5c). Small change scores were regarded as more desirable because they indicated that some leaf sugar was present after defoliation which could be used immediately for regrowth and/or maintenance.

TABLE 8.4 Defoliation intensity means for vegetative and carbohydrate characters of previously unshaded plants in experiment one at harvest one (standard errors in parentheses).

Defoliation intensity (% rLA)	root dry weight (g)	stubble dry weight (g)	leaf dry weight (g)	stubble soluble sugars (%)	stubble starch (%)	root soluble sugars (%)	root starch (%)	leaf soluble sugars (%)	leaf starch (%)	leaf number	leaf area (cm^2)
100	1.10 (0.16)	0.43 (0.04)	0.80 (0.11)	3.87 (0.17)	0	1.26 (0.32)	0	5.97 (0.71)	1.71 (0.34)	8	184.8 (29.6)
75	0.87 (0.09)	0.50 (0.08)	0.70 (0.09)	4.11 (0.48)	0	1.16 (0.17)	0	3.93 (0.56)	0.61 (0.20)	6	188.5 (17.5)
50	0.70 (0.19)	0.45 (0.11)	0.55 (0.12)	3.16 (0.75)	0	1.26 (0.09)	0	5.09 (0.70)	0.79 (0.32)	4	147.0 (29.1)
25	1.05 (0.23)	0.56 (0.14)	0.28 (0.04)	4.29 (0.62)	0	1.21 (0.21)	0	4.67 (0.43)	0.29 (0.10)	2	71.2 (3.0)
0	1.01 (0.17)	0.70 (0.18)	0	3.74 (0.23)	0	1.43 (0.13)	0	0	0	0	0

TABLE 8.5a Important statistics for the four discriminant functions for defoliation intensity from the multivariate analysis of previously unshaded plants in experiment one - vegetative and carbohydrate characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	96.95	4.57	40	13	**
2	1.83	1.54	27	12	NS
3	0.93	1.29	16	10	NS
4	0.28	0.92	7	6	NS

Table 8.5b Mean scores for the first discriminant function for defoliation intensity in experiment one.

Defoliation intensity (% rLA)	Mean score
100	15.28
75	-11.10
50	-6.60
25	19.89
0	-17.47

four largest standardised coefficients:

1. root dry weight (-20.92)
2. leaf soluble sugars (-17.13)
3. leaf dry weight (12.05)
4. stubble dry weight (-11.13)

Table 8.5c Defoliation intensity means for three dry weight characters and leaf soluble sugars (standard errors in parentheses).

Defoliation intensity (% rLA)	root dry weight (g)	leaf soluble sugars (%)	leaf dry weight (g)	stubble dry weight (g)
100	9.42 (1.72)	1.90 (1.06)	8.49 (0.31)	1.98 (0.22)
75	10.56 (3.08)	4.82 (1.18)	9.93 (2.75)	2.29 (0.67)
50	9.26 (1.70)	3.10 (0.82)	10.96 (1.05)	2.98 (0.19)
25	9.04 (1.53)	2.98 (0.53)	9.41 (1.31)	1.82 (0.45)
0	5.21 (0.95)	8.11 (0.48)	6.55 (1.26)	1.36 (0.18)

Overall, the results indicated that partially defoliated plants were equal or superior to plants defoliated completely.

In the analysis of previously shaded and unshaded plants, all material had similar root and stubble dry weights at harvest one (Table 8.6) and starch was absent in these components. Differences between treatments for some leaf characters were created. Previous shading reduced the levels of soluble sugars in the stubble of 0% rLA plants and the content of leaf starch. Leaf soluble sugar levels were similar for the 50 and 100% rLA treatments regardless of shading history.

There were two discriminant functions for the defoliation intensity x shade interaction in the multivariate analysis. The first function accounted for approximately 80% of the data dispersion (Table 8.7a) and mean scores on this function together with the four largest standardised coefficients are presented in Table 8.7b. The most important character was stubble dry weight and larger changes in it resulted in increasingly negative mean scores. Changes in root starch, leaf dry weight and leaf number were progressively less important in the function and all had positive loadings. Increases in these three characters were desirable and therefore more positive mean scores were favoured. The results indicated that a net positive score was preferable and this occurred for the 100% and 50% rLA plants which were previously unshaded and shaded, respectively. The significant interaction was due mainly to the different responses of the 50 and 100% rLA plants in the presence or absence of shade. The 0% rLA previously shaded and unshaded treatments were similar for the four characters (Table 8.7c) and they were frequently inferior to 50 and/or 100% rLA treatments for some characters. The overall importance of changes in vegetative compared with carbohydrate characters was shown again.

Defoliation intensity and experiment means at harvest one for numerous characters of plants involved in the pooled multivariate analysis are presented in Tables 8.8a and b, respectively. Some differences were created between the defoliation intensities at the start of the regrowth period for leaf area, levels of leaf starch and leaf dry weight. Stubble and root characters were similar for all defoliation intensities except for levels of root starch which were zero for 25% rLA plants and

TABLE 8.6 Defoliation intensity x shade interaction means for vegetative and carbohydrate characters of previously shaded and unshaded plants in experiment one at harvest one (standard errors in parentheses).

Shade	Defoliation intensity (% rLA)	root dry weight (g)	stubble dry weight (g)	leaf dry weight (g)	stubble soluble sugars (%)	stubble starch (%)	root soluble sugars (%)	root starch (%)	leaf soluble sugars (%)	leaf starch (%)	leaf number	leaf area (cm^2)
Unshaded	0	1.01 (0.17)	0.70 (0.18)	0	3.74 (0.27)	0	1.43 (0.13)	0	0	0	0	0
Unshaded	50	0.70 (0.19)	0.45 (0.11)	0.55 (0.12)	3.16 (0.75)	0	1.26 (0.09)	0	5.09 (0.70)	0.79 (0.32)	4	147.0 (29.1)
Unshaded	100	1.10 (0.16)	0.43 (0.04)	0.80 (0.11)	3.87 (0.17)	0	1.26 (0.32)	0	5.97 (0.71)	1.71 (0.34)	8	184.8 (29.6)
Shaded	0	0.93 (0.05)	0.53 (0.06)	0	1.83 (0.14)	0	0.84 (0.16)	0	0	0	0	0
Shaded	50	0.97 (0.10)	0.62 (0.04)	0.39 (0.05)	2.99 (0.51)	0	0.87 (0.11)	0	5.31 (0.19)	0.31 (0.14)	4	110.2 (12.0)
Shaded	100	0.82 (0.11)	0.42 (0.09)	0.92 (0.10)	3.86 (0.24)	0	1.27 (0.22)	0	5.20 (0.68)	0.32 (0.17)	8	267.2 (9.9)

TABLE 8.7a Important statistics for the two discriminant functions for the defoliation intensity x shade interaction from the multivariate analysis of previously shaded and unshaded plants in experiment one - vegetative and carbohydrate characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	79.37	3.27	20	12	*
2	20.63	2.08	9	7	NS

Table 8.7b Mean scores for the first discriminant function for defoliation intensity x shade interaction in experiment one.

	Defoliation intensity (% rLA)		
Shade	0	50	100
Unshaded	-0.05	-4.37	1.80
Shaded	-1.03	4.48	-0.83

four largest standardised coefficients:

1. stubble dry weight (-2.93)
2. root starch (2.03)
3. leaf dry weight (1.11)
4. leaf number (0.99)

Table 8.7c Defoliation intensity x shade interaction means for three vegetative characters and levels of root starch (standard errors in parentheses).

Shade	Defoliation intensity (% rLA)	stubble dry weight (g)	root starch (sqrt)	leaf dry weight (g)	leaf number
Unshaded	0	1.36 (0.18)	0.51 (0.22)	6.55 (1.26)	93.5 (16.3)
Unshaded	50	2.98 (0.19)	0.20 (0.07)	10.96 (1.05)	101.0 (8.9)
Unshaded	100	1.98 (0.22)	0.92 (0.23)	8.49 (0.31)	121.0 (10.2)
Shaded	0	0.92 (0.16)	0.19 (0.19)	3.86 (0.37)	85.5 (8.3)
Shaded	50	1.11 (0.34)	1.03 (0.08)	7.46 (0.61)	100.8 (12.2)
Shaded	100	2.35 (0.38)	0.66 (0.25)	9.40 (0.81)	112.3 (13.9)

TABLE 8.8a Defoliation intensity means for vegetative and carbohydrate characters of plants in the pooled analysis at harvest one (standard errors in parentheses).

Defoliation intensity (% rLA)	root dry weight (g)	stubble dry weight (g)	leaf dry weight (g)	stubble soluble sugars (%)	stubble starch (%)	root soluble sugars (%)	root starch (%)	leaf soluble sugars (%)	leaf starch (%)	leaf area (cm^2)
75	5.67 (1.90)	1.17 (0.28)	0.46 (0.10)	2.96 (0.50)	0	1.16 (0.14)	0.15 (0.15)	5.07 (0.68)	0.81 (0.14)	142.7 (19.7)
50	7.29 (2.74)	1.10 (0.28)	0.37 (0.09)	2.54 (0.45)	0	1.26 (0.30)	0.07 (0.04)	5.13 (0.57)	0.78 (0.17)	111.2 (19.6)
25	7.64 (2.57)	1.51 (0.37)	0.19 (0.04)	3.23 (0.50)	0	1.37 (0.14)	0	5.13 (0.50)	0.29 (0.05)	58.2 (6.0)
0	6.32 (2.08)	1,26 (0.24)	0	2.97 (0.35)	0	1.43 (0.09)	0.16 (0.09)	0	0	0

Table 8.8b Experiment means for vegetative and carbohydrate characters of plants in the pooled analysis at harvest one (standard errors in parentheses).

Experiment	root dry weight (g)	stubble dry weight (g)	leaf dry weight (g)	stubble soluble sugars (%)	stubble starch (%)	root soluble sugars (%)	root starch (%)	leaf soluble sugars (%)	leaf starch (%)	leaf area (cm^2)
1	0.91 (0.09)	0.55 (0.06)	0.38 (0.08)	3.82 (0.27)	0	1.26 (0.08)	0	3.42 (0.57)	0.42 (0.12)	101.7 (20.1)
2	12.56 (0.83)	1.96 (0.12)	0.13 (0.03)	2.02 (0.14)	0	1.35 (0.17)	0.19 (0.08)	4.25 (0.74)	0.52 (0.11)	54.4 (10.1)

detectable for the other intensities. Plants in experiment one at harvest one were less well developed than those in experiment two as indicated by their lower stubble and root dry weights (Table 8.8b). Experiment one plants also had no detectable starch in the roots. There were differences between experiments for some carbohydrate characters.

In the pooled multivariate analysis, there were significant differences between experiments and defoliation intensities, and discriminant functions for these sources of variation are presented in Table 8.9a. There was one function for experiment and the first of three functions for defoliation intensity accounted for most (88%) of the data dispersion. Mean scores on these functions, together with the several largest standardised coefficients feature in Tables 8.9b and d and appropriate univariate means are shown in Tables 8.9c and e.

The three dry weight components were the most important characters in the discriminant function for experiment and their order of importance was stubble > root > leaf (Table 8.9b). All characters had positive loadings which indicated that increasing changes in these characters resulted in larger mean scores and these were in the direction of desirability. The results (Tables 8.9b and c) indicated that plants in experiment one which had been undefoliated previously, had greater changes in the three dry weight characters than those in experiment two, which had been defoliated twice previously. For example, changes in stubble dry weight in experiment one were approximately seven times greater than those in experiment two (Table 8.9c). Furthermore, as a proportion of the stubble masses at harvest one, changes in experiment one were about 400% while those in experiment two were approximately 15%. Changes in various carbohydrate levels in the discriminant function were relatively unimportant.

For the first discriminant function for defoliation intensity, change in the levels of leaf soluble sugars was the most important character (Table 8.9d) and the 0% rLA treatment had greater changes than the partially defoliated treatments (Table 8.9e). However, for reasons similar to those given in the analysis of unshaded treatments in experiment one, low change scores for this character were desirable. Because of

TABLE 8.9a Important statistics for the discriminant functions for experiment and defoliation intensity from the multivariate analysis pooled over experiments - vegetative and carbohydrate characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
Experiment					
1	100.00	6.86	7	12	**
Defoliation intensity					
1	87.93	3.42	21	35	***
2	6.79	1.29	12	26	NS
3	5.28	1.46	5	14	NS

Table 8.9b Mean scores for the discriminant function for experiment in the pooled analysis.

Experiment	Mean score
1	1.50
2	-1.50

three standardised coefficients:

1. stubble dry weight (0.96)
2. root dry weight (0.68)
3. leaf dry weight (0.26)

Table 8.9c Experiment means for three dry weight characters (standard errors in parentheses).

Experiment	stubble dry weight (g)	root dry weight (arcsine)	leaf dry weight (log)
1	2.11 (0.25)	0.84 (0.04)	2.12 (0.13)
2	0.29 (0.17)	0.49 (0.07)	0.99 (0.09)

Table 8.9d Mean scores for the first discriminant function for defoliation intensity in the pooled analysis.

Defoliation intensity (% rLA)	Mean score
75	-2.30
50	-1.37
25	0.16
0	3.51

four largest standardised coefficients:

1. leaf soluble sugars (2.01)
2. leaf dry weight (1.74)
3. stubble dry weight (-1.28)
4. root soluble sugars (0.76)

Table 8.9e Defoliation intensity means for two dry weight and two carbohydrate characters (standard errors in parentheses).

Defoliation intensity (% rLA)	leaf soluble sugars (%)	leaf dry weight (log)	stubble dry weight (g)	root soluble sugars (%)
75	3.33 (0.89)	1.59 (0.29)	1.45 (0.49)	1.21 (0.27)
50	3.59 (0.48)	1.72 (0.27)	1.80 (0.46)	1.86 (0.38)
25	4.00 (0.66)	1.58 (0.25)	0.85 (0.43)	2.26 (0.26)
0	8.16 (0.42)	1.32 (0.24)	0.70 (0.32)	2.26 (0.31)

the positive loading for leaf soluble sugars, less positive/more negative mean scores were preferred. The second most important character in the function was leaf dry weight, despite there being similar changes for all defoliation intensities (Table 8.9e), and it had a positive loading. Changes in stubble dry weight and levels of root soluble sugars were less important in the function (Table 8.9d). Overall, desirable scores were approximately zero to small positive values and in this regard, 25% rLA and to a lesser extent 0% rLA treatments, were most satisfactory.

8.4 DISCUSSION

The findings from this study often supported the recommendation that young swards of sheep's burnet should be grazed lightly (Sheppard and Wills, 1985, 1986). However, the current experiments had a notable advantage in that they also quantified precisely the actual plant regrowth responses in vegetative and physiological terms and therefore provided a more fundamental understanding of the processes involved in regrowth. Effects of complete defoliation were often undesirable and included reductions in foliar regrowth of expanded leaves with respect to final length and dry weight, and frequently decreased stubble and root dry weights. Similar findings after intense defoliation have been reported for numerous other forage species (Brougham, 1956; Ward and Blaser, 1961; Evans, 1971, 1973; Booysen and Nelson, 1975; Grant *et al.*, 1981; Harris, 1978; Brown, 1987).

The physiological understanding of regrowth in sheep's burnet was enhanced considerably in the current studies. A valuable finding was the absence at harvest one of starch in the stubble and roots of the 3-month old plants in experiment one and the absence of stubble starch in the older and previously defoliated plants in experiment two. These findings, and the detectable but low levels of starch in the roots of most plants at harvest one in experiment two, suggested that sheep's burnet was a poor accumulator of starch. This was also inferred by the negligible changes in the levels of starch in all plant tissues in both experiments. Possible improvement in the ability of sheep's burnet to accumulate starch may enhance its regrowth potential provided starch reserves are a key source of energy.

However, in view of the low starch levels and the relatively high concentrations of soluble sugars, particularly in the leaves (for example Tables 8.8a and b), current photosynthates were probably the major energy source for foliar regrowth. This proposal was supported often by the regrowth of completely defoliated plants compared with that from all partially defoliated plants which had an existing photosynthate source from two or more fully expanded leaves. In the latter instance, new leaves were longer than those of 0% rLA plants, probably resulting from greater assimilate supply. However, the frequently superior regrowth of partially defoliated plants could also have been due to remnant soluble sugars in the tagged leaves rather than from subsequently produced photosynthate. In either case, it is likely that soluble sugars from the residual leaves were transported preferentially to the energy demanding leaf meristematic tissues rather than to other plant parts, although assimilate partitioning can vary with environment and stage of development (Wardlaw, 1968). The results were inconclusive in determining the relative contributions to foliar regrowth of current versus reserve carbohydrate. Although radioactive labelling may enhance the understanding of assimilate transport, the relative contributions of the two carbohydrate sources may never be determined fully (Blaser *et al.*, 1966).

Harvest one in each experiment was conducted one day after the imposition of defoliation treatments and it is therefore possible that there were some pronounced changes in the distribution and perhaps the composition of the remaining nonstructural carbohydrates in the plant tissues (Wardlaw, 1968; Darbyshire *et al.*, 1979; Atkinson, 1986). For example, any breakdown in the low levels of starch in the remaining tagged leaves may have inflated the estimates of the levels of soluble sugars of those leaves. Furthermore, the greater assimilate demand by the new leaf meristems compared with other plant tissues (Wardlaw, 1968; Beevers, 1969; Wareing and Patrick, 1975) could have depleted soluble sugar levels in the stubble and roots, particularly in the completely defoliated treatments. However, as the individual new leaves developed, they would have become quickly self-sufficient in their assimilate requirements and later developed export capability (Wardlaw, 1968). Frequent, earlier samplings would assist in elucidating these patterns of change.

Although starch was detected in leaves and in some stubble and root components in both experiments, the low values suggested that the presence of another, perhaps more important, reserve substance(s) should not be overlooked. The oligosaccharide fructosan, is an important carbohydrate reserve in numerous cool-season grasses and other species (Brown and Blaser, 1965; Humphreys, 1966; Blaser *et al.*, 1966; Ojima and Isawa, 1968; Booysen and Nelson, 1975; Walton, 1983) and it may have a role in the sugar physiology of sheep's burnet. However, in a recent literature review (Meier and Reid, 1982) the family Rosaceae was not listed as containing fructans in their vegetative tissues. Chromatographic analysis should enable identification of the key carbohydrate types in the species. Protein and other labile fractions (Davidson and Milthorpe, 1965) may also contribute to new growth and to respiration.

The dependence of the measured leaves on assimilates from reserves and/or current photosynthesis by other plant parts for expansion, was probably short-lived. Most of these leaves reached approximately half of their final length and leaf area, and had numerous expanding leaflets, after three to four day's growth. This suggested that, at this stage, they were able to produce adequate carbohydrates for their own growth requirements and commence export of any surplus assimilate (Wardlaw, 1968; Giaquinta, 1978). After eight to ten days, most leaves were close to full expansion and were therefore probably most active in exporting their assimilates to other physiologically active plant organs (Wardlaw, 1968). It is therefore suggested that the length of dependence on reserves for regrowth of sheep's burnet was approximately intermediate between that for grasses of 2-7 days and lucerne of 21 days, as reviewed recently (Harris, 1978). However, caution should be exercised in making such comparisons since there is such a pronounced influence of environmental conditions, particularly temperature and light, on carbohydrate metabolism and partitioning (Wardlaw, 1968; Ludlow, 1978; McWilliam, 1978). Also, extrapolation to the field situation with its often more variable environment, is risky.

The supposition that regrowth of juvenile sheep's burnet plants was dependent mainly on current photosynthate indicates that response patterns in this species could be similar to those of birdsfoot trefoil (Nelson and Smith, 1968; Smith, 1962), cicer

milkvetch (Gabrielsen *et al.*, 1985) and sainfoin (Cooper and Watson, 1968), where the retention of some leaf following defoliation is recommended. This contrasts with the carbohydrate utilisation of juvenile and mature lucerne plants following defoliation where energy for regrowth is supplied almost exclusively by the upper part of the taproot (Smith, 1962; Reynolds and Smith, 1962; Cooper and Watson, 1968; Sheaffer, 1983; Rapoport and Travis, 1984). Young sheep's burnet plants in the present investigation often had smaller roots than those in fully mature plants. Thus, it is possible that roots in mature plants have a higher carbohydrate content than those shown here, and hence a greater role in regrowth. The tolerance of mature plants to brief periods of very intense defoliation (Sheppard and Wills, 1986) partly supports this hypothesis.

The relatively low level of soluble sugars in stubble of the completely defoliated plants following shading (Table 8.6), was in agreement with the general reduction in nonstructural carbohydrates of various plant tissues which resulted from similar pretreatments (Ward and Blaser, 1961; Davies, 1965; Booysen and Nelson, 1975). The results also indicated that completely defoliated, previously shaded plants may be disadvantaged in the field compared with less shaded plants due to their lower energy status and hence regrowth ability. Shading effects were less important where partial defoliation was adopted (Table 8.6) and this provided further justification for advocating similar defoliation management.

The relatively small differences between the defoliation intensities in their overall rates of leaf extension, as defined by the function parameter $ß_2$ (Section 8.2.4.1), may have been due to several factors. These include a limit on the rate of translocation of soluble sugars, probably from adjacent leaves in the case of partially defoliated plants (Wardlaw, 1968) and from stubble in completely defoliated material. This may have hindered development of new leaves with the potential to be supplied with greater quantities of nonstructural carbohydrates. Vascular connections may restrict supply patterns to growing organs in a range of species (Wardlaw, 1968). Another factor which may explain the results was a relatively constant restraint on leaf extension by the epidermis, as indicated for several other species (Dale, 1988).

Moisture limitations in the present experiments were unlikely due to frequent watering.

An exception where β_2 estimates were important occurred in the discrimination between experiments (Table 8.3b and c) and the higher rates of extension in experiment one were probably due to the elevated temperatures during December/January compared with March/April. These results were in agreement with numerous studies which have shown superior extension rates at higher temperatures (Williams and Biddiscombe, 1965; Robson, 1972; Wilhelm and Nelson, 1978). Apart from comparing experiments, the results showed that there was little other benefit in obtaining estimates of β_2. This finding was advantageous as it suggested that incremental monitoring of leaf length was unnecessary and that the only important length measurement required when comparing early foliar regrowth in single experiments was final extension.

The presence of various numbers of fully expanded leaves compared with complete leaf removal may be associated with a range of mowing and/or grazing intensities. The normal defoliation intensities of young sheep's burnet swards under either defoliation regime are unknown. Regardless of the practical defoliation procedure, constant numbers of leaves and amounts of leaf area are not removed from every plant, nor are some plants defoliated completely while adjacent ones have no leaf removal. Despite these reservations, the method used to simulate different defoliation intensities was adopted because of its simplicity, uniformity and easy repeatability. The technique also partially simulated a grazing situation since the younger, usually more palatable and accessible leaves were removed during the imposition of the treatments and similar patterns are documented for pastures (Watkin and Clements, 1978; Rattray and Clark, 1984). For sheep's burnet however, accessibility of young leaves to livestock depends very much on the amount of stubble following defoliation, which is influenced directly by grazing management (B J Wills, pers comm.).

The palatable foliage of sheep's burnet is defoliated readily due to its semi-erect to erect growth habit, and hence accessibility. The results suggested that partial

defoliation should be a primary management objective for young swards and that complete defoliation should be avoided due to reduced foliar regrowth and levels of nonstructural carbohydrates. The recommendation would be particularly apt under stressful conditions of high temperature and low soil moisture status. For grazing, a lenient rotational system allowing satisfactory regrowth and adequate rest periods between stocking for plants to accumulate nonstructural carbohydrates, is probably the most suitable management for young swards. Similar recommendations were documented recently (Sheppard and Wills, 1985, 1986). Specification of the optimum duration between stockings, involving times required to replenish and accumulate nonstructural carbohydrates used in regrowth at different times of the year, is necessary to more accurately define management policies.

CHAPTER 9 : FOLIAR REGROWTH OF VARIOUSLY AGED AND PREVIOUSLY UNDEFOLIATED SHEEP'S BURNET DURING MOISTURE DEFICITS

9.1 INTRODUCTION

Young plants of sheep's burnet arising from a spring sowing or transplanted glasshouse prepared material may be affected adversely by moisture deficits in some seasonally dry localities. Severe leaf wilting and occasionally complete plant death may result (Douglas, unpubl.; B J Wills, pers. comm.; W Stiefel, pers. comm.). It is likely that reductions in foliar and/or root growth occur even under mild moisture deficits, and these have been reported for numerous other species (Salter and Goode, 1967; Ludlow and Ng, 1976; Chu and McPherson, 1977; Turner and Begg, 1978; Chu *et al.*, 1979; Turner, 1986).

Defoliation responses of young plants of sheep's burnet under assumed non-limiting moisture conditions were investigated in the previous study (Chapter 8). In practice however, establishing plants are likely to be exposed to one or more periods of sub-optimal soil moisture levels and it is therefore valuable to understand regrowth responses under these conditions. A strategy which may improve regrowth and enhance survival in drought-prone environments is earlier spring sowing to produce older and larger seedlings by the time soil moisture deficits occur. Larger seedlings with their greater vegetative development and perhaps levels of nonstructural carbohydrates would presumably have the potential for superior regrowth compared with smaller seedlings. Defoliation of plants under dry conditions, by reducing leaf area and consequently transpiration, may also partially overcome any growth limitations imposed by an inadequate root system and/or low soil moisture content (Jantii and Kramer, 1956; Christ, 1978; Cutler *et al.*, 1980; Wolf and Parrish, 1982).

The main objective of this study was to investigate the early foliar regrowth of variously defoliated and aged plants of sheep's burnet growing under soil moisture

deficits. A secondary objective was to determine the magnitude of water losses from the variously treated plants.

9.2 MATERIALS AND METHODS

9.2.1 LOCATION

The study was conducted in a glasshouse at the Plant Growth Unit, Massey University. Mean air temperature from September, 1987 - March, 1988 was 22-25°C. The average daily maximum air temperature during the period was 25-30°C while the corresponding daily minimum was 18-23°C. Average relative humidity at 0900 hours was 50-70%.

9.2.2 PLANT ESTABLISHMENT AND TREATMENTS

Medium sized Oregon sheep's burnet seed (Chapter 6) was sown on 23 September, 1987 at 5 mm depth into sand contained within planter bags of two different sizes. Flattened rectangular dimensions of the bags were 12 x 28 cm (small) and 12 x 42 cm (large) and the mass of air dried sand in the large bags was approximately twice that in the small bags. A slow-release fertiliser (N:P:K=15:5.2:12.5) was distributed uniformly in the medium at a rate of 5 gl^{-1}. Sand was used to facilitate later root extraction and four seeds were sown per bag. A second sowing was conducted four weeks later on 21 October to create different aged plants. Gravimetric moisture content of the sand at field capacity (defined as two days after saturation) was 11.3% (SE=0.2%) while that of air dried material was 1.1% (SE=0.1%). Available water content, defined as (mass of water at field capacity) - (mass of water at the air dry stage), was 1191 g (SE=7 g) and 844 g (SE=14 g) for large and small bags, respectively.

Two experiments, one per bag size, were separated physically in the glasshouse but run concurrently. Bags were placed on the concrete floor of the glasshouse due to the physical difficulty of supporting their weight on trolleys or on tables. Seedlings

were established under well watered conditions and were thinned randomly to one healthy seedling per bag before shading from neighbouring seedlings occurred.

Each experiment had treatments arranged in three randomised complete blocks. The experiment involving small bags had fewer treatments. The treatments were:

1. three defoliation intensities, based on the percentage of eight fully expanded leaves retained, namely
   0% residual leaf area (rLA)
   50% rLA - large bags only
   100% rLA

2. two plant ages, originating from the staggered sowing dates, namely
   young (sown 21 October, 1987)
   old (sown 23 September, 1987) - large bags only

3. two moisture regimes (post harvest one), namely
   moist - a continuation of well watered conditions such that available water content did not fall below 80% of the original value for each bag size.
   dry - watering withheld until first wilting.

Most of the investigation involved plants growing in large bags. This was because their opportunity for osmotic adjustment (Turner and Begg, 1978, 1981; Hanson and Hitz, 1982; Johnson *et al.*, 1984; Ludlow *et al.*, 1985; Sambo and Aston, 1985), if it occurs in sheep's burnet, was probably greater than for plants in small bags due to the former's relatively slower rate of decline in plant water status. Plants in small bags were included to evaluate the benefits of using larger bags in regrowth studies involving moisture deficits.

On the basis of the measurements to be taken, two groups of plants were randomised within each block. One group was used solely for non-destructive leaf extension measurements while the other group was used for destructive harvests. Details of the measurements are presented in Section 9.2.3. Plants in small bags were defoliated

on 22 December, 1987 when they had reached an arbitrary growth stage which could be suitable for grazing (about 15-25 fully expanded leaves). Growth of plants in large bags was relatively slow and imposition of defoliation intensities on these plants was delayed until the young plants reached a stage similar to those in small bags (2 February, 1988).

Following defoliation, bags were saturated uniformly by immersion in water. After removal, a 1 cm thick layer of white plastic chips was placed on the sand surface to ensure that evaporation from the surface was negligible. Blanks without plants were also included. Two days after saturation (field capacity), the "dry" treatment was commenced. When first wilting occurred plants were resaturated to observe possible compensatory leaf growth.

9.2.3 MEASUREMENTS

9.2.3.1 LEAF EXTENSION

Information on the rate and magnitude of regrowth of new leaves was obtained by measuring the lengths of the first three leaves appearing after the plants were variously defoliated. The leaves were distinguished by tagging with coloured wires and their lengths were measured every 1-2 days until the plants in the dry moisture regime first wilted. At this time, measurement on plants in the moist regime of the same age and defoliation intensity also ceased. After resaturation of the plants in the dry treatment, the tagged leaves were measured daily for four days. All measurements were conducted at approximately the same time of day to minimise possible diurnal variability in leaf extension rate (Chu and Kerr, 1977; Dale, 1988).

9.2.3.2 VEGETATIVE FEATURES AND TOTAL NONSTRUCTURAL CARBOHYDRATES

Two harvests were conducted on plants from each treatment and bag size to determine the morphological and physiological changes occurring over the foliar regrowth period. Harvest one was made one day after the defoliation intensity

treatments were imposed on 23 December, 1987 (small) and 3 February, 1988 (large). Characters measured in each experiment were:

-leaf number
- leaf area (cm^2) and dry weight (g)
- stubble and root dry weights (g)
- soluble sugar and starch levels of leaves (%)
- soluble sugar and starch levels of stubble and root (%)

Definitions of the plant parts measured as well as the procedures for determining leaf area, dry weights, and levels of nonstructural carbohydrates were presented in Chapter 3.

The above characters were measured again on plants harvested immediately after first wilting occurred. The time of harvesting varied depending on treatment and bag size and ranged from approximately 9-23 days after field capacity. Plants in the dry treatment and those of the same age and defoliation intensity growing under well watered conditions, were harvested simultaneously.

9.2.3.3 TRANSPIRATION LOSS

Bags were weighed at field capacity and then daily (small) and every 1-2 days (large), thereafter. This enabled determination of the amount of water required to replace losses from plants under the moist regime and also to determine the total magnitude of losses from plants undergoing a dry down to first wilting.

9.2.4 STATISTICAL ANALYSES

9.2.4.1 LEAF EXTENSION

Mean times of first measurement of each leaf after defoliation were determined for each treatment x experiment combination. Analyses of variance were not conducted

due to the frequently non-normal data distributions. Several log and square root transformations and an arcsine transformation failed to improve the distributions.

Plots of leaf length against time for each leaf per treatment and bag size were conducted for individual blocks. These were to suggest likely types of model functions for curves to describe the leaf extension/regrowth events over time. In all instances, an asymptotic regression model was suitable. The plots for the dry moisture regime lacked any humps or depressions which could suggest compensatory growth. The model fitted to all data sets was a three parameter monomolecular function (Richards, 1959; Landsberg, 1977) of the form

$$y = \beta_0(1 - \beta_1 e^{-\beta_2 t})$$

and fitting utilised a nonlinear least squares procedure (Section 3.6). The biological significance of the parameters together with other details on the function and its fitting were presented in Section 3.6.

