QUANTITATIVE GENETICS OF SHEEP PREFERENCE IN RED CLOVER (*TRIFOLIUM PRATENSE* L.) UNDER SPACED PLANT AND SWARD CONDITIONS

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ABSTRACT

Nine populations of diploid Red clover (*Trifolium pratense* L.) (Erect: Turkish, Hamua and Quiñiquelli; Semi-erect: Colenso, Kenland and E116; Prostrate: F.2419, Astred and Turoa) representing material from the main temperate regions of the world were used in experiments conducted at Massey University (New Zealand) and INIA-La Estanzuela (Uruguay).

Population seedlings were sampled under glasshouse conditions (one at each site) to raise representative samples for cloning for two field studies. Principal components were used to ensure representativeness of the sample.

Field designs and statistical models were developed specifically to meet the requirements for genotype evaluation under grazing conditions, and to estimate genotypic parameters of plant characteristics influencing selective grazing behaviour.

A preliminary grazing management experiment was conducted at Massey University with spaced plants (9 populations x 80 plants), where four stocking densities (2, 3, 5 and 9 sheep/18m^2 for one hour) at two times of grazing (morning or evening) were imposed on the nursery, in order to determine optimum measurement of sheep grazing preference. It was found that the preferred grazing management was to graze until an average of 40% leaf remained in the residual plant material (equivalent to a stocking density of 5 sheep/18m^2) for one hour, at either morning or evening. This achieved a 94% sampling intensity. This regime was used subsequently in the further three grazing experiments.

Two spaced-planted experiments (one at each site) were conducted in three blocks of 324 plants each (9 populations x 12 genets x 3 ramets) which were completely randomised in a 0.75m grid in each block, using the optimum grazing management. Pre-grazing plant measurements were taken on some characters (habit, leaf size, flowering and density); while pre- and post-grazing measures were taken on others (height, spread

and leafiness). Subsequently to the experimental defoliation, all plants were defoliated to a uniform 20% leaf residual, by mob stocking. The statistical design was a diffuse randomised complete block with plants nested inside populations at the whole plot level, with a split-plot in time and pooled across sites. The results demonstrated that grazing animals were grazing selectively, rather than grazing at random: the four most grazed populations were Quiñiquelli, E116, Kenland and Turoa and the least grazed were Astred and Turkish. The preferred populations had the highest levels of crude protein and digestibility, and the least grazed populations had the lowest values. Post-grazing leafiness was considered the most suitable morphological character to determine grazing preference because it was highly significant in the analyses of variance for the Population and Plant effects, and demonstrated heritability values > 0.2, allowing modest genetic progress.

A sward experiment was conducted at INIA-La Estanzuela with a subset of six populations sown in three blocks, each with three internal replicates of 12.25 m^2 each and four internal sub-samples. The same random principle was applied to give a random offer to the grazing animals, but at a plot level. The efficacy of selecting for swards in spaced plant nurseries was examined through the ratio of the correlated genetic advance in swards of selecting under spaced conditions to the direct genetic advance of selecting in sward conditions. Plant density, post-grazing leafiness, difference between pre- and post-grazing leafiness, and index of intake achieved greater genetic advance when selection was done as spaced plants: while for pre- and post-grazing height the opposite result was found. For all other characters, the best conditions to select in depended on the selection intensity achievable.

It is concluded that the breeding of Red clover to improve its grazing preference should not be based on simple morphological characters. Rather, it should be based on a measurement of forage removal such as post-grazing leafiness, and under spaced plant conditions, even considering that the final use is under sward conditions.

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CHAPTER ONE
General Introduction

The history of breeding pasture plants is relatively short in comparison with that of other species, like wheat (*Triticum aestivum L.*) and maize (*Zea mays L.*). Its origins and methodology are well identified. In the 1930's the initial breeding was done to increase yield (Vogel and Sleper, 1994). Today, in those forage species in a more advanced stage of domestication, other objectives are becoming more relevant such as persistence, production under limiting resources (high competition or poor growing environment), and high grazing pressure.

To evaluate a large number of lines or selections (as is the case in early stages of any forage breeding programme), foliage cutting is the easiest and most economical method of defoliation, but many researchers have expressed concern about the validity of the results (Hodgson, 1981; Evans *et al.*, 1992; Swift *et al.*, 1992; van Santen, 1992).

Since pastures are not an end product (except in the case of seed production, hay making or silage making), the forage breeder's goal should be to improve animal performance on pastures rather than pasture productivity *per se* (Williams, 1987; Burton, 1992; Reheul and Ghesquiere, 1994).

The grazing animal can alter the development of forage species, either by direct defoliation, treading, excretal return and/or indirectly by changing the sward structure and micro-environment (Curll and Wilkins, 1983; Grant and Marriott, 1994). All of which are arguments against having grazers in a breeding nursery, because they all increment errors and reduce heritabilities. However, despite all the problems, two aspects of plant/animal interaction are important in selecting plants for use in pasture grazing systems: (1) preferential defoliation and level of intake and (2) tolerance of (resistance to) defoliation, and recovery growth.

Preference is a complex phenomenon determined by the animal, the plants and the environment in which the plant or plant-part discrimination occurs (Marten, 1978). For this reason, it is important to identify a suitable method and/or plant characteristics with high genetic correlation with animal preference to use in pasture breeding. As no general selection criteria have yet been found for substituting the grazing animal, the inclusion of grazers is considered essential. The chosen characters need to reflect this animal reaction, and also have as high a heritability as possible.

Ivins (1952), Garner (1963), Voigt *et al.* (1970), Marten and Jordan (1974), Emile *et al.* (1992), Culvenor (1993) and Reed (1996) studied the effect of preference on animal performance, concluding that animal production was increased when grazing was done without choice on more palatable materials of the same species and under the same experimental conditions. In contrast, Marten *et al.* (1990) and Black (1990) did not confirm the results obtained by the previous authors. When choice is possible, in swards of several species, preferential grazing could be of major importance for increasing animal production.

Part of the present study aims at estimating quantitative genetic statistics to evaluate novel field and statistical designs proposed as part of this thesis. These novel designs intended to solve some practical problems of evaluating spaced plants with grazing animals in a small piece of land. A random offer of plants was given to the animals to detect if grazers were defoliating at random or if discrimination was taking place, and if that discrimination was consistent among grazings and sites. The statistical design and subsequent analyses consider all sources of variation to reduce error as much as possible, and to separate genetic effects from environment effects. Clonal replicates are used to enhance heritability estimates, as most characters are inherited quantitatively.

Heritability estimates are the most important information to decide the usefulness of the characters to be improved, and also to decide the best selection strategies and breeding methods to be applied. Genetic correlations are also considered important to explore indirect and/or multiple selection when breeders try to improve several traits at the same time. When

breeders are improving only one character they require at least that the other characters should remain stable.

Not only does the issue of grazing versus cutting constitute a dilemma for breeders, but the sowing method (spaced plants versus swards) has also been debated since the early history of plant breeding (Ahlgren, 1944; Poehlman and Borthakur, 1969).

Working with spaced plants allows the breeder to identify each plant and to see its own phenotypic value, but the genotype-sowing density interaction and genetic correlation between spaced plants and swards should be taken into account to decide if spaced plants are a suitable environment for forage breeding. The argument in favour of evaluation under sward conditions is that plant to plant interactions are active as in the farmer's field, and that selection would be done in a competitive environment similar to that in which the new cultivar will have to perform.

Falconer (1952) suggested for the first time that the same attribute could be measured in two different environments (spaced plants versus swards) and that they could be considered as if they were two different attributes, allowing estimates of genetic correlations to be made. This genetic correlation would give the necessary information to judge the best conditions to select under, based on estimates of genetic advance. The genetic correlation between performance under spaced plant conditions and under sward conditions, for the same attribute, determines the scope for extrapolation of results from one environment to the other.

McWilliam and Latter (1970) appear to be one of the few who studied this genetic correlation of spaced plant environment with sward environment with the purpose of exploring indirect selection. They found these genetic correlations to be significant for one character (autumn growth for the second year) but not for the other (winter growth for the second year). Therefore, the authors concluded that their breeding programme should include evaluation under both environments to combine virtues of both techniques.

Some approaches to the problem have involved selection of plants according to the performance of their progeny sown in swards (Taylor, 1987), or sowing spaced plants in a sward of another species (Atwood and Garber, 1942; Gibson, 1964; Dijkstra and DeVos, 1972; Van Dijk and Winkelhorst, 1978; Caradus, 1991). These are essentially phenotypic "control" or "check" experiments, and involve extra research resources. It may be more efficient to improve the selection nursery itself. In order to do so, it is useful to estimate the quantitative genetic variation acting behind the phenotype and to predict genetic advance under direct, indirect or multiple selection. Such research will also identify which characters are most useful. The work will also be a selecting experiment in its own right, and reveal plants which might lead to a new cultivar.

Red clover (*Trifolium pratense* L.) was chosen for this study for four reasons: (1) it is one of the main forage legumes in the temperate regions of the world with a wide range of adaptation to soil types and pH levels (Smith *et al.*, 1985); (2) it is one of the least persistent clovers, but with maximum production during the first two years and its persistence is reduced by grazing (Taylor and Smith, 1977); (3) it offers vast morphological variation (Claydon and Rumball, 1982) making it an ideal species to test animal preference for morphological characters such as growth habit, leaf size, plant height, plant spread, etc.; and (4) it is a cross-pollinated species with much intra-population variation to offer on top of inter-population variation.

Therefore, the overall objectives of this research were:

(1) to define procedures for including grazing animal effects in the early stages of a forage breeding programme by testing novel field and statistical designs;
(2) to investigate the influence of selective grazing on genetic evaluation;
(3) to evaluate the significance of spaced plant/sward relationships to methodology; and
(4) to establish the importance of plant growth habit/morphology in influencing selection in Red clover.

CHAPTER TWO
Literature Review

2.1 INTRODUCTION

Attention in this Review will be concentrated on plant breeding, evaluation of forage plants from a plant breeder's point of view and experimental methodology used in the studies reported in Chapters 3 to 7.

The plant breeding section reviews breeding methods for cross-pollinated species, the reproductive incompatibility system of Red clover and considerations about quantitative genetics theory regarding estimation of variance components and heritabilities, selection strategies and genetic advance, indirect selection and multiple selection to give background information to define a suitable and efficient breeding programme.

The evaluation of forage plants section considers the concern over the absence of grazing animals and plant competition from forage breeding nurseries. It also considers existing grazing-based evaluations with emphasis on selective grazing as well as on morphological, nutritional and biochemical factors influencing feeding value in forage plants.

The experimental methodologies reviewed in this section are relevant to the procedure used to create a representative sample of each of the nine Red clover populations used in the experiments, the cloning procedures available for Red clover, and the species and stock class of grazing animals with their respective management for grazing spaced plant nurseries.

2.2 PLANT BREEDING

2.2.1 BREEDING METHODS FOR CROSS-POLLINATED SPECIES

Breeding systems are mostly determined by the reproductive system and stage of development of the species (Allard, 1960; Breese and Hayward, 1972). As Red clover (*Trifolium pratense* L.) is a cross-pollinated species like many important pasture species for the temperate regions, only breeding methods for allogamous species are briefly described.

2.2.1.1 INTRODUCTIONS AND/OR ECOTYPES SELECTION

Introductions, topodemes and/or ecotypes (as defined by Lincoln and Boxshall (1987); Ricklefs (1990) and Allaby (1994)) may be used as sources of variation for the creation of a new variety. In many cases those introductions, topodemes or ecotypes without any further breeding are grown and spread as a the new variety with no history of directed artificial selection (Bolton, 1962; Poehlman, 1977; Rumbaugh *et al.*, 1988). This is mainly the case for new species, because as natural selection takes time, ready available strains are used in the early stages of domestication of the crop. However, there is a need for the combination of appropriate characteristics *via* plant breeding to meet farmers and/or industry needs.

2.2.1.2 INDIVIDUAL SELECTION

2.2.1.2.1 MASS SELECTION

Mass selection is the oldest and simplest form of selection. Open-pollinated seed from the better plants (phenotypes) in a population are selected, harvested in bulk to obtain the new cultivar, or it is used as material for the next cycle of selection. Usually, mass selection is done in circumstances which pose no specific environmental

restrictions. Because individuals are not replicated in either time or location, it is not possible to partition variability into genotype and environment components. The use of this procedure is only justifiable for characters of high heritability and under very limited resources. Individual selection is used all throughout the breeding programme (Allard, 1960; Bolton, 1962; Breese and Hayward, 1972; Poehlman, 1977; Sneep and Hendriksen, 1979; Burton, 1974, 1986, 1992; Rumbaugh *et al.*, 1988; Hallauer, 1992; Vogel and Pedersen, 1993; Gordon, 1994; Bos and Caligari, 1995).

2.2.1.2.2 CLONAL LINE SELECTION

Clonal line selection is a simple modification of mass selection, that includes clones to help select the best mother plants by reducing the environmental noise, raising the heritability estimates and allowing genetical differences to be identified (Rumbaugh *et al.*, 1988).

2.2.1.3 BACKCROSS BREEDING

Backcross breeding method is one of the most conservative ones. It involves crossing back to a recurrent parent (plant or population with good general attributes, lacking the attribute to be improved) to try to fix one or a few characters from a donor parent (plant or population with the desired attribute) without modifying the recurrent parent properties. It is usually used for adding characters controlled by a few genes, to a well adapted population. If inbreeding depression is a problem with the particular species under breeding (like Red clover), several parents should be used as recurrent and donor populations (Bolton, 1962; Rumbaugh *et al.*, 1988)

2.2.1.4 LINE SELECTION

In the line selection breeding method, introductions, good public cultivars, selected plants or a combination of all these sources will provide the basic material for

the spaced plant nursery. A large percentage of the best individual plants from the nursery are selected and sown in rows bulked by female genotype (one plant or genotype per row). To produce the progeny various mating systems could be used: open pollination from the nursery, selfing, polycross among selected plants or top-crossing. If self incompatibility is present, individual selection will continue almost to the end of the breeding programme, changing to combined selection in generations six or seven till eight or nine. If plants are able to self, combined selection is applied from the fourth to the seventh generation, and among-line selection for generations eight and nine. Best lines are bulked and the product of this breeding method is an open-pollinated line or composite (Allard, 1960; Bolton, 1962; Latter, 1964; Sneep and Hendriksen, 1979; Rumbaugh *et al.*, 1988; Hallauer, 1992; Vogel and Pedersen, 1993; Gordon, 1994).

2.2.1.5 LINE BREEDING

Line breeding is very similar to Line Selection with the difference that the source material to begin the breeding programme comes from a planned crossing exercise. F_2 is also sown as spaced plants and plants are selected individually. From F_3 to F_5, progenies are sown in rows and combined selection (best plants in best lines) is applied. For the late generation phase, among-line selection could be applied (Gordon, 1994). Varieties derived from single plants in cross-pollinated forage crops usually show inbreeding depression, so a group of the best plants or lines may be allowed to inter-mate to form an open-pollinated composite (Poehlman, 1977; Gordon, 1994).

2.2.1.6 RECURRENT SELECTION

The final product of recurrent selection can be an open-pollinated composite or a synthetic variety. If there is no test of best hybrid combinations (combining ability), it is called "open-pollinated composite" ((2.2.1.6.1) Simple Recurrent Selection). If there is a test to determine the hybrid vigour (combining ability) of possible combinations to choose parents, it is called "synthetic cultivar" ((2.2.1.6.2) Recurrent Selection for

General Combining Ability (GCA), (2.2.1.6.3) Recurrent Selection for Specific Combining Ability (SCA) and (2.2.1.6.4) Reciprocal Recurrent Selection).

2.2.1.6.1 SIMPLE RECURRENT SELECTION

Simple recurrent selection with some restrictions is also known as Recurrent restricted phenotypic selection. The first step in this method is to establish a spaced plant breeding nursery and to evaluate those plants for the desired objective/s. The spaced plant nursery may or may not be divided into smaller sections to try to reduce the environmental effect. If the area is divided, a fixed number of plants is selected from each section, but if that stratification does not take place, a percentage of the total plants is selected. Selected plants are transplanted to another area, or the non selected plants are cut, to allow the selected ones to polycross. If it is feasible, plants are selfed to produce the progeny. This method changes means by incrementing the frequency of favourable alleles through recurrent cycles and utilises all the additive genetic variance. It also keeps inbreeding depression at a minimum level if a large number of parents is polycrossed and there is not a marked loss of genetic variability. As both parents are selected, genetic gains double in comparison with mass selection when only females are selected. The seed from the polycross is space-planted again to begin the new cycle. Number of cycles depends on the breeders objectives. Individual selection is done throughout the cycles, and the product is an open-pollinated composite (Allard, 1960; Breese and Hayward, 1972; Poehlman, 1977; Sneep and Hendriksen, 1979; Burton, 1974, 1982, 1986; Rumbaugh *et al*., 1988; Vogel and Pedersen, 1993; Gordon, 1994).

2.2.1.6.2 RECURRENT SELECTION FOR GENERAL COMBINING ABILITY (GCA)

The first cycle is identical to simple recurrent selection, but instead of allowing all selected plants to polycross, they are crossed to a tester with a wide genetic base. The seed from these crosses is bulked by female genotype and sown to test the progeny as

solid stands, rows or spaced plants. Original plants are selected by the behaviour of this half-sib progeny test. Performance is measured as a deviation from overall population mean and variation among means of half-sib families is known as the GCA variance (Allard, 1960; Latter, 1964; Breese and Hayward, 1972; Rumbaugh *et al.*, 1988; Gordon, 1994).

2.2.1.6.3 RECURRENT SELECTION FOR SPECIFIC COMBINING ABILITY (SCA)

Selection cycles are identical to recurrent selection for GCA, but instead of crossing to a tester with a wide genetic base, a tester with a narrow genetic base is used. The seed from these crosses is bulked by female genotype and sown to test the progeny as solid stands, rows or spaced plants. Original plants are selected by the behaviour of this half-sib progeny test. Performance is measured as a deviation from the sum of the parental GCA, therefore, it is considered a measure of departure from additive scheme, due to dominance or epistasis (Allard, 1960 ; Latter, 1964; Breese and Hayward, 1972; Rumbaugh *et al.*, 1988; Gordon, 1994).

2.2.1.6.4 RECIPROCAL RECURRENT SELECTION (RRC)

Reciprocal Recurrent Selection selects simultaneously for GCA and SCA. It uses two unrelated populations to begin the selection programme, using one as a wide genetic base to test the other and vice versa, and both are also selfed if this is feasible. Crosses are bulked by female genotype and progeny tested to select the best parents. Selected parents are sown from the selfed seed and are allowed to polycross to begin the next cycle of selection with that seed. Individual selection is used throughout the breeding programme assisted by the information obtained in the different kinds of progeny tests (Allard, 1960; Breese and Hayward, 1972; Sneep and Hendriksen, 1979; Hallauer, 1992; Rumbaugh *et al.*, 1988; Vogel and Pedersen, 1993; Gordon, 1994).

2.2.1.7 SYNTHETIC CULTIVARS

As mentioned before in the Recurrent Selection Section, a synthetic cultivar is synthesised from genotypes which were evaluated for combining ability. Only genotypes that combine well with each other in all combinations are part of a synthetic cultivar. This method consists of a spaced plant nursery of an open-pollinated source where plants are selected by their individual phenotypic behaviour. The selected plants are grown in rows to be maintained for future selection and clones or sib-mates of the selected plants are tested for combining ability in any of the following ways: open-pollinated progeny test, top-cross test, polycross test or single cross test. The progeny of the test is sown in rows and evaluated. According to the results of the progeny test and the results of the maintenance rows, the best plants are selected and polycrossed in isolation to produce the first synthetic (Allard, 1960; Cope and Taylor, 1985; Rumbaugh *et al.*, 1988; Bos and Caligari, 1995). The following factors will determine the behaviour of the synthetic: ploidy level, amount of selfing, number of parents, inbreeding in parents, combining ability of parents, relationship among parents and generation of multiplication (Busbice, 1970; Rumbaugh *et al.*, 1988).

2.2.1.8 HYBRID CULTIVARS

Hybrid cultivars is usually known as F_1 populations used for commercial plantings (Allard, 1960). Those F_1s could be obtained by crossing clones of open-pollinated cultivars, inbred lines or genetically dissimilar populations. This breeding method makes the best use of hybrid vigour. Hybrids using inbred lines can be two-way (crossing of two inbred lines), three-way (an F_1 of two inbred lines crossed with an inbred line) and four-way (cross of two F_1 coming from crosses of inbred lines). The best possible crosses can be selected by a general combining ability (GCA) screening or a specific combining ability (SCA) screening. For this method to be successful on a large scale, it involves the use of at least one of the sterility mechanisms to produce hybrids on a large scale. Examples of those methods could be: easy mechanical emasculation on a

large scale (like detasseling in maize), cytoplasmic male sterility, self incompatibility, chemical methods of emasculation, apomixis or hybrids should be able to propagate vegetatively like in several C_4 grasses so that the F_1 could be sown directly in large areas with a fixed genotype (Allard, 1960; Sneep and Hendriksen, 1979; Burton, 1986; Gordon, 1994).

2.2.2 INCOMPATIBILITY SYSTEM IN RED CLOVER

Knowledge of the genetics and physiology of self- and cross-incompatibility is important for planning a breeding programme for a species with an incompatibility system. For plant breeders of cross-pollinated species, it is of particular importance because it reduces inbreeding and promotes cross pollination (Townsend and Taylor, 1985).

Diploid Red clover is a highly self-incompatible species and rarely produces any seed by selfing (Williams and Silow, 1933; Williams and Williams, 1947a; 1947b; Pandey, 1956; Meglic and Smith, 1992). The phenomenon is determined by a single Mendelian gene known as the S-locus (East and Mangelsdorf, 1925 *loc. cit.* Clark and Kao, 1994) with a gametophytic self-incompatibility system (Townsend and Taylor, 1985; Meglic and Smith, 1992). Gametophytic refers to the case that the self-incompatible phenotype of the pollen is determined by its own (haploid) S-genotype and not by the S-genotype of the pollen-producing plant like in the sporophytic type. An incompatible mating occurs when the S-allele of the haploid pollen matches any of the S-alleles of the diploid pistil and style. Usually, the pollen grain is able to germinate and penetrates into the style but if it is not compatible, the tube growth stops before reaching the ovary (Ascher, 1966; Pandey, 1977; Newbigin *et al.*, 1994; Clark and Kao, 1994). There is not any dominance relationship between S-alleles in the female organs, but when pollen grain is 2n like in autotetraploids, dominance, codominance and competition could occur for the S-alleles (Lewis, 1994). At least 37 S-alleles have been reported (Williams and Silow, 1933; Williams and Williams, 1947b; Pandey, 1956). The rejection

of the pollen by the female organs is not 0% or 100%, and other loci modify the strength of the reaction (de Nattancourt, 1977 *loc. cit.* Clark and Kao, 1994).

A relatively high temperature treatment (flower heads at 40^0C and stems at 25^0C) applied during anthesis could make self-incompatible plants produce seeds via pseudo-self-compatibility (Kendall and Taylor, 1969; Taylor and Giri, 1983; Taylor and Wiseman, 1987). Also a self-fertility allele (S_f) has been reported in Red clover and when present in either homozygous or heterozygous conditions, effects full fertility (Williams and Williams, 1947a; 1947b).

2.2.3 ESTIMATION OF VARIANCE COMPONENTS AND HERITABILITY

The method of variance estimation aims to separate and quantify the genotypic variance from the environmental variance (Hetzer *et al.*, 1944; Warner, 1952; Graybill *et al.*, 1956; Miller *et al.*, 1958; Allard, 1960; Bogyo, 1964; Dudley and Moll, 1969; Gordon *et al.*, 1972; Jacquard, 1983; Baker, 1986; Harville and Fenech, 1985; Rattunde *et al.*, 1991; Nyquist, 1991). A simple case where $P_i = G_i + E_i$ will be used to explain the method. The linear regression is as follows:

$$G_i - G = b_{GP}(P_i - P) \qquad (2.1)$$

where b_{GP} is the coefficient of determination (R^2) and G and P are the genotypic mean and phenotypic mean respectively, and

$$R^2 = b_{GP} = \frac{(\sigma_{GP})^2}{\sigma_G^2 \sigma_P^2} \qquad (2.2)$$

where σ_{GP} is the covariance between genotypic and phenotypic values (using Kempthorne's notation, (Kempthorne, 1960)), σ^2_P is the phenotypic variance and σ^2_G is the genotypic variance.

$$\sigma_{GP} = \sigma_{(G)(G+E)} = \sigma_G^2 + \sigma_{GE} \qquad (2.3)$$

Because G_i and E_i are independent, the covariance (σ_{GE}) equals zero and $\sigma_{GP} = \sigma^2_G$.

$$\frac{(\sigma_{GP})^2}{\sigma_G^2\sigma_P^2} = \frac{(\sigma_G^2)^2}{\sigma_G^2\sigma_P^2} = \frac{\sigma_G^2}{\sigma_P^2} = h^2 \qquad (2.4)$$

Heritability (h^2) is defined as the proportion of the variation in phenotypic values explained by the linear regression on genotypic values. As indicated in Equation (2.4), it is expressible as a variance component ratio, and that, indeed, is the more common way of expressing it.

The genetic value of an individual is the average of the phenotypic values over a large number of environments. Accordingly, the value of an environment is dependent on the genotypes utilised to test it and the same concept is valid for the value of heritability of any character (Comstock and Moll, 1963; Nyquist, 1991). There is also the problem of sampling, and therefore, the heritability estimate has its own standard error (Osborne and Paterson, 1952; Gordon *et al.*, 1972; Gordon, 1979).

When the reference population of environments is homogeneous, the genotypic variance increases in the same magnitude that the interaction decreases. If the data is collected from a single year and location, the bias will be increased by the value of the GE interaction (Comstock and Moll, 1963).

To estimate the variance components, an analysis of variance is required which sets the mean squares equal to their expectations and solves the equations for parameters contributing to the expectations. The values obtained are the estimates of the true parameters. The use of an appropriate model is the safeguard against misrepresentation of the information extractable from the data. The estimates of variance components are linear functions of mean squares, and independent in their sampling errors. The variance of the estimated variances can

be written as a function of the variances of the mean squares contributing to the estimates (Crump, 1946; 1951; Satterthwaite, 1946; Comstock and Moll, 1963; Harville and Fenech, 1985).

2.2.4 SELECTION STRATEGIES AND GENETIC ADVANCE

Four selection strategies could be used in different parts of almost any breeding method: individual selection (both parents or one parent selected), amongst-line selection, within-line selection and combined selection. The different selection strategies will be defining the general selection efficiency (rate of genetic advance) obtained for the whole breeding method.

Individual (Both parents selected): $\Delta G_{ind.} = ih^2\sigma_P$ (2.5)

Individual (One parent selected): $\Delta G_{ind.(1parent)} = \frac{ih^2\sigma_P}{2}$ (2.6)

Amongst-line: $$\Delta G_{al.} = \Delta G_{ind.}\left(\frac{1+(n-1)r_A}{\sqrt{n}\left(1+(n-1)r_P\right)^{1/2}}\right) \quad (2.7)$$

Within-line: $$\Delta G_{wl.} = \Delta G_{ind.}\left(1-r_A\right)\left(\frac{(n-1)}{n(1-r_P)}\right)^{1/2} \quad (2.8)$$

Combined: $$\Delta G_{CMB.} = \Delta G_{ind.}\left(1+\frac{(r_A-r_P)^2(n-1)}{(1-r_P)(1+(n-1)r_P)}\right)^{1/2} \quad (2.9)$$

where i = standardised selection differential, h^2 = predictive heritability, σ_P = phenotypic standard deviation, n = plants per experimental unit, r_A = genetic correlation of plants within lines, and r_P = phenotypic correlation among plants within lines (Falconer, 1960). Combined selection is always the most efficient strategy (Falconer, 1960; Gordon, 1994). Even though these ΔG's are tools to measure selection efficiency of all breeding programmes, it is also important to consider that all methods have their weak and strong points and that the most appropriate breeding method will depend on each particular

situation, mainly determined by the species, breeding objectives and resources available.

To calculate the annual genetic advance, each of the previous formulae (2.5...2.9) should be divided by the number of years to complete one generation. Methods involving progeny tests usually increase the generation interval, reducing the annual genetic advance.

2.2.5 INDIRECT SELECTION

Genotype-environment interactions could be of primary importance in a spaced plants situation in a breeder's nursery, in comparison with the normally imposed growing conditions for the plants in a pure or mixed species sward. The difference in spacing makes the reference population of environments quite different and so the estimations of genotypic values (Comstock and Moll, 1963). Traditionally, the breeder selects the plants in a spaced plant environment and the agronomist tests them under sward conditions, not surprisingly revealing many inconsistencies (Ahlgren, 1944; Ahlgren *et al.*, 1945; Lazenby and Rogers, 1962; Van Dijk and Winkelhorst, 1978).

The same trait in two different environments can be considered two different traits (Falconer, 1952; Van Vleck, 1964; Searle, 1965; McWilliam and Latter, 1970; Wiggans *et al.*, 1980; Fernando *et al.*, 1984; Rattunde *et al.*, 1991; Van Sanford *et al.*, 1993). The objective is to select in one environment to obtain a better result in another. This could be the case when the selection in one of the environments is difficult, but the decision should be based in the solution of the following formula suggested by Lerner and Cruden (1948).

$$\frac{C\Delta G_{AB}}{\Delta G_B} = \frac{i_A h_A r_{A(AB)}}{i_B h_B} \qquad (2.10)$$

where $C\Delta G_{AB}$ is the correlated genetic advance in environment B when selection is done in environment A, ΔG_B is the genetic advance in environment B, i_A is the intensity of selection in

environment A, i_B is the intensity of selection in environment B, h_A is the square root of the heritability of the character in environment A, h_B is the square root of the heritability of the character in environment B and $r_{A(AB)}$ is the genetic correlation that measures the degree of association between the genetic variations of the character in environments A and B. The $r_{A(AB)}$ is not likely to be known because A and B are environments and not characters, but $r_{P(AB)}$ (phenotypic correlation between the same character in the two environments) may be known.

$$r_{A(AB)} = \frac{(r_{P(AB)} - r_{E(AB)} e_A e_B)}{h_A h_B} \qquad (2.11)$$

where $r_{E(AB)}$ is the environmental correlation between A and B (the same as r_P with all one cultivar), e_A is the complement of the square root of heritability of A ($e_A = 1 - h_A$), and e_B is the complement of the square root of heritability of B ($e_B = 1 - h_B$). Then:

$$\frac{C\Delta G_{AB}}{\Delta G_B} = \frac{i_A (r_{P(AB)} - r_{E(AB)} e_A e_B)}{i_B h_B^2} \qquad (2.12)$$

If the ratio is greater than 1, there is more genetic advance if selection is done in A to improve B than to select directly in B (Baker, 1986; 1994).

2.2.6 MULTIPLE SELECTION

Smith (1936) suggested for the first time a method of multiple selection worked out in a logical and systematic manner. The value of plants is expressed as a linear function of their characters and by the use of "discriminant functions" developed by Fisher (Fisher, 1936), it is possible to derive the best available guide to the genetic value of each line.

Smith's index was extended by Hazel (1943) to the case when each individual has a true breeding value and the correlation of its genetic value with the observed phenotypic

expression is known. The main contribution of Hazel's paper was the definition of a method to estimate the variances and covariances required (Lin, 1978).

Some considerations follow:

(a) the phenotypic value (P_i) is partitioned into two components, a genotypic value (G_i) defined as the average over all possible environments, and an environmental contribution (E_i), i.e. the model is:

$$P_i = G_i + E_i \qquad (2.13)$$

(b) only additive (average allele) effects are part of the genotypic value in the model.
(c) with attributes being i = 1, 2, ... ,m, the genotypic importance is $H = \sum_i a_i G_i$, where a_i are constants defined by the breeder. H is also partitioned linearly.

The solution to find the selection index is the linear function $I = \sum_i b_i P_i$ which correlates best with the index H. The solving formula to find the b's is:

$$\mathbf{Pb = Ga} \qquad (2.14)$$

where **P** is the matrix of phenotypic variances and covariances, **G** is the matrix of genotypic variances and covariances and **a** is the vector of economic values or weights (Tallis, 1962; Lin, 1978; Humphreys, 1995). The solution ($\mathbf{b = P^{-1}Ga}$) of the simultaneous equations is obtained by Gaussian elimination (Humphreys, 1995).

A restricted index developed by Kempthorne and Nordskog (1959) is used when not only the best progress in H is important, but also when some G_i should remain constant or unchanged. The mathematical solution was developed by the authors and consists of a simultaneous solution subject to the condition that the covariance between the index and the linear function of genotypes is zero for the character involved to prevent any genetic change.

The Smith-Hazel index selection was named "estimated index" by Williams (1962) because the coefficients of the index are calculated using estimated parameters of the population. The author pointed out that the theoretical accuracy of the index may fail when the estimates are subject to large variation.

The indexes might be divided into three different groups according to their use:

Type 1 - to improve several characters at a time.

Type 2 - to improve one character with assistance from other characters.

Type 3 - to improve complex characters. The traits considered in the index may not include the one of interest, as in characters that are not directly measurable. Such an index can be referred to as an indirect selection index. Binet (1965, *loc. cit.* Lin, 1978) combined measurable traits to obtain genetic gains in another character not included in the index.

Cunningham (1969) suggested a method to decide which traits should be included in the index by dropping each trait in sequence. The reduction in r_{IH} (correlation between I and H) is the parameter used to consider the importance of each trait since genetic progress is proportional to this correlation. The disadvantage of this method is that it requires the calculation of a reduced index for each variable to be evaluated, plus the full rank index.

Response to multitrait selection could be predicted by extending the univariate individual selection response Equation (2.5) to several traits: $\mathbf{r} = \mathbf{P}^{-1}\mathbf{G}\mathbf{s}$ where $\mathbf{P}^{-1}$ and $\mathbf{G}$ means the same as for Equation (2.14) and $\mathbf{r}$ is a vector of selection responses and $\mathbf{s}$ is a vector of selection differentials for measured traits (Humphreys, 1995).

Other methods like "tandem" and "independent culling levels" are also used to select plants considering several attributes or environments. The tandem method is to select for each character (or the same character in different environments) at a time until each is considered improved. The method of independent culling levels or multiple goals allows the breeder to

define a certain critical level for each character (or the same character in different environments) and only the plants better for all of them are selected.

Several authors compared the efficiency of these three ways of multiple selection. The method of total score (selection index) is the most efficient, followed by independent culling levels (Bennett and Swiger, 1980) and finally by the tandem method (Hazel and Lush, 1942; Young, 1961; Finney, 1962).

Index selection is always better than tandem selection for all combinations of parameters simulated (Pesek and Baker, 1969a; 1969b). The efficiency of tandem selection is increased by selecting first the most important traits, and the efficiency of index selection is increased by calculating the coefficients more frequently (Pesek and Baker, 1969; Villanueva and Kennedy, 1993).

Elgin *et al.* (1970) compared several multiple-trait selection methods in an alfalfa trial. The independent culling levels followed the estimated index in efficiency and the least efficient was the tandem method (Elgin *et al.*, 1970; Eagles and Frey, 1974).

From the information given by the authors mentioned above, the estimated selection index is the best but certain limitations have to be considered:

(a) parameters change due to selection; for example, the genetic variance becomes smaller in each successive cycle of selection and the optimum index changes.
(b) true parameters are never known; the estimated parameters from samples are more accurate when the sample is big, but when it is small the estimates of theoretical gains could be biased.

Humphreys (1995) using selection index, compared the multivariate response with the observed response after one generation of selection among half-sib families in six populations of Perennial ryegrass, and concluded that this multivariate approach can be used to predict

breeding progress and to identify key traits and populations in which breeding objectives were most likely to be achieved.