Correlations, and hence covariances, existed between some or all of the estimates of the monomolecular function parameters (β_0, β_1, and β_2) and therefore treatments were analysed simultaneously using multivariate analyses of variance (Section 3.7). Two analyses were conducted due to the structure of the data. Firstly, all parameter estimates for each leaf were analysed jointly for all treatments in the large bag experiment. Although it was desired to assume a random effects model for all significance tests (Lindeman *et al.*, 1980; Steel and Torrie, 1980), appropriate multivariate tests for most sources of variation were not possible due to insufficient error degrees of freedom. A fixed effects model was therefore assumed for all significance tests. In the second analysis, data for treatments common to both small and large bag experiments were analysed as a multivariate analysis of variance pooled over experiments. Sources of variation were tested against the residual matrix for the same reason mentioned for the initial analysis.

All parameters were also analysed univariately. In the first analysis, fixed and mixed effects models were assumed and in the latter model only the interactions were assumed random. All first order interactions were tested against the intensity x moisture x age interaction. For the pooled analyses, fixed effects models were assumed. In addition, first order interactions were tested against the size x intensity x moisture interaction while size was tested against block(size).

9.2.4.2 VEGETATIVE FEATURES AND TOTAL NONSTRUCTURAL CARBOHYDRATES

In each experiment, there was no moisture treatment at harvest one since all bags were of similar moisture status following saturation the previous day (Section 9.2.2). By harvest two, the plants had regrown under diverging moisture conditions. The use of harvest one data as a covariate in an analysis of covariance of harvest two data was investigated but found unsuitable due to the very low R-square values (<0.20) for most characters. Therefore, the changes between the harvests were calculated for all characters as

$$\text{change} = (\text{harvest two} - \text{harvest one})$$

and the resulting data were useful in allowing a source of variation due to moisture to be included in the analyses of variance. The biological significance of the change scores for the dry weight characters was described in Section 8.2.4.2.

Two multivariate analyses of variance, as described for the leaf extension parameters (Section 9.2.4.1), were conducted on the change scores. Univariate analyses of variance were also performed. Stubble starch data were transformed to (log(0.1-X)) for the univariate and multivariate large bag analyses while a transformation of root starch data to (sqrt(X+0.8)) proved suitable for the pooled analyses. Leaf starch data for both groups of analyses were characterised by marked non-normal distributions which could not be transformed satisfactorily. They were therefore excluded from all multivariate analyses of variance. Fixed effects and partly random effects models

were assumed for all tests of significance as described for leaf extension in Section 9.2.4.1.

The time of harvest two for each treatment, expressed as days after field capacity, was the same in the moist and dry moisture regimes. Harvest times in the dry regime were analysed using analysis of variance.

9.2.4.3 TRANSPIRATION LOSS

Field capacity was nominated as time zero and all subsequent water losses were expressed relative to this baseline. At each weighing, water losses from bags without plants were subtracted from those losses from bags containing plants. The result was then subtracted from the weight at field capacity.

Three measures of water use were estimated for plants in the dry regime. Total transpiration loss per plant, expressed on a weight basis (g), was the accumulated water loss by the time of first wilting while the percentage of available water in each bag size used by the variously treated plants was calculated as total transpiration loss per plant divided by the appropriate weights of available water, namely 844 (small) and 1191 (large) g. Average transpiration rate per plant (g/day) was estimated by dividing total transpiration loss by the number of days after field capacity when harvest two was conducted. Univariate analysis of variance was conducted for each character. Net water loss data for the moist regime were combined with total transpiration loss data in an additional analysis of variance to confirm that moisture differences had been created in the study.

Multiple regression analyses were conducted to indicate the relative importance of different leaf characters on water use. Total transpiration losses and rates per plant were each regressed against leaf area, leaf dry weight and leaf number for each plant age, defoliation intensity and bag size. Data pooled across appropriate classifications were used. For the completely defoliated (0% rLA) treatments, the leaf characters represented entirely new material produced after imposing the treatments, whereas for the partially defoliated treatments the three characters included the originally

tagged leaves retained after imposing treatments. Multiple regression analyses for each plant age x defoliation intensity x bag size combination were not conducted because of insufficient data, namely three data points per combination. Standardised partial regression coefficients (Steel and Torrie, 1980) were estimated.

Further analyses were also conducted on the total transpiration loss and rate data by expressing each parameter on a per unit leaf area, leaf number and leaf dry weight basis. This facilitated comparisons between treatments at a more fundamental level than conducted previously. All rates were expressed on a per second basis and as transpiration is often relatively low at night time (Boyer and McPherson, 1975; Wolf and Parrish, 1982), a day was regarded as 12 hours (43 200 seconds) for the purpose of calculation.

The derived transpiration loss characters were then analysed jointly using multivariate analyses of variance (Section 3.7). Analyses were conducted for treatments in large bags and a pooled analysis over bag sizes was also performed. The derived transpiration rate characters were analysed similarly. In the analyses of data for large bagged plants, a fixed effects model was assumed for all tests of significance. There were insufficient error degrees of freedom to test the sources of variation due to age and defoliation intensity when using the age x intensity interaction as the residual matrix. In the pooled analyses, bag size was tested against the block(size) matrix. Intensity was unable to be tested against the size x intensity interaction matrix due to insufficient error degrees of freedom. Univariate analyses were also conducted for all characters.

9.3 RESULTS

9.3.1 LEAF EXTENSION

Length measurements on the first leaves for most treatments commenced approximately 2-3 days after defoliation. Most of the second and third leaves were first measured 4-5 and 6-7 days after defoliation, respectively.

Leaf growth rates were similar for all variously aged and defoliated plants growing under the two moisture regimes in large bags. Estimates of final extension ($ß_0$) were also similar. Supporting evidence was the non-significance of any effect in the multivariate analysis of variance of the monomolecular function parameters ($ß_0$, $ß_1$, and $ß_2$) for all leaves (Appendix). Hence, extension for all leaves and treatments could be described satisfactorily by one equation, namely,

$$y = 96.7(1 - 0.9e^{-0.3t})$$

The equation indicated that overall final leaf length ($ß_0$) averaged approximately 100 mm.

In the pooled analysis over bag sizes, there was a significant ($P<0.05$) defoliation intensity x moisture regime interaction (Table 9.1a) with all other effects being non-significant. Mean scores on the single discriminant function for this source of variation together with the three largest standardised coefficients are presented in Table 9.1b. The most important character in the function was final length of the second leaf and it was approximately twice as important as any other character. These findings were supported by the notable variability between some interaction means for this character (Table 9.1c). The means also indicated that while variously defoliated plants under dry conditions had similar leaf lengths, partially defoliated plants (100% rLA) in the moist regime produced longer leaves than completely defoliated plants (0% rLA). However, these trends were not significant statistically. Although the estimates of the $ß_1$ parameter for the second and third leaves were important in the function, they had little biological importance.

9.3.2 VEGETATIVE FEATURES AND TOTAL NONSTRUCTURAL CARBOHYDRATES

All main effects of defoliation intensity, moisture regime and plant age were highly significant ($P<0.01$) in the multivariate analysis of variance of characters measured on plants in large bags (Table 9.2a). The absence of interactions permitted a relatively simple explanation of the variability observed. The first of two

TABLE 9.1a Important statistics for the single discriminant function for defoliation intensity x moisture regime interaction from the multivariate analysis pooled over bag sizes - leaf parameters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	100.0	10.85	9	4	*

Table 9.1b Mean scores for the single discriminant function for defoliation intensity x moisture regime interaction in the pooled analysis over bag sizes.

Moisture regime	Defoliation intensity (% rLA) 0	100
dry	2.10	-1.36
moist	-5.63	4.89

three largest standardised coefficients:

1. β_0 - leaf 2 (10.13)
2. β_1 - leaf 2 (-5.45)
3. β_1 - leaf 3 (4.98)

Table 9.1c Defoliation intensity x moisture regime interaction means for some leaf parameters (standard errors in parentheses).

Moisture regime	Defoliation intensity (% rLA)	β_0-leaf 2	β_1-leaf 2	β_1-leaf 3
dry	0	96.4 (6.9)	0.87 (0.02)	0.83 (0.06)
dry	100	100.4 (8.8)	0.87 (0.04)	0.78 (0.05)
moist	0	73.8 (10.2)	0.85 (0.05)	0.78 (0.07)
moist	100	104.8 (12.0)	0.82 (0.06)	0.83 (0.05)

TABLE 9.2a Important statistics for the discriminant functions for defoliation intensity, moisture regime and plant age from the multivariate analysis of characters measured on plants growing in large bags - vegetative and carbohydrate characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
Defoliation intensity					
1	95.25	3.47	20	26	**
2	4.75	0.66	9	14	NS
Moisture regime					
1	100.00	9.62	10	13	***
Plant age					
1	100.00	6.63	10	13	**

Table 9.2b Mean scores for the discriminant functions for defoliation intensity, moisture regime and plant age for plants growing in large bags.

Defoliation intensity (% rLA)	Mean score	Moisture regime	Mean score	Plant age	Mean score
0	3.21	dry	2.13	young	1.77,
50	-1.61	moist	-2.13	old	-1.77
100	-1.60				

four largest standardised coefficients:	four largest standardised coefficients:	four largest standardised coefficients:
1. leaf scluble sugars (2.52)	1. leaf area (-2.28)	1. leaf area (3.13)
2. stubble starch (0.96)	2. leaf soluble sugars (2.13)	2. stubble starch (-2.56)
3. root dry weight (0.92)	3. stubble starch (2.11)	3. leaf dry weight (-2.34)
4. root starch (-0.89)	4. leaf dry weight (1.95)	4. leaf soluble sugars (-0.99)

discriminant functions for defoliation intensity accounted for about 95% of the total dispersion in the data and is therefore the only function discussed further. Single discriminant functions for moisture regime and plant age accounted for all respective dispersions. Mean scores on each function and the four standardised coefficients for each function are shown in Table 9.2b. The harvest one means for all characters are presented in Table 9.2c while the change score means for the most important characters (Table 9.2b) are displayed in Table 9.2d.

The change in the concentration of leaf soluble sugars between the two harvests was the most important character in the function discriminating between the three defoliation intensity means (Table 9.2b) and was approximately 2.5 times more important than any other character. Changes in root dry weight, and stubble and root concentrations of starch were about equal in their importance. Except for the negative loading on the coefficient for root starch, the important characters had positive loadings which indicated that increasingly positive mean scores represented the greatest changes between harvests. Relatively large changes occurred for the completely defoliated (0% rLA) compared with the partially defoliated plants (Table 9.2b) and this was also shown by some of the character means (Table 9.2d). For example, the concentration of leaf soluble sugars increased markedly for the 0% rLA plants, probably due mainly to the production of new leaves, while the leaf levels of soluble sugars in partially defoliated plants declined slightly between the two harvests. Of the stubble and root dry weight components, that of the roots was the most adversely affected by complete defoliation with a reduction by harvest two of approximately 40% of the weight at harvest one.

For moisture regime, the four listed characters were similarly important in the single discriminant function (Table 9.2b). Leaf area had the only negative loading indicating that larger changes in this plant character gave more negative mean scores, and these occurred under moist conditions. Under the dry regime, there were generally greater changes in leaf dry weight and levels of leaf soluble sugars and stubble starch, and these findings were also supported partly by the individual character means (Table 9.2d).

Table 9.2c Defoliation intensity, plant age and grand means for vegetative and carbohydrate characters measured on plants in large bags at harvest one (standard errors in parentheses).

Treatments/ grand mean	leaf number	leaf dry weight (g)	leaf area (cm^2)	root dry weight (g)	stubble dry weight (g)	leaf soluble sugars (%)	leaf starch (%)	stubble soluble sugars (%)	stubble starch (%)	root soluble sugars (%)	root starch (%)
Defoliation intensity (% rLA)											
0	0	0	0	16.03 (4.25)	1.41 (0.40)	0	0	5.78 (0.28)	0.65 (0.21)	2.11 (0.33)	1.01 (0.40)
50	4	0.55 (0.06)	78.2 (6.2)	12.66 (3.26)	1.19 (0.29)	4.86 (0.50)	0.84 (0.29)	5.70 (0.26)	0.35 (0.13)	2.87 (0.28)	0.42 (0.23)
100	8	1.04 (0.17)	115.2 (13.6)	9.20 (2.87)	1.04 (0.31)	5.28 (0.39)	0.85 (0.38)	6.03 (0.37)	0.39 (0.17)	3.15 (0.19)	0.57 (0.17)
Plant age											
young	4 (0.8)	0.32 (0.07)	50.5 (9.6)	3.74 (0.57)	0.44 (0.05)	2.77 (0.52)	0.08 (0.05)	5.20 (0.10)	0.09 (0.02)	2.35 (0.25)	0.11 (0.06)
old	4 (0.8)	0.73 (0.16)	78.4 (15.9)	21.53 (2.68)	1.98 (0.27)	4.00 (0.73)	1.05 (0.30)	6.47 (0.26)	0.83 (0.16)	3.07 (0.20)	1.23 (0.27)
Grand mean	4 (0.6)	0.53 (0.09)	64.5 (9.5)	12.64 (2.02)	1.21 (0.19)	3.38 (0.45)	0.56 (0.17)	5.84 (0.17)	0.46 (0.10)	2.71 (0.17)	0.67 (0.17)

Table 9.2d Defoliation intensity, moisture regime and plant age means for change scores of several vegetative and carbohydrate characters measured on plants in large bags (standard errors in parentheses).

Treatments	leaf area (cm^2)	leaf dry weight (g)	root dry weight (g)	leaf soluble sugars (%)	stubble starch (%)	root starch (%)
Defoliation intensity (% rLA)						
0	235.3 (19.7)	1.61 (0.14)	-6.34 (2.96)	4.22 (0.51)	-0.61 (0.21)	-0.87 (0.42)
50	127.4 (16.6)	1.21 (0.09)	-1.45 (2.15)	-0.31 (0.64)	-0.30 (0.13)	0.16 (0.32)
100	126.3 (30.2)	1.24 (0.23)	2.91 (1.54)	-0.50 (0.56)	-0.34 (0.18)	-0.12 (0.27)
Moisture regime						
dry	153.4 (21.5)	1.46 (0.12)	-2.04 (2.13)	2.23 (0.64)	-0.42 (0.14)	-0.46 (0.29)
moist	172.6 (22.7)	1.25 (0.15)	-1.22 (2.13)	0.05 (0.66)	-0.41 (0.15)	-0.09 (0.29)
Plant age						
young	153.0 (20.5)	1.33 (0.12)	1.60 (0.83)	1.77 (0.59)	-0.05 (0.03)	0.29 (0.15)
old	173.0 (23.6)	1.38 (0.16)	-4.85 (2.68)	0.50 (0.77)	-0.79 (0.16)	-0.84 (0.34)

Discrimination between plant ages was best achieved using changes in leaf area, while slightly less important characters were changes in leaf dry weight and the concentration of stubble starch (Table 9.2b). All three characters were considerably more important in the discriminant function than soluble sugar level changes in leaves. The coefficients and their loadings indicated that younger plants had greater changes in leaf area between the two harvests while older plants had generally larger changes in leaf dry weight and stubble starch. Older plants also had higher stubble starch levels at harvest one (Table 9.2c).

In the multivariate analysis of variance pooled over bag sizes, only the bag size x moisture regime interaction was significant (Table 9.3a). The discriminant function mean scores for this source of variation and the four largest standardised coefficients are presented in Table 9.3b. Change in leaf area was the most important character in the function as evidenced by its high standardised coefficient. Other useful but less important characters were changes in stubble soluble sugars and leaf dry weight, each with negative loadings, and root dry weight with a positive loading. With the exception of stubble soluble sugar level for the moist, large bag treatment, all other means for this character and the other important characters were positive (Table 9.3d). This indicated that there were general increases in leaf and root mass, leaf area and stubble soluble sugar concentrations following defoliation. Increases in all useful characters were therefore preferred but due to the loading differences (Table 9.3b), an agronomically acceptable score was approximately 0.5, as calculated by summing the coefficients. The closest treatment score to this estimate was that for plants growing in large bags under dry conditions. Mean scores for small bagged plants were much more negative under moist than dry conditions while the reverse pattern occurred for plants in large bags (Table 9.3b), and this probably accounted for most of the significance of the interaction.

The time of harvest two for plants in large bags was not affected significantly by treatment (Table 9.4a). However, for defoliation intensity, the differences between the means were of practical interest since they showed that the completely defoliated treatments (0% rLA), which were harvested 22 days after field capacity, were harvested later than the 50% rLA (17.3 days) and 100% rLA (15.7 days) treatments.

TABLE 9.3a Important statistics for the discriminant function for bag size x moisture regime interaction in the pooled multivariate analysis over bag sizes - vegetative and carbohydrate characters.

Discriminant function	Percent of discriminant power	F-statistic	Numerator df	Denominator df	Significance
1	100.00	10.42	10	3	*

Table 9.3b Mean scores for the discriminant function for bag size x moisture regime interaction in the pooled analysis over bag sizes.

Moisture regime	Bag size	
	small	large
dry	2.47	-0.84
moist	-7.50	5.86

four largest standardised coefficients:

1. leaf area (7.72)
2. stubble soluble sugars (-6.77)
3. leaf dry weight (-5.09)
4. root dry weight (4.69)

Table 9.3c Bag size and grand means for vegetative and carbohydrate characters measured on young plants in small and large bags at harvest one (standard errors in parentheses).

Treatments/ grand mean	leaf number	leaf dry weight (g)	leaf area (cm^2)	root dry weight (g)	stubble dry weight (g)	leaf soluble sugars (%)	leaf starch (%)	stubble soluble sugars (%)	stubble starch (%)	root soluble sugars (%)	root starch (%)
Bag size											
small	4 (1.2)	0.27 (0.08)	42.4 (12.8)	2.12 (0.21)	0.70 (0.08)	2.50 (0.80)	0.89 (0.36)	3.95 (0.33)	0.02 (0.01)	3.14 (0.19)	0.01 (0.01)
large	4 (1.2)	0.30 (0.10)	40.2 (12.5)	4.52 (0.68)	0.51 (0.06)	2.30 (0.74)	0.01 (0.01)	5.01 (0.11)	0.11 (0.03)	2.31 (0.30)	0.16 (0.08)
Grand mean	4 (0.8)	0.29 (0.06)	41.3 (8.8)	3.32 (0.43)	0.61 (0.05)	2.40 (0.53)	0.45 (0.20)	4.48 (0.20)	0.06 (0.02)	2.73 (0.20)	0.08 (0.04)

Table 9.3d Bag size x moisture regime interaction means for three vegetative characters and levels of stubble soluble sugars (standard errors in parentheses).

Bag size	Moisture regime	leaf area (cm^2)	leaf dry weight (g)	root dry weight (g)	stubble soluble sugars (%)
small	dry	212.9 (9.7)	1.13 (0.06)	1.85 (0.27)	0.28 (0.46)
small	moist	129.6 (18.9)	1.77 (0.72)	1.02 (0.62)	1.14 (0.47)
large	dry	174.8 (41.5)	1.54 (0.23)	0.88 (1.87)	0.31 (0.72)
large	moist	177.6 (38.4)	1.30 (0.25)	0.20 (0.98)	-0.74 (0.42)

TABLE 9.4a Means for several transpiration parameters and time of harvest two for variously aged defoliated plants growing in large bags under a dry moisture regime.

Plant age	Defoliation intensity (% rLA)	Total transpiration per plant (g)	% available water content	Time of harvest two (days after field capacity)	Transpiration rate per plant (g/day)
young	0	430.3	36.1	20.7	21.3
young	50	485.3	40.8	16.7	29.2
young	100	460.3	38.7	18.7	27.0
mean		458.7	38.5	18.7	25.8
old	0	433.3 b[+]	36.4 b	23.3	18.6 b
old	50	442.3 b	37.1 b	18.0	24.8 b
old	100	608.0 a	51.0 a	12.7	48.2 a
mean		494.6	41.5	18.0	30.5

[+] figures scored by different letters differ at the 5% level of significance.

Table 9.4b Means for several transpiration parameters and time of harvest two for plants growing in two bag sizes under a dry moisture regime.

Bag size	Total transpiration per plant (g)	% available water content	Time of harvest two (days after field capacity)	Transpiration rate per plant (g/day)
small	405.7	48.1 a[+]	9.2 b	44.6 a
large	445.3	37.4 b	19.7 a	24.1 b

[+] figures scored by different letters differ at the 5% level of significance.

In the pooled analysis over bag sizes, plants in the small bags were harvested significantly ($P<0.01$) earlier (9.2 days after field capacity) than those in the large bags (19.7 days) (Table 9.4b). The results for defoliation intensity were also in agreement with those for the analysis of large bag data in showing that completely defoliated plants (15.5 days) were harvested later than the 100% rLA plants (13.3 days). There was no detectable interaction between bag size and defoliation intensity.

9.3.3 TRANSPIRATION LOSS

Differences in substrate moisture contents were created in this study. In the large bags, mean net water loss from plants in the dry regime was 476.6 g which was significantly ($P<0.01$) greater than that lost by plants growing under moist conditions (93.8 g). Analysis of variance involving both bag sizes indicated a similar trend, although there was an interaction between bag size and moisture regime.

Means for transpiration parameters and time of harvest two for plants growing in large bags under a dry moisture regime are shown in Table 9.4a. Significant plant age x defoliation intensity interactions for total transpiration per plant, percentage available water content and average transpiration rate per plant were detected and could be accounted for by the variously defoliated older plants (Table 9.4a). In each instance, the 100% rLA treatment had a higher mean than the 0 and 50% rLA treatments. That is, plants in this treatment had the highest transpiration rate (48 g/day) and loss (608 g), and had used a greater proportion of the available substrate moisture (51%) by first wilting.

An analysis of both bag sizes revealed that there were significant differences between sizes for percentage available water content and transpiration rate per plant, but not for total transpiration loss per plant. Bag size means for these characters and time of harvest two are presented in Table 9.4b. Plants in small bags had a higher transpiration rate per plant and used a greater share of the available substrate moisture than those in large bags. Their mean time of harvest was also significantly earlier than the plants in large bags (Table 9.4b).

Results of the multiple regression analyses, conducted for total transpiration and rate per plant against vegetative leaf characters for 0 and/or 100% rLA plants, are shown in Table 9.5. All other regressions were non-significant indicating that there were no functional relationships between the variables. For total transpiration per plant, all parameter estimates for 0% rLA plants were significantly ($P<0.01$) different from their respective estimates for 100% rLA plants, according to pair-wise t-tests (results not presented). That is, the regression equations were completely different functions. Standardised partial regression coefficients showed that leaf area and dry weight were of equal importance in accounting for the variability in total transpiration of 0% rLA plants, although leaf dry weight had a negative loading. For 100% rLA plants, leaf dry weight was twice as important as leaf area and had a positive loading. In both analyses, leaf number was least important. For transpiration rate of 0% rLA plants, leaf dry weight accounted for most of the variability and high weight resulted in low transpiration rate. Increases in leaf area and number conferred higher rates of transpiration.

All variously defoliated and aged plants in the large bags had similar estimates of total transpiration loss and rate per unit leaf area, leaf number and leaf dry weight. Means and standard errors of the three transpiration loss and rate characters are presented in Table 9.6. In the pooled multivariate analyses of the derived transpiration loss and rate characters, the bag size x defoliation intensity interactions were significant (Table 9.7a). Mean scores on both discriminant functions for the interaction, and all standardised coefficients, are presented in Table 9.7b.

In the analysis of transpiration loss characters, transpiration loss per unit leaf dry weight was the most important character in the single discriminant function and it had a negative loading. This indicated that increasing values of this character resulted in more negative mean scores. Larger values of transpiration loss per unit leaf area and number lead to more positive scores. The trends observed in the mean scores were supported partly by the character interaction means (Table 9.7c). For example, the 0% rLA small bag treatment had the highest mean for transpiration loss per unit leaf dry weight (Table 9.7c) and this was reflected in a relatively high negative mean score (Table 9.7b). The large bag 100% rLA treatment had the

TABLE 9.5 Regression equations and associated statistics for transpiration parameters against leaf characters for 0 and 100% rLA plants.

	Total transpiration per plant for 0% rLA plants (g)			Transpiration rate per plant for 0% rLA plants (g/day)			Total transpiration per plant for 100% rLA plants (g)		
	parameter estimate	standard error	standardised estimate	parameter estimate	standard error	standardised estimate	parameter estimate	standard error	standardised estimate
intercept	882.97	127.74	0	12.49	19.14	0	177.31	124.56	0
leaf area (cm^3)	1.31	0.32	1.35	0.09	0.05	0.45	-0.72	0.38	-0.37
leaf dry weight (g)	-141.16	32.76	-1.36	-25.15	4.91	-1.13	197.69	40.72	0.88
leaf number	-10.29	2.61	-0.89	0.62	0.39	0.25	1.56	1.45	0.22
R-square	0.82			0.91			0.85		
F-test	7.78*			17.48**			9.11*		
standard error	29.27			4.39			51.80		

TABLE 9.6 Means for three transpiration loss and rate characters for plants in large bags (standard errors in parentheses).

Character	Mean
transpiration loss per leaf area (10^{-1} mg m^{-2})	2.4 (0.8)
transpiration loss per leaf number (10^{4} mg $leaf^{-1}$)	1.4 (0.8)
transpiration loss per leaf dry weight (10^{5} mg g^{-1})	2.5 (0.8)
transpiration rate per leaf area (ug cm^{-2} s^{-1})	3.3 (1.4)
transpiration rate per leaf number (10^{-2} mg $leaf^{-1}$ s^{-1})	1.9 (1.4)
transpiration rate per leaf dry weight (10^{-1} mg g^{-1} s^{-1})	3.4 (1.3)

TABLE 9.7a Important statistics for the discriminant functions for bag size x defoliation intensity interaction from the two pooled multivariate analyses over bag sizes involving transpiration loss and transpiration rate characters.

Characters	Discriminant function	Percent of discriminant Power	F-statistic	Numerator df	Denominator df	Significance
transpiration loss	1	100	57.76	3	2	*
transpiration rate	1	100	44.93	3	2	*

Table 9.7b Mean scores for the discriminant functions for bag size x defoliation intensity interaction in the pooled analyses.

Bag size	Transpiration loss parameters		Transpiration rate parameters	
	Defoliation intensity (% rLA)		Defoliation intensity (% rLA)	
	0	100	0	100
small	-33.55	-5.38	30.28	-4.01
large	-5.36	44.30	-5.47	-20.81
	standardised coefficients:		standardised coefficients:	
	1. per unit leaf area (11.05)		1. per unit leaf area (0.92)	
	2. per unit leaf number (15.47)		2. per unit leaf number (-12.25)	
	3. per unit leaf dry weight (-18.58)		3. per unit leaf dry weight (23.24)	

Table 9.7c Bag size x defoliation intensity means for three transpiration loss and rate characters (standard errors in parentheses).

Bag size	Defoliation intensity (% rLA)	TA + (10^{-1} mg m^{-2})	TF (10^{4} mg $leaf^{-1}$)	TW (10^{5} mg g^{-1})	RA (ug cm^{-2} s^{-1})	RF (10^{-2} mg $leaf^{-1}$ s^{-1})	RW (10^{-1} mg g^{-1} s^{-1})
small	0	1.9 (0.1)	0.8 (0.1)	4.0 (0.5)	4.3 (0.2)	1.7 (0.0)	8.9 (0.7)
small	100	1.3 (0.1)	0.8 (0.0)	2.2 (0.0)	3.8 (0.3)	2.3 (0.1)	6.3 (0.1)
large	0	1.8 (0.4)	0.8 (0.1)	2.6 (0.5)	2.2 (0.6)	0.9 (0.1)	3.0 (0.8)
large	100	2.7 (0.4)	1.5 (0.0)	2.4 (0.3)	3.9 (1.5)	1.9 (0.3)	3.4 (1.1)

+ TA, TF and TW = transpiration loss per leaf area, leaf number and leaf dry weight, respectively.
RA, RF and RW = transpiration rate per leaf area, leaf number and leaf dry weight, respectively.

highest means for each of the other two characters (Table 9.7c) and it also had the most positive mean score (Table 9.7b).

For the transpiration rate characters, per unit leaf dry weight was again a very useful basis for comparison since transpiration rate expressed as such was the most important character in the discriminant function. It was also approximately twice as important as rate per unit leaf number (Table 9.7b) while rate per unit leaf area was relatively unimportant. The results indicated that transpiration rate expressed on a per unit leaf area basis was relatively invariant between the treatments examined and this suggested that such a character may be approximately constant for a wide range of treatments. The positive loading for rate per unit leaf dry weight indicated that larger values for this character resulted in more positive mean scores. The 0% rLA small bag treatment had the most positive mean score (Table 9.7b) and it also had the highest transpiration rate on a leaf dry weight basis (Table 9.7c).

9.4 DISCUSSION

Different moisture regimes were created readily by the withholding of moisture supply to half of the plants while the remainder were well watered (Section 9.3.3.). The technique was therefore successful in providing distinct moisture regimes under which to investigate the regrowth of variously treated sheep's burnet plants. Similar methodology has been employed in water deficit studies on a wide range of other species (Boyer, 1970a, b; Chu and McPherson, 1977; Wolf and Parrish, 1982; Kumar *et al.*, 1984; Sambo and Aston, 1985; Ludlow *et al.*, 1985), but rarely on closely related species (Taylor *et al.*, 1982).

The similar leaf extension characteristics of the variously aged and defoliated sheep's burnet plants in large bags, plus their morphological insensitivity to moisture deficits, were inconsistent generally with the findings for other species. Leaf expansion, which is partly a function of cell extension, is acknowledged widely as one of the most sensitive plant characters to desiccation (Wardlaw, 1969; Boyer, 1970a; Ludlow and Ng, 1976; Turner and Begg, 1978, 1981; Krieg, 1983; Dale, 1988) and the current results were therefore unexpected. A possible explanation for the findings

is that the first three leaves measured for leaf extension after defoliation had all attained close to their maximum length before the water potential of the cells of each new leaf was sufficiently low to adversely affect cell division and/or extension. It is suggested that the monitoring of leaves produced later, such as the fourth or fifth leaves produced after defoliation, may have demonstrated differences in leaf extension between the moisture treatments. It may also be argued that the rate of substrate dry-down permitted some form of plant adjustment, as discussed below, which may have masked any real treatment effects. Quicker rates of dry-down might have revealed more differences between the moisture treatments but they would have been unrealistic, practically.

Changes between harvests in nonstructural carbohydrates featured prominently in distinguishing between various treatments and at least some of the changes were probably associated with the breakdown and redistribution of reserves for foliar regrowth, as has been shown in other species (Ward and Blaser, 1961; Cooper and Watson, 1968; Harris, 1978; Walton, 1983). The general decline in the concentration of starch in the stubble was particularly noteworthy in this regard, as was the reduction in the starch levels of the roots of older plants (Table 9.2d). The results suggested that, at least in the early stages of regrowth, stubble starch was an important energy source for regrowth, especially in the older plants. Presumably as the leaf area increased, current photosynthate became a more important energy source for continued regrowth (Blaser *et al.*, 1966).

As well as supplying energy for foliar regrowth, the nonstructural carbohydrates may have also been involved in osmotic adjustment (Turner and Begg, 1978; Hanson and Hitz, 1982; Johnson *et al.*, 1984; Dale, 1988). This could account partly for the higher concentrations of soluble sugars in the leaves of plants growing under dry conditions compared with those of plants growing with plentiful moisture (Table 9.2d). Relatively high levels of carbohydrates were also found in ryegrass tissues growing under a dry moisture regime (Barker, 1983). The source of the soluble sugars in the current study was uncertain, but the results indicated that the breakdown of polysaccharides to free sugars, as has been suggested for some species (Munns and Pearson, 1974; Stewart, 1971; Turner *et al.*, 1978), was probably slight. A more

likely explanation could be increased accumulation of new photosynthate in the leaves of drying plants and/or reduced translocation to other plant parts. High concentrations of soluble sugars under dry conditions appear advantageous, since they probably assist in maintaining turgor pressure and hence turgor dependent processes such as leaf expansion, to a lower water potential than possible in unadjusted plants (Hanson and Hitz, 1982). The indication of osmotic adjustment in this study may account partly for sheep's burnet's widely acknowledged tolerance to, and ability for regrowth under, low moisture deficits (McTaggart, 1935; Anon, 1951; Salmeron, 1966; Le Houerou, 1979; Sheppard and Wills, 1985). Furthermore, if sheep's burnet has genetic variability for this trait, as reported for other species (Boyer, 1983), then potential improvement of osmotic adjustment would likely prolong and/or enhance leaf area, and perhaps herbage mass, under dry conditions. Osmotic adjustment may also be another reason for the lack of differences in leaf extension characteristics between plants growing under moist and dry conditions in large bags (Section 9.3.1). The possible involvement of osmotic adjustment in plants growing in small bags was less certain.