2.3 EVALUATION OF FORAGE BREEDING MATERIAL

Correct evaluation of forage plants is of primary importance for properly targeted genetical progress. Many researchers have expressed concern about the absence of the grazing animal (Sears, 1951; Cuykendall and Marten, 1968; Frame, 1976; Hodgson, 1981; Jones and Walker, 1983; Counce *et al.*, 1984; Jones and Roberts, 1986; Evans and Williams, 1987, Williams, 1987; Evans *et al.*, 1992, Swift *et al.*, 1992; van Santen, 1992; Bouton and Hoveland, 1996) and the absence of plant competition (Ahlgren, 1944; Ahlgren *et al.*, 1945; McDonald *et al.*, 1952; Knight, 1960; Lazenby and Rogers, 1962; 1964; Poehlman and Borthakur, 1969; McWilliam and Latter, 1970; Kamastra *et al.*, 1973; Ugherughe *et al.*, 1980; Gray, 1982; McElroy and Christie, 1986; Williams, 1987; Rattunde *et al.*, 1991; Buxton and Lentz, 1993; Bouton and Hoveland, 1996) in plant breeding programmes (Pers. Comm. C.S. Hoveland (USA-University of Georgia), W.M. Williams (New Zealand-AgResearch), D.R. Woodfield (New Zealand-AgResearch), D.H. Basigalup (Argentina-INTA), R. Oram (Australia-CSIRO), R.A. Culvenor (Australia-CSIRO), and K.F.M. Reed (Australia-PVI)).

2.3.1 CONCERN OVER ABSENCE OF GRAZING ANIMAL

Cutting systems have the advantage of simplicity and low cost, and can be supplemented by adding information about nutritive value such as digestibility, etc.. They have the disadvantages (1) of uniform and sudden defoliation to an arbitrary height for all palatable and unpalatable species or plant parts, (2) that erect plants might have been more severely defoliated than prostrate plants, and (3) that all plant material is removed. In contrast, grazing animals exert treading effects, tearing, selective defoliation, defoliate to

different heights over time and return 70-90% of the ingested nutrients as dung and urine (Frame, 1976; Jones and Walker, 1983).

Jones and Walker (1983) suggested that precise simulation between grazing and cutting may not be possible, but also that it may not be necessary, and that the essential requirement for evaluation is to use cutting systems that will rank species or cultivars equally to grazing systems. Unfortunately, species by defoliation regime interactions do occur, and were evident in 4 out of 5 set of experiments reported by the authors ((Aldrich and Elliot, 1974; Camlin and Stewart, 1975; Ramírez *et al.*, 1976; Hutton *et al.*, 1978; Jones *et al.*, 1980) *loc. cit.* Jones and Walker, 1983).

Hodgson (1981) and Reed (1994) on the accumulated evidence from their reviews of cutting and grazing comparisons, concluded that cutting trials were unlikely to be a reliable guide to performance under grazing conditions.

The effects of different cutting and grazing regimes were studied by Evans and Williams (1987), Evans *et al.* (1992), and Swift *et al.* (1992). The significant interaction of cultivar x defoliation-method observed in these studies emphasises the importance of evaluating and breeding materials under a grazing environment. It was also concluded that plant breeders should consider as selection criteria the total amount eaten by the animal and not the dry matter over an arbitrary height limit. Counce *et al.* (1984) screened twenty-two alfalfa (*Medicago sativa* L.) cultivars for persistence under frequent mowing and continuous grazing. The data revealed that the results from the techniques were not correlated. On the other hand, results of evaluations on Italian ryegrass (*Lolium multiflorum* Lam.) under cutting and grazing showed that the rank order in terms of yield was the same and that the two managements were highly correlated (Jones and Roberts, 1986).

Oram and Culvenor (1994) reported the growing perception of Australian farmers that the winter-active cultivars of *Phalaris aquatica* L. do not persist as well as the old cultivar "Australian" under grazing. The authors concluded that future breeding of phalaris

must emphasise persistence under grazing.

Bouton and Hoveland (1996) conducted an experiment to compare the performance of White clover genotypes collected in Georgia pasture conditions with adapted ladino cultivars under grazing conditions. After 16 months of grazing with beef cattle, stands of the ladino types were near zero while most of the collected materials were found to possess excellent clover stands. The same authors conducted another experiment with Red clover to compare the stand survival of eight modern cultivars subjected to grazing or infrequent mowing. All entries in the grazed area showed nearly five-fold less plants per unit area than in the mowed area indicating that grazing tolerance of tested Red clover cultivars was poor. The authors concluded from these two experiments that for forage legume species, cultivar selection and testing needs to be done with the grazing animal to properly assess pasture potential and that these conditions should probably be practised as early as possible in the breeding programme.

Also, plant tolerance to treading is not the same for different species (Edmond, 1964; Clements, 1989). Edmond (1964) ranked the response of ten different species (Perennial ryegrass (*Lolium perenne* L.); Perennial ryegrass x Annual ryegrass; Cocksfoot; (*Dactylis glomerata* L.) Timothy (*Phleum pratense* L.); *Agrostis tenuis*; Yorkshire Fog (*Holcus lanatus* L.); *Poa pratensis* L.; *Poa trivialis* L.; White clover (*Trifolium repens* L.) and Red clover) to heavy and moderate treading. The ranking was different for the different levels of treading evaluated.

The list of examples could be almost endless in favour of or against the proposition that the effects of grazing and cutting are the same. The reality is that an agreement amongst researchers has not been reached yet and that perhaps it will never be reached. Some researchers prefer the simplicity of cutting, knowing that their results are debatable; others prefer to include the grazing animal, increasing the complexity of their experiments.

2.3.2 CONCERN OVER ABSENCE OF PLANT COMPETITION

Widely spaced plant growing conditions without companion species has been the most used method for evaluating breeding material because this system requires fewer seeds and estimation of individuality of plants is possible. However, evaluations done by the sole technique of spaced plants cannot infer adequate information on performance in swards (Williams, 1987; Oram and Culvenor, 1994).

Lazenby and Rogers (1962) suggested that with the lack of knowledge of the relationship between spaced plants and swards at that time, the best thing to do was to test the progenies of a large number of selections under sward conditions to decide which ones will be selected. Taylor (1987) considered it a necessity to evaluate spaced plants according to the performance of their progeny in broadcast or drilled plantings used by farmers. Also sentences like this one "it may be doubted that the source nursery is really an efficient way to identify superior plants; indeed, whether it is even very helpful." are found in the literature (Fergus and Hollowell, 1960).

Lazenby and Rogers (1964) studied the behaviour of four different varieties of Perennial ryegrass at four densities (square planted at 70, 23, 8 cm and in sward plots). The four varieties used in the experiment were selected because of differences in growth rhythms, habit of growth and tiller production under spaced plant conditions. The results were not consistent for the three years, the four cultivars or the four spacings. For example, in spaced plants the yield of cultivar S.23 was 29% more than cultivar Kent, while for the same year, the behaviour in swards was the opposite: yield of cultivar S.23 was 11% less than cultivar Kent.

McWilliam and Latter (1970) studied the behaviour of crosses between thirty Mediterranean ecotypes with two Australian and Turkish cultivars of *Phalaris aquatica* L. (*P. tuberosa* L.). The 60 F_1 families were sown as spaced plants and swards conditions. Only two traits (autumn growth for the second year and winter growth for the second year) were

measured under both environments. The rest were only measured under spaced plant conditions to explore the possibility of indirect selection. Only four out of ten had a significant genotypic correlation with the sward characters. The genetic correlation of both environments for autumn growth for the second year and winter growth for the second year were 0.78** and 0.42^{NS} respectively. Individual or family selection based on yields from spaced plants were less efficient than direct selection for sward performance, so the authors concluded that their breeding programme should include evaluation under both environments to combine virtues of both techniques.

Samuel *et al.* (1970) working with Perennial ryegrass found that the relative order of dry matter yield between spaced plants and swards was completely reversed, with S23 producing the highest yields as spaced plants, while Irish performed best under sward conditions. When fertility conditions were raised for the sward environment, the same rankings were found for spaced plants and swards. Gray (1982) found the interaction "genotype x spacing" to be highly significant in a study with ten cocksfoot clones sown at three spacings (30, 60 and 90 cm).

Kamastra *et al.* (1973) worked with Smooth Bromegrass (*Bromus inermis* L.). and did not confirm in sward conditions the advances obtained in spaced plants for quality characteristics, while Ugherughe *et al.* (1980) confirmed them for a similar situation. McElroy and Christie (1986) concluded that the use of spaced plants to improve in vitro digestibility of timothy for future use in swards is more critical than is generally assumed, and that the relative performance of genotypes is very dependent on the nursery conditions under which they are grown.

Elgersma (1990b) reported that spaced-plant traits in general (54 out of 59) showed poor phenotypic correlations to corresponding traits in drilled plots for Perennial ryegrass.

Rattunde *et al.* (1991) studied in winter rye (*Secale cereale* L.) the genetic correlation for a single trait between three different environments (spaced plants, micro-drilled plots and

large-drilled plots). The genotypic differences were significant in all plot types for all the agronomic and quality traits, but the genotypic correlations between plot types were high despite the differences in competition experienced. Based on the high heritabilities achieved in small plots and the high genotypic correlation with the other plot types, the authors concluded that greater use should be made of small plots to select for yield and quality in this species. The authors suggested that additional research should be done in other species and environments to confirm their findings.

Buxton and Lentz (1993) planted orchardgrass clones in two densities (60 cm centres and 15 cm centres) to study the effect of plant density on digestibility and plant morphology. Only a few plant density interactions were significant, indicating good correspondence between plantings for in vitro digestibility of the dry matter and width of the leaf blade.

Because competition is a complex phenomenon and is one of the many cases in which generalisation is not valid, a procedure was developed to alleviate this problem with spaced plants. The procedure involved sowing spaced plants into a sward of another species. This method included competition and individual assessment at the same time (Atwood and Garber, 1942; Davies, 1958; Gibson *et al.*, 1963; Gibson, 1964; Dijkstra and De Vos, 1972; Van Dijk and Winkelhorst, 1978). Caradus (1991) evaluated elite lines of White clover sown in Perennial ryegrass swards. Brink and Rowe (1993) used the same method with spaced plants of White clover in stands of two different bermudagrasses (*Cynodon dactylon* L.). The stolon branching behaviour of the White clover clones in monoculture was not correlated phenotypically with the behaviour in stands of either grass cultivar. They suggested that the evaluation of White clover germplasm should be in association with the future companion species.

Rhodes (1973a; 1973b) and Rhodes and Mee (1980) suggested that spaced plants should be used only to select potentially valuable characters which were easy to determine and related to sward yield.

Culvenor *et al.* (1996) and Culvenor and Oram (1996) emphasised that those who evaluate or breed pasture species need to evaluate them under realistic grazing conditions. According to their results, more productive materials of *Phalaris aquatica* L. under typical spaced plant and plot conditions where cutting or grazing is infrequent, were not more productive under fairly high grazing pressure.

2.3.3 PROCEDURES FOR GRAZING BASED EVALUATIONS

The previous sections raise many issues of controversy over appropriate research methods for testing plant-animal interactions. This section is concerned specifically with the use of grazing managements in plant evaluation, and includes a brief discussion of terminology (2.3.3.1) and a brief summary of the history of such methodology (2.3.3.2). It is concerned with the determination of both grazing preference and tolerance of grazing.

2.3.3.1 TERMINOLOGY

Hodgson (1979) specified particular definitions for different terms: **Palatable** was defined as "pleasant to the taste"; **preference** as "a general term describing the discrimination exerted by animals between areas of sward or the components of a sward canopy, and between or within samples of cut herbage"; **preference ranking** as "the ranking of a series of swards, herbage samples or morphological units, based if possible on the relative intakes determined in free-choice trials"; **diet selection** as "the removal of some components of a sward or a sample of herbage rather than others, a function of preference modified by the opportunity for selection, which is determined by the relative proportions of the preferred components in the sward, and their distribution within the canopy" and **selection ratio** as "the proportion of a component in the diet divided by the proportion of the same component in the sward canopy".

Mertens (1994) defined some of the same terms as follows: **palatability** "the characteristic of a feed indicating its acceptability, usually associated with the gustatory,

olfactory, or visual senses. Palatability affects the preference for a feed when several are available and the rate of eating and intake when a single feed is offered"; **preference** "relative acceptability of a feed when given the choice among two or more feeds that are available in a cafeteria-style feeding situation. Preference is a more specific indication of palatability that affects acceptability among feeds, but does not measure intake modification when no choice among feeds is allowed"; and **selection** "specifically defined to indicate preferential consumption among feed sub-components, such as leaves vs. stems or immature plant tops vs. mature plant bases".

Definitions given by both authors are considered to be complementary and all will be used throughout the thesis.

2.3.3.2 BRIEF HISTORY OF GRAZING BASED EVALUATIONS

Casual observation of grazing animals was the first method used by earlier investigators (Ivins, 1955). It was replaced by continuous observations of grazing animals presented with a choice of herbage species. However, time spent grazing was not an indication of the amount of herbage consumed by the animals: and the latter was considered to be a more valid measure of preference (Jones, 1952; Ivins, 1952; Ivins, 1955; Simon, 1974; Becker and Lohrmann, 1992).

A further basis of estimating preference is by eye examination of the swards pre- and post-grazing. A system of allocating marks according to the amount of herbage on offer, or the ranking of species in order of severity of grazing, are methods using this basis. The major objection to this last method is the possibility that after grazing one particular species or cultivar (presumably the most palatable) right down, the animals may then concentrate on the next most palatable, and graze it down with equal severity. Both species would then receive equal marks regardless of differences in palatability. All these methods can be considered to give a qualitative estimate of intake. To overcome this objection, several authors obtained quantitative data by the determination of an index of palatability, defined as the percentage of

the total herbage available which was actually eaten in some specified time period (Ivins, 1952; Simon, 1974; Petersen *et al.*, 1989).

During the last two or three decades, there emerged experiments to test animal preference called "cafeteria trials". These all had a similar philosophy behind them. Each material (treatment) was sown independently from the others in a randomised complete block design, and the grazing area was usually on a whole block. The grazing period varied from hours to a few days (Becker *et al.*, 1935; Ivins, 1952; Petersen *et al.*, 1958; Cowlishaw and Alder, 1960; Buckner and Burrus, 1961; Rabas *et al.*, 1970; Barnes *et al.*, 1970; Voigt *et al.*, 1970; Simons and Marten, 1971; Marten *et al.*, 1973; Simon, 1974; Marten and Jordan, 1974; Marten and Andersen, 1975; Hedges *et al.*, 1978; Burns *et al.*, 1978; Lascano *et al.*, 1988; Petersen *et al.*, 1989; Schultze-Kraft *et al.*, 1989; McGraw *et al.*, 1989; Davis, 1993).

Davies (1952, *loc. cit.* Cowlishaw and Alder, 1960) pointed out the importance of intensity of grazing in determining animal preference. If grazing is for too short a period, many plants may continue untouched by chance; and if it is too long a period, too many species will be fully grazed down. The author suggested continuous observation till the point of maximum spread in preference ranking, at which the most preferred are completely grazed and the others have been grazed to some extent. Buckner and Fergus (1960), Hedges *et al.* (1978) and McGraw *et al.* (1989) measured relative preference by a visual ranking from 1 (completely grazed) to 10 (completely rejected) and the measurements were done until the most preferred ones reached ratings of 1 or 2. Replication of the materials tested is essential and the location of each material is recommended to be random within each replicate (Jones, 1952).

The period between samplings (before and after grazing) is of importance because the measurements can underestimate the amount eaten because of new growth. The shorter this period, the better (Cowlishaw and Alder, 1960).

Hunt and Hay (1990) presented a photographic technique to measure animal preference taking frames automatically every two minutes. This technique has the advantage, remarked by the authors, that it is rapid and error-free, reliable, sequences in preference are detectable and that all stock activities can be analysed.

Mislevy *et al.* (1982) suggested a method called "mob-grazing" to evaluate the grazing tolerance of a large number of entries in the early stages of a breeding programme. This overcomes the difficulty that the most preferred materials are more severely grazed, because mob-grazing consumes most of the materials rapidly to a standard height. This situation is similar to rotational grazing, and includes the effects of treading, pulling of plants and deposition of faeces and urine. Therefore, entries surviving and producing well under these screening conditions may persist and produce well under commercial grazing conditions. The disadvantage of the technique pointed out by the authors is that the animals may group on a specific grass entry, and excessive mechanical damage and/or deposition of faeces and urine may occur with the consequence of losing highly preferred entries.

Smith *et al.* (1989), Brummer and Bouton (1991), Smith and Bouton (1993) and Bouton *et al.* (1993) determined that grazing tolerance in alfalfa can be improved by selection under continuous close grazing without sacrificing yield potential. Many factors like deep set crowns, subsurface budding, broad crowns, prolific and non-synchronous budding, extended periods of budding, maintenance of leaf area under grazing and maintenance of root carbohydrates are examples of single characters that have to be taken into account to breed grazing-tolerant alfalfa cultivars. Most of the grazing tolerant cultivars in North America were selected for one or two single characters like broad crowns and/or creeping rootedness and they have been of limited usefulness in many regions because of long winter dormancy periods, slow regrowth and variability of expression.

Under extended periods of grazing, overgrazing and thus weakening of the most preferred materials, might be a problem because if there is not an evaluation of preference, the breeder might select against those materials at the end of the grazing period (van Santen,

1992) and what the breeder was in fact measuring was grazing tolerance. This author conducted a valuable investigative experiment, as follows. Twenty-five Tall fescue (*Festuca arundinacea* Schreb.) cultivars and populations were established in two 3 x 1.5 m plots of each entry to create double plots of 9 m^2. The experimental design was a randomised complete block with six blocks. The experiment was repeated in four different pastures for 2 years, and a different stocking rate (2.5, 3.75, 5.0 and 6.25 animals/ha) was assigned to each pasture. Animal preference was rated at 3, 6, and 9 days with a visual score of 1 = 0-25%, 2 = 25-50%, 3 = 50-75% and 4 = 75-100% of defoliation. The population effect was the main explanatory effect accounting for 69 to 82% of the variability. Preference ratings within and between years agreed very closely (R^2 = 0.96 and 0.89 respectively). Preference variation could be explained by regression on maturity score (R^2 = 0.56). This result confirms that maturity and preference are related but also that there are other factors influencing animal preference at the reproductive stage of growth. This allows the breeder to increase preference without changing maturity time (van Santen, 1992).

Bittman and McCartney (1994) used the mob-grazing technique to evaluate alfalfa for production under grazing because of the growing evidence that germplasms that produce well in trials mechanically clipped may not persist and produce well under grazing. The authors preferred mob-grazing technique to continuous grazing because preferential grazing of more palatable cultivars is reduced and only resistance to grazing is evaluated.

In all these studies, however, the quantitative genetics of plant breeding selection itself has not been considered. Genetic advance under direct selection (plant tests themselves) is the product of selection intensity, heritability, and germplasm diversity (Falconer, 1960). However, selection *via* a secondary species (the grazing animal) is indirect selection with respect to the plant focus. Indirect genetic advance involves two heritabilities (one under direct and one under indirect conditions), and the genetic correlation between the two test conditions with and without animals (Falconer, 1960). Whether the preceding debates are of use to the plant breeder depends upon the levels of these selection determinants. There is a serious lack of information in this area.

2.3.4 EMPHASIS ON SELECTIVE GRAZING AND CONSEQUENCES

Since pastures are not an end product (except for seed production or hay or silage making) but a feed, pasture breeder's main goal is to improve animal performance on pastures rather than pasture productivity *per se* (Williams, 1987; Burton, 1992; Reheul and Ghesquiere, 1994).

Not many experiments have been made to directly measure animal production from different cultivars of the same species as reported by Reed (1994) where only 3 out of 11353 abstracts consulted, addressed such intraspecific comparison.

It is accepted that ruminants do not graze randomly, but select a diet among the components of herbage offered (L'Huillier *et al.*, 1984; Provenza and Balph, 1990; Black, 1990; Newman *et al.*, 1992). An example was given by Leigh and Mulham (1966a,b) where 80% of the diet selected by grazing sheep was derived from 1-5% of the forage on offer.

Culvenor (1993) reported observations of selective grazing of winter-active *Phalaris aquatica* L. cultivars in late winter where swards of cv. Australian were only lightly grazed while surrounded by heavily grazed swards of other cultivars.

Several authors studied the effect of preference on animal production. For example, Garner (1963) and Ivins (1952) stated that if stock are eating what is palatable to them, they will eat it readily and in greater quantity. Voigt *et al.* (1970) studied the performance of Hereford steers when grazing on Tall fescue of different preference ranking. The production per animal was 17% more when grazing on the most preferred than on the least preferred material.

Marten and Jordan (1974) concluded that the preference ranking in their cafeteria trial was positively related to the average daily gain by lambs grazing four legumes (Cicer milkvetch (*Astragalus cicer* L.), Alfalfa, Birdsfoot trefoil (*Lotus corniculatus* L.) and Red

clover) without choice. The differences in pasture quantity and quality did not explain the differences in performance. Marten *et al.* (1990) did not confirm the differential lamb performance when using heifers.

The effect of preference *per se* on long term intake has been studied by Black (1990) by comparing intake of untreated forages, and of the same forages after chemicals were added to reduce preference. Preference for the forages had little effect when they were fed alone but when the animals were given a choice, the untreated material was eaten in a greater proportion.

Emile *et al.* (1992) tested animal production from the grazing of Tall fescue swards selected for palatability. Plant dry matter yield in all the trials of cultivar Lubrette (highly palatable) was 92% of that of cultivar Clarine (control), while milk production on Lubrette was 110% of that of Clarine. That represents a productivity gain of 19.6%.

Reed (1996) considered palatability as one of the two important selection criteria in current improvement work on Tall fescue.

2.3.5 IMPORTANCE OF FEEDING VALUE AND LINKS TO SELECTION

The plant characteristics influencing feeding value of forages are considered in this section. "Feeding value" is itself the product of variations in intake and nutritive value. For simplicity, plant characteristics will be grouped into morphological (2.3.5.1), nutritional (2.3.5.2) and biochemical factors (2.3.5.3).

2.3.5.1 MORPHOLOGICAL FACTORS

Physical characteristics of the plants affect diet selection (as defined by Hodgson (1979) and Mertens (1994)) by modifying the opportunity for selection, which is determined

by the relative proportions of the preferred components in the sward, and their distribution within the canopy (Nelson and Moser, 1994). Most of the experimental evidence demonstrates association between sward characteristics and diet selection, but causation is difficult to prove (Hodgson, 1982). Voluntary intake is correlated with at least 24 different simple phenotypic characteristics of forages.

Hodgson (1982) suggested that before incorporating sward structural characteristics like sward height, herbage bulk density, leaf bulk density, leaf/stem ratio, live/dead ratio, habit of growth, etc. as components in breeding programmes there is a need for more studies to understand the response of grazing animals to each variable and their interactions.

The reputation of Yorkshire fog as being unpalatable was discarded by Jacques (1974), when studying the possibility of improving preference by selection and breeding. A study conducted at Massey University (Cameron, 1979) in spaced plants revealed a negative correlation of grazing preference with prolific flower head production and severe infection by crown rust (*Puccinia coronata*). For the characters mentioned above, plant to plant variability was present, making it possible to select and to improve preference in this species.

Garner (1963) explained the difference in preference between prostrate and erect habits by suggesting that erect plants were free from soil contamination and prostrate plants tended to be contaminated with soil.

Hodgson and Rodríguez Capriles (1977) conducted two experiments, one on Perennial ryegrass and the other on Pangola grass (*Digitaria decumbens* L.) and found that sward height and the proportion of green/dead material were effective in explaining herbage intake, while proportion of grass/clover in the sward or total nitrogen of the diet, were not. These results are in agreement with Buckner *et al.* (1969), using three Tall fescue cultivars, who found that cattle apparently did not select for high or low content of crude protein, silica or total sugars.

Cahn and Harper (1976) showed that the leaf marks of White clover had a clear influence on animal preference. The authors explained the behaviour as the creation of a "search image" to recognise the clover. However, Hodgson *et al.* (1989; Hodgson and Clark, 1988, unpublished data, *loc. cit.* Sheath and Hodgson, 1989) studying the influence of physical and biochemical characteristics on selection of White clover concluded that height and leaf size have a greater influence on diet selection than leaf mark or cyanogenic glucoside concentration. In general, leaf marks appeared to be unreliable characteristics in determining animal preference, because their importance is dependant on previous experiences of the animals.

Forbes (1988) found that in temperate grasses leaf surface height was the dominant variable affecting bite size, but in tropical grasses, leaf density and leaf/stem ratio had greater influence in bite size than height. Bite size was the main explanatory variable for intake while rate of biting and grazing time were mainly compensatory variables. Hodgson *et al.* (1994) considered the rate of biting also as an explanatory variable related to the canopy characteristics and not only as a compensatory variable.

Further support for the importance of canopy structure came from work on six genotypes of subterranean clover which were planted in boxes and grazed by sheep indoors in individual pens (Dynes *et al.*, 1993). The results demonstrate significant differences in the intake rates according to the genotypes, and such differences were mainly explained by genotype variation in height and bulk density (Dynes *et al.*, 1993).

Hodgson (1990b) made an effort to concentrate the current views and recent research findings to define desirable ideotypes of Perennial ryegrass and White clover for grazing systems.

The plant ideotype should posses the following characteristics:

"Large leaves and an erect growth habit to encourage high growth rate and high intake potential"

"High tillering rate to encourage rapid recovery post-grazing and contribute to high intake potential"

"Meristem protection to minimise risk of grazing or treading damage"

"Low structural strength to encourage high intake potential"

"Low concentration of aversive secondary compounds to minimise adverse intake or health effects"

"Specific structural biochemical and nutrient balance to provide high intake potential and nutritive value"

The balance of these characteristics in a breeding programme should be according to the specific objectives and particular situations because it is difficult to combine attributes of high production and high grazing tolerance (Hodgson, 1990b). Their balance will also depend on their heritabilities and their genetic correlations.

There is little evidence to define ideotypes for grazing systems in other species.

2.3.5.2 NUTRITIONAL FACTORS

Preference will not necessary help animal performance unless it is linked to nutritive value (or at least to rate of nutrient intake).

The slow progress in improving the feeding value of forages is due in part to the lack of consensus on the criteria to be used. An attempt to overcome this, involved a Delphi survey conducted by Wheeler and Corbett (1989) from a panel principally of ruminant nutritionists, from Europe, United States, New Zealand and Australia (Drs T.N. Barry, J.L. Black, J.C. Burns, G.W. Burton, A.R. Egan, A.P. Hogan, R. Jarrige, R.C. Kellaway, R.A. Leng, J.C. MacRae, G.C. Marten, J.A. Milne, D.J. Minson, R.J. Moir, J.E. Moore, G. Moseley, R.L. Reid, M.J. Ulyatt and P.J. Van Soest). Eleven parameters were ranked by the panel after three rounds of surveys (Table 2.1). Nine of them (high digestibility, easy comminution, high non-structural CHO, high crude protein, adequate mineral content, high

S-amino acid content, high lipid content, low anti-quality constituents and appropriate tannins) have to be measured in nutrition laboratories, and two (high relative palatability and erect growth habit) in the field (Wheeler and Corbett, 1989).

Table 2.1: Ranking after round three of a Delphi survey, for criteria to be used in breeding grasses and legumes of higher feeding value (Wheeler and Corbett, 1989).

Grasses	Legumes
High digestibility	High digestibility
Easy Comminution[1]	Easy Comminution
High non-structural CHO	Appropriate tannins
High crude-protein	High S-amino acid content
High S-amino acid content	High crude protein
Adequate mineral content	High non-structural CHO
High relative palatability	Adequate mineral content
Appropriate tannins	Low anti-quality constituents
Low anti-quality constituents	High relative palatability
High lipid content	High lipid content
Erect growth habit	Erect growth habit

1 rate of passage through the digestive tract.

To know if the two ranks were substantially in agreement with one another, a Spearman's rank correlation was computed. Spearman's rank correlation coefficient, denoted by r_s, is defined by:

$$r_s = 1 - \frac{6\sum d_i^2}{n^3 - n} \qquad (2.15)$$

where d_i equals the difference between the two ranks assigned to the i^{th} character and n equals the number of entities to be ranked. The correlation coefficient r_s is applicable to bivariate normal distribution and can be applied to data taken in the form of ranks or that were ranked after observation on other scale. It measures correspondence between ranks, so a measure of linear correlation is not necessary. Like r, the rank correlation can range in samples from -1 to +1 (Ostle, 1963; Steel and Torrie, 1980; Snedecor and Cochran, 1980). The value of r_s = 0.8091**, indicated that the ranks for grasses and legumes were in quite good agreement.

The four parameters chosen as most important for plant breeding to increase feeding value were: high digestibility, easy comminution, high non-structural carbohydrate content and high crude-protein content. However, before the utility of any of these can be confirmed for selection work, there is need to obtain estimates of their heritabilities, and genetic and phenotypic covariances and variances.

Digestibility can be defined as the fraction lost in the passage of feedstuff through the animal's digestive tract (Cochran and Galyean, 1994). The validity of the methods adopted to measure digestibility depends on the expected use of the data. One opinion is that, for breeding, the relative digestibility may be sometimes more important than the actual value (Weiss, 1994). Also speed and low cost of assessment are essential characteristics. The two-stage *in vitro* procedure (Tilley and Terry, 1963) was a critical development to allow plant breeders to screen large numbers of entities for improved digestibility. The development of near infrared reflectance spectroscopy (NIRS) made possible even more rapid and cheaper routine evaluation of forage digestibility (Holechek *et al.*, 1982; Moore, 1994).

Genetic variability for digestibility has been found in almost every experiment conducted to verify this objective (Vogel and Sleper, 1994). The largest variabilities were found in grasses and the smallest in legumes. An example was given by Clements (1973) where the *in vitro* digestibility of the organic matter (IVOMD) of individual plants of a broadly based population of *Phalaris tuberosa* L. at heading stage ranged from 56 to 76%.

The estimated heritability of IVOMD for this same population was 0.78 and for the same species, Oram *et al.* (1974) gave a value of 0.60. Dennis and Frandsen (1986) reported values of narrow sense heritabilities derived from parent-offspring regressions for digestibility of 0.34-0.57 and 0.38-0.79 for Perennial ryegrass and Cocksfoot respectively. Minson (1990) stated that large differences in dry matter digestibility and their heritabilities have been found among cultivars of the same species with ranges of 0.03 to 0.3 and 0.02 to 0.91 respectively.

Selection for improving total digestibility sometimes has been related to reduction in yield, but there is enough variability to improve either character without reducing the other one (Dennis and Frandsen, 1986; Clark and Wilson, 1993). A comparison of digestibility estimates for spaced plants and swards was performed by Dennis and Frandsen (1986), showing a significant phenotypic positive correlation of 0.89 and 0.64 for Perennial ryegrass and Cocksfoot respectively.

Improving the rate of passage through the digestive tract, or the ease of comminution, is a difficult task at the moment because the factors regulating this characteristic are not well understood and there is no rapid test available to screen large numbers of materials (Vogel and Sleper, 1994). One test available is the "fibrousness index" or the grinding energy, which measures the electric energy necessary to pulverise 5 g of dried material through a 1 mm mesh. Another test is an artificial mastication which cycles the forage as a slurry through a gear water pump for 10 minutes, followed by wet sieving. Yet another test is the leaf tensile strength which measures the force required to break individual leaves (Minson, 1990). Leaves of legumes are easy to break down into small particles. With grasses, the reduction in thickness of the cuticle and walls of the epidermal cells may increase the effectiveness of chewing and ruminating for stem break down (Minson and Wilson, 1994). Digesta particles must be reduced to 1 mm before they can leave the rumen. The size of these particles are the same for legumes and grasses and for sheep and cattle (Minson, 1990). Grasses with the same or more digestibility as legumes take 30% or more time of chewing to be reduced to the adequate size (Minson, 1990). The modification of the break down rate will modify the anatomical structure of the plant, because it involves chemical bonds which function to

provide mechanical strength (Minson and Wilson, 1994).

Carbohydrates not forming part of the cell wall are called non-structural carbohydrates and are composed primarily of sucrose, starch and fructosans. Their digestion is almost complete in ruminants and because of that, extensive fractionation into the constituents is not usually performed. The procedure for determining the concentration of total non-structural carbohydrates is well known, not presenting problems (Moore and Hatfield, 1994). Large variations have been found among cultivars of *Dactylis glomerata* L., *Lolium perenne* L. and *Lolium multiflorum* Lam. in levels of soluble carbohydrates. Increasing the levels of soluble carbohydrates should increase propionic acid in the rumen, reduce methane loss and increase the by-pass protein (Minson, 1990).

The crude protein level in forages based on Kjeldahl or other total N assays, is different to the amount of protein available to the animals, which occurs in the form of a-amino nitrogen (N). Apart from the differences in analytical technique, nitrogen losses also occur. Part of the forage protein escapes microbial degradation (by-pass protein) and the rest is degraded in the rumen and lost as ammonia or transformed into microbial protein (Broderick, 1994).

Crude protein content also varied among cultivars of the same species compared at the same growing stage (Minson, 1990). Genetic variation for content of crude protein has been found (Minson, 1990; Broderick and Buxton, 1991; Vogel and Sleper, 1994). Once again, Clements (1973) gave an estimated heritability of 0.59 for nitrogen content of mature herbage of *Phalaris tuberosa* L. and for the same species, Oram *et al.* (1974) gave a value of 0.54.

Although by-pass protein is perhaps the most important nitrogen-fraction to be determined, no single method has yet evolved which will yield reliable values rapidly, such as might be useful to plant breeding (Broderick, 1994).

Mousset-Declas *et al.* (1993) studied the presence of variability for quality in Red clover. The four parameters considered (dry matter (DM), crude protein (CP), water-soluble carbohydrates (WSC) and dry matter digestibility (DMD)) were significantly different among the 36 cultivars.

2.3.5.3 BIOCHEMICAL FACTORS

Examples of biochemical compounds that might affect palatability are alkaloids, which depress intake in Pearl millet (*Pennisetum americanum* Schum.) (Rouquette *et al.*, 1980), reed canarygrass (Marten *et al.*, 1973; Bush and Burton, 1994), barley (*Hordeum vulgare* L.), giant reed (*Arundo donax* L.) (Nelson and Moser, 1994) and lupin (*Lupinus* spp.) (Bush and Burton, 1994); saponins of alfalfa (Kendall and Leath, 1976); and condensed tannins, which can depress intake in Sericea lespedeza (*Lespedeza cuneata* L.) (Terrill *et al.*, 1989) and *Lotus pedunculatus* L.(Barry, 1989; Waghorn *et al.*, 1990).

The plant inheritance of alkaloid production in reed canarygrass has been studied by several authors. Bush and Burton (1994) conclude that a two gene model best fits the data. One single dominant allele controls the synthesis of alkaloids of the tryptamine and carboline group. The other dominant allele controls the synthesis of methoxylated derivates. Many genes are known to be involved in the biosynthesis of the different alkaloids in lupin (*Lupinus* spp.) and not all have the same quantitative or qualitative effect on the total content of alkaloids (Bush and Burton, 1994).

Scehovic (1991) compared several chemical and biochemical compounds of seven cultivars and hybrids of Tall fescue, which had different preference levels as evaluated in cafeteria trials. All the materials were harvested at the same time in six cuts. One third of the plant material was pressed to extract juice, another third was frozen and the other was dried at 55°C. The analysis done on each type of conservation mode will be quoted from the paper:

"Grass juice: ph, solid fraction, some volatile and non-volatile organic acids, total nitrogen,

ammonia nitrogen, volatile bases, total phenols, volatile phenols, ethers and phenol esters, soluble sugars, volatile carbonyl containing compounds and volatile sulphur compounds".
"Green matter: organic matter, dry matter, soluble sugars, soluble and volatile phenols, volatile lipophilic compounds and volatile sulphur containing compounds".
"Dried matter: organic matter, soluble sugars, total nitrogen, neutral and acid detergent fibre, triglycol lignin, true cellulose, silica, soluble phenols, total phenolic acids, ether-extract and total waxes".
"Some complementary analyses (tannins, alcohols, alkaloids, SO_2, etc.) were performed, but they did not add any valuable information".

The conclusions (Scehovic, 1991) were that organic acids, sugars and nitrogen compounds did not influence diet selection; while all the volatile compounds (except volatile phenols in grass juice) had some relation to diet selection. Volatile sulphur compounds were the only ones to have a definite negative influence on diet selection, and their emissions were altered by the presence of waxes in the cuticle because of their regulatory action on water vapour.