The much lower root mass at harvest two compared with at harvest one for completely defoliated (0% rLA) plants in large bags (Section 9.3.2), was in agreement with the findings for other species, where intense defoliation reduced root growth markedly (Humphreys, 1966; Evans, 1971). The results indicated that in addition to probable complete cessation of root elongation (Evans, 1973), there was also considerable root death and subsequent decay. The undesirable consequences of these effects, particularly under dryland conditions where sheep's burnet is best suited (Sheppard and Wills, 1985), may include reduced moisture and nutrient uptake (Jantii and Heinonen, 1957; Oswalt *et al.*, 1959), and less effective soil stabilisation.

There was some indication that, in absolute terms, root growth was depressed (and/or root death and decomposition were increased) more under the dry compared with moist regimes (Table 9.2d), but the differences were non-significant. Reduction in root mass during desiccation was also reported by Chu (1979) in his studies on grasses. However, such a comparison may be of doubtful value, particularly in view of the different root system of the grasses (fibrous) compared with that of sheep's

burnet (taproot). The current findings were at variance with those found for most of a range of cool-season forages (Bennett and Doss, 1960) where root masses at low soil moisture levels were heavier than those at higher soil moisture levels. Apart from the difference in species, reasons for discrepancies between these findings and those of the present investigation could be different aged plants, variable environmental conditions, and inconsistencies in the determination of root mass.

A possible advantage of complete defoliation suggested by the results was a delay in the time of wilting of the regrowth foliage compared with that of partially defoliated plants (Section 9.3.3). Under dry conditions, where the content of available soil moisture may be low, complete defoliation could provide a means of conserving soil moisture due to the reduced exposed leaf surface and hence transpiration loss. Similar suggestions have been made for other species (Barker and Chu, 1985; Toft *et al.*, 1987). However, complete defoliation could only be practised occasionally since frequent such treatment of other species, and probably sheep's burnet, results in considerable root death (Beard and Daniel, 1965; Evans, 1971), reduced carbohydrate status, and ultimately plant death. Removal of transpiring leaves in other species has also improved the water status of the remaining plant parts (Christ, 1978; Cutler *et al.*, 1980; Wolf and Parrish, 1982), resulting in accelerated cell expansion and hence foliar regrowth. It is suggested that a similar situation exists for sheep's burnet but further investigations are required to verify this. Decreasing leaf area reduces water and probably nutrient uptake (Russell and Shorrocks, 1959), but the suitability of sheep's burnet for low to moderately fertile sites (Sewell, 1952; Scott *et al.*, 1985) suggests that this may not be as critical as for higher fertility demanding species.

Plants growing in large bags wilted (harvest two) approximately twice as late as those growing in small bags, but they transpired similar quantities of water (Table 9.4b). This resulted in a two fold difference between the bag sizes in the estimates of average transpiration rate per plant, and the lower rate for plants in large bags could account partly for the osmotic adjustment suggested previously. A greater proportion of the available water in the small bags was transpired compared with that in the large bags (Table 9.4b) and this may have been due to variability in the extent

of root exploration in the different volumes of substrate. Although no measurements were made in this regard, root distribution in the small bags at the two harvests appeared more even throughout the medium than in the large bags. There was also frequently more root at the medium/bag interface in the small bags and this may have been an additional factor contributing to quicker moisture uptake. Temperature of the bag contents may have also influenced moisture movement in plants in the two bag sizes differently, but the extent of its involvement was unknown.

Despite the variation in total transpiration and rate per plant observed for some of the treatments in large bags, there were no differences between treatments when these parameters were expressed on a per unit leaf area, leaf number and leaf dry weight basis (Section 9.3.3). This suggested that these derived parameters may be relatively constant over a range of plant ages and defoliation intensities for a given set of environmental conditions. Hence, there should be a trade off in the practical situation between water use and dry weight gains. With relatively large leaf areas, such as undefoliated plants, there could be desirable increases in dry matter but this would also be accompanied by enhanced water use. Conversely, lower leaf areas would reduce transpiration losses but they would also restrict increases in herbage mass. These issues would be of increasing relevance in more drought prone environments.

The results supported partly the view that moisture loss was controlled mainly by environmental (solar) factors (Penman, 1956), rather than the nature and extent of the foliage. This was probably true for most plants by harvest two when they had an almost complete ground cover. However, during regrowth following treatment imposition, when leaf area was relatively low, the different canopy areas probably also had an influence on the degree of evapotranspiration (ET) from the foliar/medium surfaces, as has been reported or reviewed for crops and pasture (Humphreys, 1966; Ritchie and Burnett, 1971; Barker and Chu, 1985). The relative importance of the foliar and medium surfaces in contributing to ET in the early stages of regrowth in this study was unknown.

A factor which made interpretations difficult was the age structure of the leaves in the various treatments. In the completely defoliated plants, all leaves for which transpiration parameters were calculated, were of similar age since they were all produced between the two harvests. The leaf components of the partially defoliated treatments, however, consisted additionally of four or eight fully expanded mature leaves which remained after the treatments were imposed (Section 9.2.2). The relative transpiration efficiencies of the two leaf categories were unknown but they were probably different due to likely differences in the stomatal responses of the variously aged leaves (Jordan *et al.*, 1975; Squire and Black, 1981).

The estimated transpiration rates per unit leaf area (Tables 9.6 and 9.7c) were similar to those estimated for garden burnet by Antipov (1977), which were equivalent to 2.6-3.0 $\mu g\ cm^{-2}\ s^{-1}$ (converted from units of $mg\ cm^{-2}\ hr^{-1}$). The results indicated that two quite different estimation procedures could provide similar estimates. Transpiration rate here was calculated as an average over a period of up to 20-25 days whereas, in the other study, measurements were determined daily and there was no mention of moisture deficits. A likely disadvantage of the present method was the inclusion in the estimation procedure of a period before first wilting in which transpiration rate was probably negligible, but the length of this period was unknown. Estimation of transpiration rates per unit leaf area and weight, at intermediate stages between the two harvests, could not be undertaken because corresponding leaf area and weight data were unavailable.

This investigation was conducted under controlled environmental conditions and there has been increasing evidence recently that plants grown under such conditions respond morphologically and physiologically to moisture deficits at higher leaf water potentials (milder moisture deficits) than field grown material (Jordan and Ritchie, 1971; Begg and Turner, 1976; Turner and Begg, 1978; Ritchie, 1981; Ludlow *et al.*, 1985). The main reasons for these differences are variations in radiation level and the extent of root constriction/exploration in the growing medium (Turner and Begg, 1978). Conducting similar studies on sheep's burnet in the field would provide complementary information and enable useful comparisons to be made between the responses of glasshouse and field grown plants to dry conditions.

CHAPTER 10 : AN APPRAISAL OF THE MULTIVARIATE PROCEDURES

10.1 INTRODUCTION

Multivariate analysis of variance and multiple discriminant functions were used to discriminate amongst plant responses to treatments in this thesis. The main reason for their use was that most experiments involved either multiple measurements on the same plots or plants, or similar measurements on several occasions. Accordingly, it was assumed that covariances or correlations existed between two or more of the characters examined in any experiment and a worthwhile analysis should therefore account for them. The multivariate procedures used achieve this objective (Cooley and Lohnes, 1971; Morrison, 1976; Mardia *et al.*, 1979; Lindeman *et al.*, 1980; Harris, 1985), which differs fundamentally from univariate analysis where covariances are not accounted for. Furthermore, when covariances exist, the univariate analyses of variance are biased (Cooley and Lohnes, 1971; Kendall,1975). That is, tests on the individual means for a single character are not independent from similar tests on other characters and therefore such tests as t-tests cannot logically be applied (Kendall, 1975). A real practical concern in these circumstances is also that each response character which might be analysed univariately is in fact an unknown mixture of all the characters with which it is correlated, which complicates interpretation.

In order to determine the magnitude and direction of the covariances (correlations) involved in this thesis research, estimates were obtained for a sample of data sets from several experiments. Some of the similarities and differences in interpretation between the multivariate and equivalent univariate analyses were also ascertained from these data sets. Several problems were encountered in the use of the multivariate analyses, such as insufficient error degrees of freedom to undertake certain hypothesis tests (for example, Sections 4.2.5.2 and 7.2.4.3), and these matters are discussed. The multivariate analyses are extended for some data sets.

10.2 EXISTENCE OF CORRELATIONS

Simple (total) correlations between characters taken from earlier thesis results are presented in Tables 10.1a, b, c, d. In most instances, there was an association between at least some of the characters, with estimates being positive or negative. Correlations were consistently positive and high for the character sets in Tables 10.1a and c, while correlation estimates in Table 10.1b and d were more variable with respect to sign and magnitude. The general presence of correlations in these data sets indicated the need to use multivariate analysis procedures which take these into account. As well, the correlations indicated that significance determined from univariate analysis of variance and tests for mean separation should be interpreted with considerable caution, for reasons mentioned earlier (Section 10.1).

10.3 RANDOM VERSUS FIXED EFFECTS MODELS

10.3.1 LITERATURE REVIEW

In any experimental design, each effect (usually a treatment level) in the linear model describing the response observations may be regarded as fixed or random, depending on the population from which the sample was assumed to have been drawn (Eisenhart, 1947; Le Clerg *et al.*, 1962; Searle, 1971; Steel and Torrie, 1980). Treatment levels are regarded as fixed effects in the model when the entire population about which inferences are to be made is in the sample or experiment. When all effects are fixed, except the random error component, the corresponding model is called a fixed effects model or the Model 1 of Eisenhart (1947). In repeated experimentation, it is conceived that exactly the same treatments are being investigated and applied.

An alternative assumption about the effects in the linear model is that they are a random sample from a larger population to which the researcher wishes to make inferences based on the results of the experiment. Linear models containing all random effects are designated random effects models, or following Eisenhart (1947), Model II. The population from which samples have been drawn has been regarded

TABLE 10.1a Simple correlations between four vegetative characters measured on seedlings at Riverside (related to Table 4.8).

	X1	X2	X3	X4
X1	1.00	0.77	0.93	0.80
X2	0.77	1.00	0.82	0.82
X3	0.93	0.82	1.00	0.86
X4	0.80	0.82	0.86	1.00

X1 = foliar length (log)
X2 = root length (mm)
X3 = foliar dry weight (log)
X4 = root dry weight (log)

Table 10.1b Simple correlations between six vegetative characters measured on establishing swards at Riverside (related to Table 4.12).

	X1	X2	X3	X4	X5	X6
X1	1.00	0.06	-0.13	-0.41	-0.02	-0.53
X2	0.06	1.00	0.09	0.23	-0.23	0.42
X3	-0.13	0.09	1.00	-0.23	0.09	0.15
X4	-0.41	0.23	-0.23	1.00	0.09	0.50
X5	-0.02	-0.23	0.09	0.09	1.00	0.10
X6	-0.53	0.42	0.15	0.50	0.10	1.00

X1-X2 = herbage mass and weed content, respectively on 17/12/1985
X3-X4 = herbage mass and weed content, respectively on 5/2/1986
X5-X6 = herbage mass and weed content, respectively on 23/4/1986

Table 10.1c Simple correlations between six vegetative characters measured on sheep's burnet in a glasshouse (related to Table 6.7).

	X1	X2	X3	X4	X5	X6
X1	1.00	0.90	0.85	0.84	0.78	0.98
X2	0.90	1.00	0.81	0.84	0.81	0.92
X3	0.85	0.81	1.00	0.91	0.91	0.87
X4	0.84	0.84	0.91	1.00	0.89	0.85
X5	0.78	0.81	0.91	0.89	1.00	0.82
X6	0.98	0.92	0.87	0.85	0.82	1.00

X1 = shoot length (log)
X2 = root length (log)
X3 = leaf dry weight (log)
X4 = stem dry weight (log)
X5 = root dry weight (log)
X6 = leaf area (log)

Table 10.1d Simple correlations between nine logistic function parameters estimated for variously defoliated plants of sheep's burnet in a glasshouse (related to Table 8.1).

	X1	X2	X3	X4	X5	X6	X7	X8	X9
X1	1.00	0.54	-0.53	O.86	0.74	-0.75	0.90	-0.28	-0.33
X2	0.54	1.00	-0.84	0.53	0.45	-0.48	0.41	-0.26	-0.17
X3	-0.53	-0.84	1.00	-0.48	-0.29	0.44	-0.41	0.25	0.24
X4	0.86	0.53	-0.48	1.00	0.68	-0.79	0.85	-0.07	-0.43
X5	0.74	0.45	-0.29	0.68	1.00	-0.82	0.58	-0.20	-0.19
X6	-0.75	-0.48	0.44	-0.79	-0.82	1.00	-0.59	0.03	0.49
X7	0.90	0.41	-0.41	0.85	0.58	-0.59	1.00	-0.06	-0.45
X8	-0.28	-0.26	0.25	-0.07	-0.20	0.03	-0.06	1.00	-0.70
X9	-0.33	-0.17	0.24	-0.43	-0.19	0.49	-0.45	-0.70	1.00

X1 = β_0 - leaf 1
X2 = β_1 - leaf 1
X3 = β_2 - leaf 1
X4 = β_0 - leaf 2
X5 = β_1 - leaf 2
X6 = β_2 - leaf 2
X7 = β_0 - leaf 3
X8 = β_1 - leaf 3
X9 = β_2 - leaf 3

traditionally as infinitely large but actually the conceptual populations for random effects models can be of three kinds, depending on their size, namely infinite, finite, or finite but so large as to be deemed infinite (Mielke and McHugh, 1965; Searle and Fawcett, 1970; Searle, 1971). A special case of a finite population is when the effects occurring in the data comprise the complete population of effects, this frequently being used as a convenient representation of fixed effects (Searle, 1971). Hence, a fixed effects model may be regarded as one limit of the random effects model.

The expected values of mean squares in finite population, random effects models are not the same as those with infinite populations but in both cases the expected values are linear functions of the variance components (Searle & Fawcett, 1970). The differences between the two respective expected mean squares arise from using the variance-covariance matrix for the finite population in place of that for the infinite population and Searle & Fawcett (1970) have discussed the consequences of this for nested and crossed classifications, and mixed models. An important indication from their work is that regardless of whether the population of effects from which samples are assumed to have been drawn is infinite or finite and small, expectations of the mean squares, and hence appropriate significance tests (F-tests), will differ from those where a fixed effects model is assumed.

The designation of effects as fixed or random is usually not straightforward (Eisenhart, 1947; Le Clerg *et al.*, 1962; Searle, 1971) and "many situations arise where effects can be judged fixed in one context and random in another" (Searle, 1971). Hence, it may often be inappropriate to judge the designation of an effect in a model as being right or wrong, but rather consider the particular designation as being more or less appropriate than the alternative. There is probably justification for considerable debate on the designations of effects / variables in many experiments and some of the philosophical issues have been discussed by Eisenhart (1947), Searle (1971) and Steel and Torrie (1980). It is noteworthy that in many research situations, linear models consist of both random and fixed effects and these are referred to appropriately as mixed effects models (Le Clerg *et al.*, 1962; Searle, 1971).

In the analysis of variance, the specification of effects as fixed or random in the appropriate linear model is important because it determines the variance components of the model and hence the expectations of the mean squares (Searle, 1971; Steel and Torrie, 1980). These in turn determine the appropriate mean squares to be involved in significance tests (F-tests) for the several sources of variation in the model (Crump, 1951). Each test involves a ratio of two expected mean squares which differ in only the component of interest. In the case of experimental designs such as completely random and randomised complete blocks, and where the levels of only one factor are investigated, the appropriate mean squares involved in the F-tests for all sources of variation are the same regardless of the model assumed (Le Clerg *et al.*, 1962; Steel and Torrie, 1980).

However, in more complex experiments where two or more factors are involved, the mean square ratios in the F-tests vary depending on the model (Steel and Torrie, 1980). In fixed effects models, all sources of variation are always tested against the residual mean square (Eisenhart, 1947) while in models assuming mixed and random effects, F-tests frequently involve sources of variation tested against interactions (Le Clerg *et al.*, 1962). Since interaction mean squares are usually larger, and their associated degrees of freedom smaller than those of the residual, these tests are more conservative than their counterparts in fixed effects models (that is, they demand a higher level of variability before accepting the reality of an effect).

An example of the expectations of the mean squares obtained when assuming fixed and random effects models, and the ratios of mean squares involved in F-tests for all sources of variation in the latter model, are presented in Table 10.2. This table is adapted from one for a factorial experiment consisting of three factors of unknown nature, labelled A, B, and C (Steel and Torrie, 1980). Although the testing of interactions in the random model is relatively straightforward in the example given (Table 10.2), it can be seen that appropriate tests for main effects are more complicated and involve a combination of two mean squares in the numerator and denominator of the F-ratio. An approximation by Satterthwaite (1946) is frequently used to estimate degrees of freedom for these tests, and to estimate approximately the probabilities of these ratios.

TABLE 10.2 *Expectations of mean squares when assuming fixed and random effects models for a three factor experiment, together with mean square ratios involved for a random model (adapted from Steel and Torrie (1980)).*

Source of variation	df	Expected value of mean square		Mean square number	F-Test (random)
		Model I (fixed)	Model II (random)		
Blocks	r - 1	$\sigma^2 + abc\,\Sigma\,\rho^2/(r-1)$	$\sigma^2 + abc\sigma^2_{\rho}$	1	1/9
A	a - 1	$\sigma^2 + rbc\,\Sigma\,\alpha^2/(a-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma} + rc\sigma^2_{\alpha\beta} + rb\sigma^2_{\alpha\gamma} + rbc\sigma^2_{\alpha}$	2	2+8/5+6
B	b - 1	$\sigma^2 + rac\,\Sigma\,\beta^2/b-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma} + rc\sigma^2_{\alpha\beta} + ra\sigma^2_{\beta\gamma} + rac\sigma^2_{\beta}$	3	3+8/5+7
C	c - 1	$\sigma^2 + rab\,\Sigma\,\gamma^2/(c-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma} + rb\sigma^2_{\alpha\gamma} + ra\sigma^2_{\beta\gamma} + rab\sigma^2_{\gamma}$	4	4+8/6+7
AB	(a - 1)(b - 1)	$\sigma^2 + rc\,\Sigma\,(\alpha\beta)^2/(a-1)\,(b-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma} + rc\sigma^2_{\alpha\beta}$	5	5/8
AC	(a - 1)(c - 1)	$\sigma^2 + rb\,\Sigma\,(\alpha\gamma)^2/(a-1)\,(c-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma} + rb\sigma^2_{\alpha\gamma}$	6	6/8
BC	(b - 1)(c - 1)	$\sigma^2 + ra\,\Sigma\,(\beta\gamma)^2/(b-1)\,(c-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma} + ra\sigma^2_{\beta\gamma}$	7	7/8
ABC	(a - 1)(b - 1)(c - 1)	$\sigma^2 + r\,\Sigma\,(\alpha\beta\gamma)^2/(a-1)\,(b-1)\,(c-1)$	$\sigma^2 + r\sigma^2_{\alpha\beta\gamma}$	8	8/9
Error	(r - 1)(abc - 1)	σ^2	σ^2	9	

10.3.2 THESIS CONSIDERATIONS

Throughout this thesis it was assumed that expectations of the mean squares for all sources of variation in any experiment consisted of linear combinations of variance components. That is, random effects models (Le Clerg *et al.*, 1962; Searle, 1971) were deemed most appropriate in view of many of the research objectives. It could be argued that some treatment levels were random samples of population effects which were finite and small, but the resulting expectations of the mean squares still consist of linear combinations of variance components, and hence result in F-tests different from those when asssuming a fixed effects model (Section 10.3.1.; Searle and Fawcett, 1970). Examples of some populations assumed to be sampled in several of the experiments are now defined.

In the field trials of Chapter 4, the species sheep's burnet, birdsfoot trefoil and lucerne were regarded as random samples of selected species suitable for dryland conditions in the lower North Island of New Zealand. At a lower level, the accession of sheep's burnet studied was a random sample of the commercially available accessions of the species in New Zealand, "Granger" birdsfoot trefoil was a random sample of locally available cultivars, and the same was assumed for "Rere" lucerne in relation to locally available lucerne cultivars. Although it could be assumed that the species were fixed effects, there was justification for assuming that the outcome of the planned seedling proportions was indeed random. This was shown by the proportions of sheep's burnet and each legume at both Riverside and Flock House very often being different from those planned, and also the mixture proportions varied between the two sites (Section 4.3.2). That is, there were different treatments at each site as a result of random variation, arising mainly from environmental differences.

The three seed sizes examined in Chapters 6 and 7 were regarded as being a random sample of the arbitrary ranges of seed size (and hence seed weight) of sheep's burnet obtained from using sieves. It was important because of the present experimental objectives that seed separation was conducted into several categories to obtain a

range of seed sizes. However, it was not assumed that for other seedlots of sheep's burnet that seed had to be greater than 2.80 mm before it could be termed "large". In fact because of the variability that occurs in seed size in many species, including sheep's burnet (Chapter 6), it is possible for some seedlots that no seed would be larger than 2.80 mm. Hence, it was expected that there would be sample to sample differences in the effects for seed size in addition to experimental variation if the experiment was repeated endlessly, because of the involvement of the range of seedlots of sheep's burnet and sieve sizes available.

In the defoliation experiments described in Chapters 8 and 9, the defoliation intensities were regarded as a random sample of those intensities selected within the more severe end of the complete range of defoliation intensities which sheep's burnet can experience. The actual residual leaf area (rLA) treatments, for example 25% and 75%rLA, were not as important as the more general terms of "heavy" and "light" defoliation, respectively. That is, 25 and 75%rLA were regarded as random samples of these two broad levels of defoliation intensity and ultimately the experimental results will be used by the writer and others to discuss the effects of heavy versus light defoliation rather than 25%rLA versus 75%rLA. A fixed effects model would assume that these two treatments and the others investigated were the only ones of interest, which was not the main focus of the studies. Furthermore, such a model would also assume that the treatments would continue to be applied exactly, without error, in repeated experimentation.

The reasonable assumption of random effects models being most appropriate in this thesis research enabled inferences to be made about larger populations than the various exact treatments examined (Searle, 1971), which is usually desired in agronomic research. For example, the finding that sheep's burnet had greater early leaf area than 'Granger' birdsfoot trefoil (Section 4.3.3.) is a valid and practically useful general statement when assuming a random effects model. If the same experiment was repeated infinitely, there would undoubtedly be different samplings of the genetic variation in the germplasms of the single accession of sheep's burnet and of 'Granger' birdsfoot trefoil. However, it is desirable to refer to sheep's burnet and 'Granger' in making comparisons rather than to specific genetic samples of the

material and therefore an assumption of random effects is appropriate. That is, inferences are made about at least the complete germplasms of the single accession of sheep's burnet and of 'Granger' birdsfoot trefoil based on the results in the experiment involving a sample of the respective germplasms. In contrast, a fixed effects model would assume that the specific genetic samples of each accession/cultivar examined would be investigated in the same experiment repeated infinitely which would be impossible due to genetic variation and sampling theory (Allard, 1960; Mather and Jinks, 1971; Steel and Torrie, 1980).

A further advantage of the random effects assumption was that it introduced conservatism into declaring significant sources of variation, compared with assuming a fixed effects model (Section 10.3.1). A corollary of this is that significant sources of variation when assuming a random effects model would also be significant in a fixed effects model. However, the reverse condition is not necessarily true. An equally important issue is that if sources of variation were not significant when assuming a fixed effects model, then the same result would occur when all effects were assumed random.

The multivariate analysis of variance (Section 2.4.3.1) was one of the main multivariate techniques used to analyse the data in this thesis. However, there was frequently a problem in conducting the appropriate multivariate tests when assuming a random effects model, due to there being insufficient error degrees of freedom. Examples were described in Sections 4.2.5.2, 6.2.4.1, 7.2.4.3, 8.2.4.1 and 9.2.4.1.

The printout for multivariate analysis of variance (MANOVA) from the SAS (1982) programme, which was used to analyse all data in this thesis, includes four multivariate test statistics. These are: (1) Wilks' criterion; (2) Pillai's trace; (3) Hotelling-Lawley trace; and (4) Roy's maximum root (SAS, 1982). All tests are output, the user being unable to request only certain tests. The tests are related to the characteristic roots and vectors of the matrix $\mathbf{E}^{-1}\mathbf{H}$, and all "are commonly used" with no one test being demonstrated to be universally superior or inferior (SAS, 1982). From experience with using the SAS (1982) programme, it was found that if one multivariate test could not be performed, then no tests were undertaken by the

programme. The present problems arose from an inability to conduct the tests of Wilks' criterion, Pillai's trace and Hotelling-Lawley trace due to the appropriate equations to estimate the error degrees of freedom calculating zero or negative values. Two examples from the thesis are used to illustrate this.

In the univariate analyses of variance of t_{10}, t_{50} and t_{90} for two genotypes and three seed sizes of sheep's burnet (Section 6.2.4.1) and in similar analyses for ten vegetative and carbohydrate characters in previously shaded and unshaded sheep's burnet (Section 8.2.4.1), the sources of variation, degrees of freedom, and mean square ratios when assuming random effects models are shown in Table 10.3. Also presented is appropriate multivariate information which is required to estimate the error (denominator) degrees of freedom for tests of Wilks' criterion and Pillai's and Hotelling-Lawley traces. Table 10.3 shows that using these procedures the estimates of error degrees of freedom for the three emergence characters (a) were approximately zero or zero, and for the ten characters (b) estimates were all negative. Hence, in both cases the formulae to estimate error degrees of freedom did not give meaningful results.

These findings indicate that the various multivariate tests may not be sufficiently robust or flexible to handle situations where there are few levels of a factor, for example two. However, this should not be assumed to apply universally since the formulae for the tests (Cooley and Lohnes, 1971; SAS, 1982) indicate that estimates of degrees of freedom depend on such features as number of variables, number of factors and levels within each factor, and amount of replication. Hence, by adjusting other features, it may be possible to conduct tests for a factor with few levels.

Since the present experiments were conducted without prior knowledge of the above issues, three options were available when there were insufficient error degrees of freedom, namely:

1) omission of multivariate analyses of variance (MANOVA) in any form and concentrate instead on univariate analyses of variance where there are no similar problems in assuming random effects models (Searle, 1971; Steel and Torrie, 1980);

a) Three emergence characters (Section 6.2.4.1)

Source of variation	DF	Mean square number	Mean square ratio (random)
Block	3	1	1/5
Genotype	1	2	2/4
Seed size	2	3	3/4
Genotype x seed size	2	4	4/5
Residual	15	5	
TOTAL	23		

Some multivariate information for testing genotype:

P = rank of (**H** + **E**) = 3
Q = hypothesis df = 1
NE = df of **E** = 2
S = min (P,Q) = 1
M = 0.5 (ABS (P-Q) - 1) = 0.5
N = 0.5 (NE - P) = -0.5
Calculation of error degrees of freedom:
Wilks' criterion
F exact = -2
F approximation = 0.06
Pillai's trace = S(2N+S) = 0
Hotelling-Lawley trace = 2S * N - S + 2 = 0

b) Ten vegetative and carbohydrate characters (Section 8.2.4.1)

Source of variation	DF	Mean square number	Mean square ratio (random)
Block	3	1	1/5
Shade	1	2	2/4
Intensity	2	3	3/4
Intensity x shade	2	4	4/5
Residual	15	5	
TOTAL	23		

Some multivariate information for testing genotype:

P = rank of (**H** + **E**) = 10
Q = hypothesis df = 1
NE = df of **E** = 2
S = min (P,Q) = 1
M = 0.5 (ABS (P-Q) - 1) = 4
N = 0.5 (NE - P) = -4
Calculation of error degrees of freedom:
Wilks' criterion
F exact = -16
F approximation = -7
Pillai's trace = S(2N+S)= - 7
Hotelling-Lawley trace = 2S * N - S + 2 = -7

2) conduct MANOVA's on the condition that most sources of variation would need to be tested against the residual mean square, that is be forced to adopt mixed or fixed effects models; or

3) omit both MANOVA, because of the inability to assume a random effects model, and univariate analyses of variance, because of their bias when analysing correlated characters (Cooley and Lohnes, 1971; Kendall, 1975). That is, present just character means and their standard errors without further analyses. These statistics are valid and unique and not altered by any assumptions.

The third option was the least desirable because of the inability to conduct statistical tests and estimate confidence intervals to aid interpretation of the experimental data. Since correlations between characters existed in numerous data sets of the present study (Section 10.2), multivariate analyses of variance were essential regardless of the model assumed and therefore the second option was adopted.

The consequences of forcing a fixed effects model were examined for the two examples (Sections 6.2.4.1 and 8.2.4.1) mentioned previously. The sources of variation tested for significance in the MANOVA were the same as those presented in Table 10.3. For the three emergence characters for sheep's burnet, the MANOVA detected no significant sources of variation (Section 6.3.1.) and for the reasons discussed earlier in this section, the assumption of a random effects model would likely have given the same significance test results and hence conclusions as assuming a fixed effects model. In the second example (Table 10.3b), the defoliation intensity x shade interaction was significant in the MANOVA assuming fixed effects (Section 8.3.1). This source of variation would also have been significant when assuming a random effects model because the same mean square ratios are used for significance tests (Table 10.3).

Hence, in at least the two cases discussed, each model (fixed versus random effects) gave similar mathematical significance test results and interpretations. This might not be the case in more complex experiments consisting of several factors where the

significance test results would either be similar for the two models, or there would be more significant sources of variation when assuming a fixed effects model. It is recommended from experience gained from the present research that estimation of error degrees of freedom for multivariate tests should be calculated at the planning stage before an experiment is conducted, to avoid unnecessary problems later.

10.4 SOME COMPARISONS BETWEEN MULTIVARIATE AND UNIVARIATE TECHNIQUES

Data obtained in this thesis research are used here to make some comparisons between multivariate analysis of variance (MANOVA) / multiple discriminant functions (Sections 2.4.3.1 and 2.4.3.2) and univariate analysis of variance (ANOVA) / mean separation (Le Clerg *et al.*, 1962; Steel and Torrie, 1980). The data examining the effect of seed size on six vegetative characters (Section 6.3.2) are the first chosen for this purpose and correlations between these characters were presented in Table 10.1c. The correlations were all high and therefore multivariate analyses were appropriate, while the usual univariate analyses should be interpreted cautiously because of bias in F-tests and in t-tests for mean separation (Cooley and Lohnes, 1971; Kendall, 1975).

There were highly significant ($P<0.001$) differences between seed sizes (F-test = 4.20 with 12, 150 df) in the MANOVA and the first discriminant function for this source of variation accounted for approximately 82% of the data dispersion (Table 6.7a). On this function, the difference between large and medium seed was about 2.5 times greater than the difference between medium and small seed (Table 6.7b), which indicated that large seed was distinctly different from the other two seed sizes. The purpose of the multiple discriminant function is to discriminate maximally between the treatments, when considering all characters and their covariances within the data set (Lindeman *et al.*, 1980), and it can be seen here that large seed was distinctly different from the other two seed sizes. F-tests for seed size and mean separations for each character are presented in Table 10.4.

TABLE 10.4 Univariate F-ratios for seed size and mean separation for six vegetative characters.

	Character					
	shoot length (log)	root length (log)	leaf dry weight (log)	stem dry weight (log)	root dry weight (log)	leaf area (log)
F-ratio (df=2,80)	6.75**	6.60**	7.69***	4.91**	10.50***	13.28***
Seed size						
small	3.8b+	4.4b	2.08b	0.75b	1.27b	1.70b
medium	3.9ab	4.5ab	2.63ab	1.39a	1.93a	1.85b
large	4.0a	4.6a	2.91a	1.55a	2.32a	2.25a

+ figures scored by different letters differ at the 5% significance level.

Although the contents of Table 10.4 appear useful, they are at a disadvantage compared with the findings for the MANOVA / multiple discriminant function. The correlations found between the characters (Table 10.1c) indicate that each "character" is not pure, that is independent of the other characters. Hence, there are considerable difficulties in interpreting such information and mean discriminators like t-tests cannot logically be applied (Kendall, 1975). Mean treatment rankings of the assumed characters (Table 10.4) were similar to those shown by the mean scores on the first multiple discriminant function (Table 6.7b) described above. However, only the multivariate approach gave an unbiased, consensus opinion about treatment discrimination, which was of biological interest.