Stuedemann *et al.* (1989) stated that much of the weight-gain difference in steers grazing endophyte (*Acremonium* spp.) free and infected materials of Tall fescue can be attributed to difference in intake. Differential intake was not studied by the authors. The influence of endophyte on preference in Tall fescue was studied by van Santen (1992). The contrast between "Georgia 5 EI" (endophyte infected) and "Georgia 5 EF" (endophyte free) in preference was clear, with steers preferring the endophyte free cultivar for all stocking rates studied and the two years of the experiment.

2.4 EXPERIMENTAL METHODOLOGY

2.4.1 PLANT MATERIALS

2.4.1.1 PROCEDURES TO SAMPLE PLANT POPULATIONS

The same procedure as for creating a working or core collection (Harlan, 1972 *loc. cit.* Spagnoletti-Zeuli and Qualset, 1993; Frankel and Brown, 1984 *loc. cit.* Spagnoletti-Zeuli and Qualset, 1993) was applied when a representative sample of plants was necessary to characterise populations for genetical studies. Details are presented in the corresponding Material and Methods sections in this thesis and a brief review of different methodologies is presented here. The objective is to minimise the cost of germplasm conservation while ensuring maximum genetic diversity containing most of the alleles present in the whole collection (Crossa *et al.*, 1993: Holbrook *et al.*, 1993; Spagnoletti-Zeuli and Qualset, 1993; Diwan *et al.*, 1994).

To select a core collection from the U.S. Germplasm Collection of peanut (*Arachis hypogaea* L.) consisting of 7432 accessions, two methods were used (Holbrook *et al.*, 1993). When information was poor or unavailable, a random sample of 10% of the accessions was chosen. When the information was available, the data was sorted by country of origin and then the cluster procedure (SAS Institute, 1988) was used to sort the data and 10% of the accessions in each cluster were randomly selected. The means and ranges for the six variables considered were very similar for the entire collection and the core collection (Holbrook *et al.*, 1993).

Diwan *et al.* (1994) obtained a core collection for the United States annual *Medicago* Germplasm collection containing 3159 accessions from 36 species. A SAS macro (Jacobs, 1990 *loc. cit.* Diwan *et al.*, 1994) calculated a distance matrix for each of the *Medicago* species based on Euclidean distances between all 14 traits, to conduct cluster analysis using an unweighted pair group method with arithmetic averages. Euclidean distance of 3.0 was

used to obtain the desired core collection size (15% of the accessions). Means, variances and ranges of each trait were compared between the core collection and the main collection using a Wilcoxon rank-sum non-parametric test (SAS Institute, 1988). Differences between means of three or fewer traits were found significant for eight of the 36 species. Only two species were significantly different for each trait for variances and ranges.

Crossa *et al.* (1993) suggested that a useful strategy for forming a core collection would be to use a stratified sampling strategy subdividing the accessions into non-overlapping groups based on ecogeographical criteria. Classification techniques such as cluster analysis and ordination methods such as principal components analysis have proved to be useful for assessing genetic diversity.

Spagnoletti-Zeuli and Qualset (1993) evaluated five strategies for obtaining a core collection of 500 accessions from a collection of 3000 accessions of durum wheat (*Triticum turgidum* L. durum group). The strategies were (1) random-sampling without replacement; (2) random-systematic by chronology - sample every fourth accession in the order in which the accessions were accepted by the gene bank; (3) random-stratified by geographical origin and frequency-selecting at random 16% of the accessions of each country; (4) random-stratified by log frequency of accessions by geographical origin - same as (3) but countries with large number of accessions contributed proportionally fewer accessions to the core collection and the opposite for countries with few accessions; and (5) random-stratified by canonical variables - based on the concept that pre-existing information is available; the first three canonical variables were plotted and about 10% were randomly selected.

The first three strategies produced representative samples, but strategies four and five produced the desired effect of increasing frequencies from less-represented countries of origin for several traits. The fifth strategy was the best and was effective in increasing the phenotypic variances in the sample for most characters, due mostly to the increase of the less-frequent accessions and a decrease in the most-frequent ones, thus flattening the frequency distribution.

If necessary information is not available for the fifth strategy the others are adequate to each level of information available.

2.4.1.2 CLONING PROCEDURE

Identical copies of the same genotype of Red clover plants can be produced by cloning. Such clones can be useful in testing some issues discussed here.

Scerbakova (1936) compared the rooting and development of cuttings taken from the upper-stem, middle-stem and basal-stem. The upper cuttings rooted better than the middle ones, but both regenerated plants with only a single stem. Basal cuttings had the poorest rooting but they regenerated plants with normal stem numbers. Cuttings from the crown were also tried, and they showed the best rooting. Rooting of all the cuttings was better in sand (83.33%) than in soil (66.26%) or in water (48.33%).

Hanson (1950) found that stage of growth was important and that cuttings from actively growing vegetative plants were easier to root than cuttings from plants in the reproductive stage. He also found that temperatures between 20°C and 30°C were optimum for rooting. The influence of the length of the internode below the last node was also studied by the author, who concluded that internode length should be shorter than 1.5 cm to produce best results.

Barrales and Ludwig (1952) in their studies of photoperiodism found that with a day-length of eight hours there is a gradual decrease in stem elongation and an increase in crown bud formation. Their cloning method was to cut vertically, to include at least one bud and a portion of the crown in each cutting. To reduce wilting, the plants were kept in a cool environment with diffuse light and all the old leaves were taken from the cuttings. The method of stem cuttings (upper cuttings, middle cuttings and basal cuttings) was tried by the authors. Two nodes were included in each cutting and the upper leaf was left attached to the propagule. Two hormones (indol butyric and alpha naphthyl acetic acids) were applied to the

lower end of the cuttings to promote rooting but they were ineffective in the majority of cases. The same environmental conditions as those applied to crown cuttings were used. Individual plants were found to differ in their response to clonal propagation. Crown cuttings were the best, with 100% success in some plants, followed by basal cuttings.

Cumming and Steppler (1961) defined five possible types of propagules. Upper or tip cuttings, middle cuttings, basal cuttings, crown cuttings and leaf-bud propagules. The latter was considered by the authors to be the best type, and to show a close similarity to seedlings in growth and development. Cumming and Steppler (1961) suggested that pre-treatment of the intact plants with a short day-length environment and/or to water them with TIBA (2,3,5-triiodobenzoic acid) in the solution could increase the rate of rooted cuttings. Treatment with IAA (indolacetic acid) and increase in day length during the rooting stage were recommended. Overhead misting systems enabled the propagules to remain turgid and reduced wilting problems, without the need of shade or a cool environment.

Mirzaie-Nodoushan and Gordon (1993) used the technique developed by Barrales and Ludwig (1952) for stem cuttings, but with the improvement suggested by Cumming and Steppler (1961) of overhead misting to reduce wilting instead of a cool environment and shade. They concluded that cuttings of different portions of the plant and genotypes produced different results, not allowing a generalisation. The authors recommended the use of clones from the same portion of the plant to reduce possible variability in genetical studies. The percentage of rooted cuttings varied from 55% to 85% depending on the genotype.

2.4.2 GRAZING ANIMALS

2.4.2.1 ANIMAL SPECIES FOR GRAZING EXPERIMENTS

It is generally agreed that sheep are more selective than cattle (Cowlishaw and Alder, 1960). The difference in selective behaviour may be explained by morphological characteristics of both species. The larger jaw in cattle and the use of the tongue would

reduce the possibility of selection in comparison with sheep (Dudzinski and Arnold, 1973; Grant *et al.*, 1987).

Langlands and Sanson (1976), Grant *et al.* (1987), and Hodgson *et al.* (1991) found that, over a series of swards, the diet selected by sheep was of higher digestibility and nitrogen content than that selected by cattle. The difference was explained by the percentage of green material in the diet.

Sheep are supposed to have greater ability than cattle to differentiate between grazed clones of *Phalaris arundinacea* L. (Marten *et al.*, 1973). The selection is done at least partly on the basis of alkaloid concentration of the grass, both preferring plants having low alkaloids. However, the correlation between animal species ($r = 0.85$) was very good for those circumstances (Marten *et al.*, 1973). Grant *et al.* (1987) also found that many components which were selected or rejected by both species were similar.

Cosgrove (unpublished data, 1996) evaluated in New Zealand the relative preference (time spent grazing) between Red clover and Birdsfoot trefoil with lambs and heifers. Results for both animal species were very similar, the time spent grazing in Birdsfoot trefoil being 54.4% and in Red clover 45.6% for lambs and 53% in Birdsfoot trefoil and 47% in Red clover for heifers.

However, although the response of sheep and cattle may be similar with respect to variations in sward canopy conditions, they are not the same in detailed tests. For example, Goatcher and Church (1970a,b,c,d) in a two-choice preference test, compared the sensitivity of cattle and sheep to salty, sour, bitter and sweet tastes. Cattle discriminated first in a sensitivity series for salty, sour and sweet, but they were equal to sheep for discrimination for bitterness.

From experiments of this kind, it is possible to conclude that cattle have the ability to discriminate by taste, but that morphological characteristics of the animals might be

interfering with the ability to do so. Caution is recommended when attempting to generalise (Hodgson *et al.*, 1994).

Hunt and Hay (1990) compared preference of 16 grasses, herbs and legumes with horses, deer or calves with the photographic technique. Their results showed clearly that the three animal species exercised strong preferences and that the difference among them were also marked, for example the order of preference for deer being the inverse of that for calves.

Foot *et al.* (1996) reported that different stock classes of sheep at the same set stock have dissimilar effects on botanical composition of pastures and that the divergence is likely to be large over long time frames.

2.4.2.2 INFLUENCE OF FASTING AND DIURNAL PATTERN OF GRAZING

There are usually 3 to 5 periods of grazing during the day but the two main ones are after dawn and before dusk (Jones, 1952; Arnold, 1981; Hodgson, 1990a). Diurnal variation of the diet is found, but the evidence is conflicting (Arnold, 1981).

The normal behaviour in diet selection is altered by fasting animals, making them eat species or morphological units within species that normally they reject. Differences in palatability among species or plant parts are more important in well-fed animals than in hungry ones (Jones, 1952). Newman *et al.* (1994) showed that fasting (24 hours) not only affected grazing time and intake rate, but also altered diet composition in sheep grazing ryegrass and White clover pastures. Moseley and Antuna Manendez (1989, *loc. cit.*, Newman *et al.*, 1994) stated that a fast of only four hours is enough to alter behaviour. However, Hodgson (1990a) found that the effects of four hours of fasting can be very different depending on the time of the day (diurnal variation in grazing pattern).

2.5 CONCLUDING COMMENTS

A lack of an appropriate methodology to select for grazing preference under spaced plant conditions was obvious from the literature reviewed. In fact, only a minority of forage nurseries are grazed: and they evaluated grazing tolerance rather than grazing preference (Mislevy *et al.*, 1982; Smith *et al.*, 1989; Bouton and Hoveland, 1996). Methodologies to determine grazing preference are available, but they are mainly for large scale experiments under sward conditions (Lascano *et al.*, 1988; Schultze-Kraft *et al.*, 1989; Davis, 1993). Any extrapolation to spaced plant nurseries should consider grazing ecology issues, together with quantitative genetics, in order to determine the best characters for future breeding work.

The literature revealed that no single morphological, nutritional or biochemical factor was entirely satisfactory to explain grazing preference (Hodgson, 1982; Forbes, 1988; Wheeler and Corbett, 1989), suggesting that several characters should be measured, such as those measuring forage removal from individual plants. The nursery should also permit the assessment of other characters commonly used to describe populations.

The absence of plant competition in spaced plant nurseries was also considered a major concern by several authors (Lazenby and Rogers, 1964; Rattunde *et al.*, 1991; Buxton and Lentz, 1993). Sward conditions do provide competition, and also reflect the end-user conditions. The suitability of spaced plant selection for genetic advance in swards appears to have been evaluated only once (McWilliam and Latter, 1970), using the genetic correlation between the two environments. This, clearly, is a matter which needs more investigation.

CHAPTER THREE
Programme Outline and General Materials and Methods

3.1 INTRODUCTION

A brief outline of the complete programme is presented, as well as the materials and methods that were common for all experiments like the soil description and climate of the experimental sites and the genetical materials used.

3.2 PROGRAMME OUTLINE

A sequence of six experiments was conducted in New Zealand and Uruguay with nine Red clover (*Trifolium pratense* L.) populations: two in glasshouse conditions (3.2.1), three sown as spaced plants (3.2.2 and 3.2.3) and one sown as monoculture swards (3.2.4). Their particular objectives are presented briefly in this section, and the experiments will be discussed in full in the Chapters 4 to 7.

3.2.1 PRELIMINARY GLASSHOUSE EXPERIMENTS (NEW ZEALAND AND URUGUAY)

Objectives:

(i) to raise seedlings of the Red clover populations, and to sample representatively.

(ii) to prepare cloned material.

3.2.2 GRAZING MANAGEMENT EXPERIMENT (NEW ZEALAND)

Objectives:

(iii) to determine the optimum Stocking Density and Time of Day that enable the best discrimination among plants for grazing preference.

3.2.3 SPACED PLANT-ANIMAL INTERACTION EXPERIMENTS (NEW ZEALAND AND URUGUAY)

Objectives:

(iv) to test a grazing method to determine animal preference in a way suitable for genetical experiments.

(v) to estimate heritabilities for characters that might be selected to enhanced animal preference.

3.2.4 SWARD-ANIMAL INTERACTION EXPERIMENT (URUGUAY)

Objectives:

(vi) to test under sward conditions the same grazing method used in the spaced plant-animal interaction experiments.

(vii) to determine the genetic correlation between the estimates of preference obtained in spaced plants and swards.

(viii) to evaluate relative selection efficiencies by estimating the correlated genetic advance ratios.

3.3 GENERAL MATERIALS AND METHODS

Soils (3.3.1), climate (3.3.2) and plant materials (3.3.3) were common for all experiments and are described as follows.

3.3.1 SOIL DESCRIPTION OF EXPERIMENTAL AREAS

The experiments in New Zealand (40° 23'S, 175° 37'E and an altitude of 34 m above sea level) were located next to each other in an area of deep fertile soils in the Tiritea Stream valley of the Manawatu district. The present land use is intensive grazing systems with a potential land use of cereals, root and green fodder crops. The soil type is an undifferentiated

floodplain alluvium with a slope of 0-3% without risk of erosion. The typical soil is "Manawatu fine sandy loam" (Cowie, 1972).

In Uruguay (34° 20'S, 57° 41'W and an altitude of 81 m above sea level), the experiments were also located next to each other in an area of deep fertile soils with a slope of 0-2% without risk of erosion (Víctora, 1985). The typical soil name is "Planosol eútrico melánico" belonging to the planosoles group in an international classification (Food and Agriculture Organization (FAO)).

3.3.2 CLIMATE OF PALMERSTON NORTH (NEW ZEALAND) AND LA ESTANZUELA (URUGUAY) DURING THE EXPERIMENTAL PERIOD.

Monthly values for the period of study as well as long term averages for total rainfall and mean temperature are presented in Table 3.1 for both sites. In both cases the recording stations were less than one kilometre away from the field plots.

Table 3.1: Monthly rainfall and mean temperature for the period of study and long term averages

	New Zealand[1]	1994/1995	Uruguay[2]	1995/1996
Months	Total Rainfall (mm)	Average Temperature (°C)	Total Rainfall (mm)	Average Temperature (°C)
April	41 (81)[3]	13.0 (13.2)[3]	188 (80.8)[4]	17.0 (16.7)[4]
May	98 (89)	11.1 (10.1)	33 (79.9)	13.2 (13.6)
June	94 (97)	8.3 (7.7)	133 (64.6)	9.9 (10.4)
July	85 (89)	6.6 (7.7)	38 (77.7)	9.6 (10.5)
August	84 (89)	8.3 (7.6)	16 (78.2)	10.4 (11.2)
September	133 (75)	9.7 (9.9)	31 (83.8)	13.5 (13.1)
October	70 (88)	11.5 (12.5)	101 (107.6)	15.3 (15.8)
November	183 (78)	14.2 (15.1)	142 (103.2)	19.5 (18.6)
December	21 (94)	17.2 (18.5)	16 (95.1)	22.8 (21.7)
January	72 (79)	17.4 (18.5)	86 (95.5)	23.0 (23.2)
February	65 (67)	18.9 (18.1)	150 (118.2)	22.2 (22.1)
March	107 (69)	16.6 (16.3)	78 (129.5)	21.7 (20.1)

Sources:
(1) HortResearch-Massey University (New Zealand, 1995).
(2) National Institute of Agricultural Research (INIA-La Estanzuela, 1996)
(3) Average of 60 years (Massey University: Farms Administration, 1996)
(4) Average of 25 years (National Institute of Agricultural Research (INIA-La Estanzuela, 1996)

3.3.3 GENETICAL MATERIALS

Nine populations of Red clover were used in experiments detailed in Chapters 4, 5 and 6 (Table 3.2). A subset of six was used for the experiment detailed in Chapter 7 because of limitations in the amount of seed available. The populations were chosen because of their growth habit (equal number of erect, semi-erect and prostrate populations), ploidy level (all

2n = 14) and diverse places of origin, so as to have as many representative materials of temperate regions as possible, and to reduce the probability that the materials were related by descent (Hollowell, 1951; Crowder and Echeverri, 1961; Claydon and Rumball, 1982; OCDE, 1993). As Red clover is a cross-pollinated species, the plants within each population varied genetically, with the variation centred around their respective population mean. The population of inference, therefore, tends towards being "wide", and the plants within each have been representatively sampled (see Chapter 4).

Table 3.2: Populations used in the experiments

Population	Habit	Source
F.2367[1] Turkish	Erect	Turkey
F.2256[1] Hamua	Erect	New Zealand
Quiñiquelli	Erect	Chile
F.2378[1] Colenso	Semi-erect	New Zealand
Kenland	Semi-erect	USA
Estanzuela 116	Semi-erect	Uruguay
Astred	Prostrate	Portugal
F.2419[1]	Prostrate	Spain
F.2255[1] Turoa	Prostrate	New Zealand

1 numbers assigned by AgResearch Grasslands.

The following details are useful in evaluating the lack of bias with respect to the inference base:

Hamua — Developed by crossing and selection (line breeding) from New Zealand lines of Broad Red Clover in 1946. The actual name was given in 1964.

Turoa — Produced by crossing and selecting (line breeding) from lines of Montgomeryshire Red Clover originated in England in 1930, and

	introduced into New Zealand around 1955.
Colenso	Selected from crosses between a Moroccan ecotype and Hamua (line breeding).
F.2419	Field collection from Spain (accession).
Estanzuela 116	Selected from materials introduced from New Zealand and adapted to local conditions in 1942 (mass selection) (Boerger, 1943).
Kenland	The beginning of the development was in 1936 from several adapted southern USA strains artificially inoculated with organisms causing the southern anthracnose disease and crown rot (line selection) (Hollowell, 1951).
Quiñiquelli	Originated from individual selection from 30 ecotypes collected in the Province of Curicó (CHILE) in 1950 by Mr. Jorge Silva (mass selection) (Pers.Comm. Dr. I. Ruiz).
Turkish	Population from Turkey (accession).
Astred	Selection from parental material collected in 1968 at Crato in Portugal (line selection) (Smith and Bishop, 1993).

CHAPTER FOUR
Preliminary Glasshouse Experiments

4.1 INTRODUCTION

For any experiment there is always the dilemma of the ideal or optimum number of treatments and replicates to use, against resources and time available to meet the objectives. The experiments reported in Chapters 5, 6 and 7 are not exceptions and some compromises in plant number were intended to be solved or improved by these preliminary glasshouse experiments. Due to limited resources and time, only 900 spaced plants per Site could be grown from which to establish the experiments detailed in Chapter 6. From that number, and considering the numbers of replicates and clones needed for each of the nine Red clover populations, only 12 plants (genets) were used to represent each population. A sampling exercise was performed to obtain the most representative sample of 12 plants from 100 plants coming from a random seed sample of each population. Those selected 108 genets (12 plants x 9 populations) were cloned to supply the base materials for the subsequent experiments. There are a number of interesting aspects here: (1) seedling management; (2) representative sampling; and (3) cloning procedure.

4.2 MATERIALS AND METHODS

4.2.1 EXPERIMENTAL DETAILS

Two experiments were conducted in New Zealand and Uruguay with nine Red clover populations (Section 3.3.3). A random sample of 100 seeds per population were planted in a glasshouse. Plants were measured, to utilise Principal components to aid in unbiased sampling. Seedlings were raised, sampled and cloned in a period of five months in each site.

The management and experimental details are summarised in Table 4.1 and Plates 4.1 to 4.4.

Table 4.1: Management and experimental details

Management	New Zealand (1994)	Uruguay (1995)
Sowing media	[1]60% peat, 40% pumice and (100g agricultural lime+300g dolomite+60g Micromax[2] +300g PG[3] mix)/100 l	1/3 (peat and vermiculite), 1/3 sand and 1/3 sterilised soil
Sowing rate	4 seeds/pot[4] and thinned later to 1	4 seeds/pot[4] and thinned later to 1
Sowing date	14 May 1994	22 Mar. 1995
Weed control	By hand pulling	By hand pulling
Fungus control	Benlate[5] @ 0.5 g/l to control Pythium spp. Rhizoctonia spp. and Fusarium spp. on 25 May 1994	Topsin-M 70%[6] @ 1.0 g/l to control Erysiphe polygoni on 2 June 1995
Subsequent fertilisation	None (fertiliser already in sowing media)	Foliar fertiliser[7] weekly
Inoculation	Rhizobium leguminosarum-biovar trifolii was applied with the water	Rhizobium leguminosarum-biovar trifolii was applied with the water
Cloning date	17 Aug. 1994 28 Aug. 1994 11 Sept. 1994	4 July 1995 17 July 1995 25 July 1995
Cloning method	Leaf-bud propagule[8]	Leaf-bud propagule
Hormone	Seradix 2[9] to the lower end, and excess shaken off	Seradix 2 to the lower end, and excess shaken off
Cloning conditions	Over head misting (2 min/10 min) and 20°C temperature	Over head misting (continuously during the day) and 20°C temperature

1 Media recommended by the Plant Growth Unit Staff, Massey University.
2 The micromax constituents were: 12.0% Fe, 2.5% Mn, 1.0% Zn, 0.5% Cu, 0.1% B, 0.05% Mo and 15% of combined sulphur.
3 The PG mix constituents were: 14%N, 16%P_2O_5, 18%K_2O, 0.03%B, 0.12%Cu, 0.20% Mo, 0.16%Mn, 0.04%Zn, 0.09%Fe.
4 Plastic pots (14 cm diameter and 15 cm height)
5 Benlate = benomyl
6 Topsin M = 1,2-bis(Metoxicarbonil-2 tioureido) benceno
7 The foliar fertilizer constituents were: 12.0% N, 8.0% P, 5.0% K and micronutrients in parts per million (ppm) Mg 600, Mn 500, Zn 600, Mo 10, Ca 600, Fe 600, Cu 300, Co 10, B 600 and S 600
8 Cumming and Steppler (1960)
9 A commercial preparation containing 3 g/kg beta-indolbutyricacid in the form of dust

Plate 4.1: General view of glasshouse experiment

Plate 4.2: Cloning method: leaf-bud propagule cutting

Plate 4.3: Cloning method: rooting hormone

Plate 4.4: Cloning method: over head misting

4.2.2 CHARACTERS RECORDED

Three months after the sowing date, in each country, the following five characters were recorded (to facilitate representative sampling):

Height (HGT)	The highest point of each plant was measured to the nearest centimetre.
Leaf Number (LFNB)	The number of leaves was counted for each plant.
Habit (HBT)	An ordinal score, where: Score 1 represented the extreme prostrate habit, and 5 the extreme erect habit. Halves were used for borderline cases, and data doubled prior to analysis.
Leaf size (LSZ)	Seven circles of 2, 3, 4, 5, 6, 7, 8 cm diameter were cut in a card and used to determine the leaf size (i.e. three leaflets together) representing an interval score 1 to 7 respectively. The biggest adult leaf of each plant was passed through the circles and recorded according to which category it belonged.
Hairiness (HRN)	An ordinal score, where: Score 1 was almost without any hairs and score 7 was the hairiest. Similar to Williams, 1927.

No variables were transformed because all of them were assumed to be Normally distributed.

4.2.3 STATISTICAL ANALYSIS

4.2.3.1 EXPERIMENTAL DESIGN

The experimental design was a completely randomised design (CRD) of 9 populations of 100 plants each, pooled over two environments (New Zealand and Uruguay).

The model used to analyse the experiment was:

$$X_{ijk} = \mu + P_i + E_j + PE_{ij} + \varepsilon_{ijk}\,, \qquad (4.1)$$

where X_{ijk} = the phenotypic value of the k^{th} Plant (k = 1...s; s = 100) of the i^{th} Population (i = 1...p; p = 9) at the j^{th} Environment (j = 1...e; e = 2), μ = grand mean, P_i = effect of the i^{th} Population, E_j = effect of the j^{th} Environment, PE_{ij} = the effect of the ij^{th} interaction, and ε_{ijk} = random error ijk^{th}.

All effects of the model are considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random design may be found by equating the mean square estimates to their expectations, and solving the resultant linear function (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971), and are presented in Table 4.2.

Table 4.2: Random inference of the expectations of mean squares

S.O.V.	D.F	M.S.	Expectation of M.S.	F.Test
Environment	e-1	4	$\sigma^2_\varepsilon+s\sigma^2_{PE}+sp\sigma^2_E$	4/2
Populations	p-1	3	$\sigma^2_\varepsilon+s\sigma^2_{PE}+es\sigma^2_P$	3/2
Pop x Env	(p-1)(e-1)	2	$\sigma^2_\varepsilon+s\sigma^2_{PE}$	2/1
Error	ep(s-1)	1	σ^2_ε	

In the Table, σ^2_ε is the variance arising from ε_{ijk}, σ^2_{PE} from PE_{ij}, σ^2_P from P_i, σ^2_E from E_j.

The statistical package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows:

```
PROC GLM;
CLASS      Environment Population;
MODEL      Environment|Population / SS2;
```

4.2.3.2 SAMPLING PROCEDURE

4.2.3.2.1 PARSIMONIOUS PRINCIPAL COMPONENT ANALYSES

Principal Component Analysis was utilised for seedling discrimination in order to effect unbiased stratified randomisation of the plant populations, based on multivariate consideration of the five characters outlined earlier. The objective was to represent as much of the original population variability as possible in the sub-sample of 12 plants from each population of 100 seedlings, which themselves were from a random seed sample. Elgersma (1990a, b) has suggested that 50 random genotypes were needed to characterise a population: so the present limitation to 12 required further sophistication to ensure a representative sample. The principal component defines linear orthogonal functions of these attributes (up to five in this case), each of which provides a "local optimum" discriminator amongst the plants by maximising the principal-score variance amongst plants. This property is analogous to maximum discrimination amongst plants. The first (most discriminatory) component explains the highest percentage of the total original variance, the second accounts for the second highest variance (and is orthogonal to the first), and so on (Morrison, 1990). In the interests of parsimony, it is common practice to use only those components which cumulatively reach an arbitrary total of the explained variance (Morrison, 1990). A level of 70% was adopted here, which led to the first two components being used. From the plot of the first factor against the second, for each population, plants were chosen from the periphery and the centre, thereby maximising the retained level of the original variability in the relatively small sample of 12 plants.

4.2.3.2.2 CLUSTERED FULL PRINCIPAL COMPONENTS

However, as this Principal component analysis was available, it was decided to explore also another method (Holbrook *et al.*, 1993 and Crossa *et al.*, 1993) for selecting the 12 plants per population. In this method, the first step was again to use a principal component analysis (Section 4.2.3.2.1) but instead of using a parsimonious subset of components, all components were used, and their patterns summarised by cluster analysis. The inclusion of the five components meant that all the original variability was considered.

The problem then is to choose an appropriate clustering algorithm. The method chosen was "Ward's minimum variance method". This method is based on the within-group sums of squares rather than simple linear functions of distances. At each stage of the agglomerative clustering, the number of groups was reduced by one, by combining the two groups which gave the smallest possible increase in the total within-group sum of squares (Anderberg, 1973).

With this method, the tree or dendrogram should be cut at the 12 cluster level and one random plant should be selected from each cluster to have the 12 most representative plants.

4.2.4 VARIANCE ESTIMATE ACCORDING TO POPULATION SIZE

Expected variance depends on the number of plants contributing to the population, and, as well, estimates of this variance have been a sampling distribution and standard error. Church (1925) established the relationship between the variance of a finite population and an infinite one. In order to compare these estimates of plant variance (from the two methods), they were converted to infinite-population equivalents by rearranging the Church equation (Gordon, 1994), as follows:

$$\sigma_{\infty}^2 = \frac{\sigma_n^2}{\left(1-\frac{1}{n}\right)} \qquad (4.2)$$

where n equals the population size used as a basis to estimate the variance. The sampling error was also estimable in the usual way (Crump, 1946; Satterthwaite, 1946)

4.2.5 CLONING PROCESS

The chosen experimental design for the spaced plant-animal interaction experiment required internal replication of each plant genotype from the open-pollinated populations. This could be achieved sexually (inbreeding), or asexually (cloning). As Red clover has self-incompatibility (Williams and Silow, 1933; Williams and Williams, 1947a; 1947b; Pandey, 1956; Smith *et al.*, 1985; Meglic and Smith, 1992), selfing and sib-mating (inbreeding) are difficult to implement. But cloning is an effective way of fixing plant genotypes for this crop, and is quicker and is regarded as a very convenient way of assessing genetic parameters (Elgersma, 1990a; 1990b). The design required nine genotype copies (ramets) of each original plant genotype (genet). To be sure to obtain these 9, 15 ramets of each genet were made initially. Three stages were necessary to obtain the desired number of clones because of the size of the original plants at cloning-time, and because of the cloning method used. The methodology of leaf-bud propagule suggested by Cumming and Steppler (1961) was selected for this process because with this methodology, normal plants (ramets) similar to plants coming from seeds (genets) were generated. When the new ramets appeared to be strong enough, they were taken outside the glasshouse to harden-off for a week prior to transplanting into the field.

4.3 RESULTS

4.3.1 ANALYSIS OF VARIANCE

An analysis of variance was done to test the effect of the Environment, Population and their interaction for the five characters measured. The significance of the analysis of variance (F tests), variance components with their respective standard errors for all seedling characters are presented in Table 4.3.

Table 4.3: Significance of the analysis of variance (F tests), variance components with their respective standard errors for all seedling characters

Characters	Environment	Population	PopxEnv	Error
Height	2.85**	1.05**	0.21**	3.74
	(2.35)	(0.53)	(0.11)	(0.13)
Leaf Number	26.72**	2.12**	0.24*	17.88
	(21.85)	(1.05)	(0.19)	(0.61)
Habit	-0.02^{NS}	-0.10^{NS}	0.23**	0.38
	(0.01)	(0.06)	(0.11)	(0.01)
Leaf Size	1.25**	0.31**	0.07**	0.62
	(1.03)	(0.16)	(0.03)	(0.02)
Hairiness	-0.02^{NS}	0.55*	0.23**	1.99
	(0.02)	(0.31)	(0.11)	(0.07)

NS Not Significant
* Significant at 0.05 level
** Significant at 0.01 level

The effect of the environment was highly significant for height, leaf number and leaf size, indicating that the plants were of different size three months after the sowing date when the measurements were taken. Habit and hairiness were measured using scores and were not so much influenced by size of the plants. The effect of population was highly significant and significant for all characters except habit. Habit was not expressed so markedly in juvenile

plants. The effect of population by environment interaction was significant for all characters.

4.3.2 CHARACTERISATION OF THE POPULATIONS

The information recorded for the 900 plants in both countries was used to characterise the population means (Table 4.4).

Table 4.4: Means of all seedling characters for New Zealand (NZ) and Uruguay (ROU)

Character	Site	Grand mean	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
HGT	NZ	9.72(0.08)[1]	10.47b[2]	9.08c	11.51a	9.97b	10.42b	11.03a	8.33d	8.97c	7.72e	0.20
	ROU	7.36(0.07)	7.98b	6.77d	9.02a	6.63d	6.94cd	8.65a	7.21c	6.65d	6.40d	0.18
LFNB	NZ	16.36(0.18)	15.91b	17.39a	14.04c	16.52ab	15.15b	16.58ab	15.82	16.96ab	18.86a	0.52
	ROU	8.89(0.11)	10.21b	10.36b	5.90f	9.55c	7.27e	8.59d	8.27d	8.02de	11.80a	0.28
HBT	NZ	3.14(0.02)	3.26bc	2.89e	3.64a	3.09cd	3.42b	3.35b	2.95de	2.84e	2.85e	0.06
	ROU	2.92(0.02)	3.20b	2.82c	3.44a	3.06b	3.38a	3.04b	2.70c	2.51d	2.15e	0.06
LSZ	NZ	4.63(0.03)	4.63d	4.02f	5.85a	4.23e	5.07c	5.41b	4.77d	4.12ef	3.55g	0.07
	ROU	2.98(0.03)	3.25bc	2.89d	3.51b	2.70d	3.12c	3.86a	2.77d	2.45e	2.30e	0.08
HRN	NZ	3.79(0.05)	2.19f	3.03e	3.58d	3.06e	5.28a	3.99c	5.28a	4.54b	3.13e	0.13
	ROU	4.00(0.06)	3.41c	3.23c	3.32c	3.59c	4.16b	3.93b	6.31a	4.32b	3.71c	0.15

1 Standard errors of the grand means

2 Values within the same row followed by the same letter do not differ significantly (two tail t-test with $P \geq 0.05$).

To know if the two ranks for each character were substantially in agreement with one another, a Spearman's rank correlation was computed (Equation 2.15; Section 2.3.5.2).

The values of r_s were: 0.78, 0.77, 0.92, 0.82 and 0.85 for height, leaf number, habit, leaf size and hairiness respectively, indicating that the ranks for both countries were in quite good agreement.

Variance of all seedling characters for New Zealand and Uruguay corrected by Church equation (Equation 4.2) to take the variance of 100 plants to a variance of infinite plants are presented in Table 4.5. The purpose of presenting these variances is that they were compared with the variance of the 12 plant samples in both sites, also corrected by the same

Church equation to make them comparable at an infinite plant variance level.

Table 4.5: Variances of all seedling characters for New Zealand (NZ) and Uruguay (ROU) at an infinite plant variance level

Character	Site	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HGT	NZ	2.79	3.46	5.95	3.88	4.75	4.16	2.25	4.41	3.96
	ROU	3.72	2.07	6.76	2.07	4.04	4.84	2.16	2.96	2.19
LFNB	NZ	18.23	14.82	14.06	16.65	14.14	21.16	15.84	20.98	109.2
	ROU	8.82	9.80	4.20	5.86	5.62	7.34	5.57	10.24	13.62
HBT	NZ	0.33	0.26	0.45	0.30	0.44	0.41	0.34	0.69	0.26
	ROU	0.29	0.22	0.62	0.23	0.29	0.16	0.18	0.46	0.36
LSZ	NZ	0.48	0.35	0.94	0.55	0.59	0.77	0.64	0.58	0.41
	ROU	0.71	0.52	1.23	0.58	0.44	0.90	0.40	0.45	0.37
HRN	NZ	1.25	1.56	2.13	1.74	1.37	1.90	1.10	1.82	1.74
	ROU	2.86	1.23	2.79	2.46	2.66	2.86	0.48	2.79	1.99

These variances (Table 4.5) were compared with the variance of the samples of 12 plants corrected by Church equation (Equation 4.2) for each population, country and characters in Section 4.3.3.

4.3.3 SAMPLING PROCEDURES

4.3.3.1 PARSIMONIOUS PRINCIPAL COMPONENT ANALYSES

Standardised coefficients for factors 1 and 2 of the principal component analysis of all characters for the nine populations for each site are presented in Table 4.6.

Table 4.6: Standardised coefficients for factors 1 and 2 of the principal component analysis of all characters for the nine populations for each site

Characters	Turkish		Hamua		Quiñiquelli		Colenso		Kenland		E116		Astred		F2419		Turoa	
New Zealand	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
HGT	0.312	0.787	0.188	0.762	0.717	0.351	0.805	-0.162	-0.022	0.843	0.692	0.562	0.117	0.773	0.869	-0.136	0.815	0.277
LFNB	0.878	-0.087	0.734	-0.336	0.818	-0.305	0.237	0.773	0.858	-0.044	-0.760	0.439	0.846	-0.022	0.265	0.799	-0.229	0.697
HBT	-0.581	0.599	-0.771	0.353	-0.539	0.711	0.342	-0.719	-0.843	0.185	0.706	-0.529	-0.813	0.227	0.687	-0.492	0.726	0.027
LSZ	0.803	0.312	0.485	0.603	0.724	0.331	0.865	0.099	0.394	0.707	0.433	0.802	0.327	0.747	0.754	0.154	0.799	0.049
HRN	0.150	-0.483	0.477	0.176	-0.251	-0.562	0.236	0.457	0.297	-0.224	0.283	-0.103	-0.303	0.437	0.257	0.499	0.167	-0.752
Cumulative variation	0.37	0.64	0.33	0.57	0.41	0.64	0.33	0.60	0.34	0.60	0.36	0.65	0.32	0.60	0.38	0.62	0.38	0.61
Uruguay																		
HGT	0.404	-0.708	0.346	0.003	0.508	0.601	0.301	0.649	0.460	0.511	0.549	0.376	0.049	0.795	0.506	0.138	0.543	-0.664
LFNB	0.823	0.125	0.824	-0.208	0.836	-0.332	0.813	-0.131	0.812	-0.333	0.838	-0.111	0.834	0.109	0.868	-0.184	0.736	0.214
HBT	0.752	0.394	0.682	-0.535	0.790	-0.478	0.766	-0.316	0.670	-0.614	0.770	-0.071	0.787	-0.334	0.614	-0.671	0.725	0.409
LSZ	0.464	-0.318	0.424	0.691	0.509	0.647	0.097	0.718	0.551	0.506	0.416	0.653	0.226	0.794	0.523	0.402	0.606	-0.394
HRN	0.052	0.666	0.509	0.476	0.435	0.048	0.594	0.141	0.657	0.257	0.504	-0.655	0.604	-0.076	0.488	0.598	0.256	0.565
Cumulative variation	0.32	0.57	0.34	0.55	0.41	0.63	0.34	0.56	0.41	0.63	0.41	0.61	0.35	0.63	0.38	0.58	0.36	0.59

For Turkish, Hamua, Quiñiquelli, Kenland and Astred in New Zealand, high values of the first factor were associated with high leaf number and big leaves, while high values of the second factor were associated with tall plants. For Colenso, F2.419 and Turoa in New Zealand, high values of the first factor were associated with tall plants, while high values of the second factor were associated with high leaf number. For E116 in New Zealand, high values of the first factor were associated with erect and tall plants, while high values of the second factor were associated with big leaves.

In Uruguay, for all populations, high values of the first factor were associated with high leaf number and erect plants, while for Hamua, Quiñiquelli, Colenso and E116 high values of the second factor were associated with big leaves, for Turkish, F.2419 and Turoa, high values of the second factor were associated with hairy plants and for Kenland and Astred, high values of the second factor were associated with tall plants.

The first two factors represented on average nearly 61% of the total variance in all the populations studied for both sites, making it possible to consider only these two instead of all five.

The plot of the first factor against the second for each population showed all the plants in terms of their two components. From these scatter plots, plants were chosen from the periphery and the centre to maximise representation of the original variability in the relatively small sample of 12 plants as presented in Figures 4.1 to 4.6 for the erect, semi-erect and prostrate populations in New Zealand and Uruguay respectively.

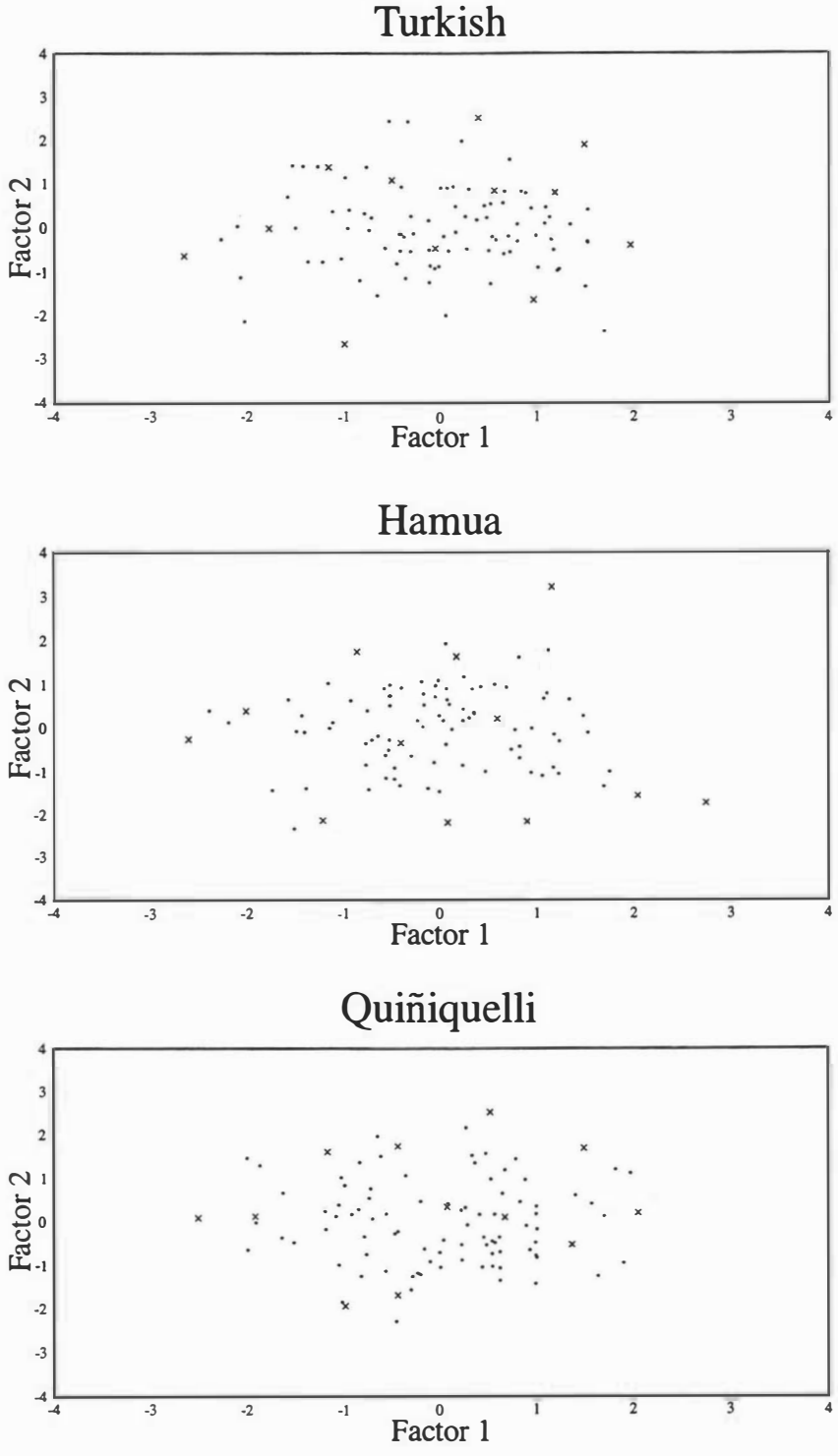

Figure 4.1: Principal component analysis: plot of the first factor against the second factor for the erect populations in New Zealand (. non-selected plants; × selected plants)

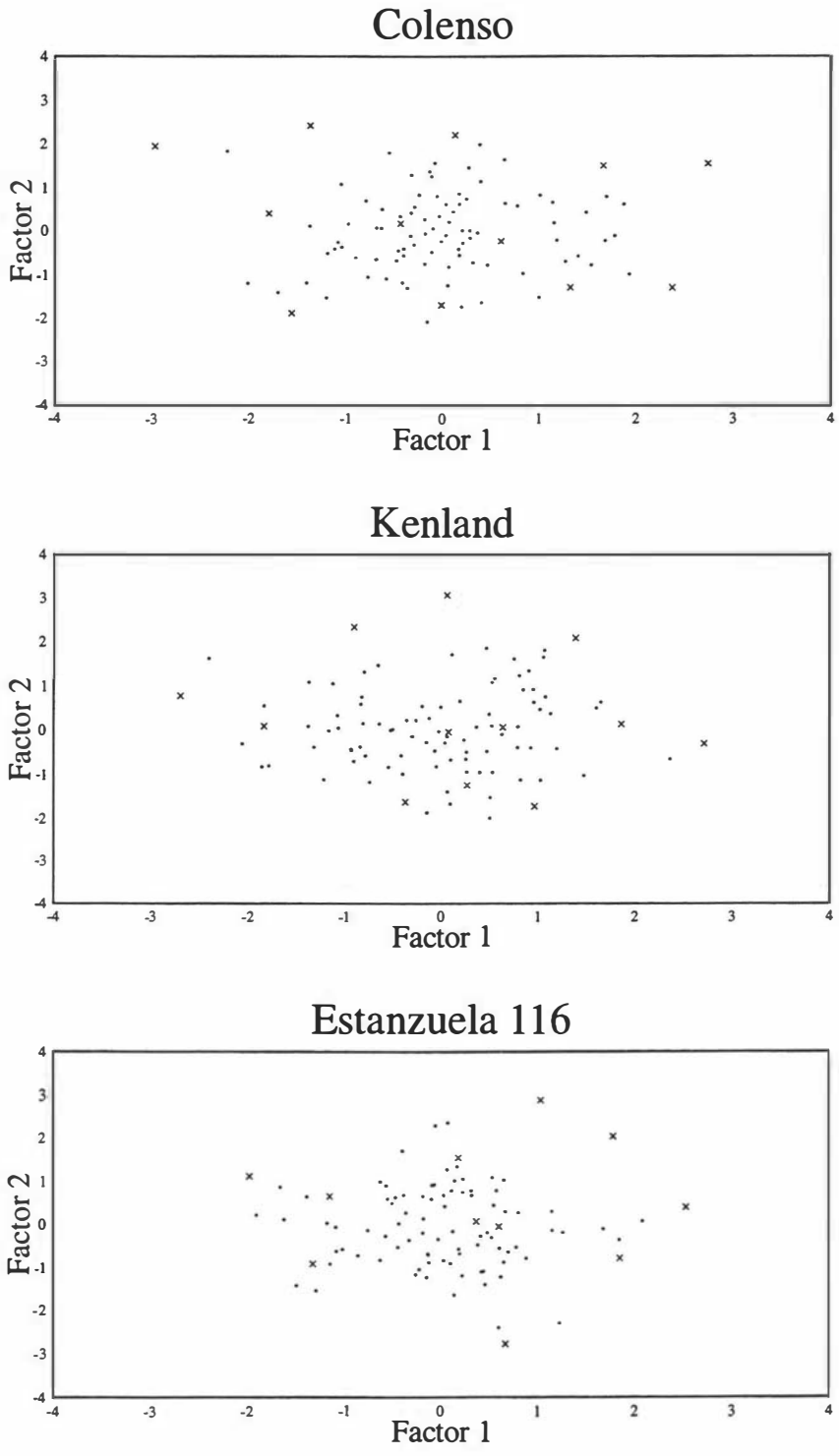

Figure 4.2: Principal component analysis: plot of the first factor against the second factor for the semi-erect populations in New Zealand (. non-selected plants; × selected plants)

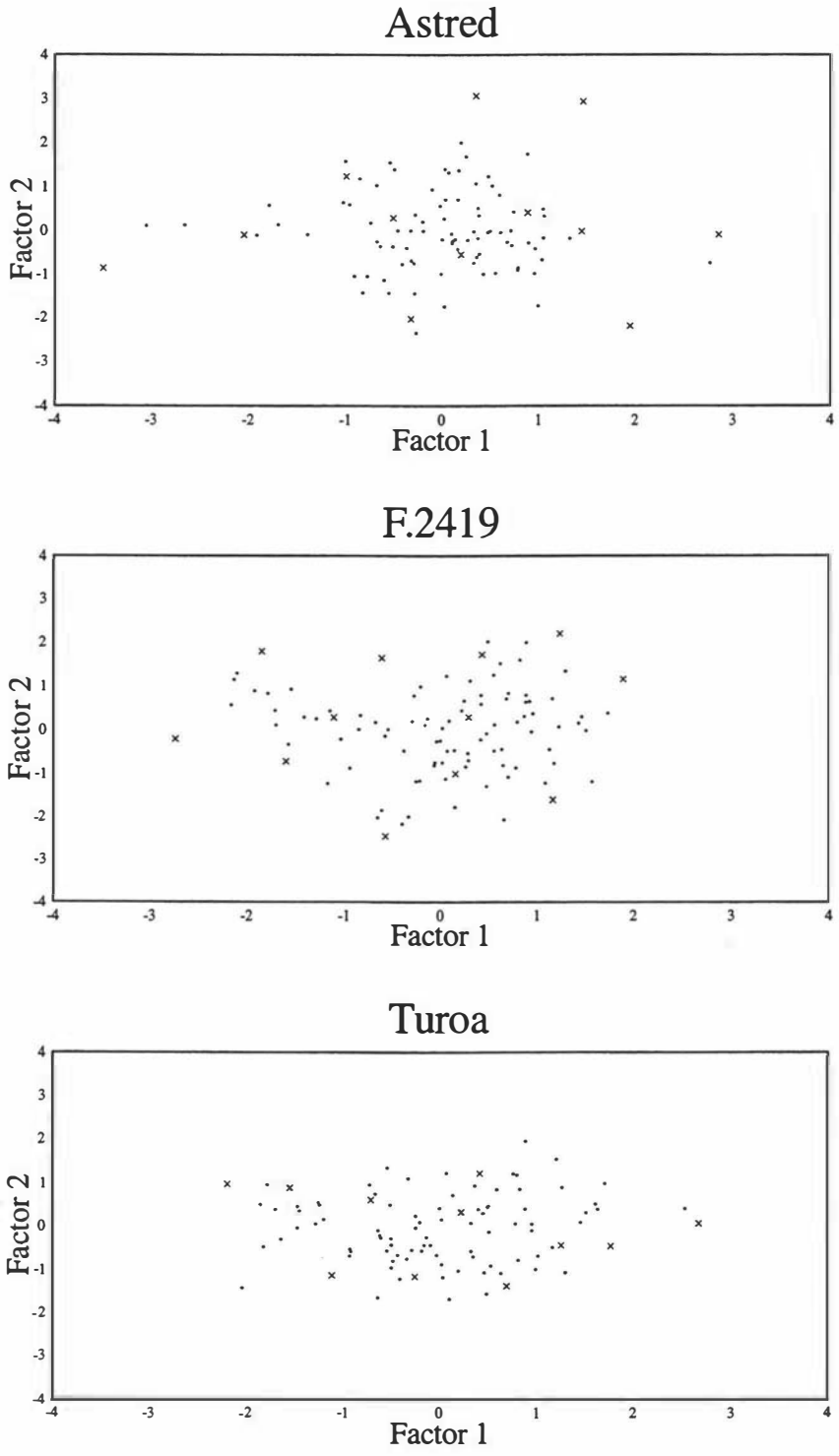

Figure 4.3: Principal component analysis: plot of the first factor against the second factor for the prostrate populations in New Zealand (. non-selected plants; × selected plants)

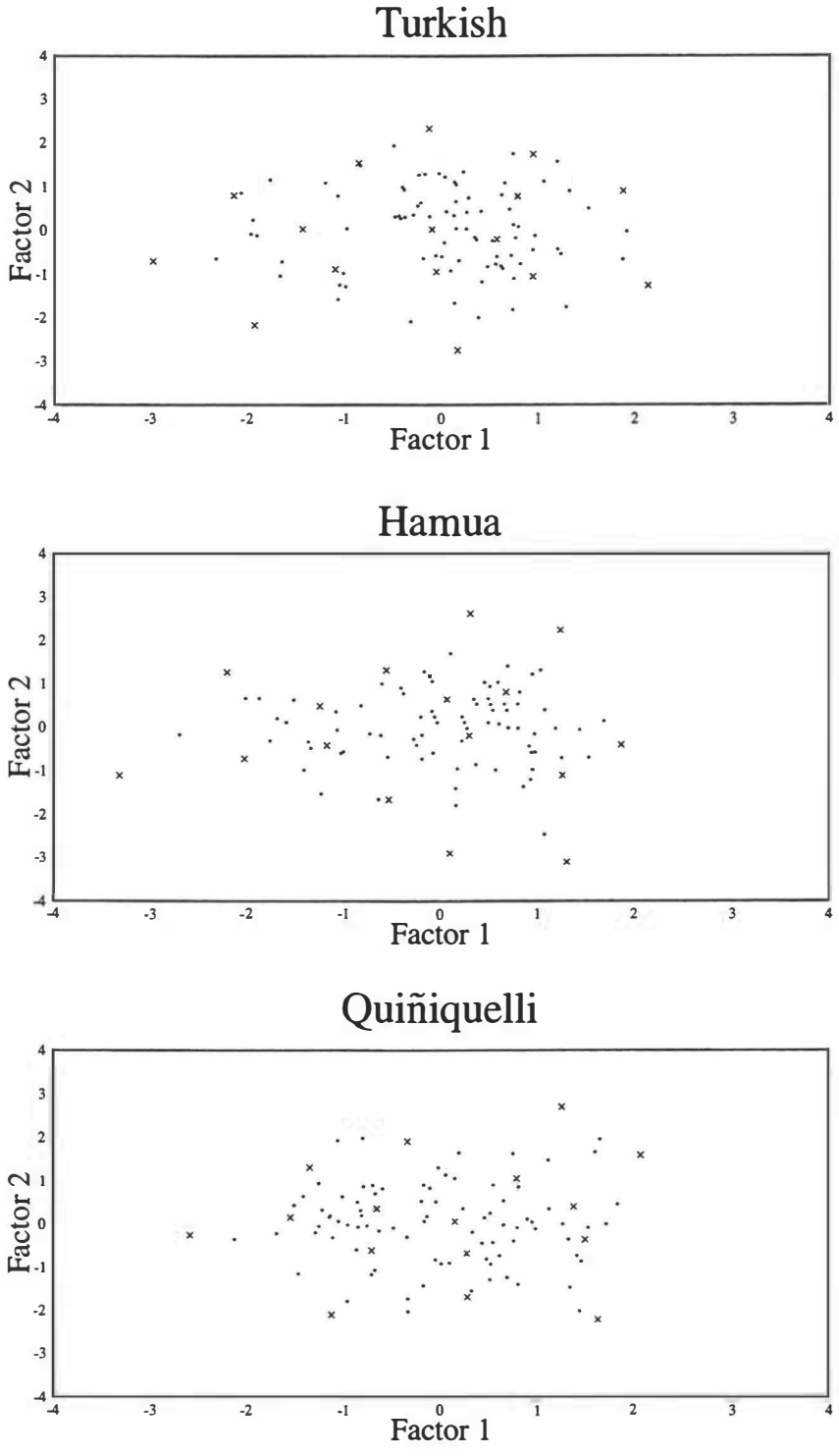

Figure 4.4: Principal component analysis: plot of the first factor against the second factor for the erect populations in Uruguay (. non-selected plants; × selected plants)

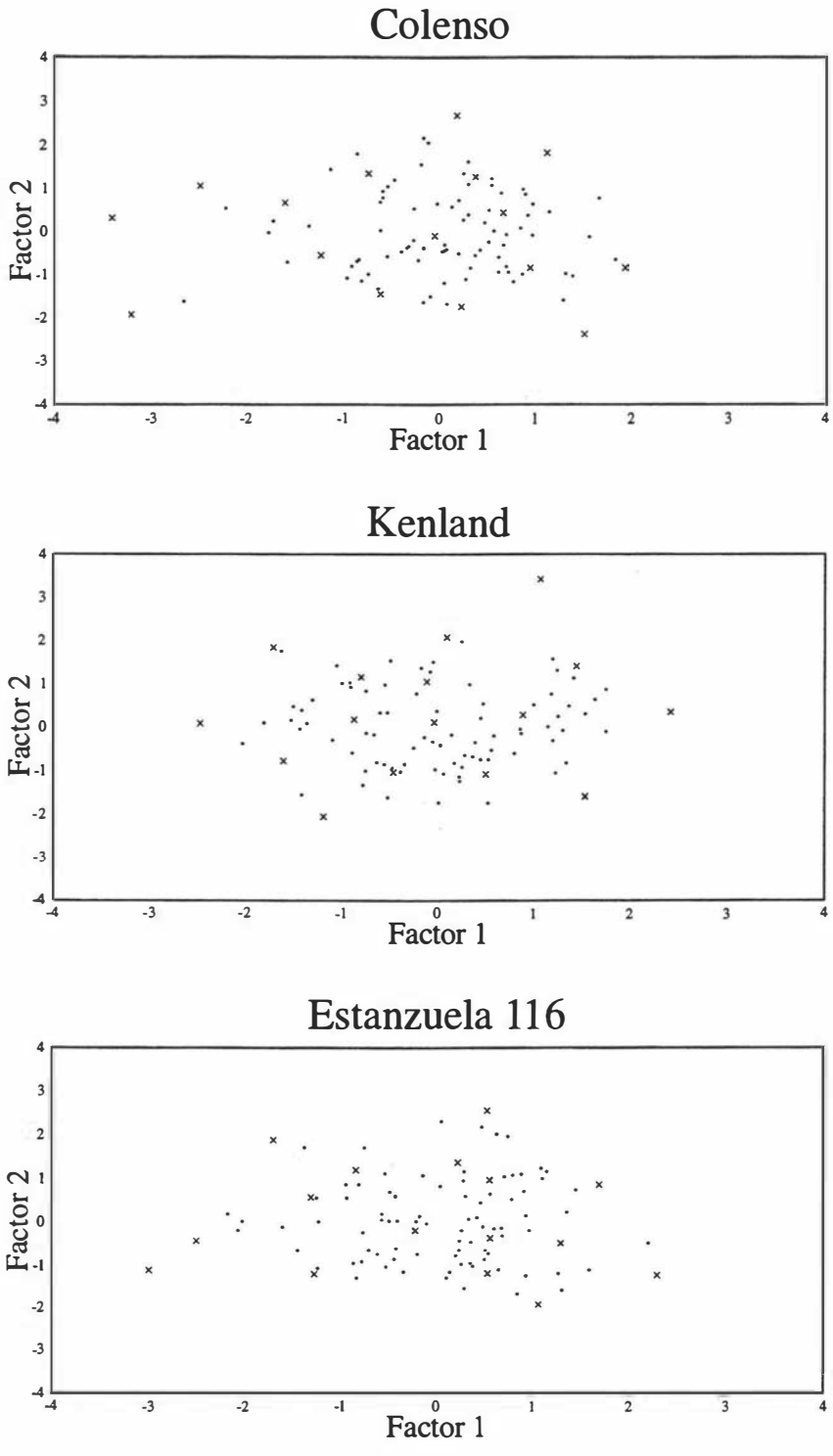

Figure 4.5: Principal component analysis: plot of the first factor against the second factor for the semi-erect populations in Uruguay (. non-selected plants; × selected plants)

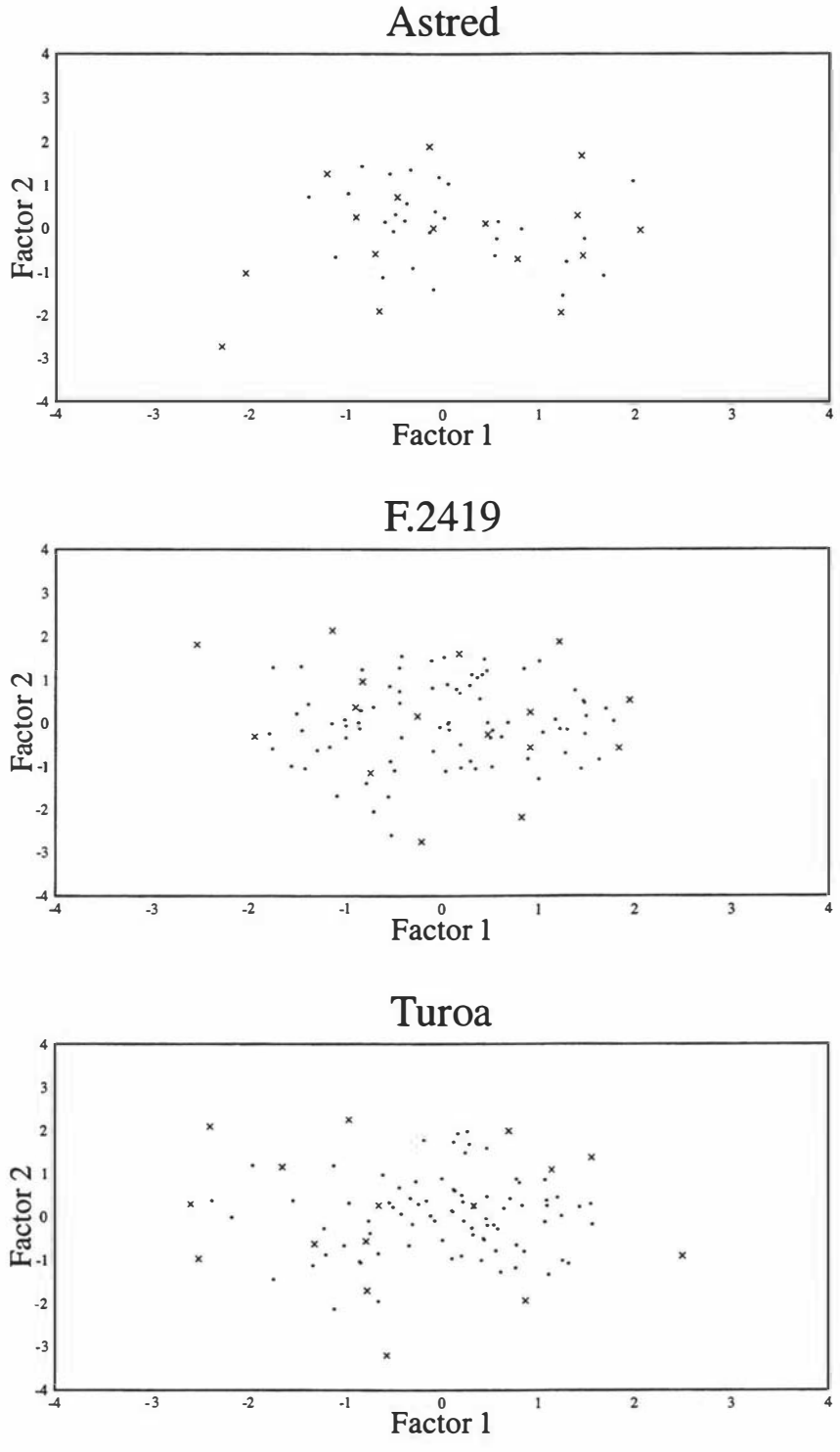

Figure 4.6: Principal component analysis: plot of the first factor against the second factor for the prostrate populations in Uruguay (. non-selected plants; × selected plants)

4.3.3.1.1 MEANS AND VARIANCES OF THE SELECTED PLANTS

Tables 4.7 and 4.8 show the means and variances of the 12 selected plants (corrected by Church equation (Equation 4.2) to take them to an infinite plant variance level) obtained by principal component analysis for the five characters in all the populations in New Zealand and Uruguay. Data in both Tables were compared with the means and variances (Tables 4.4 and 4.5) of the complete populations.

Table 4.7: Means of all characters for the selected plants in New Zealand (NZ) and Uruguay (ROU)

Character	Site	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HGT	NZ	10.58	8.83	12.08	9.92	10.92	11.25	8.92	8.67	8.33
	ROU	7.50	6.25	8.58	6.33	7.08	8.58	6.92	6.33	6.08
LFNB	NZ	15.50	18.33	12.42	16.42	16.33	18.25	17.08	17.83	25.00
	ROU	10.67	10.33	6.00	8.58	7.67	9.00	8.50	6.33	11.00
HBT	NZ	3.42	2.92	3.79	2.92	3.58	3.33	3.04	2.54	2.88
	ROU	3.50	2.75	3.50	3.37	3.50	3.12	2.67	2.67	2.21
LSZ	NZ	4.75	3.83	6.00	4.33	5.33	5.67	4.75	3.92	3.50
	ROU	3.25	2.83	3.75	2.67	3.33	4.25	2.83	2.58	2.08
HRN	NZ	2.17	3.33	3.67	3.67	5.75	3.92	5.08	4.50	3.08
	ROU	3.50	3.42	3.42	3.50	4.42	4.08	6.17	4.08	3.92

The means of all characters for all the populations in New Zealand and Uruguay, were statistically the same for the (12) selected plants and the complete (100 plants) population (Tables 4.4 and 4.7).

Table 4.8: Variances of all characters for the selected plants at an infinite plant variance level in New Zealand (NZ) and Uruguay (ROU)

Character	Site	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HGT	NZ	4.91	2.97	6.41	5.58	9.91*	12.02*	4.08	6.89	5.56
	ROU	4.25	2.69	8.24	3.22	2.58	2.24	3.74	4.89	3.58
LFNB	NZ	34.58*	22.89	15.91	25.91	26.89*	58.85*	38.74*	35.14	715.80*
	ROU	11.56	19.22*	7.17	9.74	9.22	10.33	7.42	15.89	24.33
HBT	NZ	0.53	0.70*	0.77	0.95*	0.70	1.18*	0.73*	1.02	0.30
	ROU	0.71*	0.31	0.88	0.51*	0.46	0.30*	0.18	1.06*	0.73*
LSZ	NZ	1.02*	0.81*	1.67	1.39*	1.22*	2.22*	1.19*	0.58	0.58
	ROU	0.85	1.47*	2.69*	1.06	1.22*	1.69*	0.81*	0.24	0.74*
HRN	NZ	1.31	2.89*	3.22	2.39	0.85	1.91	2.74*	2.25	2.41
	ROU	3.75	1.24	5.41*	3.25	2.08	4.24	0.47	3.91	2.74

* Variance of the 12 plants were significantly different ($P < 0.01$) from variance of the 100 plants (Snedecor and Cochran, 1980).

In 58 out of 90 cases, the variances were the same for the populations of 100 and 12 plants (Tables 4.5 and 4.8). Where differences were significant, the variance for the selected (12) plants was larger than for the population, indicating no artificial restriction in sample variation.

4.3.3.2 CLUSTERED FULL PRINCIPAL COMPONENTS

4.3.3.2.1 MEANS AND VARIANCES OF THE SELECTED PLANTS

The results of this sampling procedure are presented in Table 4.9 for the means and Table 4.10 for the variances at an infinite plant variance level (Equation 4.2).

Table 4.9: Means of each character for the selected plants in New Zealand (NZ) and Uruguay (ROU)

Character	Site	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HGT	NZ	10.83	9.17	12.50	9.33	10.50	11.42	8.67	8.92	7.42
	ROU	7.75	6.92	9.25	6.33	7.08	7.41	7.00	6.83	6.50
LFNB	NZ	13.67	16.67	14.67	16.92	15.67	17.00	16.92	16.83	26.58
	ROU	9.50	9.67	5.92	9.50	7.33	8.50	8.73	8.67	11.50
HBT	NZ	3.54	2.79	3.42	2.92	3.50	3.29	2.88	2.83	2.92
	ROU	2.75*	3.13	2.54	2.67	2.54*	3.00	3.27*	3.63*	3.58*
LSZ	NZ	4.67	4.00	6.25	4.08	5.08	5.25	4.58	4.00	3.58
	ROU	3.41	2.75	3.58	2.50	3.08	3.41	2.91	2.33	2.33
HRN	NZ	2.25	3.08	3.25	2.92	5.16	3.92	5.17	4.75	3.17
	ROU	3.58	3.08	3.50	3.00	4.33	4.25	6.27	3.83	3.58

* Mean of 12 plants were significantly different ($P < 0.05$) from mean of 100 plants (two-tail t test).

The means of each character for all the populations in New Zealand and Uruguay, were statistically the same for the (12) selected plants and the complete (100 plants) population, except for 5 means out of 90 (Tables 4.4 and 4.9).

Table 4.10: Variances of each character for the selected plants at an infinite plant variance level in New Zealand (NZ) and Uruguay (ROU)

Character	Site	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HGT	NZ	4.33	4.71	7.18	4.08	3.53	2.99	2.96	4.80	3.53
	ROU	5.11	2.28	9.30	2.79	4.62	6.81	2.99	2.34	1.72
LFNB	NZ	14.06	19.54	13.69	15.37	23.33	8.70	14.82	36.84	766.74*
	ROU	10.30	6.40	5.52	11.70	8.94	13.18	5.81	12.25	16.81
HBT	NZ	0.44	0.29	0.31	0.62	0.23	0.52	0.77	0.74	0.36
	ROU	0.52	0.28	0.88	0.48	0.20	0.31	0.22	0.59	0.49
LSZ	NZ	0.61	0.55	1.30	0.81	1.17	0.94	1.00	1.08	0.45
	ROU	1.00	0.38	1.17	0.45	1.17*	0.81	0.29	0.61	0.42
HRN	NZ	1.85	3.17	2.02	1.72	1.23	3.35	2.69*	2.76	2.53
	ROU	2.99	1.72	3.17	2.72	2.59	3.65	0.42	1.06	2.62

* Variance of the 12 plants were significantly different ($P < 0.01$) from variance of the 100 plants (Snedecor and Cochran, 1980).

All variances for the 12 plants and for the 100 plants were statistically the same, except in 3 cases out of 90 that the variance of the sample was bigger than the 100 plants (Tables 4.5 and 4.10).

4.4 DISCUSSION

4.4.1 MEANS

The Spearman's rank correlation for all characters was significant indicating that measurements could be taken at an earlier stage of growth (three comparing with four months), obtaining similar results. The advantages of this, is that it is possible to reduce the time necessary to get the same results for seedling characters and to reduce the space and facilities necessary for doing the sampling exercise. Only growth habit needs more time to be developed as in adult plants.

4.4.2 COMPARING SAMPLES vs. POPULATIONS

Almost all means of all characters in all the populations and for both countries were statistically the same for the sample of 12 plants compared with the corresponding population chosen by both methodologies.

The other statistic studied was the variance. The goal was to have the same variance in the 12 plants in comparison with the variance of 100 plants. In the experiment, all the variances were the same or higher in the sample for all characters in all populations in both countries, concluding that the sample and the methodology used was successful, and that there was lack of bias in the plant samples.

4.4.3 COMPARING SAMPLING PROCEDURES

The comparison of parsimonious principal component analyses with clustered full principal components was done after estimating the clusters for the 9 populations in each country. If both methods were similar, the 12 plants chosen by the parsimonious principal component analysis should be one in each cluster but instead, the selected plants were on average distributed over 8.1 clusters for each population in each country. Results obtained with both methods were considered quite close because stochastic sampling could easily be the cause of the differences.

CHAPTER FIVE
Grazing Management Experiment

5.1 INTRODUCTION

In the review of literature (Chapter 2), it was concluded that there is a need to develop an adequate grazing method to include the effect of the grazing animal in spaced plant nurseries in a suitable way to allow plant breeders to identify plants or populations that are being selected by the grazers and also to test those plants for grazing tolerance. If grazing is too lax, many plants will not be grazed because animals were too few or time too short for them to sample all plants, and not because there were plant properties making animals not graze certain plants or populations. If grazing is too hard, no variation will remain after grazing for breeders to work with, and only resistance to grazing will be measured. The objective of the experiment reported in this chapter was to determine the optimum Stocking Density and Time of Day that enables the best discrimination among plant phenotypes for grazing preference.

5.2 MATERIALS AND METHODS

5.2.1 EXPERIMENTAL DETAILS

The grazing management experiment was established with 720 spare plants from the 900 plants not used for the cloning exercise in the preliminary glasshouse experiment in New Zealand. The management and experimental details are presented in Table 5.1.