In the first multiple discriminant function for seed size, leaf area with a standardised coefficient of 4.85 was the most important character contributing to treatment discrimination, followed by shoot length (-1.89). An appropriate description of the function on the basis of these important characters could be a leaf area/shoot length contrast. It has been suggested in this thesis that where a discriminant function accounts for most of the data dispersion, for example greater than 80%, it would be appropriate to examine only the most important characters in future similar research aiming to discriminate the plant responses measured on these treatments. The consequences of this for interpretation were evaluated for a reduced data set by conducting a MANOVA and estimating mean discriminant scores (Section 3.7) involving leaf area and shoot length only. Results for the full and reduced character sets are presented in Table 10.5.

Separation of the three seed sizes using the reduced character set was approximately the same as using the full, original set and the discriminant function mean scores were also similar (Table 10.5). Leaf area was still more important than shoot length in contributing to treatment discrimination in the reduced character set function. Standardised coefficients for leaf area were 2.6 and 2.2 times larger than those for shoot length, in the full and reduced character set functions, respectively. The results indicated that the function Y = log(leaf area) - 2 log(shoot length) was suitable to discriminate between the seed sizes, being approximately the same as the first discriminant function involving the full character set.

TABLE 10.5 First discriminant function mean scores for seed size calculated from full and reduced character sets.

	full character (six) set	reduced character (leaf area and shoot length) set
F-statistic	4.20 (12,150df) P=0.0001	8.36 (4,158df) P=0.001
Percent dispersion accounted for by first function	82	90
Seed size	**Mean score**	**Mean score**
small	-0.64	-0.44
medium	-0.21	-0.29
large	0.84	0.73
two largest standardised coefficients	1. leaf area (4.85) 2. shoot length (-1.89)	1. leaf area (6.06) 2. shoot length (-2.74)

TABLE 10.6 Univariate F-ratios for sward types at Riverside in the establishment year.

	Character					
	X1	X2	X3	X4	X5	X6
F-ratio (df=8,16)	5.47**	2.28(NS)	0.59NS	1.45NS	0.50NS	1.57NS

X1-X2 = herbage mass and weed content, respectively on 17/12/1985
X3-X4 = herbage mass and weed content, respectively on 5/2/1986
X5-X6 = herbage mass and weed content, respectively on 23/4/1986

The herbage mass and weed content data at three times reported in Section 4.3.3 are an example of data with correlations of differing magnitude (low to medium) and direction (Table 10.1b) and they are also an example of a perennial data problem. The correlations were responsible for there being some differences in the results and interpretations between the multivariate and univariate analyses. The MANOVA was not significant (F-test = 1.55 with 48,58 df) and contrasted with one of the results from the ANOVA (Table 10.6). This is a consequence of the ANOVA significance tests being biased where correlations exist, that is, the univariate F-test does not test the supposed character independently (Cooley & Lohnes, 1971). Under these circumstances, the MANOVA results are the most meaningful. Mean separation for the character with significant differences between several sward types (X1) was presented in table 4.12, but as stated previously, these results should be interpreted cautiously (Kendall, 1975).

Implicit in the use of MANOVA and multiple discriminant functions is the desire to obtain a consensus opinion about trends or treatment differences across all measured response characters. This example demonstrates the usefulness of MANOVA in this regard. There were significant differences between treatments for only one of the six correlated characters (Table 10.6) and intuition by any researcher would have probably arrived at the same result/conclusion as MANOVA. However, apart from the problems of using univariate analyses for correlated characters (Cooley & Lohnes, 1971; Kendall, 1975) and therefore the need to use multivariate techniques, it can be envisaged that MANOVA becomes increasingly useful where an effect is significant for perhaps 50% of the characters, and particularly where there are also differences in mean separation between the characters. It is a frequent part of research reports involving several or more characters to attempt to arrive at an overall opinion in the discussion section and MANOVA can assist with this. An important advantage of the technique is that it reduces statistical and personal bias in interpretation of a character set. The associated multiple discriminant functions discriminate maximally between the plant responses measured on the treatments and they are helpful in indicating which are the more important response variables accounting for most of the variation.

There may be confusion about the way the plant response characters and the design matrix, representing the treatments, are viewed in MANOVA compared with multiple discriminant functions. It should be apparent from previous discussion that multiple discriminant function(s) and MANOVA are related very closely. MANOVA involves a search for that linear combination of the various plant responses which makes the univariate F-ratio (computed on the single combined variable) for a particular effect as large as possible (Harris, 1985). The linear function which achieves this is the multiple discriminant function (Mardia *et al.*, 1979; Lindeman *et al.*, 1980; Harris, 1985). Of course in both cases, the plant responses are the major interest, rather than the design matrix. Harris (1975; 1985) refers to the plant responses as outcome variables and elements of the design matrix, representing individual factors (treatments), as independent variables. That is, the designation of the design treatments as independent variables is the same in MANOVA and multiple discriminant functions.

10.5 REPEATED MEASUREMENTS

Consecutive measurements on the same experimental units were conducted in parts of all experiments. Examples included herbage mass and weed contents measured at three harvests (Section 4.3.3), regrowth of sheep's burnet over seven harvests (Section 5.2.3.1), six vegetative characters measured at five harvests (Section 6.2.3), range of seedling characters measured at four harvests (Section 7.2.3), and length measurements on three leaves (Sections 8.2.3.1 and 9.2.3.1).

Data of this type are often analyzed univariately as a split-plot in time or split-block in time analysis of variance (Le Clerg *et al.*, 1962; Steel and Torrie, 1980). However, a common feature of data measured on the same sampling units is that consecutive measurements are correlated (Cole & Grizzle, 1966; Morrison, 1976), resulting in correlation of experimental errors. For most biological data, common disturbances resulting from failure of data to satisfy the assumptions of ANOVA, such as independence of experimental errors are negligible (Steel and Torrie, 1980). However, it is reasonable to assume that probability levels and sensitivities of F-tests,

and hence interpretations, are adversely affected to a greater extent with larger correlations between experimental errors (Cochran, 1947; Le Clerg *et al.*, 1962).

The partial error correlations between repeated measurements were calculated for two data sets using the appropriate matrices of residual sums of squares and cross products, and are presented in Tables 10.7a and b. The estimates varied in both magnitude and direction and were often slightly different from the corresponding simple (total) correlation coefficients presented in Tables 10.1b and d. The magnitudes of the correlations, particularly some of those in Table 10.7b, indicated that account of these via the multivariate methods used in this thesis was essential. Indeed, analysing each character separately as a univariate split plot (or block) in time would be very biased (Cooley and Lohnes, 1971; Kendall, 1975).

In a multivariate analysis involving repeated measures, measurements at each time are regarded as separate characters (Morrison, 1976) to accommodate any covariances between them in the analysis. The analyses described in Chapter 7 consisted of the greatest number of such characters and the MANOVA's were straightforward in determining overall significant sources of variation. In some instances, the resulting multiple discriminant functions for significant sources of variation were useful for identifying a response character and its time of measurement, which was contributing the most to treatment discrimination. For example, in the first discriminant function for the temperature x depth x seed size interaction for Oregon sheep's burnet (Table 7.7a), total seedling dry weight at harvest one (H1) was approximately twice as important as any other variable. Hence most of the dispersion accounted for by this function could be explained by this variable. Similarly, root dry weight at H2 and shoot length at H4 were clearly most important in the discriminant functions for temperature x seed line interaction (Table 7.9a) and temperature x seed size interaction (Table 7.11a), respectively, for Oregon and Cockayne sheep's burnet.

There were some interpretational difficulties in some of the multiple discriminant functions where several characters (attributes), and at different times, were most important in contributing to treatment discrimination. This problem, however, does not negate the efficacy of analysing the data multivariately. For example, in the first

TABLE 10.7a Partial error correlations calculated from the residual matrix involving six vegetative characters measured on establishing swards at Riverside.

	X1	X2	X3	X4	X5	X6
X1	1.00	-0.09	0.07	-0.58	0.03	-0.37
X2	-0.09	1.00	-0.08	0.06	0.04	0.50
X3	0.07	-0.08	1.00	-0.51	0.20	-0.04
X4	-0.58	0.06	-0.51	1.00	0.23	0.43
X5	0.03	0.04	0.20	0.23	1.00	0.15
X6	-0.37	0.50	-0.04	0.43	0.15	1.00

X1-X2 = herbage mass and weed content, respectively on 17/12/1985
X3-X4 = herbage mass and weed content, respectively on 5/2/1986
X5-X6 = herbage mass and weed content, respectively on 23/4/1986

Table 10.7b Partial error correlations calculated from the residual matrix involving nine logistic function parameters estimated for variously defoliated plants of sheep's burnet in a glasshouse.

	X1	X2	X3	X4	X5	X6	X7	X8	X9
X1	1.00	-0.06	-0.07	0.84	0.76	-0.60	0.85	-0.29	0.08
X2	-0.06	1.00	-0.77	0.08	-0.18	0.30	-0.13	-0.60	0.65
X3	-0.07	-0.77	1.00	-0.10	0.20	-0.13	0.05	0.56	-0.43
X4	0.84	0.08	-0.10	1.00	0.77	-0.62	0.79	-0.09	0.06
X5	0.76	-0.18	0.20	0.77	1.00	-0.77	0.56	-0.12	0.09
X6	-0.60	0.30	-0.13	-0.62	-0.77	1.00	-0.36	0.00	0.14
X7	0.85	-0.13	0.05	0.79	0.56	-0.36	1.00	0.03	-0.21
X8	-0.29	-0.60	0.56	-0.09	-0.12	0.00	0.03	1.00	-0.87
X9	0.08	0.65	-0.43	0.06	0.09	0.14	-0.21	-0.87	1.00

X1 = β_0 - leaf 1
X2 = β_1 - leaf 1
X3 = β_2 - leaf 1
X4 = β_0 - leaf 2
X5 = β_1 - leaf 2
X6 = β_2 - leaf 2
X7 = β_0 - leaf 3
X8 = β_1 - leaf 3
X9 = β_2 - leaf 3

multiple discriminant function for depth x seed size interaction for Oregon sheep's burnet (Table 7.6a), four variables were most important because they had similarly high standardised coefficients. These were H1 total seedling dry weight, H4 shoot length, H2 root dry weight and H3 root length (Table 7.6a). The range of harvest times in addition to the measured characters demonstrated the considerable complexity in the data, which certainly a simpler analysis does not reveal. Furthermore, the results showed that the contribution of characters in treatment discrimination varied with harvest time. However, there are greater difficulties than in the one variable case in attempting to name and hence understand what the discriminant function is measuring biologically.

Hence, the multiple discriminant function examples presented show advantages and disadvantages arising from the method of analysis undertaken, predominantly in Chapter 7. These depend mainly on the ease of biological interpretation of the number of important characters contributing to treatment discrimination.

It was of interest to learn whether the results and hence conclusions for the MANOVA involving all characters and times would have been supported by those for MANOVA's involving the measured characters (at various times) analysed separately. Accordingly, the simple data set consisting of herbage mass and weed content, each measured at three times for nine swards at Riverside (Section 4.2.5.3), was examined. The partial error correlations for this data set were presented in Table 10.7a and in the MANOVA, there were no significant ($P<0.05$) differences between the sward types (Section 4.3.3). The same results and conclusions were obtained when the two characters at three times were analysed separately (Table 10.8). Therefore, at least in this example, the inclusion of the two measured characters and their times of measurement in the one analysis was useful as it summarised the two separate analyses.

Another approach to analysing repeated measures data reported in this thesis was to use a multivariate split-plot in time model and examples were given in Sections 4.2.5.3 and 6.2.4.2. This analysis has an advantage over the univariate split-plot (or -block) in time analysis because it incorporates the covariances between the measured

TABLE 10.8 F approximations for treatments and their significance from MANOVA's involving herbage mass and weed content at Riverside.

	Variables included in MANOVA		
	Herbage mass and weed content at three times	Herbage mass at three times	Weed content at three times
Rao's F approximation	1.55 (48,58 df)	1.49 (24,41 df)	1.52 (24,41 df)
Significance	NS	NS	NS

characters in the analysis and therefore does not suffer from the bias encountered in similar univariate analyses of correlated characters (Cooley and Lohnes, 1971; Kendall, 1975). However, the partial error correlations between successive times of measurement still remain and therefore the analyses are biased to this extent. The problem of multivariately combining several attributes which themselves should be analysed multivariately over time, awaits solution.

From experience gained in this thesis in the multivariate analysis of repeated measures, and from the previous discussion, the most consistently useful analysis with respect to simplicity and consideration of several characters jointly, and ease of interpretation, is the split-plot in time multivariate analysis of variance. In this analysis, the covariances between all measured characters are involved, the resulting multiple discriminant functions involve measured characters not distinguished by time, and time and time x treatment interaction can be tested for significance and examined further. Where correlated characters are involved, the analysis is less biased than the traditional univariate analyses.

10.6 CONCLUSIONS

Covariances (correlations) existed between the numerous response characters measured in this thesis. Univariate analysis is biased under these conditions and multivariate analysis of variance and multiple discriminant functions are the appropriate techniques to account properly for the correlations. Results and interpretations from the multivariate and univariate analyses varied depending on the size of the associations between the characters.

It is recommended that both multivariate and univariate analyses be conducted on the same data and that comparisons should be made between the results of the techniques. A refinement on conducting analysis of variance on all characters is to analyze only those characters which are important in the standardised multiple discriminant functions which account for most of the data dispersion. Univariate analyses of this reduced character set can be discussed biologically and suggestions made on the characters to be investigated in future similar research. Multivariate

analysis of variance is advantageous in obtaining an unbiased consensus opinion over many characters and it is useful for repeated measures.

The multivariate techniques used in this thesis have a valuable role in the analysis and interpretation of the results from agronomic research. The techniques are particularly relevant because frequently at least several characters are measured in experiments and these are often correlated, as shown in this thesis. Wider use of the techniques is possible due to the availability of computer programmes such as BMDP, SAS and SPSSX (Harris, 1985).

CHAPTER 11 : GENERAL DISCUSSION

11.1 INTRODUCTION

Various aspects of the agronomy of sheep's burnet were examined in the experiments described in Chapters 4 to 9 of this thesis. In line with the broad thesis objectives presented in Chapter 1, experiments investigated: a) several possible factors influencing establishment (emergence and early vegetative growth) of sheep's burnet (Chapters 4, 6 and 7); and b) regrowth responses of young plants and swards of sheep's burnet under limiting (Chapter 9) and non-limiting moisture conditions (Chapters 4, 5 and 8). The agronomic performance of sheep's burnet was also compared with one or two dryland legume species (lucerne and birdsfoot trefoil) in the field trials (Chapter 4) and in the emergence and early vegetative growth studies in controlled environments (Chapters 6 and 7). The following discussion is divided into several sections which address the main findings in, and relationships between, the experiments conducted. Sections are arranged in the order of development and management of swards of sheep's burnet.

11.2 EMERGENCE

11.2.1 FINAL EMERGENCE

Estimates of final seedling emergence of sheep's burnet were different at the field locations (27% at Riverside and 66% at Flock House) (Chapter 4) and were approximately 50% in the glasshouse experiment (Chapter 6). With the exception of the estimate at Flock House, emergence was lower than that found in the later climate room experiment (Chapter 7) where it exceeded 60%. Furthermore, in that study, emergence of the seed line from Oregon, USA was greater for larger seed (hypanthia) and ranged from 62% (small) to 81% (large). This trend was also indicated in the glasshouse study (Chapter 6) but it was less apparent and the experiment was only conducted in one environment. However, the glasshouse environment was less artificial than that in the climate room due to the alternating day/night (approximately 25°C/15°C) temperatures, which resembled the field

situation more closely. In the climate room studies, the absence of a seed size x temperature interaction indicated that large seed may result in increased emergence under a range of day and night temperatures.

The range of emergence of sheep's burnet of 30-80% was in partial agreement with findings overseas. In Spain, Salmeron (1966) found that field emergence in most cases was more than 50%, while in Sweden, emergence is usually between 30 and 60% (Nordborg, 1967b). Higher germination and/or emergence of large seed has also been found for other species such as birdsfoot trefoil (Woods and MacDonald, 1971) and 'Maku' lotus (Charlton, 1989), but not for wheat (Lafond and Baker, 1986b).

Results from the climate room studies (Chapter 7) showed that temperatures ranging from 10-25°C had a negligible effect on percentage seedling emergence of Oregon sheep's burnet under the prevailing non-limiting moisture conditions. Similar trends over a wide range of temperatures have also been reported for other species (Thompson, 1970; Cooper, 1977; Charlton *et al.*, 1986; Hampton *et al.*, 1987). These results contrasted with those found for the same seed line in the field trials where the higher emergence from sandy soils at Flock House was probably due mainly to the warmer soil temperatures there compared with those of the heavier soils at Riverside (Chapter 4). Soil moisture levels at each site appeared satisfactory for germination. The climate room studies were conducted at constant temperatures whereas material in the field trials, of course, experienced alternating night/day variations and this may also have been a contributing factor. An examination of the effects of alternating temperatures and of various moisture levels on emergence of sheep's burnet is a worthwhile broad objective for future research.

Approximately 30% of "seed" produced two seedlings (Section 2.2.5) in both the field (Chapter 4) and glasshouse studies (Chapter 6). In the latter experiment, the proportion of doubles increased with seed size for the Oregon seed line, but not for the line from the Cockayne plots in New Zealand. The pattern for the Oregon seed line was confirmed in the climate room studies (Chapter 7) at four constant temperatures, with small seed producing a much lower proportion of doubles (9%)

compared with other seed sizes (>50%). Detailed information on the conditions under which doubles are produced were undocumented previously and these studies have contributed in this regard.

There are several possible reasons for large seeds achieving higher emergence and producing a greater proportion of doubles than small seeds. Large seeds usually possess larger embryos than small seeds in many species (Bremner *et al.*, 1963; Perry, 1980) and these might have a superior ability to function under suitable environmental conditions for germination, as well as in more marginal environments. Dissection of small and large "seeds" revealed that in small seeds, the achene size was usually unequal with one achene being larger than the other. This contrasted with large seed where the two achenes were of more equal size. Hence, these findings suggested that in small seed, there was a relatively marked reduction in embryo size of one of the two achenes to the extent that it was probably non-functional. Large seeds also had the largest cotyledons (Chapter 6) and hence nutrient reserves, and these may influence the nourishment of the embryo axis. Small seeds with their lower quantities of reserves, such as nonstructural carbohydrates (Chapter 6), may be less capable of nourishing the embryonic tissues when compared with large seeds. The relative importance of embryo size versus the size of the reserve carbohydrate pool in the various seed sizes requires further elucidation. Such information would assist in explaining and understanding some of the emergence trends reported for seed size in Chapters 6 and 7.

A highlight of the climate room studies (Chapter 7) was the estimation of the minimum soil temperature required for satisfactory emergence of Oregon sheep's burnet. This was 4.9°C and it suggested that under harsh semi-arid conditions, such as those experienced in Central Otago (New Zealand Meteorological Service, 1983), autumn sowings of the species should not be conducted later than March, or perhaps April. Similarly, the figure could indicate suitable sowing times in the spring. A current recommendation in Central Otago is that minimum soil temperatures of about 10°C are required to achieve satisfactory establishment of sheep's burnet (B J Wills, pers. comm.). The present findings suggested that slightly cooler soil temperatures

may also provide satisfactory establishment, thereby enabling greater flexibility in the choice of sowing time.

Under milder conditions, and/or where irrigation is available, there should be no major difficulties in obtaining satisfactory emergence from seed sown at a shallow depth, for example 1 cm, in autumn or spring. A major exception to this was the first sowing of sheep's burnet in sandy soil at Flock House (Chapter 4). Emergence was very poor and was attributed to destruction of the seed by sand dune weevils (*Cecyropa discors* Broun.) (W. Stiefel, pers. comm.). The problem was rectified for the second sowing by using an appropriate insecticide and this practice should be a general requirement for satisfactory emergence of sheep's burnet on similar sand country.

11.2.2 EMERGENCE RATE

Soil temperature had a major influence on the rate of emergence of sheep's burnet under non-limiting moisture conditions, as well as for that of lucerne and birdsfoot trefoil. Increases in temperature resulted generally in earlier commencement of emergence, an increased rate of emergence, and a shortened duration of emergence (Chapters 4 and 7). Similar results have been reported for a wide range of other species (Woods and MacDonald, 1971; McElgunn, 1973; Hur and Nelson, 1985; Lafond and Baker, 1986a, b; Charlton *et al.*, 1986; Hampton *et al.*, 1987).

Species sown in the relatively warm soil temperatures at Flock House emerged approximately three to four days earlier than at Riverside (Chapter 4). More detailed studies in the climate room (Chapter 7) found that the emergence rate of sheep's burnet often increased with rises in temperature over the range 10-25°C. Times to reach 10% of final emergence decreased from about 12-13 days after sowing at 10°C to 3-4 days after sowing at 25°C. Similarly, the duration of emergence was reduced with increases in temperature, and varied from approximately 5-7 days at 10°C to 2-4 days at 25°C. The main practical implication of these studies was that quicker emergence of sheep's burnet can be expected from increasingly warm soils. However, higher temperatures in the field are also associated with higher evaporative

demand. Hence soil moisture deficits and possibly seedling diseases are likely to increase. Some compromise should therefore be made in the choice between soil temperatures for high emergence rate and those offering reduced chances of low soil moisture levels and pathogen attack.

Seed line, seed size and sowing depth were generally unimportant in determining the rate of emergence of sheep's burnet although there were slight interactions between some of these factors and with temperature (Chapters 6 and 7). In the glasshouse study (Chapter 6), emergence rates of the different sized seeds of the Cockayne seed line were similar while for the Oregon seed line, large seed (hypanthia) had a slightly superior emergence rate. However, this was not sufficiently high to cause differences between the seed sizes in time to reach various stages of emergence. Analysis of the emergence rates for the Oregon seed line in the climate room studies (Chapter 7) also found that seed size was relatively unimportant, although some interactions occurred with other factors. For example, at low temperatures (10° and 15°C at 5 and 10 mm depths, respectively) large seed had significantly higher emergence rates than medium or small seed. Hence, the combined results indicated that at least for the Oregon seed line, it may be advantageous to sow larger seed to ensure quicker emergence. Furthermore, interactions between seed size, sowing depth and temperature may be of increasing importance at low temperatures. The superior emergence rate found sometimes for large seed of sheep's burnet in these studies was similar to that reported for large seeds of lotus species (Woods and MacDonald, 1971; Charlton, 1989), but not to the findings for wheat where small seeds had a higher emergence rate (Lafond and Baker, 1986b).

All studies (Chapters 4 and 6) showed that sheep's burnet emerged later and hence had a slower emergence rate than lucerne. There was no evidence of significant seed dormancy in sheep's burnet. Sheep's burnet also emerged more slowly in the field trials (Chapter 4) than birdsfoot trefoil, which was approximately intermediate between sheep's burnet and lucerne. These findings were obtained from studies conducted under a range of alternating temperatures and they may therefore have general applicability in practice. Lucerne is renowned for its rapid emergence (Cooper, 1977) while birdsfoot trefoil is slow and non-competitive during

establishment (Scott and Charlton, 1983; Curtis and McKersie, 1984; Hur and Nelson, 1985), and the results supported these earlier findings. The relatively poor emergence rates of sheep's burnet suggested that it is not overly competitive at an early stage of development. Hence, the species should be sown alone or only in mixtures with other slowly to slightly faster emerging species and thorough weed control practices should also be adopted. The results provided strong quantitative support for current recommendations on seed bed preparation for establishing sheep's burnet (Sheppard and Wills, 1985, 1986; NWASCA, 1986).

11.3 EARLY VEGETATIVE GROWTH

The dominant influence of temperature continued on into the early vegetative growth stages. In an analysis of the Oregon sheep's burnet data in the climate room studies (Chapter 7), increases in temperature over the range 10-25°C resulted generally in a progressive reduction in the times to attain specific growth stages. However, this pattern was not defined clearly since there were interactions with sowing depth and seed size, indicating that a moderately complex situation existed.

The Oregon seed line had superior vegetative growth (mainly greater leaf area and root length) compared to that of the Cockayne line in the glasshouse study under alternating temperature (15/25°C) conditions (Chapter 6). The climate room studies (Chapter 7) examined this matter further and found that superiority for some plant characters depended on the temperature at which evaluations were conducted. At 20°C, Oregon sheep's burnet performed as well as or better (for example, higher total seedling dry weight very soon after emergence) than the Cockayne seed line while at 10°C, Cockayne sheep's burnet was superior for root and shoot dry weights at harvest two. This is the first report of Cockayne sheep's burnet being relatively cold active and the finding may encourage interest in the line for revegetation in cool environments. Average temperature conditions in the glasshouse study were closer to 20°C than to 10°C and this may have partly accounted for the observed superiority of the Oregon material. The results of both studies suggested that the two seed lines are adapted to different environments and they should therefore be evaluated further to identify their most suitable environments.

Large seed generally produced seedlings with greater growth for one or more plant characters. In the glasshouse study (Chapter 6), seedlings originating from large seed had superior shoot length and leaf area while under more controlled conditions (Chapter 7), seed size interacted with several other factors. These included for example, an interaction between seed size, depth and seed line for both the Oregon and Cockayne seed lines at 10 and 20°C. However, the climate room studies showed that the greatest vegetative growth was achieved by those seedlings originating from large seed and interactions were due mainly to the small and/or medium seed relationships with the other factors. The frequent dry weight advantages from using large seed were in agreement with the findings for numerous other species (Arnott, 1969; Townsend and Wilson, 1981; Curtis and McKersie, 1984; Sangakkara *et al.*, 1985) and use of large sheep's burnet seed in practice should confer a competitive advantage over those seedlings arising from small seed. Seed growers should be endeavouring to produce relatively large seed of sheep's burnet for the market and a breeding programme to improve seed size may also be a worthwhile objective.

There was an inconsistency in the relative performances of the different species in the various experiments. At Riverside (Chapter 4), vegetative growth of sheep's burnet and lucerne was similar but superior to that of birdsfoot trefoil. The main plant characters accounting for this difference were foliar and root dry weights. Leaf area per plant for sheep's burnet and lucerne was also higher than that for birdsfoot trefoil. However, in the glasshouse study (Chapter 6), Oregon sheep's burnet was inferior, equal or superior to lucerne depending on harvest time, mainly with respect to differences in leaf area and root length. Oregon sheep's burnet and lucerne were usually superior to Cockayne sheep's burnet. Inconsistencies between the two studies may be due to: differences in the age of seedlings examined (38-59 days old at Riverside versus 17-45 days old in the glasshouse study); two seed lines of sheep's burnet were examined in the glasshouse experiment while only the Oregon seed line was evaluated at Riverside; and environmental differences.

The findings from the climate room studies at 15° and 25°C (Chapter 7) showed that temperature had a differential effect on the performance of sheep's burnet relative to lucerne. For example, root and shoot lengths at some harvests were generally more

similar for sheep's burnet at the two temperatures than for lucerne. This suggested that sheep's burnet may perform more consistently over a range of temperatures although this hypothesis needs to be tested under field conditions. The finding at Riverside (Chapter 4) that sheep's burnet had early vegetative growth superior to that of birdsfoot trefoil was in agreement with practical experience in the South Island of New Zealand (B J Wills, pers. comm.). Future detailed comparisons may provide similar results. The frequently similar early vegetative growth of lucerne and sheep's burnet, particularly at Riverside (Chapter 4), suggested that a mixture of sheep's burnet and lucerne could be a satisfactory dryland option. However, a problem could be in the emergence phase where lucerne would probably emerge earlier than sheep's burnet, as found in these studies, and therefore possibly hinder development of sheep's burnet. This was not apparent in the field sowings but it has occurred in some South Island stands (B J Wills, pers. comm.). Use of a relatively low sowing rate for lucerne may overcome this difficulty.

Compensation/adjustment for a relatively slow rate of emergence was suggested for Oregon sheep's burnet since subsequent vegetative growth was comparable in some respects (for example, foliar and root dry weights and leaf area) to that of lucerne (Chapters 4 and 6). The findings of the glasshouse study suggested that the Cockayne seed line did not possess such a strategy and there are several main implications for establishment. In pure swards, Oregon sheep's burnet might establish more quickly than Cockayne sheep's burnet and hence provide earlier protective ground cover. Under mixed sward conditions, the Cockayne seed line probably would be disadvantaged relative to Oregon material due to its slow development of leaf area and root length. Furthermore, weed infestations could be a greater problem in swards of the Cockayne seed line. Comparisons between lines in swards established under similar environmental conditions should be undertaken. The growing of swards of the Cockayne seed line is not recommended (B J Wills, pers. comm.) but the findings from this thesis research indicate that the line may be useful in cool environments, as mentioned previously.

A major advantage of the multiple discriminant functions calculated frequently throughout this thesis was that they identified without bias, the plant characters which

were most important in discriminating between the various treatments. For example, in the glasshouse study (Chapter 6), six characters (shoot and root lengths, leaf area, and leaf, stem and root dry weights) were measured and the resulting analysis found that on the first discriminant function, leaf area was the most important character for distinguishing between the treatment x harvest interaction means. Root length was the second most important character and was approximately half as discriminatory as leaf area. This shows that the number of characters to be measured in any future similar experiments can be reduced and this has obvious associated advantages with savings in time, labour and money. Alternatively, more plant material could be examined for similar practical and economic inputs. The analytical approach should receive increasing use in a wide range of agronomic research.

11.4 REGROWTH RESPONSES

Foliar regrowth of variously aged and defoliated young plants of Oregon sheep's burnet was studied under a range of environments varying from field conditions in summer and autumn at Riverside (Chapters 4 and 5) to glasshouse conditions at different times of the year (Chapters 8 and 9). The relative regrowth of sheep's burnet compared to lucerne and birdsfoot trefoil was also investigated at Riverside (Chapter 4). Environmental factors such as the quality and quantity of photosynthetically active radiation were probably more important in accounting for any differences in plant regrowth responses between the field and glasshouse experiments, than plant factors. However an essential difference between the field and glasshouse studies was the nature of the residual herbage following defoliation. At Riverside, residual herbage was approximately 5-7 cm high following cutting and consisted of negligible complete leaves and unknown proportions of incomplete leaves and stubble. Conversely, in the glasshouse studies, no incomplete leaves remained after cutting and residual was stubble and 0 to 8 complete, fully expanded, leaves. The effects and relative efficiencies of these residual herbage components in contributing to regrowth under the different environmental conditions was uncertain. Furthermore, the glasshouse studies were conducted on spaced plants whereas swards were investigated in the field. A detailed examination of the influence of residual canopy structure and environmental factors, such as light and

temperature, on the regrowth of sheep's burnet would be an appropriate sequel to this research.

Swards of sheep's burnet, and those of sheep's burnet in various mixtures with lucerne or birdsfoot trefoil, had similar herbage accumulations under cutting compared with monocultures of the two legume species (Chapter 4). This suggested that sheep's burnet was relatively tolerant of cutting and that it may make a worthwhile contribution, at least in its first season, to the supply of palatable forage over the summer. Although the cutting trial was terminated formally in mid-1986 (Chapter 5), the swards were grazed periodically with sheep for some three years longer and it was found that the contribution of sheep's burnet to sward herbage mass declined markedly during this time (Foote, unpubl.). This suggested that the main use of sheep's burnet under defoliation/grazing could be as a short-term booster of palatable herbage mass. A similar conclusion was also reached from South Island studies (Daly, 1984). The lack of an advantage in herbage accumulation of mixed swards of sheep's burnet and a legume, compared with monocultures of sheep's burnet, should not discourage further investigations of such mixtures. Other possible advantages of including a legume with sheep's burnet include improved protein content and digestibility of the herbage, superior balance of minerals in the herbage, and a better seasonal spread of green herbage mass and effective ground cover.

The similar herbage accumulations between monocultures of sheep's burnet and the two legume species mentioned above indicated that, under Wairarapa conditions, establishment of sheep's burnet was not slow, as suggested under generally harsher South Island conditions (de Lacy, 1985). Furthermore, the results showed that the species could be defoliated at similar times as lucerne and birdsfoot trefoil and that there was no necessity to delay defoliation because of possible slow establishment. Responses of similarly young swards of each species to grazing, in the same trial, would provide valuable information to complement the findings of this investigation.

The detailed measurements of foliar regrowth in the autumn (Chapter 5) indicated that a suitable frequency for defoliation was approximately four weeks under the particular environmental conditions at that time. The value of this estimate would

have been enhanced if it coincided with some readily identifiable physiological feature or stage of development of the swards. This could then have been used as a criterion for scheduling defoliation in future autumns where environmental conditions could be different from those in 1986. The earlier defoliations over summer (Chapter 4) were scheduled on the basis of flowering stage of the lucerne swards and a similar criterion could be appropriate for summer management of swards of sheep's burnet.

Swards in the field trial generally grew under non-limiting soil moisture levels and were defoliated at approximately one level of partial defoliation (Chapters 4 and 5). These studies were complemented by the glasshouse experiments where regrowth following a range of defoliation intensities was investigated under limiting and non-limiting moisture conditions (Chapters 8 and 9). A general reduction in root mass was the most important morphological effect of complete defoliation (0% rLA) (Chapters 8 and 9). This was probably due to almost complete cessation of root elongation (Evans, 1973) as well as to likely root death and decay. Undesirable consequences of reduced root mass could include lowered moisture and nutrient uptake (Jantii and Heinonen, 1957; Oswalt *et al.*, 1959) and less effective soil stabilisation. However, the results of one experiment (Chapter 9) indicated that complete defoliation may also be advantageous in conserving soil moisture by lowering the amount of water transpired. This strategy could therefore have a role in dryland situations, as reported similarly by Toft *et al.* (1987), but it could only be conducted infrequently due to the deleterious effects of such a harsh treatment on probable plant survival.

Root and stubble components of old establishing plants were frequently larger (more mass) than those of plants one month younger, under the same environmental conditions (Chapter 9). They also had similar concentrations of soluble sugars and similar or slightly higher levels of starch. Hence, the absolute amounts of nonstructural carbohydrates in the two plant components were higher for the older plants and the studies indicated that these may be used for regrowth immediately following defoliation (Chapters 8 and 9). In one experiment (Chapter 8), approximately three month old, previously undefoliated plants had no detectable

starch in the stubble or roots. The low or non-existent quantities of starch found in young plants could possibly account for the relatively poor regrowth of similar plants following intense or complete defoliation observed by the author. The results suggested that, at least in young plants, complete leaf removal (0% rLA) should be avoided. Successive, complete, defoliations also allowed little or no opportunity to accumulate starch in stubble and roots and consequent leaf regrowth was inferior to that of younger previously undefoliated plants (Chapter 8).

Results from the glasshouse studies generally confirmed that the most appropriate defoliation intensities for young sheep's burnet plants were those which left some leaf area. Photosynthate from these leaves provided at least some, and perhaps most, of the foliar regrowth advantages of partial defoliation observed (Chapters 8 and 9). Here, sheep's burnet appears similar in its management requirements to birdsfoot trefoil (Nelson and Smith, 1968; Smith, 1962), cicer milkvetch (Gabrielsen *et al.*, 1985) and sainfoin (Cooper and Watson, 1968). Partial defoliation was practised in the field trial (Chapters 4 and 5) and it was therefore likely that the energy for regrowth of sheep's burnet originated mainly from current photosynthate of the remaining incomplete leaves rather than from carbohydrate reserves in the stubble and/or roots. This probably also applied to birdsfoot trefoil, but not to lucerne which obtains most of its energy for regrowth from the upper part of the tap root (Smith, 1962; Cooper and Watson, 1968; Sheaffer, 1983; Rapoport and Travis, 1984). Extrapolation of the findings on carbohydrate physiology of sheep's burnet in the glasshouse to those in the field should be treated cautiously due to probable environmental differences, particularly with respect to temperature and daylength, which may markedly influence one or more of photosynthesis, respiration and assimilate partitioning (Wardlaw, 1969; Ludlow, 1978; McWilliam, 1978; Walton, 1983).

The method for imposing defoliation intensities in the glasshouse experiments (Chapters 8 and 9) precluded making recommendations on the optimum proportion of the foliage of intact plants which should be removed at defoliation. Defoliation intensities (0-100% rLA) adopted were relatively severe since very little of the pre-cut foliage remained after imposing the treatments. Upwards of 70-80% of the

foliage was removed at cutting which represented the loss of a major proportion of photosynthetically active tissue. Hence, the trials investigated the severe end of the range of defoliation intensities which might be encountered in practice. Despite the large loss of leaf, all plants regrew following cutting, including those defoliated completely. This indicated that young plants of sheep's burnet could tolerate intense defoliation satisfactorily. However, regrowth of partially defoliated plants was superior to those defoliated completely, as discussed previously. Experiments involving a range of defoliation intensities, expressed as proportions of pre-cut herbage, would provide valuable complementary information on suitable levels of partial defoliation. Removal of up to 50% of the foliage of rangeland pasture has been suggested as the most prudent grazing management and that least likely to cause deterioration (Jameson, 1963). This may be relevant for sheep's burnet also. Grazing experiments involving various levels of defoliation intensity would provide information complementary to that obtained in these cutting experiments. Factors such as animal selectivity and treading damage (Watkin and Clements, 1978) are important in most grazing situations and are practically impossible to simulate in cutting trials.

The ability of sheep's burnet to survive and regrow in dry areas is documented widely (McTaggart, 1935; Le Houerou, 1979; Sheppard and Wills, 1985, 1986). The results for plants growing in large planter bags under glasshouse conditions (Chapter 9) suggested that a key factor conferring this advantage was osmotic adjustment. This occurs in numerous other species (Turner and Begg, 1978; Morgan, 1984; Johnson *et al.*, 1984; Blum, 1989) but its extent is dependent on the drying down time intervals. More elaborate techniques are required to test for the existence of osmotic adjustment but, if found, it might be possible to improve osmotic adjustment capabilities through plant breeding, thereby enhancing herbage production of sheep's burnet in dry environments.

11.5 SUMMARY AND RECOMMENDATIONS

1. Sheep's burnet is slow to emerge compared to lucerne and birdsfoot trefoil, which may reduce its competitive ability. Sheep's burnet should therefore only be

sown alone or at moderately high sowing rates with other slowly to slightly faster emerging species. A weed-free seed bed, or at least a markedly reduced vegetative cover, should be a major objective. Birdsfoot trefoil, and possibly lucerne, may be suitable companion species for sheep's burnet but these need to be evaluated in a wider range of environments.

2. The commercially available seed line from Oregon, USA (similar to 'Delar') and the early seed line from the Cockayne plots of Central Otago, New Zealand, should both be evaluated further, preferably in trials established from seed, to identify their most suitable environments.

3. Final emergence of Oregon sheep's burnet under non-limiting moisture conditions may be similar over a range of constant temperatures (10-25°C) or vary in the field probably largely in response to differences in soil temperature. This lack of agreement requires further investigation and it may be due to the effect of alternating temperatures. Satisfactory emergence can be achieved by sowing into moist soil with temperatures greater than 5°C. Moist substrates were used throughout the emergence studies and therefore it would now be appropriate to investigate emergence characteristics in response to variable moisture supply.

4. Large seed may improve levels of emergence and increase the proportion of "doubles", as well as enhance emergence rate and early vegetative growth. Seed growers should be encouraged to produce larger seed and the feasibility of a breeding programme to produce such seed should be evaluated. No superior optimum sowing depth was found, partly because depth interacted with both temperature and seed size. A repeat study involving a greater range of sowing depths, such as 5-30 mm in increments of 5 mm, would probably identify the sowing depths below which emergence does not occur or at least those depths which delay emergence to an undesirable extent, agronomically.

5. An insecticide to control weevils may be required for satisfactory emergence of sheep's burnet on coastal sand country.

6. Superior emergence rates and early vegetative growth can be achieved by sowing into increasingly warm soils. However, the optimum soil temperature for sowing must be a compromise between those temperatures which provide high emergence, and those which lower the risk of soil moisture deficits soon after sowing and the incidence of possible seedling diseases.

7. Young plants under mild conditions can be defoliated severely and survive satisfactorily, but the most suitable management is lenient defoliation. Under most circumstances, complete defoliation (0% rLA) should be avoided. Nonstructural carbohydrate reserves have a role in supplying energy for regrowth, although current photosynthate from residual leaves is probably the more important source in many situations. Radioactive labelling of the nonstructural carbohydrates would assist in determining the relative importance of current and reserve fractions. This information would be valuable in evaluating the merits of retaining leaf area following defoliation.

8. Herbage accumulation of swards of sheep's burnet was similar to that of lucerne and birdsfoot trefoil when defoliated partially to leave a plant height of 5-7 cm. Numerous incomplete leaves remained in this residual component, which presumably contributed to the assimilate supply of regrowth, and such a defoliation height may be suitable in general practice. Under similar defoliation management in autumn, foliar regrowth after approximately four weeks was in a suitable state for further defoliation and this frequency could be generally applicable during autumn. Determining appropriate frequencies of defoliation for young and old swards of sheep's burnet, at different times of the year, should be the next research interest in the regrowth of the species. Grazing studies involving frequencies and intensities of defoliation are a necessary sequel to the cutting trials.

9. Several selection criteria may be useful for the improvement of sheep's burnet for soil conservation and/or forage production. Large seed may ensure earlier emergence and superior early vegetative growth. The rapid development of leaf area would confer a protective ground cover advantage as well as enhanced herbage accumulation. Selection of material which is tolerant of frequent, intense defoliation

at young stages could be valuable since overgrazing is a common problem in semi-arid areas where sheep's burnet is most suitable. Rapid regrowth ability is a desirable attribute. If osmotic adjustment in sheep's burnet is confirmed in later studies, selection for increased ability would likely prolong and/or enhance leaf area, and perhaps herbage mass, under dry conditions. Sheep's burnet is in the relatively early stages of domestication and it is expected that there might be considerable genetic variation to utilise in plant improvement programmes.

10. Multivariate analysis of variance and multiple discriminant functions were valuable in simultaneously analysing the numerous characters measured in each experiment. They should be used more widely in agronomic research. Multiple discriminant functions were particularly advantageous in identifying, without bias, those plant characters which were most important in discriminating between the various treatments. This information may be used to select a smaller set of characters for future similar research.

11. The three objectives presented in Chapter 1 were achieved satisfactorily by the experiments conducted. It was endeavoured to investigate as many aspects of the establishment and regrowth of sheep's burnet as possible and the research has provided a foundation for deciding upon future research directions and objectives, as discussed previously. It is expected that sheep's burnet will remain a viable option for revegetating some dryland areas in the South Island of New Zealand and become useful similarly in the North Island.

BIBLIOGRAPHY

Ackerson, R.C., 1985: Osmoregulation in cotton in response to water stress. *Plant Physiology 77:* 309-312.

Alberda, Th., 1966: The influence of reserve substances on dry-matter production after defoliation. *Proceedings Xth International Grassland Congress, Helsinki.* pp. 140-147.

Alcock, M.B. and E.W. Morgan, 1966: The effect of defoliation on the yield of mixtures of S22 (diploid) and Tetra (tetraploid) Italian ryegrass in early establishment. *Journal of the British Grassland Society 21:* 62-64.

Allard, R.W., 1960: *Principles of plant breeding.* John Wiley & Sons, Inc. New York. 485 pp.

Anderburg, M.R., 1973: *Cluster analysis for applications.* Academic Press, New York. 359 pp.

Angus, J.F.; Cunningham, R.B.; Moncur, M.W. and D.H. MacKenzie, 1981: Phasic development in field crops. 1. Thermal response in the seedling phase. *Field Crops Research 3:* 365-378.

Anon, 1951: C.S.I.R.O. Australia. *Third Annual Report for the year ending 30 June 1951, Canberra.* pp. 15-17.

Anon, 1957: Tara Hills, North Otago. *Soil Conservation and Rivers Control Council, 15th Annual Report.*

Anon, 1958: Pastures. *Report of Department of Agriculture New South Wales, 1957.* p. 28.

Anslow, R.C., 1967: Frequency of cutting and sward production. *Journal of Agricultural Science (Cambridge) 68:* 377-384.

Antipov, N.I., 1977: Water exchange of stems, leaf petioles, and leaf blades. Translated from *Fiziologiya Rastenii 24:* 785-789.

Archer, M., 1971: Preliminary studies on the palatability of grasses, legumes and herbs to horses. *Veterinary Record 89:* 236-240.

Armstrong, D.G.; Cook, H. and B. Thomas, 1950: The lignin and cellulose contents of certain grassland species at different stages of growth. *Journal of Agricultural Science 40:* 93-99.

Armstrong, R.H.; Thomas, B. and K. Horner, 1953: The availability of calcium in three herbs of grassland. *Journal of Agricultural Science 43:* 337-342.

Arnott, R.A., 1969: The effect of seed weight and depth of sowing on the emergence and early seedling growth of *L. perenne. Journal of the British Grassland Society 24:* 104-110.

Aspinall, D., 1986: Metabolic effects of water and salinity stress in relation to expansion of the leaf surface. *Australian Journal of Plant Physiology 13:* 59-73.

Atkinson, C.J., 1986: The effect of clipping on net photosynthesis and dark respiration rates of plants from an upland grassland, with reference to carbon partitioning in *Festuca ovina. Annals of Botany 58:* 61-72.

Ayers, A.D., 1952: Seed germination as affected by soil moisture and salinity. *Agronomy Journal 44:* 82-84.

Baeumer, K. and C.T. de Wit, 1968: Competitive interference of plant species in monocultures and mixed stands. *Netherlands Journal of Agricultural Science 16:* 103-122.

Bahler, C.; Hill, Jr. R.R. and R.A. Byers, 1989: Comparison of logistic and Weibull functions: the effect of temperature on cumulative germination of alfalfa. *Crop Science 29:* 142-146.

Baker, A.M. and A. Younger, 1987: Factors affecting the leaf extension rate of perennial ryegrass in spring. *Grass and Forage Science 42:* 381-389.

Baker, C.J., 1970: The present methods of pasture establishment. *Proceedings of the New Zealand Grassland Association 31:* 52-59.

Barker, D.J., 1983: The effects of summer moisture stress and its interaction with spring cutting managements on the production and persistence of a ryegrass (*Lolium perenne L.*) sward. M.Ag.Sc. thesis, Massey University, Palmerston North.

Barker, D.J. and A.C.P. Chu, 1985: Strategies for dryland pasture management - a review. *Proceedings of the Agronomy Society of New Zealand 15:* 115-120.

Barlow, E.W.R., 1986: Water relations of expanding leaves. *Australian Journal of Plant Physiology 13:* 45-58.

Barta, A.L., 1988a: Response of alfalfa and birdsfoot trefoil to shoot removal and root anoxia. *Crop Science 28:* 275-278.

Barta, A.L., 1988b: Response of field grown alfalfa to root waterlogging and shoot removal. I. Plant injury and carbohydrate and mineral content of roots. *Agronomy Journal 80:* 889-892.

Bartlett, M.S., 1947: Multivariate analysis. *Journal of the Royal Statistical Society*

(Series B) 9: 176-197.

Bean, E.W., 1973: Seed quality: its variation, control, and importance in breeding and varietal assessment. *Annual Report of the Welsh Plant Breeding Station 1972, Aberystwyth. pp.* 194-208.

Beard, J.B. and W.H. Daniel, 1965: Effect of temperature and cutting on the growth of creeping bentgrass (*Agrostis palustris* Huds.) roots. *Agronomy Journal 57:* 249-250.

Beevers, H., 1969: Metabolic sinks. In: Eastin, J.D.; Haskins, F.A.; Sullivan, C.Y. and C.H.M. van Bavel (eds.) *Physiological Aspects of Crop Yield.* American Society of Agronomy and Crop Science Society of America, Madison, Wisconsin. pp. 169-180.

Begg, J.E. and N.C. Turner, 1976: Crop water deficits. *Advances in Agronomy 28:* 161-217.

Bennett, O.L. and B.D. Doss, 1960: Effect of soil moisture level on root distribution of cool-season forage species. *Agronomy Journal 52:* 204-207.

Berendse, F., 1981: Competition between plant populations with different rooting depths. 11. Pot experiments. *Oecologia* (Berl.) *48:* 334-341.

Beveridge, J.L. and C.P. Wilsie, 1959: Influence of depth of planting, seed size, and variety on emergence and seedling vigour in alfalfa. *Agronomy Journal 51:* 731-734.

Bewley, J.D., 1979: Physiological aspects of desiccation tolerance. *Annual Review of Plant Physiology 30:* 195-238.

Bircham, J.S. and C.J. Korte, 1984: Principles of herbage production. *New Zealand Agricultural Science 18:* 123-126.

Black, J.N., 1956: The influence of seed size and depth of sowing on pre-emergence and early vegetative growth of subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Agricultural Research 7:* 98-109.

Black, J.N., 1957a: The influence of varying light intensity on the growth of herbage plants. *Herbage Abstracts 27:* 89-98.

Black, J.N., 1957b: The early vegetative growth of three strains of subterranean clover (*Trifolium subterraneum* L.) in relation to size of seed. *Australian Journal of Agricultural Research 8:* 1-14.

Black, J.N., 1957c: Seed size as a factor in the growth of subterranean clover (*Trifolium subterraneum* L.) under spaced and sward conditions. *Australian Journal of Agricultural Research 8:* 335-351.

Black, J.N., 1958: Competition between plants of different initial seed sizes in swards of subterranean clover with particular reference to leaf area and the mean climate. *Australian Journal of Agricultural Research 9:* 299-318.

Black, J.N., 1959: Seed size in herbage legumes. *Herbage Abstracts 29:* 235-241.

Blaser, R.E.; Brown, R.H. and H.T. Bryant, 1966: The relationship between carbohydrate accumulation and growth of grasses under different microclimates. *Proceedings 10th International Grasslands Congress.* pp. 147-150.

Blum, A., 1989: Osmotic adjustment and growth of barley genotypes under drought stress. *Crop Science 29:* 230-233.

Booysen, P. deV. and C.J. Nelson, 1975: Leaf area and carbohydrate reserves in regrowth of tall fescue. *Crop Science 15:* 262-266.

Boyer, J.S., 1969: Measurement of the water status of plants. *Annual Review Plant Physiology 20:* 351-364.

Boyer, J.S., 1970a: Leaf enlargement and metabolic rates in corn, soybeans, and sunflowers at various leaf water potentials. *Plant Physiology 46:* 233-235.

Boyer, J.S., 1970b: Differing sensitivity of photosynthesis to low leaf water potential in corn and soybeans. *Plant Physiology 46:* 236-239

Boyer, J.S., 1976: Photosynthesis at low water potentials. *Philosophical Transactions of the Royal Society London B. 273:* 501-512.

Boyer, J.S., 1983: Subcellular mechanisms of plant response to low water potential. *Agricultural Water Management 7:* 239-248.

Boyer, J.S., 1985: Water transport. *Annual Review of Plant Physiology 36:* 473-516.

Boyer, J.S. and H.G. McPherson, 1975: Physiology of water deficits in cereal crops. *Advances in Agronomy 27:* 1-23.

Brash, D.W., 1985: Lucerne cultivar comparisons: herbage yields under irrigation and on dryland in Central Otago. *Proceedings of the Agronomy Society of New Zealand 15:* 121-126.

Bremner, P.M.; Eckersall, R.M. and R.K. Scott, 1963: The relative importance of embryo size and endosperm size in causing the effects associated with seed size in wheat. *Journal of Agricultural Science 61:* 139-145.

Broom, D.M. and G.W. Arnold, 1986: Selection by grazing sheep of pasture plants at low herbage availability and responses of the plants to grazing. *Australian Journal of Agricultural Research 37:* 527-538.

Brougham, R.W., 1955: A study in rate of pasture growth. *Australian Journal of Agricultural Research 6:* 804-812.

Brougham, R.W., 1956: Effect of intensity of defoliation on regrowth of pasture. *Australian Journal of Agricultural Research 7:* 377-387.

Brougham, R.W., 1958: Interception of light by the foliage of pure and mixed stands of pasture plants. *Australian Journal of Agricultural Research 9:* 39-52.

Brown, K.R., 1977: Parent seed weight, plant growth and seeding in 'Grasslands Tama' Westerwolds ryegrass. *New Zealand Journal of Experimental Agriculture 5:* 143-146.

Brown, P.W. and C.B. Tanner, 1983: Alfalfa stem and leaf growth during water stress. *Agronomy Journal 75:* 799-805.

Brown, R.F., 1987: Defoliation regrowth and dry matter partitioning in two arid zone grasses, *Aristida armata* and *Thyridolepis mitchelliana*. *Australian Journal of Agricultural Research 38:* 881-893.

Brown, R.H. and R.E. Blaser, 1965: Relationship between reserve carbohydrate accumulation and growth rate in orchardgrass and tall fescue. *Crop Science 5:* 577-582.

Brown, R.H. and R.E. Blaser, 1968: Leaf area index in pasture growth. *Herbage Abstracts 38:* 1-9.

Brown, R.H.; Cooper, R.B. and R.E. Blaser, 1966: Effects of leaf age on efficiency. *Crop Science 6:* 206-209.

Bryan, W.B., 1985: Effects of sod-seeding legumes on hill land pasture productivity and composition. *Agronomy Journal 77:* 901-905.

Bunce, J.A., 1978: Effects of water stress on leaf expansion, net photosynthesis and vegetative growth of soybeans and cotton. *Canadian Journal of Botany 56:* 1492-1498.

Bunce, J.A., 1982: Effect of water stress on photosynthesis in relation to diurnal accumulation of carbohydrates in source leaves. *Canadian Journal of Botany 60:* 195-200.

Campbell, M.H., 1979: Aerial seeding in Iran. *Agricultural Gazette of New South Wales 90:* 6-8.

Carberry, P.S. and L.C. Campbell, 1989: Temperature parameters useful for modelling the germination and emergence of pearl millet. *Crop Science 29:* 220-223.

Carleton, A.E. and C.S. Cooper, 1972: Seed size effects upon seedling vigour of three forage legumes. *Crop Science 12:* 183-186.

Carleton, A.E.; Cooper, C.S. and L.E. Wiesner, 1968: Effect of seed pod and temperature on speed of germination and seedling elongation of sainfoin (*Onobrychis viciaefolia* Scop.) *Agronomy Journal 60:* 81-84.

Castonguay, Y. and P.A. Dube, 1985: Climatic analysis of a phenological zonation: a multivariate approach. *Agricultural and Forest Meteorology 35:* 31-45.

Causton, D.R. and J.C. Venus, 1981: *The biometry of plant growth.* Edward Arnold, London. 307 pp.

Charlton, J.F.L., 1977: Establishment of pasture legumes in North Island hill country. II. Seedling establishment and plant survival. *New Zealand Journal of Experimental Agriculture 5:* 385-390.

Charlton, J.F.L., 1989: Temperature effects on germination of 'Grasslands Maku' lotus and other experimental lotus selections. *Proceedings of the New Zealand Grassland Association 50:* 197-201.

Charlton, J.F.L.; Wilson, E.R.L. and M.D. Ross, 1978: Plant introduction trials. Performance of *Lotus corniculatus* introductions as spaced plants in Manawatu. *New Zealand Journal of Experimental Agriculture 6:* 201-206.

Charlton, J.F.L. and E.R. Thom, 1984: Establishment and persistence of new herbage species and cultivars. *Proceedings of the New Zealand Institute of Agricultural Science 18:* 130-135.

Charlton, J.F.L.; Hampton, J.G. and D.J. Scott, 1986: Temperature effects on germination of New Zealand herbage grasses. *Proceedings of the New Zealand Grassland Association 47:* 165-172.

Chatterton, N.J.; Carlson, G.E.; Hart, R.H. and W.E. Hungerford, 1974: Tillering, nonstructural carbohydrates, and survival relationships in alfalfa. *Crop Science 14:* 783-787.

Christ, R.A., 1978: The elongation rate of wheat leaves. II. Effect of sudden light change on the elongation rate. *Journal of Experimental Botany 29:* 611-618.

Christiansen, S. and T. Svejcar, 1987: Grazing effects on the total nonstructural carbohydrate pools in Caucasian bluestem. *Agronomy Journal 79:* 761-764.

Chu, A.C.P., 1979: Aspects of water deficit and vegetative growth in selected pasture and forage grasses. Ph.D. thesis, Massey University, Palmerston North.

Chu, A.C.P. and J.P. Kerr, 1977: Leaf water potential and leaf extension in a sudax crop. *New Zealand Journal of Agricultural Research 20:* 467-470.

Chu, A.C.P. and H.G. McPherson, 1977: Sensitivity to desiccation of leaf extension in prairie grass. *Australian Journal of Plant Physiology 4:* 381-387.

Chu, A.C.P.; McPherson, H.G. and G. Halligan, 1979: Recovery growth following water deficits of different duration in Prairie grass. *Australian Journal of Plant Physiology 6:* 255-263.

Clarke, B.J., 1985: Cleaning seeds by fluidization. *Journal of Agricultural Engineering Research 31:* 231-242.

Clarke, C. and K.G. Moore, 1986: Effects of seed treatments on the emergence of oil seed rape seedlings from compacted soil. *Annals of Botany 58:* 363-369.

Clatterbuck, W.K. and F.T. Bonner, 1985: Utilisation of food reserves in Quercus seed during storage. *Seed Science and Technology 13:* 121-128.

Cochran, W.G., 1947: Some consequences when the assumptions for the analysis of variance are not satisfied. *Biometrics 3:* 22-38.

Cochran, W.G. and G.M. Cox, 1957: *Experimental designs.* 2nd edition. John Wiley, New York. 611 pp.

Cockayne, L., 1920a: An economic investigation of the montane tussock-grassland of New Zealand. VIII. An experiment in Central Otago concerning the relative palatability for sheep of various pasture-plants. *New Zealand Journal of Agriculture 21*: 176-188.

Cockayne, L., 1920b: An economic investigation of the montane tussock-grassland of New Zealand. IX. Further details regarding the Earnscleugh (Central Otago) palatability experiment. *New Zealand Journal of Agriculture 21:* 324-334.

Cockayne, L., 1921: An economic investigation of the montane tussock-grassland of New Zealand. X. The effect of spelling a heavily grazed pasture in Central Otago. *New Zealand Journal of Agriculture 22:* 148-157.

Cockayne, L., 1922a: An economic investigation of the montane tussock grasslands of New Zealand. XII. The regrassing experiments in Central Otago. Part II. Objects, principles and methods. *New Zealand Journal of Agriculture 25:* 1-11.

Cockayne, L., 1922b: An economic investigation of the montane tussock-grassland of New Zealand. XII. The regrassing experiments in Central Otago. Part III. Results up to May, 1922. *New Zealand Journal of Agriculture 25:* 129-144.

Cole, J.W.L. and J.E. Grizzle, 1966: Applications of multivariate analysis of variance of repeated measurements experiments. *Biometrics 22:* 810-828.

Collis-George, N. and J.E. Sands, 1962: Comparison of the effects of the physical and chemical components of soil water on seed germination. *Australian Journal of Agricultural Research 13:* 575-584.

Colman, R.L. and A. Lazenby, 1970: Factors affecting the response of some tropical and temperate grasses to fertiliser nitrogen. *Proceedings XIth International Grassland Congress, Surfers Paradise.* pp. 392-397.

Conroy, J.P.; Virgona, J.M.; Smillie, R.M. and E.W. Barlow, 1988: Influence of drought acclimation and CO2 enrichment on osmotic adjustment and chlorophyll a fluorescence of sunflower during drought. *Plant Physiology 86:* 1108-1115.

Cooley, W.W. and P.R. Lohnes, 1971: Multivariate Data Analysis. John Wiley and Sons, New York. 364 pp.

Cooper, C.S., 1966: Response of birdsfoot trefoil and alfalfa to various levels of shade. *Crop Science 6:* 63-66.

Cooper, C.S., 1977: Growth of the legume seedling. *Advances in Agronomy 29:* 119-139.

Cooper, C.S. and C.A. Watson, 1968: Total available carbohydrates in roots of sainfoin (*Onobrychis viciaefolia* Scop.) and alfalfa (*Medicago sativa* L.) when grown under several management regimes. *Crop Science 8:* 83-85.

Cooper, J.P. and N.M. Tainton, 1968: Light and temperature requirements for the growth of tropical and temperate grasses. *Herbage Abstracts 38:* 167-176.

Cornish, P.S., 1983: The contribution of a temporary watertable to surface soil water content and the establishment of surface sown seeds. *Australian Journal of Experimental Agriculture and Animal Husbandry 23:* 407-411.

Cox, J.R., 1984: Temperature, timing of precipitation and soil texture effects on germination, emergence and seedling survival of South African lovegrass. *Journal of South African Botany 50:* 159-170.

Crump, S.L., 1951: The present status of variance component analysis. *Biometrics 7:* 1-16.

Cruz, R.T.; O'Toole, J.C.; Dingkuhn, M.; Yambao, E.B.; Thangaraj, M. and S.K. De Datta, 1986: Shoot and root responses to water deficits in rainfed lowland rice. *Australian Journal of Plant Physiology 13:* 567-575.

Culleton, N. and V. McCarthy, 1983: Germination of *Lolium perenne, Bromus willdenowii* and *Trifolium pratense. Irish Journal of Agricultural Research 22:* 31-36.

Curtis, K. and B.D. McKersie, 1984: Growth potential of the axis as a determinant of seedling vigour in birdsfoot trefoil. *Crop Science 24:* 47-50.

Cutler, J.M.; Steponkus, P.L.; Wach, M.J. and K.W. Shahan, 1980: Dynamic aspects and enhancement of leaf elongation in rice. *Plant Physiology* 66: 147-152.

Cuykendall, C.H. and G.C. Marten, 1968: Defoliation by sheep-grazing versus mower-clipping for evaluation of pasture. *Agronomy Journal 60:* 404-408.

Dale, J.E., 1988: The control of leaf expansion. *Annual Review of Plant Physiology and Plant Molecular Biology 39:* 267-295.

Dalianio, C.D., 1980: Effect of temperature and seed size on speed of germination, seedling elongation and emergence of berseem and Persian clovers (*Trifolium alexandrinum* and *T. resupinatum*). *Seed Science and Technology 8:* 323-331.

Daly, M.J., 1984: Lucerne comparison: an evaluation of lucerne cv. Rere in a semi-arid high country environment. *New Zealand Journal of Experimental Agriculture 12:* 277-281.

Darbyshire, B.; Henry, R.J.; Melhuish, F.M. and R.K. Hewett, 1979: Diurnal variations in non-structural carbohydrates, leaf extension, and leaf cavity carbon dioxide concentrations in *Allium cepa* L. *Journal of Experimental Botany 30:* 109-118.

Davidson, J.L. and C.M. Donald, 1958: The growth of swards of subterranean clover with particular reference to leaf area. *Australian Journal of Agricultural Research 9:* 53-72.

Davidson, J.L. and F.L. Milthorpe, 1965: Carbohydrate reserves in the regrowth of cocksfoot (*Dactylis glomerata* L.). *Journal of the British Grassland Society 20:* 15-18.

Davies, A., 1965: Carbohydrate levels and regrowth in perennial rye-grass. *Journal of Agricultural Science 65:* 213-221.

Davies, A.; Evans, M.E. and C.J. Pollock, 1989: Influence of date of tiller origin on leaf extension rates in perennial and Italian ryegrass at 15°C in relation to flowering propensity and carbohydrate status. *Annals of Botany 63:* 377-384.

Davies, H.V. and P.T. Slack, 1981: The control of food mobilization in seeds of dicotyledonous plants. *New Phytologist 88:* 41-51.

de Wit, C.T., 1960: On competition. *Versl. landbouwk. Onderzoek, Netherlands 66:* 1-82.

de Wit, C.T. and J.P. van den Bergh, 1965: Competition between herbage plants. *The Netherlands Journal of Agricultural Science 13:* 212-221.

Dekker, R.F.H. and G.N. Richards, 1971: Determination of starch in plant material. *Journal of the Science of Food and Agriculture 22:* 441-444.

Derwyn, R.; Whalley, B.; McKell, C.M. and L.R. Green, 1966: Seedling vigour and the early nonphotosynthetic stage of seedling growth in grasses. *Crop Science 6:* 147-150.

Donald, C.M., 1958: The interaction of competition for light and for nutrients. *Australian Journal of Agricultural Research 9*: 421-432.

Donald, C.M., 1963: Competition among crop and pasture plants. *Advances in Agronomy 15:* 1-118.

Donald, C.M. and J.N. Black, 1958: The significance of leaf area in pasture measurement. *Herbage Abstracts 28:* 1-6.

Doss, B.D.; Ashley, D.A. and O.L. Bennett, 1960: Effect of soil moisture regime on root distribution of warm season forage species. *Agronomy Journal 52:* 569-572.

Doss, B.D.; Pearson, R.W. and H.T. Rogers, 1974: Effect of soil water stress at various growth stages on soybean yield. *Agronomy Journal 66:* 297-299.