Table 5.1: Management and experimental details

Management	Experimental Details
Site	Massey University (40° 23' S, 175° 37' E)
Soil type	Manawatu fine sandy loam[1]
Plant materials	9 Red clover populations (Chapter 3, Section 3.3.3)
Planting method	Spaced plants (0.75 m grid)
Planting date	27 Aug. 1994
Plot size	8.0 x 5.0 m
N° of plants/plot	45 plants (9 populations x 5 seedling genets)/plot
Treatments	4 Stocking Densities[2] and 2 Times of Day
N° of blocks	2
Weed control	Triflur 40[3] @ 1.2 kg a.i./ha before sowing, Basagran 480[4] @ 2 l/ha after sowing and by hand pulling
Sheep breed and class	Polworth ewes
Grazing dates	28 Nov. 1994; 19 Dec. 1994; 25 Jan. 1995

1 Cowie, 1972
2 Number of animals/area at any point in time (Hodgson, 1990a).
3 Triflur 40 = trifluralin
4 Basagran 480 = bentazone in the form of soluble concentrate

5.2.2 PLANT LOCATION

The locations of the 45 plants (9 populations x 5 plants = 1 Grazing Unit (GU)) were completely random inside each plot, meaning that the population GU was diffused, being defined by classifier rather than space. The random location was to give a random offer to the grazing animal and reduce the effect of external factors like location of the gate, people presence, electric fence, etc. on diet selection. The effect of plant location with reference to the electric fence was considered by setting up a concomitant dummy for covariance error adjustment. All plants next to the electric fence were assigned a one, all the next to them (but further from the fence) a two, and so on.

5.2.3 CHARACTERS RECORDED

The following measurements were made on individual plants pre-grazing:

Spread (SPR1)	Plant diameter measured in 2.0 cm units.
Height (HGT1)	Plant height measured in 1.0 cm units.
Leafiness (LFN1)	Eye estimation of percentage of leaves per plant with respect to the total plant material, in accordance with Williams, 1927 (meristic score). Ten intervals of 10% were used, from 0% to 100%, meaning no leaves and all leaves, for 0 and 100% respectively.
Visual volume (VOL)	An ordinal score 1 to 5 (and halves) as indicators of visual plant volume was determined previously to each grazing according to the biggest and smallest plants available.
Habit (HBT)	An ordinal score, where: Score 1 - plants which were completely erect and the angle between the main stem and the horizontal was in the 72°-90° interval. Score 2, 3, 4 and 5 - plants in which the fore-mentioned angle was 54°-72°, 36°-54°, 18°-36°, 5°-18° respectively. Score 6 - plants in which the fore-mentioned angle was less than 5° but the end part of stems were upward growing. Score 7 - plants which were absolutely prostrate.
Leaf size (LSZ)	Seven circles of 2, 3, 4, 5, 6, 7, 8 cm of diameter were cut in a card and used to determine leaf size representing an interval score 1 to 7 respectively. The biggest adult leaf of each plant was passed through the circles and recorded according to which category it belonged.
Flowering (FLW)	Eye estimation of percentage of flowers per plant with respect to the total plant material, in accordance with Williams, 1927.

The post-grazing measures, spread (SPR2), height (HGT2) and leafiness (LFN2) were measured in the same way as pre-grazing. Differences in spread, height, and leafiness pre- and post-grazing (DSPR, DHGT, and DLFN) were also considered in the analysis, as is commonplace in agrostology (Johnston *et al.*, 1993; Singh *et al.*, 1993; Collins *et al.*, 1993; Schmidt, 1993).

From now on, characters will be referred to with their abbreviation detailed in brackets with the description of each character.

No variables were transformed because all of them were either assumed to be Normally distributed, or transformation (arcsin $\sqrt{\text{variable}}$ for percentages (Steel and Torrie, 1980) and probit for scores did not improve normality. The normality test used was the Kolmogorov test (SAS Institute, 1988; Stephens, 1974)(Appendix 1).

Sampling intensity was determined using DSPR, DHGT and DLFN to detect whether plants had been grazed at all. If any plant had a positive value in any of these parameters, that plant was considered grazed. Counts of grazed vs. non-grazed led to estimates of sampling intensity (see Section 5.2.7)

5.2.4 SELECTION OF GRAZING DATE AND NUMBER OF SHEEP

The semi-erect populations were used as a reference to decide the date for grazing. When these populations reached on average 25 cm of HGT1, the whole experiment was grazed.

The required number of animals was calculated considering the visual estimation of herbage on offer and the herbage intake expected for sheep during one hour of grazing at that time. Destructive sampling is not an option in a spaced plant breeding nursery. Calculations were made as follows.

The visual estimated herbage mass to be removed was approximately 1000 kg DM/ha (for spaced plants of on average approximately 25 cm height), and as each plot had an effective area of 18 m^2 (6 x 3 m) the DM available was 1.8 kg DM. Sheep daily intake varies with the amount of herbage offered. For Stocking Density values of 2 and 3 sheep/18 m^2, daily intake was taken to be 1.7 kg DM and for Stocking Densities of 5 and 9 sheep/18 m^2, the values were 1.6 kg DM and 1.25 kg DM respectively (Rattray *et al.*, 1987). Assuming that the sheep graze for approximately 8 hours/day (Hodgson, 1990a), 12.5% of their daily intake is available in the experiment's 1 grazing hour in the morning or evening. The estimated DM (according to daily intake, duration of grazing and stocking density) eaten in each grazing was 0.42, 0.63, 1.00 and 1.44 kg DM, for 2, 3, 5 and 9 sheep/18 m^2 respectively during one hour.

Calculations were made for the first grazing only, and these values were re-used for the following two grazings. Sheep were allowed to graze until LFN2 in the highest Stocking Density was on average approximately 25% at time of observation. This criterion for taking animals out of the grazing areas was the one that was expected to give consistency among grazings, because the duration of grazing could not be used reliably in the short grazing period of one hour.

After the post-grazing measures were recorded, sheep were introduced again to defoliate all plots to a uniform level (approximately LFN2 of 20%).

5.2.5 STATISTICAL MODELS

Two statistical models were used to analyse the spaced plant nursery: (1) before the first grazing (5.2.5.1), and (2) the main analysis with the animal effects included (5.2.5.2).

5.2.5.1 MODEL FOR ANALYSIS OF RESULTS OBTAINED BEFORE THE FIRST GRAZING

A preliminary analysis was made to establish the base statistics of the populations before the first grazing. The reduced model omitted effects arising from different Stocking Densities and Times of Day, because these treatments had not by then been imposed. To take advantage of the physical layout in the field, the four Stocking Densities and two Times of Day were considered to be eight internal replications.

The model used to analyse the initial Populations was:

$$X_{ijkl} = \mu + B_i + R_{(i)j} + P_k + \varepsilon_{ijk} + S_{(ijk)l} \qquad (5.1)$$

where X_{ijkl} = the phenotypic value of the l^{th} Plant ($l = 1...s$; $s = 5$) of the k^{th} Population ($k = 1...p$; $p = 9$) of the j^{th} Internal repetition ($j = 1...r$; $r = 8$) of the i^{th} Block ($i = 1...b$; $b = 2$), μ = the grand mean, B_i = the effect of the i^{th} Block, P_k = the effect of the k^{th} Population, ε_{ijk} = random error associated with the experimental units, $R_{(i)j}$ = the effect of the j^{th} Internal repetition of the i^{th} Block, and $S_{(ijk)l}$ = the effect of the l^{th} Plant nested within the k^{th} Population in the j^{th} Internal repetition of the i^{th} Block.

All effects in the model were considered to be random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random designs may be found by equating the expected mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971), and are presented in Table 5.2.

Table 5.2: Random inference of the expectations of mean squares

Source	df	M.S.	Expectation of M.S.	F test
Blocks	b-1	5	$\sigma^2_S+s\sigma^2_\varepsilon+sp\sigma^2_{R(B)}+srp\sigma^2_B$	5/4
Internal Reps (B)	b(r-1)	4	$\sigma^2_S+s\sigma^2_\varepsilon+sp\sigma^2_{R(B)}$	4/2
Populations	p-1	3	$\sigma^2_S+s\sigma^2_\varepsilon+srb\sigma^2_P$	3/2
Error	(rb-1)(p-1)	2	$\sigma^2_S+s\sigma^2_\varepsilon$	2/1
Plants	bpr(s-1)	1	σ^2_S	

In the table, σ^2_ε is the variance arising from ε_{ijk}, σ^2_S from $S_{(ijk)l}$, σ^2_R from $R_{(i)j}$, σ^2_P from P_k and σ^2_B from B_i.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS       Internal_Reps Pop Block;
MODEL...=   Block Internal_Reps(Block) Pop
            Pop(Internal_Reps Block) / SS2;
```

5.2.5.2 MODEL FOR MAIN ANALYSIS

The replication internal to the blocks were now utilised via grazing treatments, and also three grazings were considered (split-plot in time). The new model became: factor (A) consisted of the nine Red clover populations; factor B consisted of four Stocking Densities (2, 3, 5 and 9 sheep/18 m^2); and factor C was Time of Day (morning or evening). This (4x2) grazing management factorial (the previous 8 internal replications) was randomised into 2 blocks, giving 16 fenced grazing units (GU) with populations arranged as a split-plot within grazing units. Plants constituted sampling units within these split-plots. Three repeated measures on the same plants (grazing times) (Gill, 1986) (factor D) defined a second split below plants.

The model used to analyse the experiment was:

$$X_{ijklno} = \mu + B_i + U_j + M_k + UM_{jk} + \tau_{ijk} + P_l + PU_{lj} + PM_{lk} + PUM_{ljk} + \delta_{ijkl} + S_{(ijkl)n} + T_o + TU_{oj} + TM_{ok} + TUM_{ojk} + TP_{ol} + TPU_{olj} + TPM_{olk} + TPUM_{oljk} + \varepsilon_{ijklno}, \quad (5.2)$$

where X_{ijklno} = the phenotypic value corresponding to the o^{th} Time (o = 1...t; t = 3) and the n^{th} Plant (n = 1...s; s = 5) of the l^{th} Population (l = 1...p; p = 9) evaluated at the k^{th} Time of Day (k = 1...m; m = 2) with the j^{th} Stocking Density (j = 1...u; u = 4) in the i^{th} Block (i = 1...b; b = 2), μ = grand mean, B_i = effect of the i^{th} Block, U_j = effect of the j^{th} Stocking Density, M_k = effect of the k^{th} Time of Day, UM_{jk} = effect of the jk^{th} interaction, τ_{ijk} = the random error associated with the Stocking Density and Time of Day P_l = effect of the l^{th} Population, PU_{lj} = effect of the lj^{th} interaction, PM_{lk} = effect of the lk^{th} interaction, PUM_{ljk} = effect of the ljk^{th} interaction, δ_{ijkl} = the random error associated with the Population level, $S_{(ijkl)n}$ = effect of the n^{th} Plant nested within the l^{th} Population in the j^{th} Stocking Density and the k^{th} Time of Day in the i^{th} Block, T_o = effect of the o^{th} Time split, TU_{oj} = effect of the oj^{th} interaction, TM_{ok} = effect of the ok^{th} interaction, TUM_{ojk} = effect of the ojk^{th} interaction, TP_{ol} = the effect of the ol^{th} interaction, TPU_{olj} = effect of the olj^{th} interaction, TPM_{olk} = effect of the olk^{th} interaction, $TPUM_{oljk}$ = effect of the $oljk^{th}$ interaction, and ε_{ijklno} = random error $ijklno^{th}$.

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971), although the present experiment does provide an example of a very complex Expectations of Mean Squares structure. The expectations of mean squares are presented in Table 5.3.

Table 5.3: Random inference of the expectations of mean squares.

Source	df	M.S	Expectation of M.S.	F test
Blocks	(b-1)	20	$\sigma^2_\varepsilon+t\sigma^2_s+st\sigma^2_\delta+stp\sigma^2_\tau+stpmu\sigma^2_B$	20/16
Stock. Density	(u-1)	19	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+t\sigma^2_s+st\sigma^2_\delta+stp\sigma^2_\tau+sbm\sigma^2_{TPU}+spb\sigma^2_{TUM}+spbm\sigma^2_{TU}+stb\sigma^2_{PUM}+stbm\sigma^2_{PU}+stbp\sigma^2_{UM}+stbmp\sigma^2_U$	(19+12+6+4)/(17+14+8+2)
Time of day	(m-1)	18	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+t\sigma^2_s+st\sigma^2_\delta+stp\sigma^2_\tau+sbu\sigma^2_{TPM}+spb\sigma^2_{TUM}+spub\sigma^2_{TM}+stb\sigma^2_{PUM}+stbu\sigma^2_{PM}+stbp\sigma^2_{UM}+stbup\sigma^2_M$	(18+12+6+3)/(17+13+7+2)
UxM	(u-1)(m-1)	17	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+spb\sigma^2_{TUM}\ t\sigma^2_s+st\sigma^2_\delta+stp\sigma^2_\tau+stb\sigma^2_{PUM}+stbp\sigma^2_{UM}$	(17+11+2)/(12+6+16)
Error a	(b-1)(um-1)	16	$\sigma^2_\varepsilon+t\sigma^2_s+st\sigma^2_\delta+stp\sigma^2_\tau$	16/11
Pop	(p-1)	15	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+t\sigma^2_s+st\sigma^2_\delta+sbu\sigma^2_{TPM}+sbm\sigma^2_{TPU}+sbmu\sigma^2_{TP}+stb\sigma^2_{PUM}+stbu\sigma^2_{PM}+stbm\sigma^2_{PU}+stbmu\sigma^2_P$	(15+12+3+4)/(14+13+5+2)
PxU	(p-1)(u-1)	14	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+t\sigma^2_s+st\sigma^2_\delta+sbm\sigma^2_{TPU}+stb\sigma^2_{PUM}+stbm\sigma^2_{PU}$	(14+2)/(12+4)
PxM	(p-1)(m-1)	13	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+t\sigma^2_s+st\sigma^2_\delta+sbu\sigma^2_{TPM}+stb\sigma^2_{PUM}+stbu\sigma^2_{PM}$	(13+2)/(12+3)
PxUxM	(p-1)(u-1)(m-1)	12	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+t\sigma^2_s+st\sigma^2_\delta+stb\sigma^2_{PUM}$	(12+1)/(11+2)
Error b	(p-1)(b-1) um	11	$\sigma^2_\varepsilon+t\sigma^2_s+st\sigma^2_\delta$	11/10
Plant (B P U M)	(s-1)pbmu	10	$\sigma^2_\varepsilon+t\sigma^2_s$	10/1
Time	(t-1)	9	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+sbu\sigma^2_{TPM}+sbm\sigma^2_{TPU}+sbmu\sigma^2_{TP}+spb\sigma^2_{TUM}+spub\sigma^2_{TM}+spbm\sigma^2_{TU}+spbmu\sigma^2_T$	(9+6+4+3)/(8+7+5+2)
TxU	(t-1)(u-1)	8	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+sbm\sigma^2_{TPU}+spb\sigma^2_{TUM}+spbm\sigma^2_{TU}$	(8+2)/(6+4)
TxM	(t-1)(m-1)	7	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+sbu\sigma^2_{TPM}+spb\sigma^2_{TUM}+spub\sigma^2_{TM}$	(7+2)/(6+3)
TxUxM	(t-1)(u-1)(m-1)	6	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+spb\sigma^2_{TUM}$	6/2
TxP	(t-1)(p-1)	5	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+sbu\sigma^2_{TPM}+sbm\sigma^2_{TPU}+sbmu\sigma^2_{TP}$	(5+2)/(4+3)
TxPxU	(t-1)(p-1)(u-1)	4	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+sbm\sigma^2_{TPU}$	4/2
TxPxM	(t-1)(p-1)(m-1)	3	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}+sbu\sigma^2_{TPM}$	3/2
TxPxUxM	(t-1)(p-1)(u-1)(m-1)	2	$\sigma^2_\varepsilon+sb\sigma^2_{TPUM}$	2/1
Error c	p(t-1){(s-1)b[1+(m-1) + (u-1) (1+(m-1))]+(b-1)mu}	1	σ^2_ε	

In the Table, σ^2_ε is the variance arising from ε_{ijklno}, σ^2_T from T_o, $\sigma^2_s$from $S_{(ijkl)n}$, σ^2_δ from δ_{ijkl}, σ^2_P from P_l, σ^2_τ from τ_{ijk}, σ^2_M from M_k, σ^2_U from U_j, σ^2_B from B_i and the various interaction variances from the respective main effects.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS     Time_of_day Stock_Density Pop Time Block Plant;
MODEL...= Block Pop|Time_of_Day|Stock_Density|Time
          Block(Time_of_Day Stock_Density)
          PopxBlock(Time_of_Day Stock_Density)
          Plant(Pop Block Time_of_Day Stock_Density) / SS2 ;
```

All the F tests were recalculated from the SAS output, because the RANDOM command followed by the option TEST was impossible to use because of limited computing resources (even on the mainframe of Massey University). Another programme called THWAITE (Gordon, unpublished) was used to estimate the F tests later. This implements the Crump (1946,1951) and Satterthwaite (1946) complex F tests and degrees of freedom. This programme also estimated the variance components and their standard errors.

5.2.6 HERITABILITY ESTIMATES

Heritabilities express the proportion of the phenotypic variance due to genetics, with several variations on explicit definitions. A restricted definitions (σ^2_{RP}) which omit several macro-environment variance components was used (Allard, 1960; Gordon *et al.*, 1972; Gordon, 1979).

$$\text{Variance of restricted phenotype} = \sigma^2_{RP} = \sigma^2_{\varepsilon} + \sigma^2_{TPUM} + \sigma^2_{TPM} + \sigma^2_{TPU} + \sigma^2_{TP} + \sigma^2_{s} + \sigma^2_{\delta} + \sigma^2_{\tau} + \sigma^2_{PUM} + \sigma^2_{PM} + \sigma^2_{PU} + \sigma^2_{P} \quad (5.3)$$

$$h^2 \text{ (Population, restricted)} = \sigma^2_P/\sigma^2_{RP} \quad (5.4)$$

$$h^2 \text{ (Plant, restricted)} = (\sigma^2_S \text{ x } (g))/\sigma^2_{RP} \quad (5.5)$$

$$h^2 \text{ (Overall, restricted)} = (\sigma^2_{P+}\sigma^2_S \text{ x } (g))/\sigma^2_{RP} \quad (5.6)$$

The variance of plants was a confounding of two sources: genetic segregation and environmental. These were partitioned by using the estimates of genetic fractions (g) from the neighbouring experiment (estimated using clonal replicates), conducted with the same populations at the same time. It was assumed that no bias would arise thereby in the statistics in this experiment (see Chapter Six, Section 6.2.6).

The standard error of heritabilities were obtained following Osborne and Paterson (1952) (Appendix 2 and 3 for Model Equations 5.1 and 5.2 respectively). One tail t tests were performed for the heritabilities, by dividing them by their respective standard error and using the degrees of freedom of the error term.

5.2.7 ANALYSIS OF SAMPLING INTENSITY

Plants were classified as grazed or not grazed as explained in Section 5.2.3, and averaged into classifications "Stocking Density" and "Time of Day" for use in a two-way contingency table. The purpose of this test was to detect if the sampling pressure that the plants were being exposed to for each grazing treatment were the same across classifications.

Observed values were tested with the null hypothesis that all cells have the same probability (marginal probability for Stocking Density x marginal probability for Time of Day x total observations), against the alternative hypothesis that the probabilities were different to being the same for each cell.

Marginal probabilities in a two-way contingency table could be tested by themselves as if they were a one-way Table with 2 or 4 cells for Time of Day and Stocking Density respectively. For this case, the null hypothesis was that all cells have the same probability (0.5 and 0.5 for morning and evening respectively and 0.25 for each Stocking Density), against the alternative hypothesis that the probabilities were different to being the same for each cell.

The test criterion was:

$$\chi^2 = \sum \frac{(Observed - Expected)^2}{Expected} \qquad (5.7)$$

with (r-1)(c-1) degrees of freedom, being r = rows and c = columns (Steel and Torrie, 1980).

5.2.8 TEST FOR TIME CORRELATION

The same spaced plants were harvested three times in successive periods, possibly causing failure of the assumption of independence of the error effects and biasing the expectation of the mean squares, the F tests, etc. A possible solution to this problem is to calculate the value of the repeat-correlation, then to adjust the split-plot-in-time analysis for that correlation. The correlation across time was calculated according to the following formulae (developed from Gill, 1986; Gordon, 1994).

MS_δ = Error b = $\sigma^2_\varepsilon + t\sigma^2_s + st\sigma^2_\delta$ (5.8)

MS_ε = Error c = σ^2_ε (5.9)

θ_δ = Time covariance (5.10)

$\sigma^2_\varepsilon = \sigma^2$ - Time covariance (5.11)

$\sigma^2 = \sigma^2_\varepsilon + \sigma^2_\delta$ (5.12)

Corr. Across Time = $\sigma^2_\delta / \sigma^2_\delta + \sigma^2_\varepsilon$ (5.13)

The t value to test the significance of the time correlation was calculated in the following way (Steel and Torrie, 1980):

$$t = \frac{r}{\sqrt{(1-r^2)/(n-2)}} \qquad (5.14)$$

where r = correlation across Time and n = total number of spaced plants in each grazing

period.

5.3 RESULTS

5.3.1 BASE CONDITION OF THE POPULATIONS BEFORE GRAZING TREATMENTS WERE IMPOSED

The base properties of the populations before grazing treatments were imposed were described by seven characters (HGT1, SPR1, LFN1, VOL, LSZ, HBT and FLW). The purpose of this section is to present pure plant properties to describe the population and to compare them in the discussion section with the same attributes measured after the grazing treatments were imposed. Analysis of variance (5.3.1.1), means and standard errors (5.3.1.2) and heritabilities (5.3.1.3) were used to characterise the populations without the effect of the grazing animal.

5.3.1.1 ANALYSIS OF VARIANCE

The significance of the analysis of variance (F tests), variance components with their respective standard errors for characters measured before the first grazing are presented in Table 5.4.

Table 5.4: Significance of the analysis of variance (F test), variance components with their respective standard errors for characters measured before the first grazing

Characters	Blocks	Internal Reps.	Populations	Error	Plants
HGT1	5.24** (4.34)	0.09 ^{NS} (0.24)	21.67** (9.83)	0.77 ^{NS} (0.68)	21.03 (1.24)
SPR1	7.19** (6.07)	-0.40 ^{NS} (0.74)	105.31** (47.68)	1.86 ^{NS} (2.91)	95.41 (5.62)
LFN1	3.71** (3.41)	1.80* (1.32)	105.50** (47.65)	-2.09 ^{NS} (2.43)	94.76 (5.58)
VOL	0.02** (0.01)	0.00 ^{NS} (0.00)	0.16** (0.07)	0.01* (0.01)	0.24 (0.01)
LSZ	0.13** (0.12)	0.04** (0.02)	0.75** (0.34)	0.04* (0.02)	0.86 (0.05)
HBT	-0.00 ^{NS} (0.00)	0.01* (0.01)	0.58** (0.26)	0.02 ^{NS} (0.02)	0.64 (0.04)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

The Population effect was highly significant for all characters, meaning that all were useful in detecting differences and describing the populations. Blocks effect was highly significant for all except HBT and FLW, while the Internal Repetitions effect was only significant for LFN1, HBT and highly significant for LSZ. Blocks and Internal Repetitions effects were useful partitions, and, if they had not been removed they would have increased the plot Error effect of the model. Error effect was only significant for VOL and LSZ.

5.3.1.2 ANALYSIS OF MEANS

As shown by the Population effect being highly significant for all attributes, mean separation was used to describe the populations. Grand means and means per Population with their respective standard errors for all characters measured before the first grazing are presented in Table 5.5.

Table 5.5: Grand means and means per Population with their respective standard errors for all characters measured before the first grazing

Character	Grand Mean	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
HGT1	16.1(0.25)[1]	15.3d[2]	13.6e	20.9b	14.9de	17.2c	25.2a	9.7g	15.8dc	11.8f	0.56
SPR1	53.0(0.52)	45.7d	48.7cd	58.9b	48.6cd	51.7c	61.5b	73.6a	49.7c	38.2e	1.14
LFN1	68.0(0.51)	61.6e	60.4e	75.9b	62.1de	72.0c	82.8a	80.6a	64.4d	52.3f	1.03
VOL	3.1(0.02)	2.8d	3.0c	3.6a	2.9cd	2.9c	3.7a	3.3b	3.0c	2.4e	0.06
LSZ	4.0(0.05)	3.5f	3.7ef	5.2a	3.9de	4.8b	4.2c	4.1cd	4.0cd	2.1g	0.10
HBT	3.0(0.04)	2.9d	3.2c	2.3e	3.3c	2.5e	1.8f	4.5a	3.1cd	3.6b	0.10
FLW	0.1(0.01)	0.01bc	0.01bc	0.00c	0.03bc	0.03bc	0.53a	0.09b	0.06bc	0.01bc	0.03

1 Standard errors of the grand means

2 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$).

As indicated by FLW, all these measurements were made with the plants in a vegetative stage and flowering was just starting. The biggest population was E116, being the tallest, second in SPR1, the most leafy and with the largest volume. This population was followed in size by Quiñiquelli and the smallest was Turoa.

Quiñiquelli had the biggest leaves followed by Kenland and E116, while Turoa was the population with smallest leaves. Astred was the most prostrate and E116 was the most erect population.

5.3.1.3 HERITABILITIES

For breeding purposes, the proportion of variation due to genetics (h^2) in its several definitions (Equations 5.4...5.6, Section 5.2.6) is of primary importance, to assess if plant breeders are able to make genetic progress through selection. Here, we are examining the characters without the influence of grazing animals. This provides a purely plant-focused indication of which characters will respond most to selection. The heritabilities of the different characters are presented in Table 5.6.

Table 5.6: Heritability values

Characters	Genetic fractions (g)	Population Restricted[1]	Plant Restricted[2]	Overall Restricted[3]
HGT1	0.55	0.50**(0.11)	0.27**(0.06)	0.77**(0.06)
SPR1	0.27	0.52**(0.11)	0.13**(0.03)	0.65**(0.09)
LFN1	0.00	0.53**(0.11)	0.00^{NS} (0.00)	0.53**(0.11)
VOL	0.31[5]	0.39**(0.11)	0.18**(0.04)	0.57**(0.08)
LSZ	0.35	0.46**(0.11)	0.18**(0.04)	0.64**(0.08)
HBT	0.66	0.47**(0.11)	0.34**(0.08)	0.81**(0.05)

NS Non significant
** Significant at 0.01 level
1...3 Equations (5.4...5.6) in Section 5.2.6
4 Volume was not measured in the spaced plant-animal interaction experiment so an average of all genetic fractions of all characters measured pre-grazing was used. This average was considered the best guess, considering the other alternatives of being zero (no genetic variation) or one (all genetic variation).

All heritability values were highly significant ($P < 0.01$) for all characters and all definitions except the plant heritabilities for LFN1 because the genetic fraction for them was zero.

The overall restricted heritability that is in fact the most commonly mentioned in the literature is high for HGT1, SPR1, LSZ and HBT; medium to high for LFN1 and VOL.

5.3.2 EVALUATION OF NEED FOR DATA ADJUSTMENT

Before beginning with the analysis of the results after the grazing treatments were imposed, assessment of the necessity of adjustment for repeated measurements (5.3.2.1) and for location of the plants with reference to the electric fence was necessary (5.3.2.2). Results for these issues are considered next.

5.3.2.1 REPEATED MEASUREMENTS ANALYSIS.

The correlation across time was calculated for each character (Table 5.7) to determine if there was any adjustment necessary for repeated measures effects.

Table 5.7: Correlation across time (n=720)[1]

Characters	Correlation across time
HGT1	0.11**
SPR1	0.05^{NS}
LFN1	0.00^{NS}
VOL	0.05^{NS}
LSZ	0.04^{NS}
HBT	0.00^{NS}
FLW	0.02^{NS}
HGT2	0.06^{NS}
SPR2	0.04^{NS}
LFN2	0.05^{NS}

1 Total number of spaced plants in each grazing period
2 t value (Section 5.2.8)
NS Non significant
** Significant at 0.01 level

Only one of the correlations across Time (HGT1) was significant, and it was of such a low value (0.11), that it was considered not necessary to adjust by such correlation.

5.3.2.2 PLANT LOCATION ANALYSIS

The significance of the F tests of the plant location with reference to the fence (concomitant dummy) with HGT2, SPR2 and LFN2 and DHGT, DSPR and DLFN were studied as a concomitant model with PROC GLM of SAS statistical package (SAS Institute, 1988). The location (concomitant dummy) was not significant for any post-grazing or differences pre- and post-grazing concomitant analysis (F tests).

Simple regressions between HGT2, SPR2, LFN2, DHGT, DSPR, DLFN at a time with location were performed, all R^2 being not significant and less than 0.01. Therefore, considering the effect on the error partition (concomitant analysis) and the overall effect on the simple regressions, location was considered not to affect the subsequent results and was not considered further.

5.3.3 MAIN ANALYSIS OF RESULTS

Grazing managements were studied in three successive grazings, and ten plant attributes were measured. Analyses were done according to the design model in Methods (Section 5.2.5.2). Analysis of variance (5.3.3.1), means and standard errors (5.3.3.2), heritabilities (5.3.3.3), and sampling intensities (5.3.3.4) were tools used to analyse the experiment.

5.3.3.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was done. The purpose of this analysis was to detect if grazing managements were having a significant effect on the measured attributes and to separate genetics from environment, to determine their usefulness for plant breeding. The results of the analysis of variance (F tests), variance component with their respective standard errors for all characters are presented in Table 5.8.

Table 5.8: Significance of the analysis of variance (F tests), variance components with their standard error for all characters

Character	Blocks	Stock. Density	Time of Day	UxM[1]	Error a	Pop.	PxU	PxM	PxUxM	Error b	Plant (BPUM)	Time	TxU	TxM	TxUxM	TxP	TxPxU	TxPxM	TxPx UxM	Error c
HGT1	1.52*	-0.31^{NS}	-0.10^{NS}	-0.02^{NS}	1.33*	17.44**	-0.44^{NS}	-0.42^{NS}	-0.39^{NS}	2.47**	15.33**	76.55**	0.77*	0.07^{NS}	0.22^{NS}	4.63**	0.15^{NS}	-0.18^{NS}	0.53^{NS}	19.78
	(1.46)	(0.48)	(0.21)	(0.85)	(0.99)	(8.54)	(0.57)	(0.28)	(1.09)	(1.22)	(1.32)	(54.70)	(0.54)	(0.14)	(0.26)	(1.64)	(0.38)	(0.20)	(0.51)	(0.78)
SPR1	0.08^{NS}	0.87^{NS}	0.49^{NS}	-5.22^{NS}	7.53**	52.23**	-0.01^{NS}	-0.81^{NS}	-0.30^{NS}	3.24^{NS}	47.00**	60.04**	0.59^{NS}	-0.13^{NS}	1.98*	16.30**	-1.29^{NS}	-0.80^{NS}	1.93*	63.55
	(1.17)	(0.83)	(0.36)	(2.29)	(4.45)	(26.06)	(1.67)	(0.81)	(2.90)	(3.05)	(4.10)	(44.00)	(1.20)	(0.52)	(1.46)	(5.55)	(1.01)	(0.59)	(1.68)	(2.50)
LFN1	0.22*	-0.04^{NS}	-0.04^{NS}	-0.08^{NS}	0.15^{NS}	-25.13^{NS}	0.09^{NS}	0.53*	-0.15^{NS}	0.29^{NS}	-15.64^{NS}	2.28^{NS}	-0.38^{NS}	-0.29^{NS}	1.17^{NS}	86.34**	-0.16^{NS}	-1.01^{NS}	1.46^{NS}	84.61
	(0.23)	(0.31)	(0.15)	(0.55)	(0.23)	(9.83)	(0.58)	(0.38)	(0.86)	(0.51)	(1.33)	(8.99)	(0.70)	(0.32)	(1.16)	(29.02)	(1.38)	(0.70)	(2.01)	(3.33)
VOL	-0.00^{NS}	-0.00^{NS}	0.00^{NS}	-0.01^{NS}	0.04**	0.03*	-0.00^{NS}	-0.01^{NS}	0.00^{NS}	0.01^{NS}	0.13**	0.00^{NS}	0.01*	0.00^{NS}	0.00*	0.06**	0.00^{NS}	0.01*	-0.00^{NS}	0.21
	(0.00)	(0.01)	(0.00)	(0.01)	(0.02)	(0.02)	(0.00)	(0.00)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.00)	(0.00)	(0.02)	(0.00)	(0.00)	(0.00)	(0.01)
LSZ	0.00^{NS}	-0.01^{NS}	-0.01^{NS}	0.02*	-0.01^{NS}	0.49**	0.01^{NS}	0.01^{NS}	-0.04^{NS}	-0.10^{NS}	0.43**	0.01^{NS}	0.02*	-0.00^{NS}	0.00^{NS}	0.16**	-0.01^{NS}	0.02*	0.01^{NS}	0.69
	(0.00)	(0.01)	(0.00)	(0.01)	(0.01)	(0.25)	(0.01)	(0.01)	(0.02)	(0.01)	(0.04)	(0.02)	(0.01)	(0.00)	(0.00)	(0.06)	(0.01)	(0.01)	(0.02)	(0.03)
HBT	0.00^{NS}	-0.01^{NS}	-0.00^{NS}	-0.00^{NS}	0.00^{NS}	0.62**	-0.02^{NS}	-0.02^{NS}	0.03^{NS}	0.00^{NS}	0.50**	0.15**	0.01*	0.00^{NS}	0.01*	0.08**	0.01^{NS}	0.00^{NS}	0.00^{NS}	0.49
	(0.00)	(0.01)	(0.00)	(0.01)	(0.01)	(0.29)	(0.02)	(0.01)	(0.03)	(0.02)	(0.04)	(0.12)	(0.01)	(0.00)	(0.01)	(0.03)	(0.01)	(0.01)	(0.01)	(0.02)
FLW	0.03^{NS}	0.02^{NS}	0.01^{NS}	-0.06^{NS}	0.12**	0.15^{NS}	-0.01^{NS}	-0.01^{NS}	-0.00^{NS}	0.07^{NS}	-0.07^{NS}	10.06**	0.27*	0.01^{NS}	0.19**	1.67**	0.10^{NS}	0.02^{NS}	0.04^{NS}	4.60
	(0.04)	(0.12)	(0.03)	(0.08)	(0.07)	(0.38)	(0.05)	(0.03)	(0.07)	(0.07)	(0.07)	(7.32)	(0.21)	(0.06)	(0.12)	(0.59)	(0.08)	(0.05)	(0.10)	(0.18)
HGT2	0.16^{NS}	5.99*	-0.60^{NS}	0.01^{NS}	1.36*	15.70**	-0.45^{NS}	-0.62^{NS}	0.30^{NS}	1.81*	14.63**	42.82**	1.54*	1.12*	0.76**	5.04**	0.29^{NS}	-0.03^{NS}	-0.31^{NS}	27.40
	(0.36)	(4.55)	(0.39)	(1.00)	(0.99)	(7.81)	(0.63)	(0.27)	(1.13)	(1.18)	(1.44)	(31.45)	(1.07)	(0.98)	(0.52)	(1.81)	(0.39)	(0.23)	(0.50)	(1.08)
SPR2	-1.23^{NS}	12.04**	0.81^{NS}	-8.46^{NS}	10.87**	52.55**	0.30^{NS}	-1.41^{NS}	-2.00^{NS}	3.40^{NS}	47.00**	138.16**	-0.37^{NS}	-1.47^{NS}	7.10**	9.59**	-0.25^{NS}	1.15^{NS}	2.00^{NS}	74.07
	(0.82)	(7.67)	(0.44)	(3.33)	(6.06)	(25.18)	(1.59)	(0.78)	(2.68)	(3.21)	(4.33)	(98.63)	(2.75)	(1.14)	(4.08)	(3.82)	(1.29)	(1.26)	(1.90)	(2.92)
LFN2	0.20^{NS}	256.07**	15.22^{NS}	-33.12^{NS}	13.42**	12.37*	7.52**	4.56**	-5.93^{NS}	6.66**	10.00**	15.02^{NS}	-38.85^{NS}	-12.69^{NS}	115.01**	13.58**	-1.81^{NS}	-1.70^{NS}	7.40**	130.42
	(1.95)	(158.59)	(13.57)	(21.40)	(7.24)	(9.75)	(2.87)	(2.45)	(2.50)	(3.08)	(3.57)	(14.40)	(30.91)	(18.71)	(58.64)	(5.02)	(2.64)	(1.52)	(4.11)	(5.14)
DHGT	0.48**	5.55*	-0.43^{NS}	0.85*	-0.09^{NS}	0.17^{NS}	0.08^{NS}	0.24^{NS}	-0.63^{NS}	1.15**	0.26^{NS}	4.48*	1.42*	0.46^{NS}	0.58*	0.96**	-0.53^{NS}	-0.29^{NS}	1.08*	27.25
	(0.42)	(4.11)	(0.25)	(0.74)	(0.13)	(0.36)	(0.28)	(0.24)	(0.50)	(0.54)	(0.66)	(3.74)	(0.96)	(0.50)	(0.51)	(0.42)	(0.47)	(0.29)	(0.77)	(1.07)
DSPR	0.11^{NS}	12.13**	1.42*	-2.10^{NS}	1.04**	-0.85^{NS}	0.28^{NS}	0.30^{NS}	-0.54^{NS}	0.29^{NS}	-1.19^{NS}	16.99**	-0.70^{NS}	-1.04^{NS}	5.33**	2.35**	0.49^{NS}	0.23^{NS}	-0.39^{NS}	58.31
	(0.25)	(7.69)	(1.02)	(1.07)	(0.70)	(0.46)	(0.45)	(0.38)	(0.62)	(0.72)	(1.32)	(12.26)	(1.89)	(0.81)	(2.97)	(1.14)	(0.84)	(0.60)	(1.11)	(2.30)
DLFN	2.18^{NS}	251.48**	17.05^{NS}	-42.35^{NS}	12.35**	-23.23^{NS}	8.01**	4.46*	-8.52^{NS}	8.42**	-12.59^{NS}	-8.53^{NS}	-41.13^{NS}	-18.47^{NS}	130.76**	105.8**	-4.13^{NS}	-2.51^{NS}	11.26*	213.86
	(3.38)	(153.86)	(12.77)	(23.22)	(6.88)	(14.05)	(3.00)	(2.39)	(3.17)	(3.58)	(4.45)	(11.15)	(35.96)	(19.81)	(67.20)	(35.90)	(4.07)	(2.49)	(6.58)	(8.43)

1 Character abbreviations are detailed in Equation 5.2

NS Non significant; * Significant at 0.05 level; ** Significant at 0.01 level

Stocking Density and Time of Day had no significant effects upon plant characteristics measured pre-grazing. This is the expected result for the first grazing because those treatments had not at this stage been imposed, and also implies that the uniformity grazing was a success in setting up conditions for grazings two and three. The effect of Stocking Density was significant for all characters measured post-grazing and the differences, but Time of Day was only significant for DSPR.