Douglas, G.B., 1985: Ecology and the influence of environmental factors on sheep's burnet and closely related species. Internal Report No. 151. Soil Conservation Centre, Aokautere, Ministry of Works and Development, Palmerston North. 25 pp.

Douglas, J.A., 1970: The Cockayne plots of Central Otago. *Proceedings of the New Zealand Ecological Society 17:* 18-24.

Douglas, J.A. and J.W. Kinder, 1973: Production and composition of various lucerne and grass mixtures in a semi-arid environment. *New Zealand Journal of Experimental Agriculture 12:* 23-27.

Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. and F. Smith, 1956: Colorimetric method for determination of sugars and related substances. *Analytical Chemistry 28:* 350-356.

Duke, J.A., 1981: *Handbook of legumes of world economic importance.* Plenum Press, New York. 345 pp.

Easton, H.S. and W. Stiefel, 1983: Lucerne cultivars: interaction with site, management and grass. *Proceedings of the New Zealand Grassland Association 44:* 120-126.

Eisenhart, C., 1947: The assumptions underlying the analysis of variance. *Biometrics 3:* 1-21.

Erickson, L.C., 1946: The effect of alfalfa seed size and depth of seeding upon the subsequent procurement of stand. *Journal of the American Society of Agronomy 38:* 964-973.

Evans, P.S., 1970: Root growth of *Lolium perenne* L. I. Effect of plant age, seed weight and nutrient concentration on root weight, length, and number of apices. *New Zealand Journal of Botany 8:* 344-356.

Evans, P.S., 1971: Root growth of *Lolium perenne* L. II. Effects of defoliation and shading. *New Zealand Journal of Agricultural Research 14:* 552-562.

Evans, P.S., 1973: Effect of seed size and defoliation at three development stages on root and shoot growth of seedlings of some common pasture species. *New Zealand Journal of Agricultural Research 16:* 389-394.

Evert, E.F. and R.L. Hartman, 1984: Additions to the vascular flora of Wyoming. *Great Basin Naturalist 44:* 482-483.

FAO, 1965: *Soil erosion by water: Some measures for its control on cultivated lands.* FAO Agricultural Development Paper No. 81. Food and Agriculture Organization of the United Nations, Rome. 284 pp.

Field, T.R.O.; Pearson, C.J. and L.A. Hunt, 1976: Effects of temperature on the growth and development of alfalfa. *Herbage Abstracts 46:* 145-150.

Fisher, A.G.; Brick, M.A.; Riley, R.H. and D.K. Christensen, 1987: Dryland stand establishment and seed production of revegetation species. *Crop Science 27:* 1303-1305.

Francis, S.M. and M.L. Smetham, 1985: Pasture utilisation and its effect on herbage quality. *Proceedings of the New Zealand Grassland Association 46:* 221-225.

Fransen, S.C. and C.S. Cooper, 1976: Seed weight effects upon emergence, leaf development, and growth of the sainfoin (*Onobrychis* spp.). *Crop Science 16:* 434-437.

Frasier, G.W.; Woolhiser, D.A. and J.R. Cox, 1984: Emergence and seedling survival of two warm-season grasses as influenced by the timing of precipitation: a greenhouse study. *Journal of Range Management 37:* 7-11.

Friend, D.J.C.; Helson, V.A. and J.E. Fisher, 1962: The rate of dry weight accumulation in Marquis wheat as affected by temperature and light intensity. *Canadian Journal of Botany 40:* 939-955.

Gabrielsen, B.C.; Smith, D.H. and C.E. Townsend, 1985: Cicer milkvetch and

alfalfa as influenced by two cutting schedules. *Agronomy Journal 77:* 416-422.

Garcia-Huidobro, J.; Monteith, J.L. and G.R. Square, 1982: Time, temperature and germination of pearl millet. *Journal of Experimental Botany 33:* 288-296.

Gates, C.T., 1955: The responses of young tomato plants to a brief period of water shortage. I. The whole plant and its principal part. *Australian Journal of Biological Science 8:* 196-214.

Gay, P.E.; Grubb, P.J. and H.J. Hudson, 1982: Seasonal changes in the concentrations of nitrogen, phosphorus and potassium, and in the density of mycorrhiza, in biennial and matrix-forming perennial species of closed chalkland turf. *Journal of Ecology 70:* 571-593.

Giaquinta, R., 1978: Source and sink leaf metabolism in relation to phloem translocation, carbon partitioning and enzymology. *Plant Physiology 61:* 380-385.

Gifford, R.M. and L.T. Evans, 1981: Photosynthesis, carbon partitioning and yield. *Annual Review of Plant Physiology 32:* 485-509.

Given, D.R., 1982: Checklist of dicotyledons naturalised in New Zealand. 13. Rosaceae. *New Zealand Journal of Botany 20:* 221-226.

Gleeson, A.C. and C.A. McGilchrist, 1980: Mixture diallel experiments with unequal proportions of genotypes. *Journal of Agricultural Science (Cambridge) 95:* 525-532.

Glenday, A.C. and S.O. Fejer, 1956: The use of discriminant functions in the selection of pasture plants, with particular reference to the *Lolium* species. *Proceedings 7th International Grasslands Congress.* pp. 461-470.

Goloff, A.A., Jr. and F.A. Bazzaz, 1975: A germination model for natural seed populations. *Journal of Theoretical Biology 52:* 259-283.

Goodman, S.M. and W.R. Scott, 1985: Seedling emergences of wheat as affected by sowing depth, seed size and sowing date. *Proceedings of the Agronomy Society of New Zealand 15:* 65-69.

Gordon, I.L.; Balaam, L.N. and N.F. Derera, 1979: Selection against sprouting damage in wheat. II. Harvest ripeness, grain maturity and germinability. *Australian Journal of Agricultural Research 30:* 1-17.

Grant, S.A.; Barthram, G.T. and L. Torvell, 1981: Components of regrowth in grazed and cut *Lolium perenne* swards. *Grass and Forage Science 36:* 155-168.

Guy, M.C.; Watkin, B.R. and D.A. Clark, 1981: Effect of season, stocking rate,

and grazing duration on diet selected by hoggets grazing mixed grass-clover pastures. *New Zealand Journal of Experimental Agriculture 9:* 141-146.

Hafenrichter, A.L.; Foster, R.B. and J.L. Schwendiman, 1965: Effect of storage at four locations in the west on longevity of forage seeds. *Agronomy Journal 57:* 143-147.

Haissig, B.E. and R.E. Dickson, 1979: Starch measurement in plant tissue using enzymatic hydrolysis. *Physiologia Plantarum 47:* 151-157.

Hall, R.L., 1974a: Analysis of the nature of interference between plants of different species. I. Concepts and extension of the de Wit analysis to examine effects. *Australian Journal of Agricultural Research 25*: 739-747.

Hall, R.L., 1974b: Analysis of the nature of interference between plants of different species. II. Nutrient relations in a Nandi Setaria and Greenleaf Desmodium association with particular reference to potassium. *Australian Journal of Agricultural Research 25:* 749-756.

Hall, R.L., 1978: The analysis and significance of competitive and non-competitive interference between species. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 11. CSIRO, Australia. pp. 163-174.

Hall, R.L., 1974: Analysis of the nature of interference between plants of different species. II. Nutrient relations in a Nandi *Setaria* and Greenleaf *Desmodium* association with particular reference to potassium. *Australian Journal of Agricultural Research 25:* 749-756.

Halmer, P., 1985: The mobilization of storage carbohydrates in germinated seeds. *Physiologie Vegetale 23:* 107-125.

Hampton, J.G., 1981: The relationship between field emergence, laboratory germination, and vigour testing of New Zealand seed wheat lines. *New Zealand Journal of Experimental Agriculture 9:* 191-197.

Hampton, J.G.; Charlton, J.F.L.; Bell, D.D. and D.J. Scott, 1987: Temperature effects on the germination of herbage legumes in New Zealand. *Proceedings of the New Zealand Grassland Association 48:* 177-183.

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

Harper,J.L., 1967: A Darwinian approach to plant ecology. *Journal of Ecology 55:* 247-270.

Harris, R.J., 1975: A Primer of Multivariate Statistics. Academic Press, London. 332 pp.

Harris, R.J., 1985: *A primer of multivariate statistics 2nd Edition.* Academic Press,

London. 576 pp.

Harris, W., 1972: Shading, defoliation, temperature, growth stage and residual fertility effects on competition between *Rumex acetosella*, *Trifolium repens* and *Lolium (multiflorum x perenne). New Zealand Journal of Agricultural Research 15:* 687-705.

Harris, W., 1971: The effects of density, cutting height and white clover (*Trifolium repens* L.) on the structure of a ryegrass (*Lolium* spp.) population. *Journal of Agricultural Science, Cambridge 77:* 385-395.

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., 1978: Defoliation as a determinant of the growth, persistence and composition of pasture. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 5. CSIRO, Australia. pp. 67-85.

Harris, W. and J.R. Sedcole, 1974: Competition and moisture stress effects on genotypes in three grass populations. *New Zealand Journal of Agricultural Research 17:* 443-454.

Harris, W. and V.J. Thomas, 1973: Competition among pasture plants. III. Effects of frequency and height of cutting on competition between white clover and two ryegrass cultivars. *New Zealand Journal of Agricultural Research 16:* 49-58.

Haskins, F.A. and H.J. Gorz, 1975: Influence of seed size, planting depth, and companion crop on emergence and vigour of seedlings in sweetclover. *Agronomy Journal 67:* 652-654.

Haslemore, R.M. and P.G. Roughan, 1976: Rapid chemical analysis of some plant constituents. *Journal of the Science of Food and Agriculture 27:* 1171-1178.

Hayes, P., 1975: The influence of seed weight on seedling growth in perennial ryegrass, tall fescue and Yorkshire fog. *Record of Agricultural Research 23:* 33-43.

Haynes, R.J., 1980: Competitive aspects of the grass-legume association. *Advances in Agronomy 33:* 227-261.

Healy, A.J., 1984: *Standard common names for weeds in New Zealand.* New Zealand Weed and Pest Control Society, 2nd Edition. 207 pp.

Heinrichs, D.H., 1963: Creeping alfalfas. *Advances in Agronomy 15:* 317-337.

Herberer, J.A.; Below, F.E. and R.H. Hageman, 1985: Drying method effect on leaf chemical constituents of four crop species. *Crop Science 25:* 1117-1119.

Herriott, J.B.D., 1958: The establishment of herbage species in Great Britain. *Herbage Abstracts 28:* 73-82.

Hill, J. and Y. Shimamoto, 1973: Methods of analysing competition with special reference to herbage plants. 1. Establishment. *Journal of Agricultural Science Cambridge 81:* 77-89.

Hodgson, J., 1979: Nomenclature and definitions in grazing studies. *Grass and Forage Science 34:* 11-18.

Hoglund, J.H.; Crush, J.R.; Brock, J.L.; Ball, P.R. and R.A. Carran, 1979: Nitrogen fixation in pasture. XII. General discussion. *New Zealand Journal of Experimental Agriculture 7:* 45-51.

Hope, K., 1968: *Methods of multivariate analysis.* Gordon and Breach Science Publishers, New York. 288 pp.

Hotelling, H., 1931: The generalization of Student's ratio. *Annals of Mathematical Statistics 2:* 360-378.

Hotelling, H., 1936: Relations between two sets of variates. *Biometrika 28:* 321-377.

Hsiao, T.C., 1973: Plant responses to water stress. *Annual Review Plant Physiology 24:* 519-570.

Hsiao, T.C. and E. Acevedo, 1974: Plant responses to water deficits, water-use efficiency, and drought resistance. *Agricultural Meteorology 14:* 59-84

Hsu, F.H.; Nelson, C.J. and W.S. Chow, 1984: A mathematical model to utilize the logistic function in germination and seedling growth. *Journal of Experimental Botany 35:* 1629-1640.

Hume, D.E. and T.J. Fraser, 1985: Establishing and managing recent cultivars in arable dryland pastures. In: Burgess, R.E. and J.L. Brock (eds.) *Using Herbage Cultivars.* Grassland Research and Practice Series No.3, New Zealand Grassland Association, Palmerston North. pp. 45-48

Humphreys, L.R., 1966: Pasture defoliation practice: a review. *Journal of the Australian Institute of Agricultural Science 32:* 93-105.

Hunter, J.R. and A.E. Erickson, 1952: Relation of seed germination to soil moisture tension. *Agronomy Journal 44:* 107-109.

Hur, S.N. and C.J. Nelson, 1985: Temperature effects on germination of birdsfoot trefoil and Seombadi. *Agronomy Journal 77:* 557-560.

Hussaini, S.H.; Goodman, M.M. and D.H. Timothy, 1977: Multivariate analysis and the geographical distribution of the world collection of finger millet.

Crop Science 17: 257-263.

Incoll, L.D.; Long, S.P. and M.R. Ashmore, 1981: SI units in publications in plant science. In: Smith, H. (ed.) *Commentaries in Plant Science.* Vol. 2. Oxford, Pergamon Press. pp. 83-96.

ISTA, 1976: International Rules for Seed Testing. *Seed Science and Technology 4:* 1-180.

ISTA, 1985: International Rules for Seed Testing. *Seed Science and Technology 13:* 300-513.

Ivens, G.W. and F. Lowe, 1980: A study of competition between seedlings of gorse (*Ulex europeaus* L.) and perennial ryegrass (*lolium perenne* L.) by means of replacement series experiments. *Weed Research 20:* 181-191.

Jameson, D.A., 1963: Response of individual plants to harvesting. *Botanical Review 29:* 532-594.

Jamieson, P.D., 1985: Soil moisture extraction patterns from irrigated and dryland arable crops in Canterbury. *Proceedings of the Agronomy Society of New Zealand. 15:* 1-6.

Jantii, A. and R. Heinonen, 1957: Effect of defoliation and soil moisture on grassland re-growth. *Journal of the British Grassland Society 12:* 56-61.

Jantii, A. and P.J. Kramer, 1956: Regrowth of pastures in relation to soil moisture and defoliation. *Proceedings 7th International Grasslands Congress.* pp. 33-43.

Johnson, R.C.; Nguyen, H.T. and L.I. Croy, 1984: Osmotic adjustment and solute accumulation in two wheat genotypes differing in drought resistance. *Crop Science 24:* 957-962.

Jolliffe, P.A.; Minjas, A.N. and V.C. Runeckles, 1984: A reinterpretation of yield relationships in series experiments. *Journal of Applied Ecology 21:* 227-243.

Jolliffe, P.A. and J. Hoddinott, 1988: The assessment of net dry matter partitioning by stepwise multivariate analysis of variance. *Canadian Journal of Botany 66:* 805-808.

Jordan, G.L. and M.R. Haferkamp, 1989: Temperature responses and calculated heat units for germination of several range grasses and shrubs. *Journal of Range Management 42:* 41-45.

Jordan, W.R. and J.T. Ritchie, 1971: Influence of soil water stress on evaporation, root absorption and internal water status of cotton. *Plant Physiology 48:* 783-788.

Jordan, W.R.; Brown, K.W. and J.C. Thomas, 1975: Leaf age as a determinant in stomatal control of water loss from cotton during water stress. *Plant Physiology 56:* 595-599.

Kahre, L., 1983: Germination sensu lato. *Advances in Research and Technology of Seeds 8:* 35-83.

Kanemasu, E.T.; Bark, D.L. and E.C. Choy, 1975: Effect of soil temperature on sorghum emergence. *Plant and Soil 43:* 411-417.

Kasperbauer, M.J. and T.G. Sutton, 1977: Influence of seed weight on germination, growth and development of tobacco. *Agronomy Journal 69:* 1000-1002.

Kaufmann, M.L. and A.D. McFadden, 1960: The competitive interaction between barley plants grown from large and small seeds. *Canadian Journal of Plant Science 40:* 623-629.

Kendall, M., 1975: Multivariate Analysis. Charles Griffin and Co. Ltd., London. 210 pp.

Kendall, M.G. and A. Stuart, 1958: The Advanced Theory of Statistics. Volume 1. Charles Griffin and Company, Ltd., London.

Kerr, J.P. and H.G. McPherson, 1978: Evapotranspiration and physiological responses to water stress of several pasture and crop species. *Proceedings of the New Zealand Grassland Association 39:* 70-78.

Khan, S.M. and A.M. Wilson, 1984: Nonstructural carbohydrates and dehydration tolerance of blue grama seedlings. *Agronomy Journal 76:* 637-642.

Kim, K.S. and J.B. Beard, 1988: Comparative turfgrass evapotranspiration rates and associated plant morphological characteristics. *Crop Science 28:* 328-331.

Koller, D., 1964: The survival value of germination - regulating mechanisms in the field. *Herbage Abstracts 34:* 1-7.

Kozov, N.I., 1965: Trials with *Poterium sanguisorba* in Rostov Province. *Zap. Leningrad Sel'khoz Inst. 98:* 58-62. (In *Herbage Abstracts 36:* 1175).

Kolstrup, E. and T.A. Wijmstra, 1977: A palynological investigation of the moershoofd, Hengelo and Denekamp Interstadials in the Netherlands. *Geologie en Mijnbouw 56:* 85-102.

Kriedemann, P.E., 1986: Stomatal and photosynthetic limitations to leaf growth. *Australian Journal of Plant Physiology 13:* 15-31.

Krieg, D.R., 1983: Photosynthetic activity during stress. *Agricultural Water Management 7:* 249-263.

Kumar, U.D.J.; Saraswathy, R. and V.S.R. Das, 1984: Differential performance of *Cleome gynandra* L. (C4) and *C. speciosa* L. (C3) under water, stress and recovery. *Environmental and Experimental Botany 24:* 305-310.

de Lacy, H., 1985: Sheep's burnet gets top marks from drought victims. *New Zealand Farmer 106:* 20-23.

Lafond, G.P. and R.J. Baker, 1986a: Effects of genotype and seed size on speed of emergence and seedling vigour in nine spring wheat cultivars. *Crop Science 26:* 341-346.

Lafond, G.P., and R.J. Baker, 1986b: Effects of temperature, moisture stress, and seed size on germination of nine spring wheat cultivars. *Crop Science 26:* 563-567.

Lam, A. and M.S. Ridout, 1985: Observations on variation in size and quality of ryegrass (*Lolium* spp.) seeds and effect on seedling emergence. *Seed Science and Technology 13:* 675-682.

Lambert, D.A., 1982: A study of growth in swards of timothy and meadow fescue. III. The effect of two levels of nitrogen under two cutting treatments. *Journal of Agricultural Science 59:* 25-32.

Lambert, M.G.; Rhodes, A.P.; Barker, D.J. and J.S. Bircham, 1985: Establishing and managing improved plants in hill country. In: Burgess, R.E. and J.L. Brock (eds.) *Using herbage cultivars.* Grassland Research and Practice Series No. 3, New Zealand Grassland Association, Palmerston North. pp. 31-34.

Lancashire, J.A., 1984: The physical boundaries of temperate pasture species in New Zealand. *Proceedings of the New Zealand Institute of Agricultural Science 18:* 98-110.

Lancashire, J.A. and J.L. Brock, 1983: Management of new cultivars for dryland. *Proceedings of the New Zealand Grassland Association 44:* 61-73.

Landsberg, J.J., 1977: Some useful equations for biological studies. *Experimental Agriculture 13:* 273-286.

Langer, R.H.M., 1973: Grass species and strains. In: Langer, R.H.M. (ed.) *Pastures and Pasture Plants.* Reed, Wellington. pp. 65-84.

Lawan, M.; Barnett, F.L.; Khaleeq, B. and R.L. Vanderlip, 1985: Seed density and seed size of pearl millet as related to field emergence and several seed and seedling traits. *Agronomy Journal 77:* 567-571.

Leach, G.J., 1978: The ecology of lucerne pastures. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 19. pp. 290-308. CSIRO, Australia.

Leafe, E.L.; Stiles, W. and S.E. Dickinson, 1974: Physiological processes influencing the pattern of productivity of the intensively managed grass sward. *Proceedings XIIth. International Grassland Congress, Moscow.* Vol 1, Part 1. pp. 442-457.

LeClerg, E.L.; Leonard, W.H. and A.G. Clark, 1962: *Field Plot Technique.* 2nd edition. Burgess Publishing Company, Minnesota. 373 pp.

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

Le Houerou, H.N., 1974: Principles, methods and techniques for range management and fodder production in the Mediterranean. FAO Range Management and Forage and Fodder Crops Improvement Study No. 2/Rev.1. FAO, Rome.

Le Houerou, H.N., 1979: Resources and potential of the native flora for fodder and sown pasture production in the arid and semi-arid zones of North Africa. In: Goodin, J.R. and D.K. Northington (eds.) *Arid Lands and Plant Resources, Proceedings of the International Arid Lands Conference on Plant Resources, Texas.* pp. 384-401.

Levy, E.B. and E.A. Madden, 1933: The point method of pasture analysis. *New Zealand Journal of Agriculture 46:* 267-279.

Lewis, E.J. and J.A. Garcia, 1979: The effect of seed weight and coleoptile tiller development on seedling vigour in tall fescue (*Festuca arundinacea*). *Euphytica 28:* 393-402.

Lindeman, R.H.; Merenda, P.F. and R.Z. Gold, 1980: *Introduction to Bivariate and Multivariate Analysis.* Scott, Foresman and Company, USA. 444 pp.

Lovato, A., 1981: Germination of seeds. *Advances in Research and Technology of Seeds 6:* 86-120.

Ludlow, M.M., 1978: Light relations of pasture plants. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 3, pp. 35-49. CSIRO, Australia.

Ludlow, M.M. and T.T. Ng, 1976: Effect of water deficit on carbon dioxide exchange and leaf elongation rate of *Panicum maximum* var. *trichoglume. Australian Journal of Plant Physiology 3:* 401-413.

Ludlow, M.M.; Fisher, M.J. and J.R. Wilson, 1985: Stomatal adjustment to water deficits in three tropical grasses and a tropical legume grown in controlled conditions and in the field. *Australian Journal of Plant Physiology 12:* 131-149.

Lunn, W.A., 1951: Regrassing experiments on depleted country in Central Otago.

New Zealand Journal of Agriculture 83: 363-368.

Machin, D. and B. Sanderson, 1977: Computing maximum-likelihood estimates for the parameters of the de Wit competition model. *Applied Statistics 26*: 1-8.

Macpherson, A., 1910: Regrassing experiments. Report on grassing denuded country, Mackenzie Plains and Central Otago. *New Zealand Journal of Agriculture 1:* 477-479.

Macpherson, A., 1911: Revegetation of depleted country. *New Zealand Journal of Agriculture 3:* 391-393.

Macpherson, A., 1912: Revegetating experiments. Tests in connection with depleted country, Central Otago. *New Zealand Journal of Agriculture 4:* 308-311.

Macpherson, A., 1920: Pasture in the Mackenzie Country. *New Zealand Journal of Agriculture 20:* 288.

McCree, K.J., 1986: Whole-plant carbon balance during osmotic adjustment to drought and salinity stress. *Australian Journal of Plant Physiology 13:* 33-43.

McCree, K.J.; Kallsen, C.E. and S.G. Richardson, 1984: Carbon balance of sorghum plants during osmotic adjustment to water stress. *Plant Physiology 76:* 898-902.

McElgunn, J.D., 1973: Germination response of forage legumes to constant and alternating temperatures. *Canadian Journal of Plant Science 53:* 797-800.

McGillivray, R., 1929: The Mackenzie Country grasslands. Progress of natural regeneration and experimental sowings. *New Zealand Journal of Agriculture 39:* 73-78.

McGinnies, W.J., 1960: Effects of moisture stress and temperature on germination of six range grasses. *Agronomy Journal 52:* 159-162.

McGraw, R.L. and P.R. Beuselinck, 1983: Growth and seed yield characteristics of birdsfoot trefoil. *Agronomy Journal 75:* 443-446.

McIlroy, R.J., 1967: Carbohydrates of grassland herbage. *Herbage Abstracts 37:* 79-87.

McKersie, B.D.; Tomes, D.T. and S. Yamamoto, 1981: Effect of seed size on germination, seedling vigour, electrolyte leakage and establishment of birdsfoot trefoil (*Lotus corniculatus* L.). *Canadian Journal of Plant Science 61:* 337-343.

McLean, A. and S. Wikeem, 1985: Rough fescue response to season and intensity

of defoliation. *Journal of Range Management 38:* 100-103.

McTaggart, A., 1935: A study of the inter-relation of species in pasture mixtures subjected to differential treatment in the Canberra (F.C.T.) area. *Journal Commonwealth Scientific and Industrial Research Australia 8:* 177-186.

McWilliam, J.R., 1978: Response of pasture plants to temperature. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 2, pp. 17-34. CSIRO, Australia.

McWilliam, J.R. and P.M. Dowling, 1970: Factors influencing the germination and establishment of pasture seed on the soil surface. *Proceedings of the 11th International Grassland Congress.* pp. 578-583.

McWilliam, J.R.; Clements, R.J. and P.M. Dowling, 1970: Some factors influencing the germination and early seedling development of pasture plants. *Australian Journal of Agricultural Research 21:* 19-32.

Maranville, J.W. and M.D. Clegg, 1977: Influence of seed size and density on germination, seedling emergence and yield of grain sorghum. *Agronomy Journal 69:* 329-330.

Mardia, K.V.; Kent, J.T. and J.M. Bibby, 1979: Multivariate Analysis. Academic press Inc. (London) Ltd. 521 pp.

Marten, G.C., 1985: Nutritional value of the legume in temperate pastures of the United States. In: R.F. Barnes et al. (ed.) *Forage Legumes for Energy-Efficient Animal Production.* Proceedings of a trilateral workshop held in Palmerston North, New Zealand. April 30-May 4 1984. US Department of Agriculture, Agricultural Research Service. pp. 204-212.

Martin, M.P.L.D., 1984: Interference of fathen (*Chenopodium album*) with lucerne (*Medicago sativa*) during establishment. *New Zealand Journal of Agricultural Research 27:* 593-596.

Masiunas, J.B. and P.L. Carpenter, 1984a: Radicle growth of grasses and legumes in response to temperature. *Horticultural Science 19:* 298-300.

Masiunas, J.B. and P.L. Carpenter, 1984b: The effect of temperature and polyethylene glycol-induced osmotic stress on radicle growth of *Lespedeza stipulacea, Lolium multiflorum*, and *Bouteloua curtipendula. Journal of the American Society for Horticultural Science 109:* 67-69.

Mather, K. and J.L. Jinks, 1971: *Biometrical Genetics.* Second edition. Chapman and Hall, London.

Mayer, A.M. and A. Poljakoff-Mayber, 1982: *The germination of seeds.* Pergamon Press, Oxford, 3rd edition. 211 pp.

Medvedev, P., 1969: Seed production characteristics of burnet. *Luga Pastbishcha 5:* 34. (In: *Herbage Abstracts 40:* 127).

Meier, H. and J.S.G. Reid, 1982: Reserve polysaccharides other than starch in higher plants. In: Loewus, F.A. and W. Tanner (eds.) *Plant Carbohydrates.* Encyclopedia of Plant Physiology (New Series). Vol. 13A. Springer-Verlag, Berlin. pp. 418-471.

Mielke, P.W. Jr. and R.B. McHugh, 1965: Non-orthogonality in the two-way classification for the mixed effects finite population model. *Biometrics 21*: 308-323.

Mercier, C., 1985: Biosynthesis and accumulation of polysaccharides in seeds and tubers. *Physiologie Vegetale 23:* 231-242.

Minson, D.J., 1985: Nutritional value of tropical legumes in grazing and feeding systems. In: R.F. Barnes et al. (ed.) *Forage Legumes for Energy-Efficient Animal Production.* Proceedings of a trilateral Workshop held in Palmerston North, New Zealand, April 30-May 4, 1984. US Department of Agriculture, Agricultural Research Service. pp. 192-196.

Mitchell, K.J., 1953: Influence of light and temperature on the growth of ryegrass (*Lolium* spp.). II. The control of lateral bud development. *Physiologia Plantarum 6:* 425-443.

Mitchell, K.J., 1954: Growth of pasture species. I. Short rotation and perennial ryegrass. *New Zealand Journal of Science and Technology (A) 36:* 193-206.

Mitchell, K.J. and S.T.J. Coles, 1955: Effects of defoliation and shading on short rotation ryegrass. *New Zealand Journal of Science and Technology (A) 36:* 586-604.

Moore, T.C., 1979: *Biochemistry and Physiology of Plant Hormones.* Springer-Verlag, New York.

Morgan, J.M., 1984: Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology 35:* 299-319.

Morrison, D.F., 1976: Multivariate Statistical Methods. McGraw-Hill Kogakusha, Ltd., Tokyo. Second edition. 415 pp.

Muendel, H.H., 1986: Emergence and vigour of soybean in relation to initial seed moisture and soil temperature. *Agronomy Journal 78:* 765-769.

Munns, R. and C.J. Pearson, 1974: Effect of water deficit on translocation of carbohydrate in *Solanum tuberosum. Australian Journal of Plant Physiology 1:* 529-537.

Musgrave, D.J., 1976: Effect of ground cover and herbicide application on

establishment of oversown lucerne. *New Zealand Journal of Experimental Agriculture 4:* 447-449.

Musgrave, D.J., 1982: Lucerne establishment-oversowing and overdrilling. In: Wynn-Williams, R.B. (ed.) *Lucerne for the 80's.* Agronomy Society of New Zealand. Special Publication No. 1. pp. 21-31.

Musgrave, D.J.; Clare, R.J. and W.L. Lowther, 1975: The potential for oversowing lucerne on steepland soils in North and Central Otago. *Proceedings of the New Zealand Grassland Association 36:* 89-96.

Mytton, L.R., 1973: The effect of seed weight on the early growth and nodulation of white clover. *Annals of Applied Biology 73:* 329-338.

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

Nelson, C.J. and D. Smith, 1968: Growth of birdsfoot trefoil and alfalfa. III. Changes in carbohydrate reserves and growth analysis under field conditions. *Crop Science 8:* 25-28.

Nelson, C.J. and D. Smith, 1969: Growth of birdsfoot trefoil and alfalfa. IV. Carbohydrate reserve levels and growth analysis under two temperature regimes. *Crop Science 9:* 589-591.

Nelson, C.J. and D. Smith, 1972: Changes in carbohydrates and nitrogen concentrations during storage of heat and freeze dried tissues. *Journal of Agriculture and Food Chemistry 20:* 125-128.

Nemati, N., 1978: Range improvement practices in Iran. In: Hyder, D.N. (ed.) *Proceedings of the First International Rangeland Congress, Denver, Colorado, USA, August 14-18 1978.* Society for Range Management, Denver, Colorado.

New Zealand Meteorological Service, 1983: Summaries of climatological observations to 1980. *New Zealand Meteorological Service Miscellaneous Publication 177.* 172 pp.

New Zealand Standards, 1980: Methods of testing soils for civil engineering purposes. Part 1. Soil classification and chemical tests. Standards Association of New Zealand. pp. 15-17.

Noor, M., 1978: Introduction trials of range plants at Sari, sub-alpine zone. *The Pakistan Journal of Forestry 28:* 153-156.

Nordborg, G., 1963: Studies in *Sanguisorba officinalis* L. *Botaniska Notiser 116:* 267-288.

Nordborg, G., 1967a: Embryological studies in the *Sanguisorba minor* complex (Rosaceae). *Botaniska Notiser 120:* 104-114.

Nordborg, G., 1967b: The genus *Sanguisorba* section *Poterium.* Experimental studies and taxonomy. *Opera Botanica 16:* 1-166.

Nordborg, G., 1968: Pubescence in the *Sanguisorba* group (Rosaceae). *Botaniska Notiser 121:* 641-651.

Nordmeyer, A.H. and M.R. Davis, 1977: Legumes in high-country development. *Proceeaings of the New Zealand Grassland Association 38:* 119-125.

Norrington-Davies, J., 1967: Application of diallel analysis to experiments in plant competition. *Euphytica 16:* 391-406.

Norrington-Davies, J., 1968: Diallel analysis of competition between grass species. *Journal of Agricultural Science (Cambridge) 71:* 223-231.

Noy-Meir, I., 1975: Stability of grazing systems: an application of predator-prey graphs. *Journal of Ecology 63:* 459-481.

NWASCA (National Water and Soil Conservation Authority) 1986: Sheep's burnet. *Streamland 45:* Water and Soil Directorate, MWD, Wellington. 4 pp.

NWASCO (National Water and Soil Conservation Organisation), 1982: Sanguisorba. *Streamland 6:* Water and Soil Division, MWD, Wellington. 4 pp.

Ogata, G.; Richards, L.A. and W.R. Gardner, 1960: Transpiration of alfalfa determined from soil water content changes. *Soil Science 89:* 179-182.

Ojima, K. and T. Isawa, 1968: The variation of carbohydrates in various species of grasses and legumes. *Canadian Journal of Botany 46:* 1507-1511.

Okajima, H. and D. Smith, 1964: Available carbohydrate fractions in the stem bases and seed of timothy, smooth bromegrass, and several other northern grasses. *Crop Science 4:* 317-320.

Orchard, T.J., 1977: Estimating the parameters of plant seedling emergence. *Seed Science and Technology 5:* 61-69.

Oswalt, D.L.; Bertrand, A.R. and M.R. Teal, 1959: Influence of nitrogen fertilization and clipping on grass roots. *Soil Science Society of America Proceedings 23:* 228-230.

Overall, J.E. and C.J. Klett, 1972: *Applied Multivariate Analysis.* McGraw-Hill, Inc., New York. 500 pp.

Palmer, T.P. and R.B. Wynn-Williams, 1982: Cost savings in lucerne production.

In: Wynn-Williams, R.B. (ed.) *Lucerne for the 80's.* Agronomy Society of New Zealand. Special Publication No. 1. pp. 43-45.

Parker, W.J.; Tillman, R.W. and D.I. Gray, 1989: Management considerations in nitrogen fertiliser usage on a Wairarapa sheep farm. *Proceedings of the Workshop on Nitrogen in New Zealand Agriculture and Horticulture.* Fertiliser and Lime Research Centre, Massey University. pp. 65-78.

Penman, H.L., 1956: Evaporation: an introductory survey. *Netherlands Journal of Agricultural Science 4:* 9-29.

Percival, E.G.V., 1952: The carbohydrate constituents of herbage. *British Journal of Nutrition 6:* 104-109.

Perry, D.A., 1976: Seed vigour and seedling establishment. *Advances in Research and Technology of Seeds 2:* 62-85.

Perry, D.A., 1980: Seed vigour and seedling establishment. *Advances in Research and Technology of Seeds 5:* 25-40.

Pineiro, J. and W. Harris, 1978a: Performance of mixtures of ryegrass cultivars and prairie grass with red clover cultivars under two grazing frequencies. I. Herbage production in the establishment year. *New Zealand Journal of Agricultural Research 21:* 83-92.

Pineiro, J. and W. Harris, 1978b: Performance of mixtures of ryegrass cultivars and prairie grass with red clover cultivars under two grazing frequencies. II. Shoot populations and natural reseeding of prairie grass. *New Zealand Journal of Agricultural Research 21:* 665-673.

Poehlman, J.M., 1979: *Breeding field crops.* AVI Publishing Company, Inc. Westport, Connecticut. 483 pp.

Powell, A.A., 1988: Seed vigour and field establishment. *Advances in Research and Technology of Seeds 11:* 29-61.

Preiss, J., 1982: Regulation of the biosynthesis and degradation of starch. *Annual Review of Plant Physiology 33:* 431-454.

Press, S.J., 1972: *Applied multivariate analysis.* Holt, Rinehart and Winston, Inc., New York, London. 521 pp.

Proctor, M.C.F. and G. Nordborg, 1968: *Sanguisorba.* In: Tutin, T.G.; Heywood, V.H.; Burgess, N.A.; Moore, D.M.; Valentine, D.H.; Walters, S.M. and D.A. Webb (eds.) *Flora Europaea Vol. 2.* Cambridge University Press. pp. 33-34.

Purohit, K.M. and G. Panigrahi, 1984: The genus *Sanguisorba* (Rosaceae) in India. *Blumea 30:* 51-68.

Qualls, R. and C.S. Cooper, 1968: Germination, growth, and respiration rates of birdsfoot trefoil at three temperatures during the early non-photosynthetic stage of development. *Crop Science 8:* 758-760.

Rao, C.R., 1952: *Advanced statistical methods in biometric research.* Wiley, New York. 390 pp.

Rapoport, H.F. and R.L. Travis, 1984: Alfalfa root growth, cambial activity, and carbohydrate dynamics during the regrowth cycle. *Crop Science 24:* 899-903.

Ratkowsky, D.A., 1983: *Nonlinear regression modelling. A unified practical approach.* Marcel Dekker, Inc. New York. 276 pp.

Rattray, P.V. and D.A. Clark, 1984: Factors affecting the intake of pasture. *New Zealand Agricultural Science 18:* 141-146.

Raven, A.M. and A. Thompson, 1961: The availability of iron in certain grass, clover and herb species. III. Burnet, chicory and narrow-leaved plantain. *Journal of Agricultural Science 56:* 229-234.

Reid, D., 1959: Studies on the cutting management of grass-clover swards. I. The effect of varying the closeness of cutting on the yields from an established grass-clover sward. *Journal of Agricultural Science 53:* 299-312.

Reynolds, J.H. and D. Smith, 1962: Trend of carbohydrate reserves in alfalfa, smooth bromegrass, and timothy grown under various cutting schedules. *Crop Science 2:* 333-336.

Rhodes, I., 1968: The growth and development of some grass species under competitive stress. 1. Competition between seedlings, and between seedlings and established plants. *Journal of the British Grassland Society 23:* 129-136.

Rhodes, I., 1970: Competition between herbage grasses. *Herbage Abstracts 40:* 115-121.

Rhodes, I. and W.R. Stern, 1978: Competition for light. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 5. CSIRO, Australia. pp. 175-189.

Richards, F.J., 1959: A flexible growth function for empirical use. *Journal of Experimental Botany 10:* 290-300.

Richards, J.H. and M.M. Caldwell, 1985: Soluble carbohydrates, concurrent photosynthesis and efficiency in regrowth following defoliation: a field study with *Agropyron* species. *Journal of Applied Ecology 22:* 907-920.

Ritchie, J.T., 1974: Atmospheric and soil water influences on the plant water balance. *Agricultural Meteorology 14:* 183-198.

Ritchie, J.T., 1981: Water dynamics in the soil-plant-atmosphere system. *Plant and Soil 58:* 81-96.

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

Robertson, K.R., 1974: The genera of Rosaceae in the southeastern United States. *Journal of the Arnold Arboretum 55:* 344-401.

Robson, M.J., 1972: The effect of temperature on the growth of S.170 tall fescue (*Festucà arundinacea*) I. Constant temperature. *Journal Applied Ecology 9:* 643-653.

Rogler, G.A., 1954: Seed size and seedling vigour in crested wheatgrass. *Agronomy Journal 46:* 216-220.

Russell, R.S. and V.M. Shorrocks, 1959: The relationship between transpiration and the absorption of inorganic ions by intact plants. *Journal of Experimental Botany 10:* 301-316.

Rys, G.J.; Smith, N. and M.W. Slay, 1989: Alternative forage species for Hawke's Bay. *Proceedings of the New Zealand Agronomy Society 18:* (in press).

Salim, M.H.; Todd, G.W. and A.M. Schlehuber, 1965: Root development of wheat, oats and barley under conditions of soil moisture stress. *Agronomy Journal 57:* 603-607.

Salmeron, J.G., 1966: Studies of the botany, ecology, biology and pasciology of the principal existing species in the spontaneous pasture grounds of the mountainlands of our semi-arid regions. Poterium. Final report of project No. E25/FS/1. Instituto Forestal de Investigaciones y Experiencias, Madrid.

Salter, P.J. and J.E. Goode, 1967: *Crop responses to water at different stages of growth.* Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Maidstone, Kent.

Sambo, E.Y. and M.J. Aston, 1985: Evidence for osmotic adjustment in *Phalaris tuberosa* L. cvv. Australian and Sirosa. *Australian Journal of Plant Physiology 12:* 481-486.

Sangakkara, R.; Roberts, E. and B.R. Watkin, 1982: Grass species used and pasture establishment practices in central New Zealand. *New Zealand Journal of Experimental Agriculture 10:* 359-364.

Sangakkara, R.; Roberts, E. and B.R. Watkin, 1985: Relationships between seed characters and seedling growth of three herbage grasses. *Seed Science and Technology 13:* 219-225.

SAS, 1982: SAS *user's guide.* Statistical Analysis System Institute. Cary, North

Carolina, USA.

Satterthwaite, F.E., 1946: An approximate distribution of estimates of variance components. *Biometrics 2:* 110-114.

Schimpf, D.J.; Flint, S.D. and I.G. Palmblad, 1977: Representation of germination curves with the logistic function. *Annals of Botany 41:* 1357-1360.

Schnyder, H. and C.J. Nelson, 1987: Growth rates and carbohydrate fluxes within the elongation zone of tall fescue leaf blades. *Plant Physiology 85:* 548-553.

Schnyder, H. and C.J. Nelson, 1988: Diurnal growth of tall fescue leaf blades. I. Spatial distribution of growth, deposition of water, and assimilate import in the elongation zone. *Plant Physiology 86:* 1070-1076.

Schnyder, H.; Nelson, C.J. and W.G. Spollen, 1988: Diurnal growth of tall fescue leaf blades. II. Dry matter partitioning and carbohydrate metabolism in the elongation zone and adjacent expanded tissue. *Plant Physiology 86:* 1077-1083.

Schulze, E.-D., 1986: Whole-plant responses to drought. *Australian Journal of Plant Physiology 13:* 127-141.

Schwass, R.H. and W.A. Jacques, 1956: Root development in some common New Zealand pasture plants. VIII. The relationship of top growth to root growth in perennial ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*) and tall fescue (*Festuca arundinacea*). *New Zealand Journal of Science and Technology (A) 38:* 109-119.

Scott, D. and J.F.L. Charlton, 1983: Birdsfoot trefoil (*Lotus corniculatus*) as a potential dryland herbage legume in New Zealand. *Proceedings of the New Zealand Grassland Association 44:* 98-105.

Scott, D.; Keoghan, J.M.; Cossens, G.G.; Maunsell, L.A.; Floate, M.J.S.; Wills, B.J. and G.B. Douglas, 1985: Limitations to pasture production and choices of species. In: Burgess, R.E. and J.L. Brock (eds.) *Using Herbage Cultivars.* Grassland Research and Practice Series No. 3. New Zealand Grassland Association. pp. 9-15.

Scott, D.J. and J.G. Hampton, 1985: Aspects of seed quality. In: Hare, M.D. and J.L. Brock, (eds.) *Producing Herbage Seeds.* Grassland Research and Practice Series No. 2. New Zealand Grassland Association, Palmerston North. pp. 43-52.

Scott, R.C., 1932: Pasture improvement. Experiments conducted in co-operation with the South Australian Advisory Dairy Board. *Journal Department of Agriculture South Australia 35:* 968-978.

Scott, R.S. and W.L. Lowther, 1980: Competition between white clover

'Grasslands Huia' and *Lotus pedunculatus* 'Grasslands Maku'. *New Zealand Journal of Agricultural Research 23:* 501-507.

Scott, S.J.; Jones, R.A. and W.A. Williams, 1984: Review of data analysis methods for seed germination. *Crop Science 24:* 1192-1199.

Seaney, R.R. and P.R. Henson, 1970: Birdsfoot trefoil. *Advances in Agronomy 22:* 119-157.

Searle, S.R., 1966: *Matrix algebra for the biological sciences, including applications in statistics.* Wiley, New York. 296 pp.

Searle, S.R., 1971: Topics in variance component estimation. *Biometrics 27:* 1- 76.

Searle, S.R. and R.F. Fawcett, 1970: Expected mean squares in variance components models having finite populations. *Biometrics 26*: 243-254.

Sears, P.D., 1961: Pasture establishment. *Proceedings of the Ruakura Farmer's Conference 13:* 133-141.

Sewell, T.G., 1952: Tussock grasslands investigations. *Proceedings of the New Zealand Grassland Association 14:* 123-137.

Shaykewich, C.F., 1973: Proposed method for measuring swelling pressure of seeds prior to germination. *Journal of Experimental Botany 24:* 1056-1061.

Sheaffer, C.C., 1983: Seeding year harvest management of alfalfa. *Agronomy Journal 75:* 115-118.

Sheaffer, C.C.; Marten, G.C. and D.L. Rabas, 1984: Influence of grass species on composition, yield, and quality of birdsfoot trefoil mixtures. *Agronomy Journal 76:* 627-632.

Sheard, R.W., 1973: Organic reserves and plant growth. In: Butler, G.W. and R.W. Bailey (eds.) *Chemistry and Biochemistry of Herbage, Volume 2.* Academic Press, London. pp. 353-357.

Sheath, G.W., 1978: Growth studies on defoliated *Lotus pedunculatus* cv. 'Grasslands Maku'. Unpublished Ph.D. thesis, Massey University, Palmerston North.

Sheppard, J.S. and B.J. Wills, 1985: Sheep's burnet - a forage herb for soil conservation in New Zealand. *New Zealand Agricultural Science 19:* 115-118.

Sheppard, J.S. and B.J. Wills, 1986: Management and uses of *Sanguisorba minor* (sheep's burnet). In: van Kraayenoord, C.W.S. and R.L. Hathaway (eds.) *Plant Materials Handbook for Soil Conservation. Volume 2: Introduced Plants.* Technical Note No. H12. NWASCA. pp. 282-286.

Siegel, S., 1956: *Nonparametric statistics for the behavioural sciences.* McGraw-Hill, Inc. Kogakush Ltd. 312 pp.

Sievwright, H.M., 1956: Improving fescue tussock grassland in the Mackenzie Country. *New Zealand Journal of Agriculture 92:* 122-127.

Silvertown, J.W. and J.B. Dickie, 1980: Seedling survivorship in natural populations of nine perennial chalk grassland plants. *New Phytologist* 88: 555-558.

Sinclair, D.F. and A.C. Gleeson, 1984: On the analysis of replacement series diallel experiments. *Journal of Agricultural Science (Cambridge) 102*: 531-537.

Slaughter, J.C., 1988: Mobilization of reserves during germination. *Advances in Research and Technology of Seeds 11:* 62-80.

Slayter, R.O., 1969: Physiological significance of internal water relations to crop yield. In: Eastin *et al.* (eds.) *Physiological Aspects of Crop Yield.* American Society of Agronomy. pp. 53-83.

Smetham, M.L., 1973: Grazing management. In: Langer, R.H.M. (ed.) *Pastures and Pasture Plants.* Reed, Wellington, New Zealand. pp. 179-228.

Smith, D., 1950: Seasonal fluctuations of root reserves in red clover, *Trifolium pratense* L. *Plant Physiology 25:* 702-710.

Smith, D., 1962: Carbohydrate root reserves in alfalfa, red clover, and birdsfoot trefoil under several management schedules. *Crop Science 2:* 75-78.

Smith, D., 1971: Efficiency of water for extraction of total nonstructural carbohydrates from plant tissue. *Journal of the Science of Food and Agriculture 22:* 445-447.

Smith, D., 1973: Influence of drying and storage on nonstructural carbohydrate analysis of herbage tissue - a review. *Journal of the British Grasslands Society 28:* 129-134.

Smith, D., 1981: Removing and analyzing total nonstructural carbohydrates from plant tissue. University of Wisconsin, College of Agriculture and Life Sciences Publication R-2107. University of Wisconsin, Madison (cited by Richards, J.H. and M.M. Caldwell, 1985).

Smith, D. and B.W. Struckmeyer, 1974: Grass morphology and starch accumulation in leaves of alfalfa plants grown at high and low temperatures. *Crop Science 14:* 433-436.

Smith, R.G. and W. Stiefel, 1978: Pasture and lucerne research on sand country. *Proceedings of the New Zealand Grassland Association 39:* 61-69.

Smoliak, S.; Johnston, A. and M.R. Hanna, 1972: Germination and seedling growth of alfalfa, sainfoin, and cicer milkvetch. *Canadian Journal of Plant Science 52:* 757-762.

Snaydon, R.W. and E.H. Satorre, 1989: Bivariate diagrams for plant competition data : modifications and interpretation. *Journal of Applied Ecology 26*: 1043-1057.

Spitters, C.J.T., 1980: Competition effects within mixed stands. In: *Opportunities for Increasing Crop Yields.* Edited by Hurd, R.G.; Biscoe, P.V. and C. Dennis. Pitmans publishers, London. pp. 219-231.

Spitters, C.J.T. and J.P. van den Bergh, 1982: Competition between crops and weeds. A system approach. *In: Biology and Ecology of Weeds.* Holzner, W. and M. Numata (eds.). Monographie Biologicae, W. Junk Publications, The Hague pp 137-147.

Spollen, W.G. and C.J. Nelson, 1988: Characterization of fructan from mature leaf blades and elongation zones of developing leaf blades of wheat, tall fescue and timothy. *Plant Physiology 88:* 1349-1353.

Squire, G.R. and C.R. Black, 1981: Stomatal behaviour in the field. In: Jarvis, P.G. and T.A. Mansfield (eds.) *Stomatal Physiology.* Cambridge University Press, London. pp. 223-245.

Stapledon, R.G., 1948: The nutritive influence of the herbs of grassland. *Farming 2:* 86-89.

Steel, R.G.D. and J.H. Torrie, 1980: *Principles and procedures of statistics.* McGraw-Hill, Inc. Second Edition. 633 pp.

Stern, W., 1962: Light measurements in pastures. *Herbage Abstracts 32:* 91-96.

Stewart, C.R., 1971: Effect of wilting on carbohydrates during incubation of excised bean leaves in the dark. *Plant Physiology 48:* 792-794.

Stewart, M.J., 1979: The roles of burnet reviewed. Unpublished report. New Zealand Ministry of Agriculture and Fisheries. 12 pp.

Stickler, F.C. and C.E. Wassom, 1963: Emergence and seedling vigour of birdsfoot trefoil as affected by planting depth, seed size and variety. *Agronomy Journal 55:* 78.

Stroud, D.O.; Hart, R.H.; Samuel, M.J. and J.D. Rodgers, 1985: Western wheatgrass responses to simulated grazing. *Journal of Range Management 38:* 103-108.

Stroup, W.W. and J. Stubbendieck, 1983: Multivariate statistical methods to determine changes in botanical composition. *Journal of Range Management*

36: 208-212.

Suckling, F.E.T., 1966: *Hill pasture improvement.* DSIR/Newton King.

Suckling, F.E.T., 1975: Pasture management trials on unploughable hill country at Te Awa. III. Results for 1959-69. *New Zealand Journal of Experimental Agriculture 3:* 351-436.

Sydes, C.L. and J.P. Grime, 1984: A comparative study of development using a simulated rock crevice. *Journal of Ecology 72:* 937-946.

Taylor, A.A.; de-Felice, J. and D.C. Havill, 1982: Nitrogen metabolism in *Poterium sanguisorba* during water stress. *New Phytologist 90:* 19-25.

Tennent, R.B., 1935: A summary of the Northburn regrassing experiments conducted by the late Dr L. Cockayne. *Proceedings of the New Zealand Grassland Association 4:* 1-12.

Thakur, C., 1957: Grassland in soil conservation. *Journal of Soil and Water Conservation in India 6:* 43-46

Thomas, H., 1980: Terminology and definitions in studies of grassland plants. *Grass and Forage Science 35:* 13-23.

Thomas, R.L., 1966: The influence of seed weight on seedling vigour in *L. perenne. Annals of Botany 30:* 111-121.

Thomas, V.J., 1970: A mathematical approach to fitting parameters in a competition model. *Journal of Applied Ecology 7:* 487-496.

Thompson, P.A., 1970: Characterisation of the germination responses to temperature of species and ecotypes. Nature, London 225: 827-831.

Thompson, P.A., 1981: Ecological aspects of seed germination. *Advances in Research and Technology of Seeds 6:* 9-42.

Tinney, F.W.; Aamodt, O.S. and H.L. Ahlgren, 1937: Preliminary report of a study on methods used in botanical analyses of pasture swards. *Journal of the American Society of Agronomy 29:* 835-840.

Tischler, C.R. and P.W. Voigt, 1983: Effects of planting depth on vegetative characteristics of three forage grasses at 14 days post emergence. *Crop Science 23:* 481-484.

Toft, N.L.; McNaughton, S.J. and N.J. Georgiadis, 1987: Effects of water stress and simulated grazing on leaf elongation and water relations of an East African grass, *Eustachys paspaloides. Australian Journal of Plant Physiology 14:* 211-226.

Townsend, C.E., 1977: Recurrent selection for high seed weight in cicer milkvetch. *Crop Science 17:* 473-476.

Townsend, C.E. and A.M. Wilson, 1981: Seedling growth of cicer milkvetch as affected by seed weight and temperature regime. *Crop Science 21:* 405-409.

Townsend, C.E.; Christensen, D.K. and A.D. Dotzenko, 1978: Yield and quality of cicer milkvetch forage as influenced by cutting frequency. *Agronomy Journal 70:* 109-113.

Trenbath, B.R., 1978: Models and the interpretation of mixture experiments. *In:* Wilson, J.R. (ed.). *Plant Relations in Pastures.* Chapter 10. CSIRO, Australia. pp. 145-162.

Troughton, A., 1957: *The Underground Organs of Herbage Grasses.* Commonwealth Agricultural Bureaux, Farnham Royal, England. 163 pp.

Turner, J.F., 1949: The metabolism of the apple during storage. *Australian Journal of Scientific Research 2:* 138-153.

Turner, N.C., 1986: Crop water deficits: a decade of progress. *Advances in Agronomy 39:* 1-51.

Turner, N.C., 1986: Adaptation to water deficits: a changing perspective. *Australian Journal of Plant Physiology 13:* 175-190.

Turner, N.C. and J.E. Begg, 1978: Responses of pasture plants to water deficits. In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 4, pp. 50-66. CSIRO, Australia.

Turner, N.C. and J.E. Begg, 1981: Plant water relations and adaptation to stress. *Plant and Soil 58:* 97-131.

Turner, N.C. and M.M. Jones, 1980: Turgor maintenance by osmotic adjustment: a review and evaluation. In: Turner, N.C. and P.J. Kramer (eds.) *Adaptation of plants to water and high temperature stress.* Wiley Interscience, New York. pp. 87-103.

Turner, N.C.; Begg, J.E. and M.L. Tonnet, 1978: Osmotic adjustment of sorghum and sunflower crops in response to water deficits and its influence of the water potential at which stomata close. *Australian Journal of Plant Physiology 5:* 597-608.

Twamley, B.E., 1967: Seed size and seedling vigour in birdsfoot trefoil. *Canadian Journal of Plant Science 47:* 603-609.

Tyree, M.T. and P.G. Jarvis, 1982: Water in tissues and cells. In: Lange, O.L.; Nobel, P.S.; Osmond, C.B. and H. Ziegler (eds.) *Encyclopaedia of Plant Physiology New Series, Volume 12B. Physiological Plant Ecology II.*

Springer-Verlag, Berlin. pp. 35-77.

Valassis, V.; Hedrick, D.W. and D.D. Hill, 1957: The performance of several improved forage species on Laughlin-like soils in western Oregon. *Journal of Range Management 10:* 94-98.

van den Bergh, J.P., 1968: An analysis of yields of grasses in mixed and pure stands. *Verslagen landbouwkundig onderzoekingen, Nederlands 74:* 1-71.

van Kraayenoord, C.W.S., 1986: Characteristics of plants for soil conservation. In: van Kraayenoord, C.W.S. and R.L. Hathaway (eds.) *Plant Materials Handbook for Soil Conservation Vol. 1: Principles and Practices.* Water and Soil Directorate, MWD, Wellington. pp. 17-20.

Vartha, E.W. and J.H. Hoglund, 1983: What is the make-up of a dryland pasture? *Proceedings of the New Zealand Grassland Association 44:* 204-210.

Volenec, J.J., 1986: Nonstructural carbohydrates in stem base components of tall fescue during regrowth. *Crop Science 26:* 122-126.

Volenec, J.J., 1988: Herbage growth and carbohydrate metabolism of diploid and tetraploid alfalfa. *Crop Science 28:* 128-132.

Walton, P.D., 1983: *The production and management of cultivated forages.* Reston Publishing Company, Virginia, USA.

Ward, C.Y. and R.E. Blaser, 1961: Carbohydrate food reserves and leaf area in regrowth of orchardgrass. *Crop Science 1:* 366-370.

Ward, F.E., 1923: Regrassing experiments of Canterbury back-country. *New Zealand Journal of Agriculture 26:* 358-362.

Wardlaw, I.F., 1968: The control and pattern of movement of carbohydrates in plants. *Botanical Review 34:* 79-105.

Wardlaw, I.F., 1969: The early stages of grain development in wheat: response to water stress in a single variety. *Australian Journal of Biological Sciences 24:* 1047-1055

Wareing, P.F. and J. Patrick, 1975: Source-sink relations and the partition of assimilates in the plant. In: Cooper, J.P. (ed.) *Photosynthesis and Productivity in Different Environments.* Cambridge University Press. pp. 481-499.

Warrington, I.J.; Dixon, T.; Robotham, R. and D.A. Rook, 1978: Lighting systems in major New Zealand controlled environment facilities. *Journal of Agricultural Engineering Research 23:* 23-26.

Watkin, B.R. and R.J. Clements, 1978: The effects of grazing animals on pastures.

In: Wilson, J.R. (ed.) *Plant Relations in Pastures.* Chapter 18, pp. 273-289. CSIRO, Australia.

Watson, D.J., 1947: Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties, and within and between years. *Annals of Botany, N.S. 11:* 41-76.

Webb, C.J.; Sykes, W.R. and P.J. Garnock-Jones, 1988: *Flora of New Zealand. Volume IV. Naturalised pteridophytes, gymnosperms, dicotyledons.* Botany Division, DSIR, New Zealand. pp. 1136-1137.

Weinmann, H., 1948: Underground development and reserves of grasses. A review. *Journal of the British Grassland Society 3:* 115-140.

Weinmann, H., 1961: Total available carbohydrates in grasses and legumes. *Herbage Abstracts 31:* 255-261.

Wester, D.B.; Dahl, B.E. and P.F. Cotter, 1986: Effects of pattern and amount of simulated rainfall on seedling dynamics of weeping lovegrass and Kleingrass. *Agronomy Journal 78:* 851-855.

Westgate, M.E. and J.S. Boyer, 1985: Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. *Planta 164:* 540-549.

White, J.G.H., 1973: Improvement of hill country pastures. In: Langer, R.H.M. (ed.) *Pastures and pasture plants.* A.H. and A.W. Reed, Wellington. pp. 259-290.

White, J.G.H., 1982: Lucerne grazing management for the 80's. In: Wynn-Williams, R.B. (ed.) *Lucerne for the 80's.* Agronomy Society of New Zealand (Inc.). pp. 111-114.

Whiteman, P.C., 1969: The effects of close grazing and cutting on the yield, persistence and nitrogen content of four tropical legumes with Rhodes grass at Samford, south-eastern Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry 9:* 287-294.

Widdup, K.H.; Keoghan, J.M.; Ryan, D.L. and H. Chapman, 1987: Breeding *Lotus corniculatus* for south island tussock country. *Proceedings of the New Zealand Grassland Association 48:* 119-124.

Wilhelm, W.W. and C.J. Nelson, 1978: Leaf growth, leaf aging and photosynthetic rate of tall fescue genotypes. *Crop Science 18:* 769-772.

Wilkin, R., 1877: Grasses and forage plants best adapted to New Zealand. *New Zealand Country Journal 1:* 3-12.

Williams, C.N. and E.F. Biddiscombe, 1965: Extension growth of grass tillers in the field. *Australian Journal of Agricultural Research 16:* 14-22.

Wills, B.J., 1983: Forage plants for the semi-arid high country and rangelands of New Zealand. In: Robertson, B.T. (ed.) *Proceedings of the 1983 Hill and High Country Seminar*, Centre for Resource Management Special Publication No. 26. pp. 59-65.

Wills, B.J., 1984: Alternative plant species for revegetation and soil conservation in the tussock grasslands of New Zealand. *Tussock Grasslands and Mountain Lands Institute Review 42:* 49-58.

Wills, B.J., 1986: Plant materials for revegetation and reclamation in semi-arid areas. In: van Kraayenoord, C.W.S. and R.L. Hathaway (eds.) *Plant Materials Handbook for Soil Conservation. Volume 1: Principles and Practices.* Section 7. NWASCA. pp. 79-93.

Wills, B.J. and J.S.C. Begg, 1986: The Cockayne plots of Central Otago - a 1985 evaluation. *New Zealand Journal of Ecology 9:* 41-55.

Wills, B.J.; Sheppard, J.S. and J.S.C. Begg, 1987: Evaluation of alternative dryland pasture plants and browse shrubs for soil conservation in drought-prone Otago grasslands. *Proceedings of the New Zealand Grassland Association 48:* 115-118.

Wilson, A.M., 1984: Nonstructural carbohydrates and root development in blue grama seedlings. *Journal of Range Management 37:* 28-30.

Wilson, A.M. and D.D. Briske, 1979: Seminal and adventitious root growth of blue grama seedlings on the Central Plains. *Journal of Range Management 32:* 209-213.

Wolf, D.D. and D.J. Parrish, 1982: Short-term growth responses of tall fescue to changes in soil water potential and to defoliation. *Crop Science 22:* 996-999.

Woods, L.E. and H.A. MacDonald, 1971: The effects of temperature and osmotic moisture stresses on the germination of *Lotus corniculatus. Journal of Experimental Botany 22:* 575-585.

Wright, D.L.; Blaser, R.E. and J.M. Woodruff, 1978: Seedling emergence as related to temperature and moisture tension. *Agronomy Journal 70:* 709-712.

Wright, N., 1962: Effects of management practices on forage yield and percent protein in blue panicgrass, *Panicum antidotale* Retz. *Agronomy Journal 54:* 413-416.

Wynn-Williams, R.B., 1982: Lucerne establishment - conventional. In:

Wynn-Williams R.B. (ed.) *Lucerne for the 80's.* Agronomy Society of New Zealand Special Publication No. 1. pp. 11-19.

APPENDICES

CHAPTER 4

Table A4.1 Soil fertility attributes at Flock House and Riverside (July, 1985) (mean of replicates).

Location	pH	Ca	K	P	Mg	S	Phosphate retention
Flock House	6.1	7	3	38	15	3	13
Riverside	5.6	6	8	17	17	9	42

Table A4.2 Characteristics of seed used in field trials at Riverside and Flock House.

Species	Purity (%)	Germination (%)	Hard seed (%)	1,000 seed weight (g)
sheep's burnet	99.7	87	-	7.40
birdsfoot trefoil	99.7	70	2	1.22
lucerne	100.0	80	1	2.59

Table A4.3a Summary of results of multivariate analysis of variance pooled over locations involving times to reach various stages of emergence.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Location	56.24	3, 2	*
Location (block)	0.89	12, 16	NS
Species	10.92	6, 12	***
Species x location	1.01	6, 12	NS

Table A4.3b Between group structure and standardised coefficients for the single discriminant function for locations involving times to reach various stages of emergence.

Character	between group structure	standardised coefficient
t_{10} (days)	1.00	1.60
t_{50} (days)	1.00	12.48
t_{90} (days)	1.00	-3.26

Table A4.3c Between group structure and standardised coefficients for the first discriminant function for species involving times to reach various stages of emergence.

Character	between group structure	standardised coefficient
t_{10} (days)	0.99	1.95
t_{50} (days)	1.00	1.79
t_{90} (days)	0.91	0.93

Table A4.4a Summary of results of split plot multivariate analysis of variance over harvests involving vegetative characters at Riverside.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	1.55	8, 2	NS
Species	7.92	8, 2	NS
Species x block	0.99	16, 46	NS
Harvest	17.00	12, 8	***
Harvest x species	0.96	24, 54	NS

Table A4.4b Between group structure and standardised coefficients for the first discriminant function for species involving vegetative characters.

Character	between group structure	standardised coefficient
foliar length (log)	0.87	-0.67
root length (mm)	0.98	0.27
foliar dry weight (log)	0.98	6.84
root dry weight (log)	0.87	-1.68

Table A4.4c Between group structure and standardised coefficients for the first discriminant function for harvests involving vegetative characters.

Character	between group structure	standardised coefficient
foliar length (log)	0.99	2.10
root length (mm)	0.97	0.68
foliar dry weight (log)	1.00	4.25
root dry weight (log)	0.92	-1.51

CHAPTER 5

Table A5.1a Summary of results of multivariate analysis of variance involving herbage masses of complete leaves, incomplete leaves and stubble, and remainder.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	1.13	6, 20	NS
Harvest	4.44	18, 29	***

Table A5.1b Between group structure and standardised coefficients for the first discriminant function for harvests involving herbage masses of three plant components.