The effects of Populations and Plants were highly significant for all characters except LFN1 and FLW and the differences pre- and post-grazing (DHGT, DSPR and DLFN).

Time effect was not significant for LFN1, LFN2, DLFN, VOL and LSZ, meaning that those characters behaved consistently across grazings. From the first order interactions: UxM (Stocking Density by Time of Day) was only significant for LSZ and DHGT; PxU (Population by Stocking Density) and PxM (Population by Time of Day) were only significant for LFN2 and DLFN and LFN1 for PxM; TxU (Time by Stocking Density) was not significant for characters measuring spread and leafiness; TxM (Time by Time of Day) was only significant for HGT2; and TxP (Time by Population) was highly significant for all characters, meaning that Populations did not behave consistently across grazings. The second and third order interactions were all not significant with some exceptions mainly in the TxUxM (Time by Stocking Density by Time of Day) effect.

5.3.3.2 ANALYSIS OF MEANS

Means were used to describe the plant materials for each grazing period, for grazing management effects, for each population, and for their interactions.

Grand means, coefficients of variation and means per Grazing Date with their respective standard errors for all characters are presented in Table 5.9.

Table 5.9: Grand means, coefficients of variation and means per Grazing Date with their respective standard errors for all characters

Characters	Grand Mean	Coefficient of variation	Grazing Dates 28/11	19/12	25/01	S. errors
HGT1	21.6 (0.15)[1]	48.8	16.1c[2]	17.0b	31.9a	0.17
SPR1	59.9 (0.30)	24.9	53.0c	58.0b	68.7a	0.30
LFN1	67.4 (0.25)	17.1	68.0	70.5	63.7	0.35
VOL	3.0 (0.02)	22.6	3.1	2.9	3.0	0.02
LSZ	4.1 (0.03)	32.5	4.0	4.3	4.1	0.03
HBT	3.5 (0.03)	37.7	3.0b	3.8a	3.7a	0.03
FLW	1.9 (0.08)	187.7	0.1b	0.2b	5.7a	0.08
HGT2	20.1 (0.21)	49.7	15.2c	17.3b	27.8a	0.20
SPR2	55.6 (0.32)	30.8	44.0c	54.8b	68.0a	0.33
LFN2	48.0 (0.42)	43.8	48.4	51.6	43.9	0.43
DHGT	1.5 (0.14)	408.4	0.8b	-0.3c	4.1a	0.20
DSPR	4.3 (0.20)	212.5	8.9a	3.2b	0.8c	0.29
DLFN	19.4 (0.50)	119.5	19.7	18.9	19.8	0.56

1 Standard errors of the grand means

2 Values across grazing dates, followed by the same letter do not differ significantly ($P \geq 0.05$).

FLW and the three differences (DHGT, DSPR and DLFN) had high coefficients of variation, while the other characters vary from 17% to 50%. VOL, LSZ, LFN1, LFN2 and DLFN had an F test that was not significant, therefore no mean separation was performed, the means being statistically the same. For HBT, the first grazing is on average more erect than the second and third grazing and for FLW, the third grazing is in a more advanced flowering stage than grazings one and two. All the other characters were significantly different for each grazing, the third grazing being the one performed with bigger plants as indicated by HGT1 and SPR1.

The means and standard errors per Stocking Density for all characters are shown

in Table 5.10.

Table 5.10: Means and standard errors per Stocking Densities for all characters

Characters	Stocking Density				S. errors
	2	3	5	9	
HGT1	21.8	22.1	20.7	22.0	0.72
SPR1	60.6	59.7	58.4	60.8	1.54
LFN1	67.8	67.6	67.4	66.9	0.34
VOL	3.0	3.0	2.9	3.0	0.11
LSZ	4.0	4.2	4.1	4.0	0.06
HBT	3.4	3.5	3.6	3.5	0.07
FLW	2.2	1.7	1.6	2.4	0.20
HGT2	23.1a[1]	21.5a	18.7b	17.2b	0.72
SPR2	59.6a	57.0ab	53.9ab	51.7b	1.81
LFN2	63.8a	57.1b	43.2c	27.8d	1.98
DHGT	-1.3d	0.6c	2.0b	4.8a	0.25
DSPR	1.0c	2.7bc	4.5b	9.0a	0.62
DLFN	4.0d	10.5c	24.2b	39.1a	1.92

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

As mentioned before, all characters measured pre-grazing had no significant F test, therefore means were the same. F tests were significant for the three post-grazing measurements and differences between pre- and post-grazing measurements, therefore mean separation was performed. From the three post-grazing measurements, LFN2 was the one that defined contrasts best for Stocking Density. All Stocking Densities were significantly different from one another for LFN2. The reduction in SPR2 was the same for Stocking Densities 2, 3 and 5 and Stocking Densities 3, 5 and 9. The reduction in HGT2 was the same for Stocking Densities 2 and 3 and for 5 and 9.

The grazing target of allowing animals to graze till LFN2 reached on average 25% for the highest Stocking Density treatment was considered achieved, LFN2 for that treatment being equal to 27.8%.

The state of the plants after the grazing treatments were imposed are shown in Plates 5.1 to 5.4 for the four Stocking Densities.

Plate 5.1: State of spaced plants after the 2 sheep/plot treatment was imposed

Plate 5.2: State of spaced plants after the 3 sheep/plot treatment was imposed

Plate 5.3: State of spaced plants after the 5 sheep/plot treatment was imposed

Plate 5.4: State of spaced plants after the 9 sheep/plot treatment was imposed

F tests were significant for DHGT, DSPR and DLFN and all mean differences were significantly different from each other at each Stocking Density.

Means and standard errors per Time of Day for all characters are presented in Table 5.11.

Table 5.11: Means and standard errors per Time of Day for all characters

Characters	Morning	Evening	S. error
HGT1	21.4	21.9	0.51
SPR1	59.4	60.3	1.09
LFN1	67.3	67.5	0.24
VOL	2.9	3.0	0.08
LSZ	4.1	4.1	0.04
HBT	3.5	3.5	0.05
FLW	1.9	2.1	0.14
HGT2	20.0	20.2	0.51
SPR2	55.9	55.2	1.27
LFN2	45.3	50.7	1.39
DHGT	1.4	1.7	0.18
DSPR	3.5b[1]	5.1a	0.43
DLFN	22.1	16.9	1.36

1 Values within the same row, followed by different letter differ significantly ($P < 0.05$).

F tests were not significant for all characters except DSPR. The difference between pre- and post-grazing spread was larger in the evening than in the morning.

Population means are statistics useful for cultivar evaluation. The means and standard errors per Population for all characters are presented in Table 5.12.

Table 5.12: Means and standard errors per Population for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S.error
HGT1	23.7bc[1]	21.6d	25.5ab	21.9cd	24.4b[1]	26.7a	13.1f	21.7d	16.3e	0.66
SPR1	55.8d	57.4cd	63.9b	57.8cd	59.1c	59.6c	77.5a	58.3cd	49.3e	1.03
LFN1	64.3	68.2	67.5	66.6	65.8	67.3	70.5	66.4	70.1	0.42
VOL	3.0b	3.0b	3.3a	3.0b	3.0b	3.0b	3.0b	2.9b	2.5c	0.05
LSZ	4.2b	4.1b	5.2a	4.1b	5.1a	4.1b	3.3c	4.1b	2.8d	0.10
HBT	3.0d	3.5c	2.9d	3.5c	3.0d	2.6e	5.3a	3.5c	4.1b	0.09
FLW	2.8	1.9	0.8	2.5	2.0	3.0	2.3	2.2	0.4	0.15
HGT2	22.9a	19.9b	22.8a	21.0b	23.8a	23.6a	11.8d	20.2b	14.9c	0.65
SPR2	51.3d	54.0cd	58.7b	54.3cd	54.0cd	55.3c	73.0a	54.4c	44.9e	1.07
LFN2	48.0c	50.9bc	43.5d	50.4bc	43.7d	40.2e	54.8a	49.1bc	51.2b	1.05
DHGT	0.78	1.67	2.68	0.94	0.57	3.02	1.29	1.5	1.44	0.44
DSPR	4.45	3.37	5.19	3.48	5.04	4.34	4.49	3.92	4.42	0.50
DLFN	16.28	17.3	24.0	16.2	22.1	27.1	15.8	17.4	18.9	1.12

1 Values within the same row, followed by the same letter do not differ significantly
($P \geq 0.05$).

LFN1, FLW and the three differences (DHGT, DSPR and DLFN) had F tests not significant so all means were considered statistically the same. For the other characters, mean separation was performed.

The populations offered to the grazing animals were quite varied with HGT1 ranging from 26.7 cm to 13.1 cm, SPR1 from 77.5 cm to 49.3 cm, LSZ from 5.2 (= 6.2 cm) to 2.8 (= 3.8 cm) and HBT from 5.3 (approx. 10° from the main stems to the horizontal) to 2.6 (approx. 60° from the main stems to the horizontal); while VOL had statistically significant differences, but they were minor, ranging from 2.5 to 3.3.

The target to graze every time that the semi-erect plants reached on average 25 cm of HGT1 was achieved, as shown by the results that average grazing HGT1 for that group was 24.3 cm.

Post-grazing, the tallest plants were found in Kenland, E116 and Turkish while

the shortest ones were found in Astred. Astred had the largest diameter, and Turoa the smallest. The most leafy population post-grazing was Astred and the least leafy was E116.

Time by Population interaction was highly significant for all characters so the population means for each Grazing Date are presented in Table 5.13.

Table 5.13: Population means by Grazing Date for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
28/11/94										
HGT1	15.3de[1]	13.6e	20.9b	14.9de	17.2c	25.2a	9.7g	15.7cd	11.8f	0.62
SPR1	45.7d	48.7cd	58.9b	48.6cd	51.7c	61.5b	73.6a	49.7c	38.2e	1.18
LFN1	61.6e	60.4e	75.9b	62.1de	72.0c	82.8a	80.6a	64.4d	52.3f	0.99
VOL	2.8d	3.0c	3.6a	2.9cd	3.0c	3.7a	3.3b	3.0c	2.4e	0.06
LSZ	3.5f	3.7ef	5.2a	3.9de	4.8b	4.2c	4.1cd	4.0cde	2.1g	0.10
HBT	2.9d	3.2c	2.3e	3.3c	2.5e	1.8f	4.5a	3.1cd	3.6b	0.10
FLW	0.0bc	0.0bc	0.0c	0.0bc	0.0bc	0.5a	0.1b	0.1bc	0.0bc	0.03
HGT2	14.6de	13.5e	19.5b	14.4de	17.1c	21.7a	9.9f	15.3d	10.8f	0.56
SPR2	37.0d	41.5c	48.3b	40.7c	42.2c	49.9b	63.4a	41.4c	32.1e	1.17
LFN2	49.9bc	50.5ab	42.6d	52.4ab	45.5cd	36.9e	55.0a	49.6bc	53.0ab	1.72
DHGT	0.8bc	0.1bc	1.4b	0.5bc	0.1bc	3.5a	-0.2c	0.5bc	1.0bc	0.47
DSPR	8.8bcd	7.3de	10.6ab	8.0cde	9.4abcd	11.6a	10.2abc	8.3bcde	6.1e	0.87
DLFN	11.7d	9.9d	33.3b	9.7d	26.5c	45.9a	25.6c	14.9d	-0.8e	2.08
19/12/94										
HGT1	19.7ab	17.2c	20.1ab	18.0bc	19.2bc	21.7a	7.9e	18.0bc	11.8d	0.83
SPR1	55.1c	56.3c	62.0b	57.2c	56.8c	55.3c	77.4a	56.4c	45.4d	1.38
LFN1	68.9de	78.3b	63.3g	73.3c	65.6fg	59.6h	67.3ef	71.8cd	86.4a	1.06
VOL	3.0ab	3.0ab	3.1a	3.1ab	2.8cd	2.6de	3.0abc	2.9bc	2.4e	0.07
LSZ	4.7b	4.2c	5.4a	4.5bc	5.4a	4.1c	3.2d	4.2c	3.1d	0.14
HBT	2.9e	3.7c	3.1e	3.6cd	3.2de	2.9e	6.2a	3.5cd	4.6b	0.14
FLW	0.1b	0.0b	0.0b	0.1b	0.1b	0.8a	0.1b	0.2b	0.0b	0.09
HGT2	21.3a	17.9b	19.1b	18.0b	19.9ab	19.9ab	9.5d	18.9b	11.7c	0.77
SPR2	50.9c	53.1c	57.4b	53.7bc	53.2bc	52.3c	77.1a	53.4bc	42.2d	1.51
LFN2	48.4cd	55.8b	46.4de	51.5c	44.7de	42.9e	60.1a	51.6bc	62.6a	1.51
DHGT	-1.7d	-0.7cd	1.0ab	-0.0bc	-0.7cd	1.8a	-1.5d	-0.9cd	0.1bc	0.51
DSPR	4.2a	3.2a	4.6a	3.5a	3.6a	3.0a	0.3b	3.0a	3.2a	0.80
DLFN	20.5ab	22.5a	16.9b	21.8a	20.9ab	16.7b	7.2c	20.2ab	23.8a	1.69
25/01/95										
HGT1	36.2ab	33.9bcd	35.5abc	32.9cd	36.8a	33.1cd	21.5f	31.7d	25.6e	0.93
SPR1	66.7cd	67.2cd	70.7b	67.6bcd	68.8bc	61.7e	81.4a	69.4bc	64.5de	1.17
LFN1	62.3d	65.9b	63.1cd	64.4c	59.7e	59.2e	63.7cd	63.0cd	71.6a	0.53
VOL	3.2ab	3.1abc	3.3a	3.1bc	3.1abc	2.8de	2.8d	2.9cd	2.6e	0.08
LSZ	4.3b	4.3b	5.0a	3.9b	5.1a	4.1b	2.7d	4.0b	3.3c	0.13
HBT	3.2f	3.6cd	3.4def	3.6cde	3.3ef	3.1f	5.2a	3.9bc	4.2b	0.11
FLW	8.4a	5.6d	2.4e	7.2abc	6.0cd	7.6ab	6.7bcd	6.4bcd	1.1f	0.45
HGT2	32.9ab	28.3cd	29.9bc	30.5bc	34.5a	29.5cd	15.9f	26.6d	22.3e	1.10
SPR2	66.3cd	67.6bcd	70.5b	68.6bc	66.7bcd	63.9de	78.4a	69.1bc	60.6e	1.38
LFN2	45.6a	46.4a	41.5b	47.4a	40.9bc	40.9bc	49.2a	45.9a	37.7c	1.28
DHGT	3.2ab	5.6a	5.7a	2.4b	2.3b	3.8ab	5.6a	5.1a	3.3ab	0.90
DSPR	0.4bcd	-0.4cd	0.4bcd	-1.0d	2.1abc	-1.7d	2.9ab	0.3cd	3.9a	0.92
DLFN	16.7cd	19.6bc	21.8b	17.1cd	18.8bc	18.5bcd	14.5d	17.1cd	33.9a	1.46

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$).

HGT1 and HGT2 had a close behaviour in population ranking so they are described together. E116 was the tallest population for the first and second grazing but fifth tallest for the last grazing, while Kenland was the tallest for this grazing. Astred was the shortest population for the three grazings, followed by Turoa.

SPR1 and SPR2 also had a similar behaviour, Astred always being the population with the largest diameter and Turoa the population with the smallest diameter. The extremes behaved consistently but changes in ranking occurred in the middle of the ranking.

LFN1 and LFN2 did not have a similar behaviour, so their results are discussed separately. For LFN1, Astred and E116 were the most leafy populations before the first grazing (LFN1), while Turoa was for the second and third grazing. For LFN2, Astred and Turoa were the most leafy post-grazing for the first and second grazings, but Astred, Colenso, Turkish, Hamua and F 2419 were for the last grazing.

The highest DHGT was achieved with E116 for grazings one and two, and with Quiñiquelli, Hamua and Astred for the third grazing. The largest DSPR was obtained in E116 for the first grazing, Quiñiquelli for the second grazing and Turoa for the last grazing. For the other extreme, the smallest DSPR was achieved with Turoa, Astred and E116 for grazings one, two and three respectively. DLFN was maximum for E116 for the first grazing but for Turoa for the other two grazings.

Biggest VOL were obtained with E116 and Quiñiquelli, Quiñiquelli and Colenso and Quiñiquelli and Turkish for the first, second and third grazings respectively. Quiñiquelli had the biggest leaves for the first grazing while Kenland and Quiñiquelli had them for the last two grazings. Turoa had the smallest leaves for the first two grazings, but Astred had the smallest leaves for the last grazing. Astred was the most prostrate population for all grazings, while the most erect were E116 for the first grazing and Turkish and E116 for the last two grazings. Plants were just starting to flower during the

first two grazings where E116 was the population with more flowers. The third grazing was with plants in a more advanced reproductive state, Turkish being the population with greatest flower development and Turoa the least.

Time by Stocking Density interaction was significant for 7 out of 13 characters so the Stocking Density means for each Grazing Date are presented in Table 5.14.

Table 5.14: Stocking Density means by Grazing Date for all characters

Character	2	3	5	9	S. error
28/11/94					
HGT1	15.94a[1]	16.47a	16.41a	15.39a	0.36
SPR1	52.27	52.7	53.7	53.13	0.78
LFN1	67.03	68.58	68.89	67.56	1.15
VOL	3.07a	3.06a	3.11a	3.02a	0.04
LSZ	3.73b	4.05ab	4.18a	3.84ab	0.11
HBT	2.94a	3.04a	3.07a	3.07a	0.10
FLW	0.08a	0.08a	0.09a	0.09a	0.01
HGT2	17.02a	15.98a	15.53a	12.32b	0.52
SPR2	47.25	46.01	44.41	38.48	1.29
LFN2	69.97	54.39	44.36	24.75	3.90
DHGT	-1.08c	0.49b	0.88b	3.07a	0.35
DSPR	5.01	6.69	9.29	14.66	1.15
DLFN	-2.94	14.19	24.53	42.81	3.64
19/12/94					
HGT1	17.80a	17.12a	15.96a	17.34a	0.81
SPR1	58.94	58.25	55.34	59.34	2.22
LFN1	71.26	70.30	70.00	70.42	0.89
VOL	3.01a	2.85a	2.70a	2.91a	0.12
LSZ	4.41ab	4.44a	4.11b	4.28ab	0.09
HBT	3.64a	3.94a	3.74a	3.69a	0.12
FLW	0.18a	0.15ab	0.08b	0.21a	0.02
HGT2	20.66a	17.92ab	15.19b	15.58b	1.18
SPR2	60.53	56.96	51.11	50.61	3.02
LFN2	65.98	64.51	45.53	30.28	3.11
DHGT	-2.86c	-0.80b	0.77ab	1.76a	0.48
DSPR	-1.59	1.29	4.23	8.73	1.42
DLFN	5.28	5.79	24.47	40.14	3.21
25/01/95					
HGT1	31.75a	32.74a	29.67a	33.26a	1.32
SPR1	70.33	68.15	66.17	69.79	2.16
LFN1	64.84	63.99	63.13	62.76	0.91
VOL	3.03a	3.01a	2.76a	3.12a	0.20
LSZ	3.99b	4.18a	4.04ab	4.01ab	0.05
HBT	3.76ab	3.53b	3.87a	3.69ab	0.07
FLW	6.26ab	4.77b	4.75b	7.03a	0.59
HGT2	31.58a	30.55a	25.43b	23.64b	0.86
SPR2	71.12	68.11	66.37	66.09	1.98
LFN2	55.22	52.53	39.62	28.35	1.95
DHGT	0.23c	2.19bc	4.38b	9.61a	0.89
DSPR	-0.58	0.04	-0.15	3.70	0.81
DLFN	9.64	11.47	23.60	34.42	2.35

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$).

SPR1, LFN1, SPR2, LFN2, DSPR and DLFN had no significant F tests for the first order interaction of Stocking Density by Grazing Time, therefore means were statistically the same.

HGT1 and VOL were statistically the same at each Grazing Time among Stocking Densities, while LSZ, HBT and FLW had minor differences among Stocking Densities at each Grazing Time.

HGT2 and DHGT had significant reductions at each Stocking Density for each Grazing Time and grazings were done as indicated by HGT1 and HGT2 with taller plants in the second and third grazings.

HGT1, LFN1 and LSZ had no significant F test for the second order interaction of TxUxM (Grazing Time by Stocking Density by Time of Day), therefore means were not separated. For the other characters, the interactions were explored for Stocking Density by Grazing Date and Time of Day (Table 5.15).

Table 5.15: Stocking Density means by grazing Date and Time of Day

Character	Morning					Evening				
	2	3	5	9	S. error	2	3	5	9	S. error
28/11/94										
SPR1	51.89a[1]	51.73a	53.76a	52.58a	0.85	52.64a	53.67a	53.64a	53.69a	1.24
VOL	2.95c	3.09b	3.17a	3.03b	0.01	3.19a	3.02a	3.04a	3.01a	0.08
HBT	3.03a	3.06a	2.99a	3.02a	0.17	2.84a	3.02a	3.14a	3.11a	0.13
FLW	0.06a	0.10a	0.08a	0.06a	0.02	0.11a	0.06a	0.10a	0.12a	0.02
HGT2	16.28a	16.19a	17.02a	14.59a	0.83	17.76a	15.77ab	14.04b	10.06c	0.67
SPR2	46.38a	45.42a	45.66a	42.13a	2.20	48.13a	46.60a	43.16a	34.82b	1.36
LFN2	64.00a	40.89a	44.39a	37.00a	6.67	75.94a	67.89a	43.33b	12.50c	3.65
DHGT	-0.74a	0.91a	-0.46a	0.70a	0.39	-1.41c	0.07bc	2.22b	5.44a	0.49
DSPR	5.51a	6.31a	8.10a	10.44a	2.28	4.51c	7.07bc	10.49b	18.87a	0.97
DLFN	3.39b	27.28ab	23.67ab	30.06a	5.35	-9.28c	1.11c	25.39b	55.56a	4.27
19/12/94										
SPR1	56.34a	57.64a	55.75a	58.66a	1.86	61.53a	58.85a	54.93a	60.02a	4.31
VOL	2.79a	2.88a	2.64a	2.87a	0.13	3.23a	2.82a	2.75a	2.94a	0.22
HBT	3.79a	4.09a	3.77a	3.69a	0.22	3.49a	3.79a	3.72a	3.70a	0.14
FLW	0.12a	0.19a	0.12a	0.14a	0.03	0.24a	0.11ab	0.03b	0.27a	0.04
HGT2	18.88a	18.17a	14.36a	15.09a	1.20	22.44a	17.67a	16.03a	16.08a	2.07
SPR2	59.12a	58.69a	50.59a	49.53a	2.15	61.93a	55.23a	51.33a	51.69a	6.04
LFN2	60.07a	64.33a	35.61b	22.61b	3.01	71.89a	64.68a	55.44ab	37.94b	3.94
DHGT	-2.03c	-0.66bc	1.74a	1.41ab	0.49	-3.70b	-0.94ab	-0.21ab	2.10a	0.86
DSPR	-2.77c	-1.04c	4.87b	9.13a	0.66	-0.40a	3.62a	3.60a	8.33a	3.01
DLFN	8.96c	5.67c	34.44b	49.39a	2.71	1.61b	5.91b	14.50ab	30.89a	5.10
25/01/95										
SPR1	71.44a	69.22a	65.22a	68.76a	2.75	69.22a	67.07a	67.12a	70.83a	3.74
VOL	2.96a	3.07a	2.71a	3.02a	0.25	3.11a	2.94a	2.80a	3.23a	0.34
HBT	3.90a	3.37c	3.84b	3.68a	0.03	3.61a	3.69a	3.90a	3.70a	0.11
FLW	6.46a	4.94a	3.88a	6.17a	0.76	6.06a	4.60a	5.63a	7.90a	0.96
HGT2	30.11a	30.77a	24.45ab	23.68b	1.51	33.06a	30.34ab	26.41bc	23.60c	0.90
SPR2	73.55a	69.11a	65.75a	65.09a	2.44	68.69a	67.11a	66.99a	67.10a	3.49
LFN2	55.56a	52.28a	39.82b	26.61c	2.41	54.89a	52.78a	39.42ab	30.08b	3.44
DHGT	0.08c	3.19b	4.30b	8.79a	0.57	0.38b	1.20b	4.45ab	10.44a	1.63
DSPR	-1.69a	0.11a	-0.53a	3.67a	1.42	0.53b	-0.03b	0.22b	3.73a	0.53
DLFN	9.39c	11.11c	23.85b	36.94a	2.42	9.89b	11.82ab	23.35ab	31.89a	4.45

1 Values within the same row and box, followed by the same letter do not differ significantly ($P \geq 0.05$).

SPR1, VOL, HBT and FLW were statistically the same for Stocking Densities by Grazing Date and Time of Day, but grazing was done with bigger plants for the second and third grazings. Post-grazing measurements decrease when Stocking Densities increase, while differences between pre- and post-grazing measurements increase when Stocking Density increase by Grazing Date and Time of Day.

5.3.3.3 HERITABILITIES

To consider the usefulness of the measured characters for selection purposes, heritabilities should be known. If all variation was due to environment, no genetic progress could be achieved, but if a large enough portion was due to genetics, reasonable genetic advance could be obtained. (This issue will be taken up more strongly in the spaced plant-animal interaction experiment in Chapter 6).

The heritabilities of the different characters are presented in Table 5.16.

Table 5.16: Heritability values for all characters.

Character	Genetic fractions (g)	Population Restricted[1]	Plant Restricted[2]	Overall Restricted[3]
HGT1	0.55	0.30**(0.10)	0.14**(0.02)	0.44**(0.08)
SPR1	0.27	0.29**(0.10)	0.07**(0.01)	0.36**(0.09)
LFN1	0.00	0.00^{NS}(0.05)	0.00^{NS}(0.00)	0.00^{NS}(0.05)
VOL	0.31[4]	0.07^{NS}(0.05)	0.09**(0.01)	0.16**(0.05)
LSZ	0.35	0.29**(0.11)	0.09**(0.02)	0.38**(0.09)
HBT	0.66	0.36**(0.11)	0.20**(0.04)	0.56**(0.08)
FLW	0.00	0.02^{NS}(0.06)	0.00^{NS}(0.00)	0.02^{NS}(0.06)
HGT2	0.50	0.25**(0.09)	0.12**(0.02)	0.36**(0.08)
SPR2	0.34	0.28**(0.10)	0.09**(0.01)	0.37**(0.09)
LFN2	0.00	0.07^{NS}(0.05)	0.00^{NS}(0.00)	0.07^{NS}(0.05)
DHGT	0.00	0.01^{NS}(0.01)	0.00^{NS}(0.00)	0.01^{NS}(0.01)
DSPR	0.00	0.00^{NS}(0.01)	0.00^{NS}(0.00)	0.00^{NS}(0.01)
DLFN	0.00	0.00^{NS}(0.04)	0.00^{NS}(0.00)	0.00^{NS}(0.04)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1...3 Equations (5.4...5.6) in Section 5.2.6
4 Volume was not measured in the spaced plant-animal interaction experiment so an average of all genetic fractions of all characters measured pre-grazing was used. This average was considered the best guess, considering the other alternatives of being zero (no genetic variation) or one (all genetic variation).

Genetic fractions for the following characters: LFN1, FLW, LFN2, DHGT, DSPR and DLFN were zero, meaning there was no additional genetic variation arising from plant segregation.

HBT and HGT1 were characters with medium to high values of heritability while SPR1, HGT2, SPR2 and LSZ had medium values and VOL had low values of

heritability. LFN1, LFN2, the three differences and FLW had heritabilities not significantly different from zero. Plant heritabilities were always smaller than population heritabilities and all of them were medium to low values. Except for VOL, the population variance component was always larger than the plant variance component.

According to the results of this experiment, genetical progress could only be obtained by selecting in height and spread pre- or post-grazing, VOL, LSZ and HBT.

5.3.3.4 SAMPLING INTENSITY ANALYSIS

The analysis of sampling intensity was one of the criteria suggested (Section 5.2.7) to detect the best grazing method to be used in subsequent experiments for grazing discrimination in a plant breeding nursery. If any of the post-grazing characters was reduced in comparison to their respective pre-grazing character, the plant was considered grazed, therefore sampled. In Table 5.17, the sampling rates per Stocking Density and Time of Day are presented.

Table 5.17: Sampling intensity by Stocking Density and Time of Day

	Stocking Density						
Time of Day	2	3	5	9	Total	%	χ^2
Morning	**200**	**240**	**255**	**260**	955	88.4	0.97
	(4.2)[1]	**(0.3)**	**(2.4)**	**(3.6)**			
Evening	**174**	**202**	**250**	**269**	895	82.9	0.97
	(14.2)	**(3.7)**	**(1.5)**	**(6.2)**			
Total	374	442	505	529	1850	100	1.94
%	69.3	81.9	93.5	98.0	100		
χ^2	16.93	0.91	3.91	9.56	31.31		

1 contribution of each cell to the final χ^2 for the interaction

The interaction of Stocking Density by Time of Day was significant with a χ^2 of 36.09. Marginal probabilities of this two-way contingency table were analysed as if they were a one-way table with 2 cells for Time of Day and four cells for Stocking Density (Section 5.2.7). The Time of Day effect was non significant with a χ^2 of 1.94, while the Stocking Density effect was significant with a χ^2 of 31.31, meaning that sampling intensity was the same either in the morning or evening, but was significantly different according to Stocking Density.

5.4 DISCUSSION

5.4.1 SELECTION OF BEST GRAZING METHOD

The objective of this experiment was to determine the optimum **Stocking Density** and **Time of Day** that enables the best discrimination among plants for preference. The decision was based on F tests, means and sampling intensity analysis.

The post-grazing measurements and the differences pre- and post-grazing were all significant for Stocking Density. For Time of Day, only DSPR was significant. Considering the significant results of the analysis of variance (F tests) and that the sampling intensities of the two Times of Day were not significantly different, it was concluded that Stocking Density was the most relevant grazing management to decide which is the best grazing method and that either moming or evening grazings could be used for grazing purposes.

The sampling intensity for the four Stocking Densities were significantly different. Stocking Densities of 5 and 9 sheep/plot had 94% and 98% of the plants sampled respectively (Table 5.17). Both sampling intensities are high. While the 98% is better, it has a high risk of losing all variation due to overgrazing. The 9 sheep/plot Stocking Density (98%) treatment reduces LFN2 from 66.9% to 27.8% in 60 minutes.

Assuming that grazing animals eat at a constant rate during one and a half hours, if the grazing is extended for seven, nineteen or twenty seven minutes after one hour, LFN2 would be reduced to 20%, 15% or 10% respectively. For a general recommendation for practical breeding purposes, an accidental delay in taking the grazing animals out of the spaced plant breeding nursery of only a few minutes could ruin the selective measurements for that grazing time. This is considered too risky, and a sampling intensity of 94% (5 sheep/plot (18 m^2)) equivalent to allow animals to graze until LFN2 was on average approximately 40% (see Table 5.10) is recommended for future use for grazing breeding nurseries with sheep.

5.4.2 COMPARISON OF HERITABILITIES BEFORE THE FIRST GRAZING WITH THE MAIN ANALYSIS INCLUDING ANIMAL EFFECTS

The inclusion of the grazing animal might have several advantages and disadvantages as detailed earlier in the review of literature (2.3.1). One of the disadvantages studied in this experiment is the reduction in heritability values due to the inclusion of a new source of variation (grazing animals) in the plant evaluation process. The reduction in heritability will directly affect the genetic advance achievable in all of its several definitions (Chapter 2, Section 2.2.4), like for example $\Delta G = i\, h^2\, \sigma_P$ where ΔG = genetic advance, i = intensity of selection, h^2 = heritability and σ_P = phenotypic standard deviation. The overall restricted heritability values before the first grazing compared with the same characters for main analysis are presented in Figure 5.1.

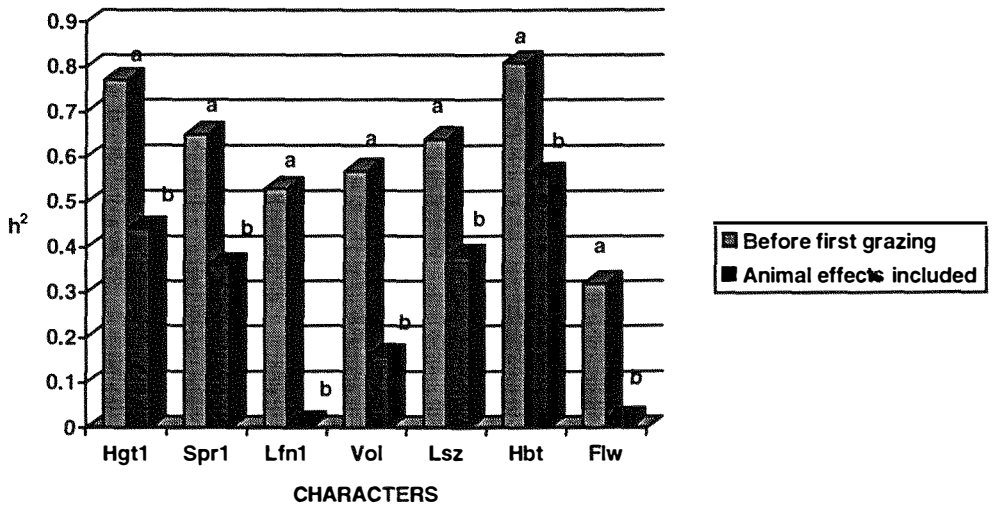

Figure 5.1: Overall restricted heritabilities of all characters before the first grazing and main analysis including the animal effects (bars of the same character followed by different letter differ significantly ($P < 0.05$)).

The effect of the grazing animal has been to introduce new non-genetic (non-plant) variation into the measurements, i.e. increasing "noise" and thereby reducing the heritability values. All the reductions were significant ($P < 0.05$). The overall average reduction in heritability was 0.34 with a range of 0.25 to 0.53. This is a severe price to pay in a forage breeding programme for the inclusion of the grazing animal, but perhaps, (and that "perhaps" has to be judged for each species and breeding programme objective) it is the only way to make genetic advance in the right direction.