Character	between group structure	standardised coefficient
incomplete leaves and stubble (g)	0.89	1.94
complete leaves (g)	-0.79	-1.47
remainder (g)	-0.52	-0.02

CHAPTER 6

Table A6.1a Summary of results of multivariate analysis of variance of logistic function parameters describing seedling emergence for lucerne and all sheep's burnet treatments.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	2.16	9, 39	*
Treatment	2.84	18, 46	**

Table A6.1b Between group structure and standardised coefficients for the first discriminant function for treatments involving logistic function parameters.

Parameter	between group structure	standardised coefficient
β_0	-0.95	-1.13
β_1	-0.45	-1.16
β_2	0.76	1.98

Table A6.2a Summary of results of multivariate analysis of variance of logistic function parameters describing seedling emergence for all sheep's burnet treatments.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	2.28	9, 32	*
Seed line	4.30	3, 13	*
Seed size	0.55	6, 26	NS
Seed line x seed size	2.91	6, 26	*

Table A6.2b Between group structure and standardised coefficients for the first discriminant function for seed line x seed size interaction involving logistic function parameters.

Parameter	between group structure	standardised coefficient
β_0	-0.94	-1.20
β_1	-0.69	-1.81
β_2	0.03	2.27

Table A6.3a Summary of results of multivariate analysis of variance of times to reach various stages of emergence for all treatments.

		Wilks' Criterion	
Source of variation	F-test	df	Significance
Block	2.26	9, 39	*
Treatment	5.65	18, 46	***

Table A6.3b Between group structure and standardised coefficients for the first discriminant function for treatments involving times to reach various stages of emergence.

Character	between group structure	standardised coefficient
t_{10} (days)	0.99	640.12
t_{50} (days)	0.99	-1515.50
t_{90} (days)	0.98	946.43

Table A6.4 Summary of results of multivariate analysis of variance of times to reach various stages of emergence for all sheep's burnet treatments.

		Wilk's Criterion	
Source of variation	F-test	df	Significance
Block	2.10	9, 32	(NS)
Seed line	1.91	3, 13	NS
Seed size	2.01	6, 26	NS
Seed line x seed size	1.68	6, 26	NS

Table A6.5a Summary of results of split plot multivariate analysis of variance over harvests involving six vegetative characters for lucerne and sheep's burnet treatments.

		Wilk's Criterion	
Source of variation	F-test	df	Significance
Block	1.95	18, 37	*
Treatment	5.89	36, 60	***
Block x treatment	1.28	108, 460	*
Harvest	13.29	24, 67	***
Harvest x treatment	1.29	144, 470	*

Table A6.5b Between group structure and standardised coefficients for the first three discriminant functions for harvest x treatment interaction involving six vegetative characters.

	DF1		DF2		DF3	
Character	between group structure	standardised coefficient	between group structure	standardised coefficient	between group structure	standardised coefficient
shoot length (log)	0.25	-1.97	0.88	1.38	-0.31	-5.45
root length (log)	-0.30	-2.70	0.73	0.72	0.21	1.45
leaf dry weight (log)	0.36	-0.81	0.31	-0.43	-0.34	-0.49
stem dry weight (log)	0.04	-1.37	0.66	1.60	-0.07	0.71
root dry weight (log)	0.59	1.97	0.03	-0.61	-0.33	-0.91
leaf area (log)	0.56	6.18	0.80	0.68	0.05	4.88

DF = discriminant function

Table A6.6a Summary of multivariate analysis of variance over harvests involving six vegetative characters for all sheep's burnet treatments.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	2.24	18, 29	*
Seed line	30.25	6, 75	***
Seed size	4.20	12, 150	***
Seed line x seed size	0.39	12, 20	NS
Error	1.22	90, 428	NS
Harvest	22.99	24, 263	***
Harvest x seed line	2.24	24, 263	**
Harvest x seed size	0.92	48, 373	NS

Table A6.6b Between group structure and standardised coefficients for the first discriminant functions for harvest x seed line interaction and seed size involving vegetative characters.

	harvest x seed line interaction		seed size	
Character	between group structure	standardised coefficient	between group structure	standardised coefficient
shoot length (log)	0.86	-0.84	0.97	-1.89
root length (log)	-0.61	-2.74	0.96	-0.27
leaf dry weight (log)	0.89	-0.69	0.91	-0.11
stem dry weight (log)	0.71	-0.95	0.84	-0.47
root dry weight (log)	0.83	1.72	0.93	1.38
leaf area (log)	0.97	5.29	0.99	4.85

CHAPTER 7

Table A7.1 Treatment conditions in controlled environment room at Plant Physiology Division, DSIR.

Daylength (hrs)	12
Temperature (±0.5°C)	
24.6.86-14.7.86	20/20 (Day/night
21.7.86-12.9.86	10/10
16.9.86-20.10.86	15/15
20.10.86-7.11.86	25/25
Relative Humidity (±5% RH)	
24.6.86-14.7.86	70
22.7.86-12.9.86	70
16.9.86-20.10.86	70
20.10.86-7.11.86	70
Photosynthetic Photon Flux Density (umol m^{-2} sec^{-1})*	
Pre-expt	706
Post-expt	667
Mean	687
Photosynthetic Irradiance ($W m^{-2}$)**	
Pre-expt	150
Post-expt	140
Mean	145

* Licor LI 185 Meter with LI 190S Quantum Sensor
** Licor LI 185 Meter with LI 190SE Flat Response Photosynthetic Irradiance Sensor

Table A7.2 R-square estimates of total dry weight per plot against seedling number at each temperature and harvest.

Temperature (°C)	Harvest 1	Harvest 2		Harvest 3		Harvest 4	
		shoot (mg)	root (mg)	shoot (mg)	root (mg)	shoot (mg)	root (mg)
10	0.77	0.79	0.63	0.81	0.68	0.68	0.63
15	0.68	0.68	0.47	0.76	0.73	0.69	0.65
20	0.48	0.57	0.23	0.80	0.68	0.69	0.60
25	0.80	0.81	0.37	0.84	0.52	0.63	0.46

Table A7.3 Parameters and their standard errors for the logistic functions describing cumulative emergence (totalled over blocks) over time for all Oregon sheep's burnet treatments at four temperatures.

Temperature (°C)	Seed size	Depth (mm)	$\hat{\beta}_0$	$SE(\hat{\beta}_0)$	$\hat{\beta}_1$	$SE(\hat{\beta}_1)$	$\hat{\beta}_2$	$SE(\hat{\beta}_2)$	Error df
10	small	5	109.25	7.83	3.01	0.32	0.79	0.12	5
10	medium	5	97.46	3.50	3.11	0.32	0.74	0.09	8
10	large	5	97.36	3.93	3.42	0.51	1.23	0.20	5
10	small	10	108.10	3.26	4.42	0.23	1.37	0.09	3
10	medium	10	96.09	2.42	3.45	0.36	1.05	0.12	7
10	large	10	96.95	2.74	3.33	0.42	1.38	0.18	5
15	small	5	96.18	2.06	4.14	0.50	1.84	0.23	5
15	medium	5	92.61	4.45	1.98	0.56	1.43	0.38	5
15	large	5	92.20	3.08	3.50	0.72	2.46	0.51	4
15	small	10	102.75	12.10	2.74	0.46	0.87	0.21	4
15	medium	10	98.53	1.26	5.07	0.30	1.99	0.12	4
15	large	10	95.90	1.43	5.89	0.56	2.51	0.25	5
20	small	5	95.36	2.47	4.43	0.75	2.26	0.39	4
20	medium	5	90.02	8.98	3.05	1.35	2.36	1.12	2
20	large	5	97.28	5.61	2.67	0.56	1.88	0.42	2
20	small	10	95.28	4.78	2.24	0.44	1.24	0.25	4
20	medium	10	97.49	6.66	2.52	0.59	1.77	0.45	2
20	large	10	98.48	5.58	4.44	0.92	2.18	0.49	2
25	small	5	98.53	4.07	3.43	0.90	3.16	0.93	1
25	medium	5	97.22	3.65	3.87	2.09	4.06	2.13	1
25	large	5	94.98	3.88	3.69	2.79	4.27	2.82	2
25	small	10	101.60	1.59	5.19	0.40	2.59	0.21	2
25	medium	10	101.96	0.77	5.06	0.13	3.00	0.08	1
25	large	10	96.25	3.59	3.86	0.60	2.56	0.41	2

Table A7.4 Parameter estimates and their standard errors from regression analyses of $1/t_{50}$ vs. temperature for each Oregon sheep's burnet treatment.

Seed size	Depth (mm)	$\hat{\beta}_0$	$SE(\hat{\beta}_0)$	$\hat{\beta}_1$	$SE(\hat{\beta}_1)$
small	5	-0.07	0.03	0.01	0.00
medium	5	-0.07	0.01	0.01	0.00
large	5	-0.06	0.02	0.01	0.00
small	10	-0.04	0.02	0.01	0.00
medium	10	-0.05	0.03	0.01	0.00
large	10	-0.04	0.02	0.01	0.00

Table A7.5a Summary of results of multivariate analysis of variance of numerous vegetative characters for Oregon sheep's burnet at four temperatures.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Temperature	32.51	54, 69	***
Temperature (block)	1.69	144, 182	***
Depth	18.76	18, 23	***
Seed size	8.99	36, 46	***
Temperature x depth	1.17	54, 69	NS
Temperature x seed size	1.85	108, 139	***
Depth x seed size	2.15	36, 46	**
Temperature x depth x seed size	1.35	108, 139	*

Table A7.5b Between group structure and standardised coefficients for up to the first two discriminant functions, involving vegetative characters for Oregon sheeep's burnet, for temperature x seed size, depth x seed size interaction.

	temperature x seed size interaction				depth x seed size interaction	
	DF1		DF2			
Character	between group structure	standardised coefficient	between group structure	standardised coefficient	between group structure	standardised coefficient
emergence (%)	0.57	0.00	-0.08	0.83	0.43	0.31
doubles (%)	0.29	1.34	0.68	1.10	-0.98	-0.79
time of final emergence (days)	0.78	3.35	0.31	0.09	0.56	-0.14
H1 shoot length (mm)	0.09	-0.12	0.03	1.62	-0.64	1.28
H2 shoot length (mm)	0.49	1.79	0.58	0.48	0.89	0.64
H3 shoot length (mm)	-0.20	1.13	-0.34	1.06	-0.91	0.33
H4 shoot length (mm)	-0.27	-0.74	-0.01	-0.18	-0.56	-1.81
H1 root length (mm)	0.08	-0.03	-0.49	-1.64	-0.72	-1.17
H2 root length (mm)	-0.33	-0.06	-0.48	-0.13	0.97	1.29
H3 root length (mm)	0.03	0.04	-0.72	-1.12	-0.99	-1.51
H4 root length (mm)	0.47	1.02	-0.67	-0.22	-0.65	0.54
H1 total seedling dry weight (mg)	0.92	2.46	0.00	1.22	0.39	1.85
H2 shoot dry weight (mg)	0.06	0.07	0.94	-0.11	-0.61	-0.92
H3 shoot dry weight (mg)	-0.09	-0.11	-0.42	0.40	-0.99	-0.01
H4 shoot dry weight (mg)	0.03	-0.22	-0.59	-0.23	0.68	-0.37
H2 root dry weight (mg)	-0.57	-0.52	0.74	1.71	0.86	1.76
H3 root dry weight (mg)	0.54	0.84	-0.72	-0.82	-0.88	0.37
H4 root dry weight (mg)	0.34	0.62	-0.64	-1.03	0.96	0.45

DF = discriminant function

Table A7.5b contd.

Character	temperature x depth x seed size interaction			
	DF1		DF2	
	between group structure	standardised coefficient	between group structure	standardised coefficient
emergence (%)	-0.66	-0.34	0.03	-0.77
doubles (%)	-0.23	0.47	-0.82	-2.07
time of final emergence (days)	0.10	1.06	0.42	0.13
H1 shoot length (mm)	-0.69	0.27	0.62	0.71
H2 shoot length (mm)	0.00	0.55	0.16	0.01
H3 shoot length (mm)	0.17	1.68	0.47	-0.48
H4 shoot length (mm)	0.27	-0.55	-0.36	-0.42
H1 root length (mm)	-0.71	-1.21	0.42	0.00
H2 root length (mm)	0.13	1.21	0.88	1.46
H3 root length (mm)	-0.02	-0.45	0.06	-0.18
H4 root length (mm)	0.46	1.13	-0.10	0.44
H1 total seedling dry weight (mg)	0.86	3.02	-0.39	-1.01
H2 shoot dry weight (mg)	-0.23	-0.35	-0.63	-0.52
H3 shoot dry weight (mg)	-0.30	0.00	0.32	-0.23
H4 shoot dry weight (mg)	-0.41	-0.93	-0.56	-1.07
H2 root dry weight (mg)	0.05	1.05	-0.85	0.04
H3 root dry weight (mg)	0.10	0.99	0.68	0.59
H4 root dry weight (mg)	0.17	0.26	0.71	1.44

Table A7.6a Summary of results of multivariate analysis of variance of numerous vegetative characters for Oregon and Cockayne sheep's burnet at two temperatures.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Temperature	148.97	18, 29	***
Temperature (block)	1.80	72, 116	**
Seed line	14.78	18, 29	***
Depth	15.78	18, 29	***
Seed size	4.82	36, 58	***
Temperature x seed line	7.03	18, 29	***
Temperature x depth	2.60	18, 29	*
Temperature x seed size	1.66	36, 58	*
Seed line x seed size	1.48	36, 58	(NS)
Seed line x depth	1.08	18, 29	NS
Depth x seed size	1.54	36, 58	(NS)
Temperature x depth x seed size	0.92	36, 58	NS
Temperature x seed line x depth	1.55	18, 29	NS
Temperature x seed line x seed size	1.51	36, 58	(NS)
Seed line x depth x seed size	1.64	36, 58	*

Table A7.6b Between group structure and standardised coefficients for the single or first discriminant functions, involving vegetative characters for Oregon and Cockayne sheep's burnet, for four interactions.

	temperature x seed line interaction		temperature x depth interaction	
Character	between group structure	standardised coefficient	between group structure	standardised coefficient
emergence (%)	1.00	-0.14	1.00	-0.22
doubles (%)	-1.00	0.86	-1.00	0.53
time of final emergence (days)	1.00	-0.80	1.00	-1.88
H1 shoot length (mm)	-1.00	0.38	-1.00	-0.58
H2 shoot length (mm)	1.00	-0.06	-1.00	-0.22
H3 shoot length (mm)	1.00	1.03	-1.00	-1.06
H4 shoot length (mm)	1.00	-0.88	-1.00	-2.20
H1 root length (mm)	-1.00	-1.24	1.00	0.33
H2 root length (mm)	1.00	0.63	-1.00	0.54
H3 root length (mm)	-1.00	-0.41	1.00	0.23
H4 root length (mm)	-1.00	-0.44	-1.00	-0.48
H1 total seedling dry weight (mg)	1.00	1.63	1.00	1.63
H2 shoot dry weight (mg)	1.00	-1.15	-1.00	-2.44
H3 shoot dry weight (mg)	1.00	0.45	1.00	1.09
H4 shoot dry weight (mg)	-1.00	-0.36	1.00	0.62
H2 root dry weight (mg)	1.00	2.86	1.00	2.59
H3 root dry weight (mg)	1.00	0.59	1.00	0.65
H4 root dry weight (mg)	-1.00	-0.08	1.00	-0.23

Table A7.6b (contd.)

	temperature x seed size interaction		depth x seed size x seed line interaction	
Character	between group structure	standardised coefficient	between group structure	standardised coefficient
emergence (%)	0.62	0.12	-0.39	-0.44
doubles (%)	-0.69	0.04	0.88	1.26
time of final emergence (days)	0.09	0.14	-0.21	-1.68
H1 shoot length (mm)	0.99	0.06	-0.91	0.58
H2 shoot length (mm)	0.84	0.59	-0.91	0.04
H3 shoot length (mm)	-0.84	-0.36	-0.98	-0.53
H4 shoot length (mm)	-0.93	-2.84	0.96	-0.94
H1 root length (mm)	0.96	-0.07	-0.77	-1.19
H2 root length (mm)	-0.99	0.61	0.94	0.90
H3 root length (mm)	0.67	0.17	0.15	0.12
H4 root length (mm)	0.94	0.54	-0.24	-0.38
H1 total seedling dry weight (mg)	0.79	1.38	0.99	2.51
H2 shoot dry weight (mg)	0.99	-0.49	-0.94	-1.97
H3 shoot dry weight (mg)	0.55	0.05	-0.99	0.50
H4 shoot dry weight (mg)	-0.52	-0.82	0.74	-0.66
H2 root dry weight (mg)	-0.94	0.04	-0.71	1.99
H3 root dry weight (mg)	0.97	1.01	-0.30	0.57
H4 root dry weight (mg)	0.84	0.93	0.16	0.61

Table A7.7a Summary of results of multivariate analysis of variance of numerous vegetative characters for sheep's burnet and lucerne at two temperatures

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Temperature	74.53	17, 28	***
Temperature (block)	1.88	68, 112	**
Treatment	7.10	85, 140	***
Depth	17.25	17, 28	***
Temperature x depth	0.59	17, 28	NS
Temperature x treatment	2.66	85, 140	***
Depth x treatment	0.94	85, 140	NS
Temperature x depth x treatment	1.04	85, 140	NS

Table A7.7b Between group structure and standardised coefficients for up to the first two discriminant functions, involving vegetative characters for sheep's burnet and lucerne, for depth and temperature x treatment interaction.

Character	Depth		temperature x treatment interaction			
			DF1		DF2	
	between group structure	standardised coefficient	between group structure	standardised coefficient	between group structure	standardised coefficient
emergence (%)	-1.00	0.20	0.56	0.38	0.78	0.81
time of final emergence (days)	1.00	2.30	0.92	3.37	-0.06	0.00
H1 shoot length (mm)	1.00	1.13	-0.63	0.18	0.63	0.90
H2 shoot length (mm)	1.00	2.14	0.88	2.24	-0.28	-0.47
H3 shoot length (mm)	1.00	2.28	0.49	1.09	0.83	2.07
H4 shoot length (mm)	1.00	0.39	0.29	-0.32	0.53	0.88
H1 root length (mm)	-1.00	-0.72	-0.90	-1.28	0.39	-0.05
H2 root length (mm)	-1.00	0.04	-0.37	0.14	0.51	0.10
H3 root length (mm)	-1.00	-0.38	0.79	0.91	0.43	0.12
H4 root length (mm)	-1.00	0.61	0.20	0.70	-0.55	0.14
H1 total seedling dry weight (mg)	-1.00	0.48	-0.80	-0.08	0.55	0.39
H2 shoot dry weight (mg)	1.00	-0.76	-0.35	-0.72	0.67	-0.31
H3 shoot dry weight (mg)	-1.00	-0.64	0.82	-0.69	-0.13	-0.62
H4 shoot dry weight (mg)	-1.00	-0.17	-0.03	0.16	0.00	-0.92
H2 root dry weight (mg)	-1.00	0.24	0.29	0.83	0.80	0.33
H3 root dry weight (mg)	-1.00	-0.07	0.52	0.55	-0.79	-0.78
H4 root dry weight (mg)	-1.00	-0.23	0.19	0.10	-0.14	0.66

DF = discriminant function

CHAPTER 8

Table A8.1a Summary of results of multivariate analysis of variance of monomolecular function parameters describing leaf extension for three leaves of plants defoliated to 0, 25, 50, 75 and 100% rLA - experiment one.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	0.98	27, 12	NS
Defoliation intensity	2.60	36, 17	*

Table A8.1b Between group structure and standardised coefficients for the first discriminant function for intensity involving monomolecular function parameters.

Parameter	between group structure	standardised coefficient
β_0 - leaf 1	0.91	5.02
β_0 - leaf 2	0.88	-2.77
β_0 - leaf 3	0.84	-0.70
β_1 - leaf 1	0.96	2.33
β_1 - leaf 2	0.92	0.06
β_1 - leaf 3	-0.31	1.70
β_2 - leaf 1	-0.87	0.15
β_2 - leaf 2	-0.94	-0.64
β_2 - leaf 3	-0.78	0.28

Table A8.2a Summary of results of multivariate analysis of variance of monomolecular function parameters describing leaf extension for three leaves of defoliated plants which were previously shaded and unshaded.

		Wilks' Criterion	
Source of variation	F-test	df	Significance
Block	0.77	27, 21	NS
Shade	0.33	9, 7	NS
Defoliation intensity	2.95	18, 14	*
Shade x intensity	1.30	18, 14	NS

Table A8.2b Between group structure and standardised coefficients for the first discriminant function for shade x intensity interaction involving monomolecular function parameters.

Parameter	between group structure	standardised coefficient
β_0 - leaf 1	1.00	3.10
β_0 - leaf 2	0.98	1.01
β_0 - leaf 3	1.00	-0.93
β_1 - leaf 1	0.99	0.73
β_1 - leaf 2	0.96	-2.00
β_1 - leaf 3	-0.96	-0.98
β_2 - leaf 1	-0.94	0.58
β_2 - leaf 2	-1.00	-0.38
β_2 - leaf 3	-0.98	-0.72

Table A8.3a Summary of results of multivariate analysis of variance pooled over experiments of monomolecular function parameters describing leaf extension for three leaves of variously defoliated unshaded plants.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Experiment	9.38	9, 10	***
Block (experiment)	0.96	54, 56	NS
Defoliation intensity	2.01	27, 30	*
Intensity x experiment	0.88	27, 30	NS

Table A8.3b Between group structure and standardised coefficients for the single or first discriminant functions for experiment and defoliation intensity - pooled analysis.

	experiment +		defoliation intensity	
Parameter	between group structure	standardised coefficient	between group structure	standardised coefficient
β_0 - leaf 1	1.00	0.25	0.82	0.56
β_0 - leaf 2	1.00	-0.13	0.93	2.99
β_0 - leaf 3	1.00	1.55	0.57	-2.76
β_1 - leaf 1	1.00	-0.18	0.96	0.77
β_1 - leaf 2	-1.00	-0.22	0.89	0.65
β_1 - leaf 3	1.00	0.51	0.96	-0.95
β_2 - leaf 1	1.00	0.27	-0.85	0.05
β_2 - leaf 2	1.00	-0.15	-0.99	1.36
β_2 - leaf 3	1.00	1.57	-0.96	-2.75

+ single discriminant function

Table A8.4a Summary of results of multivariate analysis of variance of vegetative and carbohydrate characters for unshaded plants variously defoliated - experiment one.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	1.90	30, 9	NS
Defoliation intensity	4.57	40, 13	**

Table A8.4b Between group structure and standardised coefficients for the first discriminant function for defoliation intensity involving vegetative and carbohydrate characters.

Character	between group structure	standardised coefficient
root dry weight (g)	0.40	-20.92
stubble dry weight (g)	-0.06	-11.13
leaf dry weight (g)	0.18	12.05
stubble soluble sugars (%)	0.14	4.53
stubble starch (sqrt %)	0.62	-4.87
root soluble sugars (%)	0.02	-3.61
root starch (sqrt %)	0.53	9.37
leaf soluble sugars (%)	-0.79	-17.13
leaf starch (%)	-0.68	-0.61
leaf number	0.99	8.30

Table A8.5a Summary of results of multivariate analysis of variance of vegetative and carbohydrate characters for shaded and unshaded plants variously defoliated - experiment one.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	0.28	30, 18	NS
Shade	3.22	10, 6	(NS)
Defoliation intensity	4.49	20, 12	**
Shade x intensity	3.27	20, 12	*

Table A8.5b Between group structure and standardised coefficients for the first discriminant function for shade x intensity interaction involving vegetative and carbohydrate characters.

Character	between group structure	standardised coefficient
root dry weight (g)	-0.72	-0.39
stubble dry weight (g)	-0.97	-2.93
leaf dry weight (g)	-0.74	1.11
stubble soluble sugars (%)	-0.81	0.07
stubble starch (sqrt %)	0.82	-0.21
root soluble sugars (%)	0.85	0.23
root starch (sqrt %)	0.98	2.03
leaf soluble sugars (%)	-0.73	-0.46
leaf starch (%)	0.41	-0.02
leaf number	0.99	0.99

Table A8.6a Summary of results of multivariate analysis of variance pooled over experiments for vegetative and carbohydrate characters of variously defoliated unshaded plants.

Source of variation	Wilks' Criterion F-test	df	Significance
Experiment	6.86	7, 12	**
Block (experiment)	0.95	42, 60	NS
Defoliation intensity	3.42	21, 35	***
Intensity x experiment	1.21	21, 35	NS

Table A8.6b Between group structure and standardised coefficients for the single or first discriminant functions for experiment and defoliation intensity - pooled analysis.

Character	Experiment +		Defoliation intensity	
	between group structure	standardised coefficient	between group structure	standardised coefficient
root dry weight (arcsine)	1.00	0.68	-0.86	-0.43
stubble dry weight (g)	1.00	0.96	-0.81	-1.28
leaf dry weight (log)	1.00	0.26	-0.88	1.74
stubble soluble sugars (%)	1.00	0.21	-0.66	-0.62
root soluble sugars (%)	-1.00	-0.17	0.78	0.76
leaf soluble sugars (%)	-1.00	0.25	0.96	2.01
leaf starch (%)	-1.00	-0.25	0.92	0.51

+ single discriminant function

CHAPTER 9

Table A9.1 Summary of results of multivariate analysis of variance of monomolecular function parameters describing leaf extension for three leaves of variously defoliated plants growing in large bags.

		Wilks' Criterion	
Source of variation	F-test	df	Significance
Block	0.38	18, 28	NS
Defoliation intensity	1.65	18, 28	NS
Age	1.63	9, 14	NS
Moisture	2.00	9, 14	NS
Intensity x age	1.29	18, 28	NS
Intensity x moisture	1.29	18, 28	NS
Age x moisture	0.97	9, 14	NS
Intensity x age x moisture	1.08	18, 28	NS

Table A9.2a Summary of results of multivariate analysis of variance pooled over bag sizes of monomolecular function parameters describing leaf extension for three leaves of variously defoliated plants.

		Wilks' Criterion	
Source of variation	F-test	df	Significance
Bag size	1.66	9, 4	NS
Block (size)	1.31	36, 17	NS
Defoliation intensity	3.33	9, 4	NS
Moisture	1.19	9, 4	NS
Size x intensity	0.56	9, 4	NS
Size x moisture	4.96	9, 4	(NS)
Moisture x intensity	10.85	9, 4	*
Intensity x size x moisture	2.01	9, 4	NS

Table A9.2b Between group structure and standardised coefficients for the single discriminant function for moisture x intensity interaction in the pooled analysis.

Parameter	between group structure	standardised coefficient
β_0 - leaf 1	1.00	-0.76
β_0 - leaf 2	1.00	10.13
β_0 - leaf 3	1.00	-4.64
β_1 - leaf 1	1.00	1.05
β_1 - leaf 2	-1.00	-5.45
β_1 - leaf 3	1.00	4.98
β_2 - leaf 1	-1.00	0.65
β_2 - leaf 2	1.00	4.37
β_2 - leaf 3	1.00	-1.75

Table A9.3a Summary of results of multivariate analysis of variance of vegetative and carbohydrate characters for variously defoliated plants growing in large bags.

		Wilks' Criterion	
Source of variation	F-test	df	Significance
Block	1.79	20, 26	(NS)
Defoliation intensity	3.47	20, 26	**
Age	6.63	10, 13	**
Moisture	9.62	10, 13	***
Intensity x age	1.36	20, 26	NS
Intensity x moisture	0.78	20, 26	NS
Age x moisture	0.57	10, 13	NS
Intensity x age x moisture	0.33	20, 26	NS

Table A9.3b Between group structures and standardised coefficients for the single or first discriminant functions for defoliation intensity, moisture level and plant age involving vegetative and carbohydrate characters for plants in large bags.

Character	defoliation intensity +		moisture level		plant age	
	between group structure	standardised coefficient	between group structure	standardised coefficient	between group structure	standardised coefficient
leaf number	0.99	0.42	-1.00	0.50	-1.00	-0.81
leaf dry weight (g)	0.99	0.18	1.00	1.95	-1.00	-2.34
leaf area (cm^2)	0.99	-0.22	-1.00	-2.28	-1.00	3.13
root dry weight (g)	-0.88	0.92	-1.00	0.37	1.00	-0.21
stubble dry weight (g)	-0.67	-0.75	1.00	-0.62	1.00	0.73
leaf soluble sugars (%)	0.99	2.52	1.00	2.13	1.00	-0.99
stubble soluble sugars (%)	-0.99	0.21	1.00	1.74	1.00	-0.08
stubble starch (log)	0.98	0.96	1.00	2.11	-1.00	-2.56
root soluble sugars (%)	0.89	-0.20	1.00	-0.22	1.00	0.30
root starch (%)	-0.97	-0.89	-1.00	-0.36	1.00	-0.34

+ = first discriminant function

Table A9.4a Summary of results of multivariate analysis of variance pooled over bag sizes for vegetative and carbohydrate characters of variously defoliated plants.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Bag size	6.22	10, 3	(NS)
Block (size)	1.94	40, 13	(NS)
Defoliation intensity	6.34	10, 3	(NS)
Moisture	1.16	10, 3	NS
Size x intensity	3.20	10, 3	NS
Size x moisture	10.42	10, 3	*
Moisture x intensity	2.99	10, 3	NS
Intensity x size x moisture	1.28	10, 3	NS

Table A9.4b Between group structure and standardised coefficients for the single discriminant function for bag size x moisture regime interaction, involving vegetative and carbohydrate characters.

Character	between group structure	standardised coefficient
leaf number	1.00	-4.67
leaf dry weight (g)	-1.00	-5.09
leaf area (cm^2)	1.00	7.72
root dry weight (g)	1.00	4.69
stubble dry weight (g)	1.00	-1.63
leaf soluble sugars (%)	-1.00	0.69
stubble soluble sugars (%)	-1.00	-6.77
stubble starch (%)	1.00	3.04
root soluble sugars (%)	-1.00	1.99
root starch (sqrt)	1.00	-0.92

Table A9.5a Summary of results of multivariate analysis of variance for plants growing in large bags, involving transpiration loss per unit leaf area, leaf number and leaf dry weight.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	0.72	6, 16	NS
Defoliation intensity	2.46	6, 16	(NS)
Plant age	0.49	3, 8	NS
Intensity x age	0.89	6, 16	NS

Table A9.5b Summary of results of multivariate analysis of variance for plants growing in large bags, involving transpiration rate per unit leaf area, leaf number and leaf dry weight.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Block	0.47	6, 16	NS
Defoliation intensity	1.98	6, 16	NS
Plant age	0.41	3, 8	NS
Intensity x age	1.31	6, 16	NS

Table A9.6a Summary of results of multivariate analysis of variance pooled over bag sizes involving transpiration loss per unit leaf area, leaf number and leaf dry weight.

	Wilks' Criterion		
Source of variation	F-test	df	Significance
Bag size	801.94	3, 2	**
Block (size)	4.79	12, 6	*
Defoliation intensity	826.29	3, 2	**
Intensity x size	57.76	3, 2	*

Table A9.6b Summary of results of multivariate analysis of variance pooled over bag sizes involving transpiration rate per unit leaf area, leaf number and leaf dry weight.

		Wilks' Criterion	
Source of variation	F-test	df	Significance
Bag size	353.12	3, 2	**
Block (size)	3.50	12, 6	(NS)
Defoliation intensity	315.73	3, 2	**
Intensity x size	44.93	3, 2	*

Table A9.6c Between group structure and standardised coefficients for the single discriminant function for the intensity x size interaction in the pooled analysis - transpiration loss characters.

Character	between group structure	standardised coefficient
transpiration per leaf area (g cm^{-2})	1.00	11.05
transpiration per leaf number (g $number^{-1}$)	1.00	15.47
transpiration per leaf dry weight (g g^{-1})	1.00	-18.58

Table A9.6d Between group structure and standardised coefficients for the single discriminant function for the intensity x size interaction in the pooled analysis - transpiration rate characters.

Character	between group structure	standardised coefficient
transpiration rate per leaf area (g $d^{-1}cm^{-2}$)	1.00	0.92
transpiration rate per leaf number (g $d^{-1}number^{-1}$)	1.00	-12.25
transpiration rate per leaf dry weight (g $d^{-1}g^{-1}$)	1.00	23.24