CHAPTER SIX
Spaced Plant-Animal Interaction Experiments

6.1 INTRODUCTION

Forage breeding nurseries are rarely grazed and when grazing is done, it is usually as a defoliation tool and no systematic procedure is followed. On the other hand, all grazing management experiments where systematic procedures are followed, are done on a larger scale and on swards. There is little information on the extrapolation of that information to small areas and to spaced plants. The objectives of these experiments reported here were: (i) to test a grazing method to determine animal preference in a way suitable for genetical experiments and (ii) to estimate heritabilities for characters that might be selected to enhance animal preference. If the first objective is achieved, a detailed grazing management will be available for forage breeding nurseries; if the second objective is achieved, useful characters to quantify animal effects will be available.

6.2 MATERIALS AND METHODS

A spaced plant-animal interaction experiment was conducted in New Zealand during 1994/5 and also in Uruguay during 1995/96, with the cloned material from the selected 108 genets (9 population x 12 plants) per Site from the preliminary glasshouse experiments.

6.2.1 EXPERIMENTAL DETAILS

The management and experimental details are summarised in Table 6.1.

Table 6.1: Management and experimental details

Management	New Zealand	Uruguay
Site	Massey University (40°23'S,175°37'E)	La Estanzuela (34°20'S, 57°41'W)
Soil type	Manawatu fine sandy loam[1]	Planosoles[2]
Planting method	Spaced plants (0.75 m grid)	Spaced plants (0.75 m grid)
Planting date	8 Oct. 1994	22 Aug. 1995
Treatments	9 Red clover populations (Chapter 3, Section 3.3.3)	9 Red clover populations (Chapter 3, Section 3.3.3)
N° of blocks	3	3
Weed control	Triflur 40[3] @ 1.2 kg a.i./ha before sowing, Basagran 480[4] @ 2 l/ha after sowing and by hand pulling	Round up[5] @ 3.5 l/ha before sowing, Basagran 480[4] @ 2 l/ha after sowing and by hand pulling
Insect control	None	Karate 50[6] @ 0.1 l/ha (29 Aug.)
Fertilisation	None	60 kg P_2O_5/ha before sowing
Sampling for nutritional data	12 Mar. 1995	10 Jan. 1996
Sheep breed and class	Perendale ewes	Polworth ewes
Grazing dates	19 Jan. 1995 13 Feb. 1995 15 Mar. 1995 19 Apr. 1995	13 Nov. 1995 13 Dec. 1995 18 Jan. 1996 21 Feb. 1996

1 Cowie, 1972
2 Víctora, 1985
3 Triflur 40 = trifluralin
4 Basagran 480 = Bentazone in the form of soluble concentrate
5 Round up = glyfosate
6 Karate 50 = lamda cialotrina

6.2.2 FIELD DESIGN

The field design used was the same in New Zealand and Uruguay, involving three blocks each of 324 plants (9 populations x 12 genets x 3 ramets) and an area of 14.75 x 14.75 m per block because of the presence of 1 m between the plants and the electric fence. The 324 plants were completely randomised in each block, and each block had a different random layout. Randomisation was not only for the routine reasons of gate location, fence proximity, people presence, etc., but also to provide unbiased free-choice cafeteria conditions for the animals. The effect of plant location with reference to the electric fence was considered by setting up a concomitant dummy for covariance error adjustment (similar to the grazing management experiment (Section 5.2.2)). Even though this effect was not significant for the grazing management experiment, it was tested again in these experiments.

6.2.3 CHARACTERS RECORDED

The same characters were recorded in New Zealand and Uruguay. Spread (SPR1, SPR2), Height (HGT1, HGT2), Leafiness (LFN1, LFN2) where "1" means pre-grazing and "2" means post-grazing, differences pre- and post-grazing (DHGT, DSPR, DLFN), Habit (HBT), Leaf size (LSZ), and Flowering (FLW), were measured in the same way as in the preliminary grazing experiment (Chapter 5, Section 5.2.3). Three additional characters also were examined, namely plant density (DST), and two nutritional characters: crude protein (PRT) and digestibility (DGT). PRT and DGT were recorded only once at each site, and were selected because of the results obtained in a Delphi survey done by Wheeler and Corbett (1989) (Section 2.3.5.2). The analyses were done with NIRS (near infrared reflectance spectroscopy) methodology (Shenk and Westerhaus, 1994).

Plant density (DST) Visual ordinal score of number of stems in each plant from 1 to 5 (5 being the most dense). Equivalent to the score used by Williams (1927) of very lax, lax, intermediate, dense and very dense. Halves were employed for borderline cases.

Material for the chemical analyses was provided by taking randomly five to ten stems with their attached leaves per plant. Material was cut near the crown and the samples (stems and leaves) were dried at 60°C for 48 hours in an oven, and ground through a 1 mm sieve in a cyclone grinder. Because of the amount of material and the cost of the analysis, the three clones of each plant in each block were pooled together for analysis and only one set of samples was taken from each country. A total of 324 samples (9 populations x 12 genets (plants) x 3 blocks) were analysed.

Another additional combined character of the three differences (centred and standardised) was intake (INTK), which was calculated as follows:

$$\begin{aligned}\text{Intake} = &[(\text{DHGT} + (\text{Mean}_{\text{HGT2}} - \text{Mean}_{\text{HGT1}}))/\text{s.e. of difference}] + \\ &[(\text{DSPR} + (\text{Mean}_{\text{SPR2}} - \text{Mean}_{\text{SPR1}}))/\text{s.e. of difference}] + \\ &[(\text{DLFN} + (\text{Mean}_{\text{LFN2}} - \text{Mean}_{\text{LFN1}}))/\text{s.e. of difference}] \end{aligned} \quad (6.1)$$

where standard error of the difference = $\sqrt{\left(\sigma^2_{Hgt1} + \sigma^2_{Hgt2} - 2\,\text{cov}_{Hgt1,Hgt2}\right)}$ (6.2)

for HGT, and similarly for the other two characters.

No variables were transformed because all of them were found to be Normally distributed, except FLW which was non-normal in only 36% of cases. Normality was studied in 2160 tests (Combinations of Site, Time, Block and Populations for 10 characters presented in Appendix 4). The transformations tried (arcsin $\sqrt{\text{variable}}$ for percentages (Steel and Torrie, 1980) and probit (Bartlett, 1947) for scores, did not improve normality. The normality test used was the Shapiro-Wilk test (SAS Institute, 1988; Stephens, 1974). For the cases

where the distribution was not normal, it was not considered a serious error, as the true probability of the 5% F tests and 5% t tests was expected to lie approximately between 4% and 7% (Cochran, 1947).

The homogeneity of the error variances for each character in each site was studied to consider pooling both experiments in the analysis. DST, SPR1, HGT2, SPR2 and LFN2 were not homogeneous at this overall level. The test used was a modification of the Neyman-Pearson likelihood ratio test by Bartlett (Steel and Torrie, 1980). An assumption of this test is that the distribution is normal. If this is not the case for the overall level, the test might be detecting a non-normal distribution rather than heterogeneity of variance (Steel and Torrie, 1980). For the characters that showed heterogeneity of variance, the test was done again population by population to compare the error variance for each site. At this level, all variances were homogeneous for all characters.

Sampling intensity was determined using DSPR, DHGT and DLFN to detect whether plants had been grazed. If any plant had a positive value in any of them, that plant was considered grazed. The number of plants sampled at each Grazing Time, Block, Population and Site was considered another variable called Sampling Intensity.

Number of plants recorded at each Grazing Time, Block, Population and Site were considered another variable called persistence.

The last two variables (sampling intensity and persistence) were transformed (arcsin $\sqrt{\text{variable}}$) to improve normality because they were developed from binomial proportions (Steel and Torrie, 1980; Snedecor and Cochran, 1980).

6.2.4 SELECTION OF GRAZING DATE AND NUMBER OF SHEEP.

The semi-erect populations were used as a reference to decide the date for grazing. When those populations reached on average 25 cm of HGT1, the whole

experiment was grazed.

The required number of animals was calculated considering the visual estimation of herbage offered (destructive sampling is not an option in a spaced plant breeding nursery) and the herbage intake expected for sheep during one hour of grazing at that time.

The estimated herbage mass to be removed was approximately 1000 kg DM/ha, and as each plot had an effective area of 162.56 m^2 (12.75 x 12.75 m) the DM available was 16.3 kg DM. From the grazing management experiment (Chapter 5), it was decided to graze with a Stocking Density equivalent to 5 sheep/18 m^2 per hour. For approximately two hours and an area of 162.56 m^2, the Stocking Density used was 18 sheep.

Calculations were made for the first grazing only and this Stocking Density was kept for the following three grazings. Sheep were allowed to graze until LFN2 was on average approximately 40%. This criterion for taking animals out of the grazing areas was the one that gave consistency among grazings and sites, because the duration of grazing could not be used in a fixed way in such a short grazing of approximately two hours.

Sheep were introduced subsequently to defoliate to a uniform level in all the plots after the post-grazing measurements were taken with a high Stocking Density of 50 sheep/block until a target of 20% leaf remaining was achieved.

The selective grazing with 18 sheep, the state of the spaced plants after the selective grazing, a general view of the cleaning-off grazing and state of plants after the cleaning-off grazing are shown in Plates 6.1 to 6.4 respectively.

Plate 6.1: Selective grazing with 18 sheep

Plate 6.2: State of spaced plants after selective grazing

Plate 6.3: General view of cleaning-off grazing

Plate 6.4: State of spaced plants after the cleaning-off grazing

6.2.5 STATISTICAL MODELS

Four statistical models were used to analyse the spaced plant nursery. One was for the analysis of results per site (6.2.5.1), another was to analyse the persistence and sampling intensity results (6.2.5.2), another was to analyse the results obtained before the first grazing and for the nutritional characters (6.2.5.3), and the fourth was for an analysis pooled across sites (6.2.5.4).

The last layer of all models (ramets or clones) permitted separation of genetics from non-genetics in the plants (genets) effect, which was important in these experiments. However, the main-frame computers at both Massey University and INIA had insufficient memory to handle the full model. Subsequently, a programme was written by Dr. I.L. Gordon which obtained the clone-sums-of-squares just prior to meaning of clones within plants (genets) and producing a new data-file from these plant means. The pooled clones variance was thereby obtained, and the reduced model was now within the capabilities of the main-frame. The models discussed next are those of this reduced rank.

6.2.5.1 MODEL FOR ANALYSIS OF RESULTS PER SITE

The experimental design was a diffuse randomised complete block with plants nested inside populations, and with a split-plot in time. The diffuse description was used because each experimental unit (Population x Block) was not in the same contiguous physical area, its plants being randomised throughout the whole block. Not having all clones of the same plant and all plants of the same population next to each other as is the usual way, was not considered a major problem because the area was very small (14.75 x 14.75 m) per block on the same soil with the same management history, reducing field heterogeneity. Randomisation of the plants gives several advantages as stated in Section 6.2.2. However, the experimental unit is still defined as an external replicate (Block) of a treatment (Population), in the usual way.

The model used to analyse the experiment was:

$$X_{ikln} = \mu + B_i + P_k + \delta_{ki} + S_{(ik)l} + T_n + TP_{kn} + \varepsilon_{ikln} \quad (6.3)$$

where X_{ikln} = the phenotypic value of the plant corresponding to the n^{th} Time ($n = 1...t$; $t = 4$) of the l^{th} Plant ($l = 1...s$; $s = 12$) of the k^{th} Population ($k = 1...p$; $p = 9$) evaluated in the i^{th} Block ($i = 1...b$; $b = 3$) μ = the grand mean, B_i = the effect of the i^{th} Block, P_k = the effect of the k^{th} Population, δ_{ki} = random error associated with the main plots, $S_{(ik)l}$ = the effect of the l^{th} Plant nested within the k^{th} Population in the i^{th} Block, T_n = the effect of the n^{th} Time, TP_{kn} = the effect of the kn^{th} interaction, and ε_{ikln} = random error $ikln^{th}$.

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971). The expectations of mean squares are presented in Table 6.2.

Table 6.2: Random inference of the expectations of mean squares

S.O.V.	D.F.	M.S.	Expectation of M.S.	F.Test
Blocks	(b-1)	7	$\sigma^2_\varepsilon + t\sigma^2_S + st\sigma^2_\delta + tps\sigma^2_B$	7/5
Population	(p-1)	6	$\sigma^2_\varepsilon + bs\sigma^2_{TP} + t\sigma^2_S + st\sigma^2_\delta + tbs\sigma^2_P$	(6+1)/(5+2)
Error a	(b-1)(p-1)	5	$\sigma^2_\varepsilon + t\sigma^2_S + st\sigma^2_\delta$	5/4
Plant	pb(s-1)	4	$\sigma^2_\varepsilon + t\sigma^2_S$	4/1
Time	(t-1)	3	$\sigma^2_\varepsilon + bs\sigma^2_{TP} + bsp\sigma^2_T$	3/2
TxP	(t-1)(p-1)	2	$\sigma^2_\varepsilon + bs\sigma^2_{TP}$	2/1
Error b	p(t-1)[(b-1) + b(s-1)]	1	σ^2_ε	

In the table, σ^2_ε is the variance arising from ε_{ikln}, σ^2_T from T_n, σ^2_S from $S_{(ik)l}$, σ^2_P from P_k, σ^2_B from B_i and the various interaction variances from the respective interaction effects.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS      Blocks Population Plant Time;
MODEL...=  Blocks | Population Plant(Population Block) Time
           TimexPopulation / SS2;
```

All the F tests were recalculated from the SAS output, because the RANDOM command followed by the option TEST was impossible to use because of limited computing resources (even on the mainframe of Massey University). Also, another programme called THWAITE (Gordon, unpublished) was considered better to estimate complex F tests later. This programme implements the Crump (1946, 1951) and Satterthwaite (1946) complex F tests and degrees of freedom. This programme also estimated the variance components and their standard errors.

6.2.5.2 MODEL FOR ANALYSIS OF PERSISTENCE AND SAMPLING INTENSITY PER SITE

The experimental design was a randomised complete block, with populations at the main plot level, and with a split-plot in time.

The model used to analyse the experiment was:

$$X_{ikn} = \mu + B_i + P_k + \delta_{ki} + T_n + TP_{kn} + \varepsilon_{ikn} \qquad (6.4)$$

where X_{ikn} = the phenotypic value of the Population corresponding to the n^{th} Time (n = 1...t; t = 4) of the k^{th} Population (k = 1...p; p = 9) evaluated in the i^{th} Block (i = 1...b; b = 3) μ = the grand mean, B_i = the effect of the i^{th} Block, P_k = the effect of the k^{th} Population, δ_{ki} = random error associated with the main plots, T_n = the effect of the n^{th} Time, TP_{kn} = the effect of the kn^{th} interaction, and ε_{ikn} = random error ikn^{th}.

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971). The expectations of mean squares are presented in Table 6.3.

Table 6.3: Random inference of the expectations of mean squares

S.O.V.	D.F.	M.S.	Expectation of M.S.	F.Test
Blocks	(b-1)	6	$\sigma^2_\varepsilon+t\sigma^2_\delta+tp\sigma^2_B$	6/4
Population	(p-1)	5	$\sigma^2_\varepsilon+b\sigma^2_{TP}+t\sigma^2_\delta+tb\sigma^2_P$	(5+1)/(4+2)
Error a	(b-1)(p-1)	4	$\sigma^2_\varepsilon+t\sigma^2_\delta$	4/1
Time	(t-1)	3	$\sigma^2_\varepsilon+b\sigma^2_{TP}+bp\sigma^2_T$	3/2
TxP	(t-1)(p-1)	2	$\sigma^2_\varepsilon+b\sigma^2_{TP}$	2/1
Error b	p(t-1)(b-1)	1	σ^2_ε	

In the table, σ^2_ε is the variance arising from ε_{ikn}, σ^2_T from T_n, σ^2_P from P_k, σ^2_B from B_i and the various interaction variances from the respective interaction effects.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS      Blocks  Population Time;
MODEL...=  Blocks | Population Time  TimexPopulation  /  SS2;
```

All the F tests were recalculated from the SAS output as detailed in Section 6.2.5.1.

6.2.5.3 MODEL FOR ANALYSIS OF RESULTS OBTAINED BEFORE THE FIRST GRAZING AND NUTRITIONAL CHARACTERS

A simpler analysis was done (no split-plot in Time) to characterise the populations before the first grazing and for the third grazing individually, because the latter was the grazing when the nutritional characters were sampled. The experimental design was: an environment (sites) pooling of blocked genotypes, and plants nested

within plots.

The model used was:

$$X_{ijkl} = \mu + E_j + B_{i(j)} + P_k + PE_{jk} + \varepsilon_{ki(j)} + S_{(ijk)l} \quad (6.5)$$

where X_{ijkl} = the phenotypic value of the l^{th} Plant ($l = 1...s$; $s = 12$) of the k^{th} Population ($k = 1...p$; $p = 9$) evaluated in the j^{th} Site ($j = 1...e$; $e = 2$) of the i^{th} Block ($i = 1...b$; $b = 3$), μ = the grand mean, E_j = the effect of the j^{th} Site, $B_{i(j)}$ = the effect of the i^{th} Block nested in the j^{th} Site, P_k = the effect of the k^{th} Population, PE_{jk} = the effect of the jk^{th} interaction, $\varepsilon_{ki(j)}$ = random error associated with the Population level and $S_{(ijk)l}$ = the effect of the l^{th} Plant nested within the k^{th} Population in the i^{th} Block of the j^{th} Site.

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations.

The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971), and are presented in Table 6.4.

Table 6.4: Random inference of the expectations of mean squares

S.O.V.	D.F	M.S.	Expectation of M.S.	F.Test
Site	e-1	6	$\sigma^2_S + s\sigma^2_\varepsilon + bs\sigma^2_{PE} + ps\sigma^2_B + pbs\sigma^2_E$	(6+2)/(5+3)
Block(E)	e(b-1)	5	$\sigma^2_S + s\sigma^2_\varepsilon + ps\sigma^2_B$	5/2
Population	(p-1)	4	$\sigma^2_S + s\sigma^2_\varepsilon + bs\sigma^2_{PE} + bse\sigma^2_P$	4/3
PxE	(p-1)(e-1)	3	$\sigma^2_S + s\sigma^2_\varepsilon + bs\sigma^2_{PE}$	3/2
Error	e(b-1)(p-1)	2	$\sigma^2_S + s\sigma^2_\varepsilon$	2/1
Plant (PBE)	pbe(s-1)	1	σ^2_S	

In the table, σ^2_S from $S_{(ijk)l}$, σ^2_P from P_k, σ^2_ε from ε_j, σ^2_B from $B_{i(j)}$ and the various interaction variances from the respective interaction effects.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS      Site Blocks Population Plant;
MODEL...=  Site | Population Site(Blocks) Site(Blocks Population) / SS2;
```

All the F tests were recalculated from the SAS output as detailed in Section 6.2.5.1.

6.2.5.4 MODEL FOR ANALYSIS OF RESULTS OF EXPERIMENTS POOLED ACROSS SITES

The main analyses were done by pooling the data obtained in New Zealand and Uruguay. The experimental design was the diffuse randomised complete block with plants nested inside Populations, at the whole plot level, with a split-plot in time and pooled across sites. The diffuse name was given for the reasons explained in Section 6.2.5.1.

The model used to analyse the experiment was:

$$X_{ijkln} = \mu + E_j + B_{i(j)} + P_k + PE_{jk} + \delta_{ki(j)} + S_{(ijk)l} + T_n + TP_{kn} + TE_{jn} + TPE_{jkn} + \varepsilon_{ijkln} \quad (6.6)$$

where X_{ijkln} = the phenotypic value of the plant corresponding to the n^{th} Time ($n = 1...t$; $t = 4$) of the l^{th} Plant ($l = 1...s$; $s = 12$) of the k^{th} Population ($k = 1...p$; $p = 9$) evaluated in the i^{th} Block ($i = 1...b$; $b = 3$) of the j^{th} Site ($j = 1...e$; $e = 2$), μ = the grand mean, $B_{i(j)}$ = the effect of the i^{th} Block nested in the j^{th} Site, E_j = the effect of the j^{th} site, P_k = the effect of the k^{th} Population, PE_{jk} = the effect of the jk^{th} interaction, $\delta_{ki(j)}$ = random error associated with the Population level, $S_{(ijk)l}$ = the effect of the l^{th} Plant nested within the k^{th} Population in the i^{th} Block of the j^{th} Site, T_n = the effect of the n^{th} Time, TP_{kn} = the effect of the kn^{th} interaction, TE_{jn} = the effect of the jn^{th} interaction, TPE_{jkn} = the effect of the jkn^{th} interaction and ε_{ijkln} = random error $ijkln^{th}$

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.*, 1962; Searle, 1971). The expectations of mean squares are presented in Table 6.5.

Table 6.5: Random inference of the expectations of mean squares

S.O.V.	D.F	M.S	Expectation of M.S.	F.Test
Site	e-1	11	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}+bsp\sigma^2_{TE}+et\sigma^2_S+st\sigma^2_\delta+tbs\sigma^2_{PE}+tps\sigma^2_B+tpbs\sigma^2_E$	(11+7+2)/(10+8+3)
Block(E)	e(b-1)	10	$\sigma^2_\varepsilon+et\sigma^2_S+st\sigma^2_\delta+tps\sigma^2_B$	10/7
Population	(p-1)	9	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}+bse\sigma^2_{TP}+et\sigma^2_S+st\sigma^2_\delta+tbs\sigma^2_{PE}+tbse\sigma^2_P$	(9+2)/(8+4)
PxE	(p-1)(e-1)	8	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}+et\sigma^2_S+st\sigma^2_\delta+tbs\sigma^2_{PE}$	(8+1)/(7+2)
Error a	e(b-1)(p-1)	7	$\sigma^2_\varepsilon+et\sigma^2_S+st\sigma^2_\delta$	7/6
Plant (PBE)	pbe(s-1)	6	$\sigma^2_\varepsilon+et\sigma^2_S$	6/1
Time	(t-1)	5	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}+bse\sigma^2_{TP}+bsp\sigma^2_{TE}+bsep\sigma^2_T$	(5+2)/(4+3)
TxP	(t-1)(p-1)	4	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}+bse\sigma^2_{TP}$	4/2
TxE	(t-1)(e-1)	3	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}+bsp\sigma^2_{TE}$	3/2
TxPxE	(t-1)(p-1)(e-1)	2	$\sigma^2_\varepsilon+bs\sigma^2_{TPE}$	2/1
Error b	pe(t-1)[(b-1)+b(s-1)]	1	σ^2_ε	

In the table, σ^2_ε is the variance arising from ε_{ijkln}, σ^2_T from T_n, σ^2_S from $S_{(ijk)l}$, σ^2_P from P_k, σ^2_E from E_j, σ^2_B from $B_{i(j)}$ and the various interaction variances from the respective interaction effects.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS      Site Population Blocks Plant Time;
MODEL...=  Blocks(Site) Site | Population | Time
           PopulationxBlocks(Site) Plant(Blocks Site Population) / SS2;
```

All the F tests were recalculated from the SAS output as detailed in Section 6.2.5.1.

6.2.6 HERITABILITY ESTIMATES

Heritabilities were calculated according to the appropriate model in a restricted definition (Allard, 1960; Gordon *et al.* 1972; Gordon, 1979) for the Population, Plant and Overall effect according to the following formulae.

$$h^2 \text{ (Population)} = \sigma^2_P / \sigma^2_{\Sigma \text{ all variances containing population or plant effects}} \quad (6.7)$$

$$h^2 \text{ (Plant)} = (\sigma^2_S \text{ x } (g))/ \sigma^2_{\Sigma \text{ all variances containing population or plant effects}} \quad (6.8)$$

$$h^2 \text{ (Overall)} = (\sigma^2_P + \sigma^2_S \text{ x } (g))/ \sigma^2_{\Sigma \text{ all variances containing population or plant effects}} \quad (6.9)$$

The Plant variance contained confounded effects of genetic segregation and the environment. To estimate the direct influence of genetics in the Plant effect, clones were used. Cloning was made following the procedure detailed in Section 4.2.5. As clones are identical genetically, the variation amongst ramets is considered due to environment. Genetic fractions (**g**) were calculates as follows.

$$g = \frac{\sigma_S^2 - \sigma^2_{clone/\bar{n}}}{\sigma_S^2} \quad (6.10)$$

where $\bar{n}$ = harmonic mean of actual number of ramets.

The standard errors of heritabilities were obtained from Gordon *et al.* (1972) for the first model to analyse the results by Site (Equation 6.3). The standard errors of heritabilities for the results obtained before the first grazing and quality attributes (Equation 6.5) were obtained following Osborne and Paterson (1952) (Appendix 5), and the same methodology was followed to estimate the standard errors of heritabilities for the main pooled model (Equation 6.6) (Appendix 6).

One tail t tests were performed for the heritabilities, dividing them by their respective standard error and using the error degrees of freedom.

6.2.7 ASSESSMENT OF PARSIMONIOUS MULTIPLE REGRESSIONS

Multiple regression analysis was performed to explore the possibility of explaining grazing variables (post-grazing measurements and differences between pre- and post-grazing measurements) by pre-grazing characters. Always the full rank model is the best regression, but in the interest of parsimony, all possible equations of lower ranks were assessed according to the criteria suggested by C.L. Mallows (Draper and Smith, 1981):

$$Cp = RSSp / s^2 - (n - 2p) \qquad (6.11)$$

where RSSp is the residual sum of squares from a model containing p parameters, p is the number of parameters in the model including β_0, and s^2 is the residual mean square from the largest equation postulated containing all the z's.

6.2.8 TEST FOR TIME CORRELATION

The same spaced plants were harvested four times in successive grazings, possibly causing failure of the assumption of independence of the error effects and biasing the expectations of the mean squares, F tests, etc.. For the model detailed in Section 6.2.5.4, the correlation across time was calculated according to the following formulae (developed from Gill, 1986; Gordon, 1994).

$$MS_\delta = \text{Error a} = \sigma^2_\varepsilon + et\sigma^2_S + st\sigma^2_\delta \qquad (6.12)$$

$$MS_\varepsilon = \text{Error b} = \sigma^2_\varepsilon \qquad (6.13)$$

$$\theta_\delta = \text{Time covariance} \qquad (6.14)$$

$$\sigma^2_\varepsilon = \sigma^2 - \text{Time covariance} \qquad (6.15)$$

$$\sigma^2 = \sigma^2_\varepsilon + \sigma^2_\delta \qquad (6.16)$$

$$\text{Corr. Across Time} = \sigma^2_\delta / (\sigma^2_\varepsilon + \sigma^2_\delta) \qquad (6.17)$$

The t value to test the significance of the time correlation was calculated in the following way (Steel and Torrie, 1980).

$$t = \frac{r}{\sqrt{(1-r^2)/(n-2)}} \tag{6.18}$$

where r = correlation across time and n = total number of spaced plants in each grazing period.

6.2.9 METHODS USED TO ESTIMATE CORRELATIONS

Multivariate analyses of variance (MANOVA) on character-sets were performed to obtain the matrices of sums of squares (ss) and sums of cross products (sscp) at the Population- and Plant-partition levels for all morphological and nutritional characters (Section 6.3.5.4); and for all morphological characters pooled across environments (Section 6.3.6.4). This was done to obtain the essential statistics for estimating the genotypic correlations (r_g) amongst characters, as follows.

$$r_g = \frac{\left[sscp_{1,2\,POP} + \left(sscp_{1,2\,PLANT} * \sqrt{g_1 * g_2}\right)\right]}{\sqrt{\left[ss_{1\,POP} + \left(ss_{1\,PLANT} * g_1\right)\right] * \left[ss_{2\,POP} + \left(ss_{2\,PLANT} * g_2\right)\right]}} \tag{6.19}$$

Also, the phenotypic correlations (r_p) were obtained as follows.

$$r_P = \frac{sscp_{1,2}}{\sqrt{ss_1 * ss_2}} \tag{6.20}$$

6.2.10 SELECTION INDEX

The solution to find the selection index is the linear function $I = \sum_i b_i P_i$ which correlates best with the index H. The solving formula to find the b's is **Pb = Ga** where **P** is the matrix of phenotypic variances and covariances, **G** is the matrix of genotypic variances and covariances and **a** is the vector of weights (Lin, 1978). The solution ($\mathbf{b} = \mathbf{P^{-1}Ga}$) of the simultaneous equations is obtained by Gaussian elimination.

Baker (1986) suggested that broad sense estimates are appropriate for dealing with problems concerning selection among inbred genotypes, while narrow sense (only additive variance considered) are more appropriate for problems concerning selection in random mating populations, as in this case. In our particular index, the genetic portion is all the genetic partition of the variance (overall-broad sense) and not the additive portion. So the index presented in Section 6.3.6.5 is only an exploratory index and is not intended to be used further for breeding purposes.

6.3 RESULTS

6.3.1 EVALUATION OF NEED FOR DATA ADJUSTMENT

Before beginning with the analysis of the results, the requirement for data adjustment due to repeated measurements (6.3.1.1) and for plant location with reference to the fence (6.3.1.2) was assessed. Results for these issues are considered next.

6.3.1.1 REPEATED MEASUREMENTS ANALYSIS

The correlation across time (Equations 6.12...6.17) for the full model of the genetical experiment was calculated for each character (Table 6.6) to determine if there was any adjustment necessary for repeated measures effects.

Table 6.6: Correlation across time for the full model of the genetical experiment (n=324)[1]

Characters	Corr. across time[2]
HGT1	-0.109*
SPR1	-0.091 NS
LFN1	-0.032 NS
DST	-0.019 NS
LSZ	-0.077 NS
HBT	-0.152**
FLW	-0.021 NS
HGT2	-0.095 NS
SPR2	-0.099 NS
LFN2	-0.017 NS

1 Total number of spaced plants in each grazing period
2 t value (Section 6.2.8)
NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

Even though HGT1 and HBT correlations were significantly different from zero, it was decided not to correct the data because both correlations were very small (-0.11 and -0.15 for HGT1 and HBT respectively), and therefore could be safely disregarded.

6.3.1.2 PLANT LOCATION ANALYSIS

The significance of the F tests of plant location (concomitant dummy) with post-grazing (HGT2, SPR2 and LFN2) and differences pre- and post-grazing measurements (DHGT, DSPR and DLFN) at a time were studied with Proc GLM of SAS statistical package (SAS Institute, 1988). The location (concomitant dummy) was not significant for any post-grazing or differences pre- and post-grazing concomitant analysis (F tests).

Simple regressions between HGT2, SPR2, LFN2, DHGT, DSPR, DLFN at a time with location were performed, all R^2 being not significant and all six simple regressions with R^2's < 0.01.

Therefore, considering the effect on the error partition (concomitant analysis) and the overall effect on the simple regressions, location was considered as not affecting the subsequent results and was not considered further.

6.3.2 RESULTS BY SITE

Results were analysed by Site to obtain information for each country directly without pooling them.

6.3.2.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was done. Results of the analysis of variance (F tests), variance components with their respective standard errors for all characters are presented in Table 6.7 for the New Zealand study.

Table 6.7: Significance of the analysis of variance (F tests), variance components with their respective standard errors for all characters measured in New Zealand

Characters	Blocks	Populations	Error a	Plants	Time	TimexPop.	Error b
HGT1	0.19**	35.28**	-1.64^{NS}	20.41**	30.05**	5.84**	10.87
	(0.22)	(18.42)	(0.19)	(1.90)	(25.09)	(1.77)	(0.50)
SPR1	1.80**	40.15**	-2.56^{NS}	42.12**	87.80**	13.38**	32.61
	(1.98)	(22.04)	(0.67)	(4.14)	(72.98)	(4.12)	(1.50)
LFN1	9.48**	29.39**	-0.78^{NS}	8.28**	48.41**	4.19**	33.37
	(9.54)	(15.33)	(0.24)	(1.42)	(39.99)	(1.48)	(1.53)
DST	0.00*	0.19**	-0.00^{NS}	0.09**	-0.00^{NS}	0.06**	0.17
	(0.01)	(0.10)	(0.00)	(0.01)	(0.01)	(0.03)	(0.01)
LSZ	0.01*	0.46**	-0.03^{NS}	0.38**	0.10**	0.10**	0.33
	0.01	(0.24)	(0.01)	(0.04)	(0.09)	(0.03)	(0.02)
HBT	0.01**	0.82**	-0.03^{NS}	0.43**	0.01*	0.06**	0.17
	(0.01)	(0.42)	(0.01)	(0.04)	(0.01)	(0.02)	(0.01)
FLW	0.27**	1.13^{NS}	-0.12^{NS}	1.13**	10.65**	6.99**	8.99
	(0.29)	(1.56)	(0.06)	(0.30)	(9.36)	(2.09)	(0.41)
HGT2	0.33**	36.53**	-1.52^{NS}	20.26**	42.77**	6.32**	12.46
	(0.38)	(19.13)	(0.22)	(1.92)	(35.53)	(1.92)	(0.57)
SPR2	1.23**	42.73**	-2.74^{NS}	49.46**	160.49**	17.62**	30.83
	(1.46)	(23.94)	(0.82)	(4.70)	(132.72)	(5.33)	(1.42)
LFN2	7.09**	23.56**	0.15^{NS}	16.60**	102.65**	6.02**	46.38
	(7.37)	(12.96)	(0.91)	(2.37)	(84.48)	(2.11)	(2.14)
DHGT	0.77**	0.76*	-0.11^{NS}	0.55*	1.65**	1.15**	11.07
	(0.79)	(0.56)	(0.06)	(0.30)	(1.48)	(0.42)	(0.51)
DSPR	0.02^{NS}	1.16**	-0.27^{NS}	1.53**	16.13**	0.99**	23.58
	(0.06)	(0.77)	(0.13)	(0.67)	(13.32)	(0.48)	(1.09)
DLFN	1.09*	55.12**	0.08^{NS}	15.25**	40.63**	8.81**	54.96
	(1.37)	(29.09)	(0.90)	(2.46)	(34.11)	(2.99)	(2.53)
INTK	0.08**	0.49**	-0.01^{NS}	0.20**	-0.01^{NS}	0.10**	1.09
	(0.08)	(0.26)	(0.01)	(0.04)	(0.01)	(0.04)	(0.05)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

The Population, Plant and TimexPop effects were significant for all characters. Blocks was significant for all characters except DSPR. Time effect was significant for all except INTK and DST. Error a effect was non significant for all characters.

Results of the analysis of variance (F tests), variance components with their respective standard errors for all characters are presented in Table 6.8 for the Uruguay study.

Table 6.8: Significance of the analysis of variance (F tests), variance components with their respective standard errors for all characters measured in Uruguay

Characters	Blocks	Populations	Error a	Plants	Time	TimexPop.	Error b
HGT1	0.41**	17.84**	-0.44^{NS}	8.75**	2.78**	3.14**	10.24
	(0.46)	(9.40)	(0.20)	(0.94)	(2.59)	(0.99)	(0.51)
SPR1	4.10**	27.88**	-4.20^{NS}	47.16**	4.28*	12.33**	49.73
	(4.18)	(15.64)	(0.49)	(4.93)	(4.78)	(3.96)	(2.49)
LFN1	0.31*	18.42**	-1.34^{NS}	16.54**	95.44**	0.46*	36.14
	(0.40)	(9.40)	(0.33)	(2.15)	(78.06)	(0.42)	(1.81)
DST	0.03**	0.07**	-0.01^{NS}	0.17**	0.20**	0.04**	0.23
	(0.03)	(0.04)	(0.01)	(0.02)	(0.17)	(0.01)	(0.01)
LSZ	0.01**	0.41**	-0.02^{NS}	0.25**	0.32**	0.07**	0.34
	0.01	(0.21)	(0.00)	(0.03)	(0.27)	(0.02)	(0.02)
HBT	0.00*	0.51**	-0.02^{NS}	0.30**	0.03**	0.03**	0.19
	(0.00)	(0.26)	(0.01)	(0.03)	(0.03)	(0.01)	(0.01)
FLW	0.32**	1.27**	-0.26^{NS}	2.03**	14.07**	1.83**	9.85
	(0.33)	(0.89)	(0.05)	(0.39)	(11.68)	(0.61)	(0.49)
HGT2	1.43**	3.36**	-0.14^{NS}	3.14**	2.23**	0.74**	6.04
	(1.45)	(1.82)	(0.09)	(0.39)	(1.91)	(0.26)	(0.30)
SPR2	11.51**	31.60**	-3.28^{NS}	42.63**	6.91**	7.49**	36.70
	(11.62)	(16.92)	(0.51)	(4.28)	(6.42)	(2.46)	(1.84)
LFN2	17.75**	24.55**	-2.14^{NS}	19.55**	18.66**	2.59**	72.99
	(17.86)	(12.77)	(0.44)	(3.23)	(15.66)	(1.34)	(3.66)
DHGT	0.25**	6.52**	-0.21^{NS}	2.97**	-0.10^{NS}	1.56**	9.56
	(0.27)	(3.49)	(0.09)	(0.46)	(0.11)	(0.53)	(0.48)
DSPR	2.13**	3.72**	-0.03^{NS}	0.62*	0.38^{NS}	2.59**	18.82
	(2.18)	(2.26)	(0.15)	(0.50)	(0.61)	(0.90)	(0.94)
DLFN	16.08**	26.08**	-1.31^{NS}	15.35**	88.29**	3.77**	80.27
	(16.27)	(13.79)	(0.63)	(3.08)	(72.63)	(1.74)	(4.03)
INTK	0.33**	1.00**	-0.04^{NS}	0.36**	-0.03^{NS}	0.23**	1.60
	(0.33)	(0.53)	(0.01)	(0.07)	(0.01)	(0.08)	(0.08)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

The results were similar to these from the New Zealand study. Blocks, Population, Plant and TimexPop effects were all significant for all characters, while Time had DHGT, DSPR and INTK as exceptions. Error a effect was non significant for all characters.

6.3.2.2 ANALYSIS OF MEANS

Grand means for each Site for all characters are presented in Section 6.3.6.2. Population effects were significant and highly significant for all characters except FLW, therefore mean separation could be assessed to describe populations in New Zealand. Means and standard errors of all characters per Population measured in New Zealand are presented in Table 6.9.

Table 6.9: Means and standard errors per Population in New Zealand for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
HGT1	30.92a[1]	26.87b	25.04c	27.03b	26.40b	20.55d	14.60f	16.13e	15.01f	0.31
SPR1	46.89c	53.81b	52.84b	54.62b	54.99b	38.66d	61.92a	53.81b	46.65c	0.74
LFN1	55.50f	64.56b	59.60d	62.11c	57.92e	55.37f	63.01c	58.62de	73.17a	0.45
DST	2.11f	2.95bc	2.21ef	2.75c	2.37de	1.66g	3.11b	2.49d	3.84a	0.08
LSZ	3.40b	3.34b	4.13a	3.44b	4.09a	3.13c	2.29d	2.38d	2.39d	0.06
HBT	2.46e	3.25d	3.34d	3.22d	3.31d	3.23d	5.10a	4.93b	4.57c	0.06
FLW	5.84	5.09	2.11	4.80	4.97	6.07	5.69	5.20	1.19	0.23
HGT2	28.31a	24.45b	20.32c	24.04b	23.90b	16.42d	11.68f	14.08e	11.57f	0.38
SPR2	43.23d	50.49b	46.98c	50.49b	49.55b	33.65f	57.27a	48.82bc	40.03e	0.82
LFN2	35.97cd	42.91a	30.89ef	39.86b	33.63de	28.59fg	37.62bc	34.72d	27.78g	0.91
DHGT	2.66cd	2.49cd	4.95a	3.11bc	2.55cd	5.00a	2.92bc	2.12d	3.47b	0.24
DSPR	3.66de	3.38e	6.11ab	4.13de	5.44bc	6.67a	4.67cd	5.20bc	6.61a	0.34
DLFN	19.53f	21.70ef	28.74b	22.25ef	24.29de	27.04bc	25.39cd	23.94de	45.40a	0.91
INTK	-0.63d	-0.57d	0.64b	-0.36cd	-0.14c	0.57b	-0.14c	-0.33cd	1.59a	0.10

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

The tallest population pre-grazing was Turkish and the shortest was Astred. The situation was reversed for SPR1, Astred being the one with greatest diameter and E116, Turkish and Turoa the ones with least. Turoa was the most leafy and dense while E116 was the least leafy and dense. The largest leaves were found in Quiñiquelli and Kenland while the smallest were found in the prostrate populations. HBT indicated as expected that the prostrate populations were the most prostrate ones, but the semi-erect and erect ones were similar. E116 was the population that flowered most and Turoa least. HGT2 and SPR2 followed the same pattern as for their respective pre-grazing measurements. LFN2 did not follow the same pattern as LFN1, Turoa having least leaves post-grazing and Hamua the most. DHGT and DSPR had a similar ranking among populations, E116, Turoa and Quiñiquelli being the ones suffering the largest reductions in height and spread. DLFN was largest in Turoa and lowest in Turkish. INTK was highest in Turoa, followed by E116 and Quiñiquelli.

Means and standard errors of all characters per Grazing Date measured in New Zealand are presented in Table 6.10.

Table 6.10: Means and standard errors per Grazing Date in New Zealand for all characters

Character	19/01/1995	13/02/1995	15/03/1995	19/04/1996	S. error
HGT1	28.41a	25.06b	20.97c	15.53d	0.18
SPR1	61.08a	56.51b	49.22c	39.44d	0.32
LFN1	71.19a	58.07c	55.25d	59.86b	0.32
DST	---	---	2.67	2.55	0.02
LSZ	3.39a	3.43a	3.17b	2.71c	0.03
HBT	3.88a	3.71b	3.71b	3.55c	0.02
FLW	7.08a	7.18a	3.95b	0.00c	0.17
HGT2	27.09a	22.09b	16.48c	11.97d	0.20
SPR2	62.01a	50.40b	42.51c	31.93d	0.31
LFN2	47.25a	38.30b	28.16c	24.89d	0.38
DHGT	1.48c	3.20b	4.67a	3.63b	0.19
DSPR	-0.87c	6.51b	7.02ab	7.74a	0.27
DLFN	23.97c	19.77d	27.13b	35.09a	0.41
INTK	0.02	0.12	0.09	0.04	0.06

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

--- Density was not measured in the first two grazings

The biggest plants were at the first grazing, where spaced plants should be strong to be grazed for the first time, so as not to be pulled out of the soil while grazing. For the second to fourth grazing, there was a reduction in HGT1 and SPR1, but a similar LFN1 was offered to the animals. The first two grazings were at a reproductive stage while the fourth was entirely at a vegetative stage. Density was only measured for the last two grazings and changed little. LSZ declined with grazings three and four, probably due to a general reduction

in size of the plants as indicated by HGT1 and SPR1. HGT2 and SPR2 also followed the same pattern as HGT1 and SPR1, but again LFN2 did not. The three differences were greater in the third and fourth grazings, while INTK was statistically the same for the four grazing dates.

Means and standard errors of all characters for the first order interaction of Population by Grazing Date are presented in Table 6.11a (first two grazings) and Table 6.11b (last two grazings) for New Zealand.

Table 6.11a: Means and standard errors per Population for the first two grazings in New Zealand for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
19/01/95										
HGT1	37.96a[1]	30.68d	33.32c	31.61d	35.60b	28.15e	18.61g	19.01g	20.77f	0.43
SPR1	57.21d	58.41cd	66.29b	60.89c	67.19b	52.70e	74.18a	65.62b	47.24f	0.87
LFN1	70.14d	74.77b	69.65d	72.27c	68.43e	65.23f	69.49de	69.35de	81.39a	0.38
DST	—	—	—	—	—	—	—	—	—	—
LSZ	3.57b	3.69b	4.36a	3.77b	4.12a	3.00d	2.28e	2.42e	3.29c	0.08
HBT	2.71d	3.37c	3.53c	3.45c	3.50c	3.51c	5.38a	5.31a	4.11b	0.13
FLW	8.43c	4.32d	1.53e	5.09d	10.65b	16.20a	8.93bc	7.78c	0.79e	0.57
HGT2	39.25a	31.10cd	29.28d	31.23c	35.74b	24.06e	16.17g	19.30f	17.64fg	0.64
SPR2	59.24c	60.59c	66.90b	62.72c	70.68ab	52.71d	74.37a	66.99b	43.87e	1.39
LFN2	49.68ab	52.18a	44.44d	50.74ab	48.48bc	46.16cd	49.63ab	47.96bc	36.02e	1.16
DHGT	-1.08c	-0.25c	4.22a	0.56c	0.07c	4.37a	2.44b	-0.25c	3.25ab	0.57
DSPR	-2.04cd	-2.19cd	-0.62bc	-1.83bcd	-3.50d	0.43b	-0.11bc	-1.37bcd	3.37a	0.82
DLFN	20.46c	22.59bc	25.21b	21.53c	19.94c	19.40c	19.86c	21.39c	45.37a	1.13
INTK	-0.75e	-0.47de	0.51b	-0.42cde	-0.79e	0.19bc	-0.10cd	-0.47de	2.45a	0.20
13/02/95										
HGT1	34.52a	29.98b	28.72b	30.63b	29.94b	22.42c	15.51e	17.99d	15.84e	0.65
SPR1	50.85d	59.56bc	57.44c	62.07b	58.81bc	40.66e	65.98a	61.38b	51.83d	1.10
LFN1	52.59e	63.84b	57.45cd	59.21bc	54.77cde	51.67e	58.75c	53.65de	70.70a	1.60
DST	—	—	—	—	—	—	—	—	—	—
LSZ	3.56b	3.47b	4.39a	3.66b	4.57a	3.40b	2.25d	2.63c	2.94c	0.11
HBT	2.28d	3.24c	3.19c	3.16c	3.15c	3.32c	5.25a	5.10a	4.69b	0.10
FLW	10.18a	9.17ab	4.58d	8.24b	6.62c	6.48c	8.75b	8.87b	1.71e	0.43
HGT2	32.76a	28.08b	23.74c	27.80b	28.31b	17.54d	12.92e	15.82d	11.84e	0.67
SPR2	47.33ef	55.57b	49.99de	56.22b	51.13cd	34.62g	60.33a	53.60bc	44.76f	1.04
LFN2	38.10c	47.59a	34.91cd	42.18b	35.83c	31.67d	44.54ab	38.20c	31.67d	1.25
DHGT	1.76c	1.97c	5.29ab	3.09bc	1.64c	6.12a	2.59c	2.42c	4.00abc	0.81
DSPR	3.52c	3.98c	8.03ab	5.85bc	7.69ab	8.19ab	5.65bc	8.61a	7.08ab	0.91
DLFN	14.49c	16.25c	22.69b	17.04bc	18.94bc	19.68bc	14.21c	15.60c	39.03a	1.91
INTK	-0.75d	-0.56d	0.81b	-0.18d	-0.05cd	0.64bc	-0.43d	-0.08d	1.65a	0.24

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

Table 6.11b: Means and standard errors per Population for the last two grazings in New Zealand for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
15/03/95										
HGT1	31.41a[1]	26.99b	21.72d	26.53bc	24.73c	17.99e	11.99g	15.19f	12.21g	0.61
SPR1	45.78c	54.13b	47.86c	53.43b	53.43b	31.72d	61.64a	48.94c	46.00c	1.33
LFN1	48.06e	58.24b	53.67d	56.48bc	53.33d	49.54e	55.37cd	53.80d	68.70a	0.89
DST	2.32de	3.06b	2.18e	2.94b	2.57cd	1.55f	2.84bc	2.45de	4.14a	0.11
LSZ	3.64b	3.34bc	4.26a	3.51bc	4.45a	3.18c	2.09de	2.30d	1.76e	0.13
HBT	2.32e	3.10d	3.35c	3.08d	3.29cd	3.06d	5.37a	4.89b	4.96b	0.08
FLW	4.77c	6.85a	2.33de	5.88ab	2.59d	1.57e	5.09bc	4.17c	2.27de	0.33
HGT2	25.00a	22.41b	16.03d	21.49b	19.41c	13.91e	9.40g	11.75f	8.80g	0.51
SPR2	39.36e	49.35b	40.29de	47.82bc	44.37cd	24.26f	55.91a	42.08de	38.57e	1.44
LFN2	32.36bc	39.54a	22.79def	36.99ab	27.27cd	17.50f	28.33cd	26.76cde	21.44ef	1.93
DHGT	6.41a	4.58cd	6.11ab	5.04bc	5.32abc	5.20bc	2.59e	3.44de	3.42e	0.39
DSPR	6.42cd	4.79d	7.80abc	5.61cd	9.06ab	9.62a	5.74cd	6.86bcd	7.43abc	0.79
DLFN	15.70d	18.70d	30.88bc	19.49d	26.06c	32.26b	27.04bc	27.04bc	47.27a	1.89
INTK	-0.35d	-0.76d	0.67ab	-0.50d	0.45bc	0.89ab	-0.51d	-0.18cd	1.17a	0.22
19/04/95										
HGT1	19.79a	19.82a	16.03b	19.37a	15.31bc	13.63cd	12.28de	12.33de	11.22e	0.58
SPR1	33.71e	43.15b	39.27d	42.09bc	40.52cd	29.57f	45.88a	39.30d	41.51bcd	0.87
LFN1	51.20f	61.40c	57.38e	60.46cd	55.14e	55.05e	68.43b	57.69de	71.90a	0.96
DST	1.90e	2.84b	2.24d	2.57c	2.16d	1.78e	3.38a	2.53c	3.54a	0.07
LSZ	2.85cd	2.87c	3.49a	2.82cd	3.20ab	2.92bc	2.53d	2.18e	1.57f	0.11
HBT	2.53d	3.28b	3.28b	3.17bc	3.30b	3.05c	4.41a	4.42a	4.50a	0.06
FLW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
HGT2	16.24a	16.21a	11.89b	15.62a	12.14b	9.75c	8.22d	9.47c	8.01d	0.41
SPR2	26.97f	36.46ab	30.09e	35.19bc	32.00de	22.11g	38.48a	32.58cde	32.93cd	0.93
LFN2	23.75cd	32.32a	20.91de	29.54ab	22.92cd	18.14e	27.96b	25.97bc	21.99d	1.21
DHGT	3.55ab	3.66ab	4.20a	3.74ab	3.17ab	4.29a	4.06ab	2.86b	3.20ab	0.42
DSPR	6.74c	6.94c	9.35a	6.91c	8.52ab	8.62a	7.40bc	6.71c	8.58ab	0.40
DLFN	27.45e	29.26de	36.48c	30.93d	32.22d	37.55bc	40.46b	31.71d	49.91a	1.02
INTK	-0.68d	-0.49cd	0.57b	-0.36cd	-0.16c	0.55b	0.47b	-0.59cd	1.07a	0.16

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

Despite significant TimexPopulation interactions (Table 6.7), all characters being significant and highly significant, the ranking of the populations for all characters except FLW was almost invariable for the four grazings. For example, Turkish was the tallest population in the first (37.9 cm), second (34.52 cm), and third grazings (31.41 cm), and for the last grazing it was second (19.79 cm). Consistently, Astred had the largest diameter, Turoa was the most leafy, Quiñiquelli had the biggest leaves and Astred was the most prostrate. E116 was the earliest to flower in the first grazing, followed by Turkish for the second grazing, and Hamua in the third. HGT2 and SPR2 behaved in a similar pattern to their pre-grazing measurements while LFN2 did not, Hamua being maximum for all grazings. DHGT was larger for Quiñiquelli and E116 for grazings one, two and four and Turkish for the third grazing. DSPR was larger for Turoa and Astred for the first and second grazing while E116 was for the last two grazings. For all grazings, Turoa, followed by Quiñiquelli and E116, were the ones with greater values of INTK.

Population effects were highly significant for all characters in Uruguay, so mean separation was assessed to describe the populations. Means and standard errors of all characters per Population measured in Uruguay are presented in Table 6.12.

Table 6.12: Means and standard errors per Population in Uruguay for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
HGT1	23.63b[1]	20.21d	23.12bc	17.87e	25.21a	21.94c	10.22g	19.46d	15.40f	0.42
SPR1	42.76c	38.68e	42.64c	36.86f	45.34b	44.00bc	56.45a	40.56d	37.27ef	0.51
LFN1	66.63c	69.15ab	60.83e	66.59c	64.87d	55.48f	59.37e	67.60bc	70.35a	0.61
DST	2.93a	2.97a	2.46c	2.68b	2.91a	2.07d	2.98a	2.92a	3.10a	0.07
LSZ	3.89cd	4.00bc	5.12a	3.65e	5.06a	4.06b	3.29g	3.81d	3.45f	0.06
HBT	3.14ef	3.33de	3.05f	3.40cd	3.15ef	3.16ef	5.50a	3.55c	3.80b	0.07
FLW	5.18b	3.67c	1.64d	4.24c	3.98c	6.04a	5.23b	3.66c	1.36d	0.24
HGT2	14.85a	12.78b	12.32b	11.16c	14.60a	13.06b	8.12d	12.37b	10.71c	0.34
SPR2	33.91b	31.34cd	30.44d	30.18d	34.23b	34.98b	50.14a	33.22bc	31.28d	0.70
LFN2	28.85d	30.99bc	20.65f	29.08d	26.17e	19.75f	36.94a	32.08b	29.63d	0.70
DHGT	8.79b	7.47c	11.09a	6.75c	10.62a	8.95b	2.11e	7.18c	4.68d	0.33
DSPR	8.85c	7.38de	12.81a	6.77de	11.16b	9.30c	6.38e	7.57d	6.33e	0.45
DLFN	37.77bc	38.35b	40.73a	37.59bc	38.74ab	35.93c	22.47d	35.66c	41.08a	0.88
INTK	0.35c	-0.06d	1.55a	-0.33de	1.06b	0.36c	-2.24f	-0.27d	-0.59e	0.11

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

The tallest population was Kenland followed by Turkish and the shortest were Astred and Turoa. Astred was the population with the widest diameter and the smallest diameter was found in Turoa. Turoa and Hamua were the most leafy. E116 was the population with fewer stems, followed by Quiñiquelli and Colenso. Quiñiquelli and Kenland had the biggest leaves, and Astred and Turoa the smallest ones. Again, the three prostrate populations were the most prostrate (as expected), but again there was not a clear difference between semi-erect and erect populations. E116 flowered most and Turoa least. HGT2 and SPR2 again followed a similar ranking to HGT1 and SPR1, and again LFN2 did not. Quiñiquelli and Kenland had greatest DHGT, DSPR, DLFN and INTK.

Population variations were similar in the New Zealand and Uruguay studies (Tables 6.9 and 6.12). Turkish was the tallest and Astred the shortest in both studies while Astred was the population with largest diameter and Turoa the smallest diameter for both sites. Turoa was the most leafy population and E116 the least. Quiñiquelli and Kenland had the

biggest leaves while Turoa and Astred had the smallest leaves for both sites. HBT and FLW also behaved consistently across sites. HGT2 and SPR2 had a similar behaviour to their respective pre-grazing measurement in both sites. LFN2 is the first character that behaved differently, Turoa being the population having least leaves post-grazing and Hamua the most for New Zealand and Quiñiquelli and E116 having the least and Astred having the most for Uruguay. The largest DHGT, DSPR, DLFN and INTK were observed in Quiñiquelli, E116 and Turoa for New Zealand and Quiñiquelli and Kenland for Uruguay.

Means and standard errors of all characters per Grazing Date are presented in Table 6.13 for Uruguay.

Table 6.13: Means and standard errors per Grazing Date in Uruguay for all characters

Character	13/11/1995	13/12/1995	18/01/1996	21/02/1996	S. error
HGT1	17.75c[1]	19.71b	22.89a	19.98b	0.21
SPR1	44.40a	44.21a	42.01b	40.94c	0.40
LFN1	73.82a	69.39b	50.09d	61.16c	0.34
DST	3.33a	2.85b	2.29d	2.45c	0.03
LSZ	4.36b	4.62a	3.45d	3.68c	0.03
HBT	3.73a	3.52c	3.28d	3.62b	0.03
FLW	0.00c	1.40b	8.15a	7.84a	0.20
HGT2	10.71c	12.20b	14.75a	11.99b	0.15
SPR2	35.27b	37.20a	31.52d	33.14c	0.35
LFN2	27.31b	33.47a	28.02b	22.53c	0.57
DHGT	7.04c	7.57b	8.24a	8.08a	0.21
DSPR	9.15a	7.18b	9.28a	9.17a	0.29
DLFN	46.54a	36.04c	22.35d	38.86b	0.52
INTK	0.03a	0.05a	0.09a	-0.04a	0.08

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

The third grazing was done with the tallest average; the second and fourth were similar and the smallest plants were found in the first grazing. SPR1 was larger for the first two grazings followed by the third and fourth. The grazing with most leaves was the first one, which occurred at a vegetative stage indicated by the absence of flowers. Grazings three and four were less leafy and had more flowers. HGT2 and SPR2 followed a similar pattern to HGT1 and SPR1. LFN2 was different, grazing three being the one with least leaves for LFN1 and the second most leafy for LFN2. DHGT was greater for grazings three and four, while DSPR was the same for the first, third and fourth grazings. DLFN was greater for the first grazing and the smallest for the third. INTK was the same for all grazing dates.

Means and standard errors of all characters for the first order interaction of Population by Grazing Date are presented in Table 6.14a (first two grazings) and Table 6.14b (last two grazings) for Uruguay.

Table 6.14a: Means and standard errors per Population for the first two grazings in Uruguay for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
13/11/95										
HGT1	17.96d[1]	17.11d	20.93b	16.98d	19.58c	23.00a	11.55f	17.25d	15.34e	0.35
SPR1	40.52e	43.11cde	44.18cd	40.95e	45.92c	52.76a	49.35b	42.18de	40.64e	0.94
LFN1	76.25bcd	78.70a	70.14e	77.29abc	74.72d	64.42f	68.70e	75.67cd	78.45ab	0.74
DST	3.52bc	3.79ab	2.80e	3.51bc	3.41c	2.45f	3.10d	3.52bc	3.87a	0.10
LSZ	3.95cd	3.99cd	5.68a	3.81d	5.17b	4.86b	4.29c	4.11cd	3.39e	0.11
HBT	3.49cd	3.73bc	3.03e	3.63c	3.44cd	3.17de	5.29a	3.75bc	4.04b	0.12
FLW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HGT2	10.95cd	11.57bc	11.87ab	10.14d	11.52bc	12.49a	7.25e	10.26d	10.30d	0.28
SPR2	31.96d	34.54cd	33.82cd	31.94d	36.98bc	40.44ab	42.39a	33.27d	32.06d	1.21
LFN2	26.94cd	32.13ab	22.43e	27.29cd	25.28de	18.08f	34.26a	30.56abc	28.87bcd	1.28
DHGT	7.01cd	5.54e	9.04b	6.84d	8.06bc	10.51a	4.30f	6.99cd	5.05ef	0.41
DSPR	8.56c	8.57c	10.30b	9.01bc	8.94bc	12.32a	6.96d	8.91c	8.76c	0.46
DLFN	49.31a	46.57ab	47.89ab	50.00a	49.44a	46.34ab	34.44c	45.12b	49.70a	1.30
INTK	0.11cde	-0.39f	0.74b	0.18cd	0.41bc	1.28a	-1.69g	-0.11def	-0.27ef	0.14
13/12/95										
HGT1	24.02b	20.32de	22.28bc	17.83f	26.24a	21.94cd	10.46h	19.15ef	14.64g	0.60
SPR1	43.91cd	40.82de	45.53bc	38.04ef	47.87b	46.84bc	63.50a	37.58f	33.39g	1.06
LFN1	72.92ab	74.75a	68.38c	70.27bc	70.74bc	61.02d	63.55d	71.20bc	70.76bc	0.96
DST	3.01abc	3.17a	2.62e	2.77cde	3.05ab	2.16f	3.18a	2.90bcd	2.67de	0.08
LSZ	4.68b	4.65b	5.71a	4.20c	5.85a	4.29c	3.77d	4.50b	3.82d	0.06
HBT	3.03d	3.38bc	3.28bcd	3.39bc	3.20cd	3.39bc	5.18a	3.36bc	3.49b	0.08
FLW	1.30cd	0.30de	0.28de	0.88cde	1.57bc	5.68a	2.62b	0.30de	0.00e	0.39
HGT2	15.06a	12.82c	11.59d	11.30d	13.96b	12.83c	9.26f	12.49c	10.31e	0.29
SPR2	36.35bcd	35.01cd	31.45e	33.33de	36.99bc	39.04b	57.70a	33.73cde	31.10e	1.11
LFN2	34.77b	37.64b	24.21d	35.83b	30.37c	23.33d	43.05a	37.14b	34.34b	1.19
DHGT	8.95b	7.59bc	10.73a	6.61c	12.28a	9.14b	1.23e	6.95c	4.33d	0.52
DSPR	7.56c	6.05d	14.10a	4.80de	10.88b	7.91c	6.03d	4.58e	2.29f	0.47
DLFN	38.15bc	37.18bc	44.44a	34.41c	40.37ab	37.76bc	20.64d	34.67c	36.42bc	1.56
INTK	0.49bc	-0.03cd	2.16a	-0.57e	1.70a	0.54b	-2.30g	-0.53de	-1.19f	0.18

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

Table 6.14b: Means and standard errors per Population for the last two grazings in Uruguay for all characters

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	El 16	Astred	F.2419	Turoa	S. error
18/01/96										
HGT1	28.06a[1]	24.48b	27.63a	19.53d	28.28a	22.29c	9.50e	22.28c	17.89d	0.60
SPR1	42.22b	35.30de	38.59cd	33.81e	40.46bc	36.30de	59.60a	39.94bc	36.29de	1.12
LFN1	51.67abc	55.46a	47.50cd	52.98ab	50.69bc	41.96e	45.63de	52.74ab	53.13ab	1.41
DST	2.38b	2.40b	2.03cd	2.15bc	2.36b	1.74d	2.75a	2.44ab	2.39b	0.11
LSZ	3.22c	3.56b	4.38a	3.23c	4.23a	3.26c	2.38d	3.18c	3.14c	0.09
HBT	2.76d	2.87d	2.71d	3.19c	2.76d	2.89d	5.94a	3.29c	3.59b	0.09
FLW	9.96b	8.15bc	2.38d	9.82b	7.78c	9.32bc	12.01a	8.96bc	4.22d	0.66
HGT2	17.96a	15.46b	14.24bc	13.23cd	18.32a	14.86bc	8.33e	15.25b	12.40d	0.59
SPR2	32.01b	26.47de	25.99e	26.96de	30.12bc	30.48bc	52.27a	30.68bc	29.02cd	0.97
LFN2	29.26b	30.43ab	19.24d	31.61ab	29.07b	20.69cd	34.31a	32.86ab	23.78c	1.39
DHGT	10.10b	9.06bc	13.69a	6.38de	9.96b	7.77cd	1.17f	7.03cde	5.55e	0.64
DSPR	10.21b	8.73bcd	13.13a	7.11d	10.34b	6.91d	7.33cd	9.26bc	8.89bcd	0.67
DLFN	22.41cd	25.73bc	28.55ab	21.73cd	21.62cd	22.05cd	11.32e	19.88d	30.22a	1.47
INTK	0.52b	0.40b	1.99a	-0.57c	0.45b	-0.35c	-2.19d	-0.31c	0.22b	0.17
21/02/96										
HGT1	24.57a	19.28cd	21.67b	17.39d	26.73a	20.06bc	8.95f	19.91bc	14.04e	0.66
SPR1	44.55bc	33.82e	42.12c	33.07e	47.13b	36.70de	53.51a	43.12bc	39.70cd	1.54
LFN1	65.58ab	64.69ab	56.57c	61.82b	63.33ab	50.83d	56.40c	67.44a	67.04a	1.37
DST	2.78a	2.24cd	2.8bc	1.99de	2.81a	1.78e	2.83a	2.63ab	2.80a	0.09
LSZ	3.68b	3.62b	4.65a	3.17cd	4.98a	3.51bc	2.36e	3.13d	2.85d	0.11
HBT	3.30d	3.23d	3.17d	3.34d	3.22d	3.17d	5.75a	3.81c	4.30b	0.10
FLW	9.85ab	7.93bc	4.12d	8.33bc	6.57c	11.31a	8.21bc	7.69c	6.67c	0.65
HGT2	15.47a	11.24b	11.51b	10.20b	14.62a	12.22b	7.59c	12.01b	10.99b	0.59
SPR2	35.46b	27.78d	30.37cd	27.17d	32.84bc	27.27d	48.45a	35.22b	32.59bc	1.33
LFN2	24.04bcd	21.27cd	16.05f	20.34de	19.95def	16.63ef	35.48a	26.54b	25.18bc	1.31
DHGT	9.11b	8.04bc	10.96a	7.19c	12.17a	7.85bc	1.36e	7.90bc	3.04d	0.42
DSPR	9.09bc	6.04bc	13.85a	5.90bc	14.48a	9.41b	5.06c	7.89bc	7.11bc	1.27
DLFN	41.48a	43.43a	41.86a	41.48a	43.52a	34.20b	20.92c	40.90a	41.85a	1.84
INTK	0.30b	-0.18bc	1.29a	-0.47bc	1.69a	-0.35bc	-2.91d	-0.12b	-0.99c	0.26

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

Again, similarly to New Zealand, the first order interaction of TimexPopulation was significant and highly significant (Table 6.8) for all characters, but ranking of populations with reference to the grazing dates were in quite good agreement for all characters except flowering. The first grazing was done with all populations in a vegetative state. E116 had the most flowers in the second and fourth grazings and Astred in the third grazing.

E116 and Kenland were the tallest populations pre- and post-grazing for the first, and second grazings respectively. Turkish was the tallest populations pre- and post-grazing for the last two grazings. The most leafy population for the first, second and third grazings was Hamua, while F.2419 and Turoa were for the last grazing. Astred was the most leafy population post-grazing for all grazings. The most dense population was Turoa for the first grazing, Astred and Hamua for the second grazing, and Astred for the third and fourth grazings. Quiñiquelli and Kenland had the biggest leaves (for all grazings) and Astred was the most prostrate population. The biggest differences pre- and post-grazing and intake were obtained in E116 for the first grazing, Quiñiquelli for the second and third grazings, and Quiñiquelli and Kenland for the last grazing.

Comparing results per Grazing Date between Sites was considered not so important as comparisons based on reproductive state: (a) grazings one and two in New Zealand were compared with grazings three and four in Uruguay where plants were flowering and, (b) grazings three and four in New Zealand were compared with grazings one and two in Uruguay where plants were in a vegetative state (Tables 6.10 and 6.13).

(a) Plants were taller, more spread, more leafy and with bigger leaves, and more prostrate in New Zealand than in Uruguay pre- and post-grazing. Differences in height, spread and leafiness pre- and post-grazing were greater in Uruguay than in New Zealand, and INTK was of a similar magnitude.

(b) Plants were of similar height and spread pre- and post-grazing for New Zealand and Uruguay. Leafiness pre- and post-grazing were larger in Uruguay. Plants were more dense,

with bigger leaves and differences were larger in Uruguay than in New Zealand. Similar HBT and INTK were found in both sites.

6.3.2.3 HERITABILITIES

For breeding purposes, the portion of the variation due to genetics (h^2) in its several definitions (Equations 6.7…6.9) is important to determine if genetic progress could by achieved through selection. Heritability values and their standard errors of all characters for New Zealand are presented in Table 6.15a and for Uruguay in Table 6.15b.

Table 6.15a: Heritabilities and standard errors for all characters measured in New Zealand

Characters	Genetic fraction (g)[1]	Population Restricted[2]	Plant Restricted[3]	Overall Restricted[4]
HGT1	0.55	0.50**(0.13)	0.16** (0.04)	0.66** (0.10)
SPR1	0.27	0.32**(0.13)	0.09** (0.02)	0.41** (0.11)
LFN1	0.00	0.39**(0.13)	0.00^{NS} (0.00)	0.39**(0.13)
DST	0.00	0.37**(0.14)	0.00^{NS} (0.00)	0.37**(0.14)
LSZ	0.35	0.37**(0.13)	0.11** (0.02)	0.48** (0.11)
HBT	0.66	0.56**(0.13)	0.20** (0.06)	0.76** (0.08)
FLW	0.00	0.06^{NS}(0.08)	0.00^{NS}(0.00)	0.06^{NS}(0.08)
HGT2	0.50	0.49**(0.13)	0.14** (0.04)	0.63** (0.10)
SPR2	0.34	0.31* (0.12)	0.13** (0.03)	0.43** (0.10)
LFN2	0.00	0.25* (0.04)	0.00^{NS} (0.00)	0.25* (0.04)
DHGT	0.00	0.06^{NS}(0.04)	0.00^{NS} (0.00)	0.06^{NS}(0.04)
DSPR	0.00	0.04^{NS}(0.03)	0.00^{NS} (0.00)	0.04^{NS}(0.03)
DLFN	0.00	0.41**(0.13)	0.00^{NS} (0.00)	0.41**(0.13)
INTK	0.00	0.26* (0.10)	0.00^{NS} (0.00)	0.26* (0.10)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Equation 6.10
2...4 Equations 6.7...6.9 (Section 6.2.6)

LFN1, DST, FLW, LFN2, DHGT, DSPR, DLFN, and INTK had genetic fractions equal to zero, meaning that the environmental variance was greater than the genetical variance at the Plant partition level, therefore, Plant restricted heritabilities were all equal to zero for those characters.

Considering the Overall restricted heritabilities, three characters had a medium to high value (HGT1, HGT2 and HBT), six characters had a medium value (SPR1, LFN1, SPR2, DLFN, DST and LSZ), two characters with medium to low values (LFN2 and INTK) and three were non significantly different from zero values (DHGT, DSPR and FLW).

Table 6.15b: Heritabilities and standard errors for all characters measured in Uruguay

Characters	Genetic fraction (g)[1]	Population Restricted[2]	Plant Restricted[3]	Overall Restricted[4]
HGT1	0.36	0.45**(0.13)	0.08** (0.02)	0.53** (0.11)
SPR1	0.46	0.21* (0.10)	0.16** (0.02)	0.37** (0.08)
LFN1	0.00	0.26**(0.10)	0.00^{NS} (0.00)	0.26**(0.10)
DST	0.18	0.14* (0.08)	0.06**(0.01)	0.20**(0.07)
LSZ	0.11	0.39**(0.13)	0.03** (0.01)	0.41** (0.12)
HBT	0.65	0.50**(0.13)	0.20** (0.05)	0.69** (0.08)
FLW	0.00	0.09^{NS}(0.06)	0.00^{NS}(0.00)	0.09^{NS}(0.06)
HGT2	0.00	0.26* (0.10)	0.00^{NS} (0.00)	0.26* (0.10)
SPR2	0.38	0.27* (0.11)	0.14** (0.02)	0.42** (0.09)
LFN2	0.00	0.21* (0.09)	0.00^{NS} (0.00)	0.21* (0.09)
DHGT	0.00	0.32**(0.12)	0.00^{NS} (0.00)	0.32**(0.12)
DSPR	0.00	0.15* (0.08)	0.00^{NS} (0.00)	0.15* (0.08)
DLFN	0.00	0.21* (0.09)	0.00^{NS} (0.00)	0.21* (0.09)
INTK	0.00	0.32**(0.12)	0.00^{NS} (0.00)	0.32**(0.12)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Equation 6.10
2...4 Equations 6.7...6.9 (Section 6.2.6)

LFN1, FLW, HGT2, LFN2, DHGT, DSPR, DLFN, and INTK had genetic fractions equal to zero. No variation was present due to genetics at the Plant partition level, therefore, Plant restricted heritabilities were also zero.

Considering the Overall restricted heritabilities, two characters had a medium to high value (HGT1 and HBT), three characters had a medium value (SPR1, SPR2 and LSZ), eight characters with medium to low values (LFN1, DST, HGT2, LFN2 DHGT, DSPR, DLFN and INTK) and the heritability of FLW was non significantly different from zero.

6.3.3 PERSISTENCE AND SAMPLING INTENSITY

6.3.3.1 ANALYSIS OF VARIANCE

Results of the analysis of variance (F tests), variance components with their respective standard errors for Sampling intensity and Persistence in New Zealand and Uruguay are presented in Table 6.16.

Table 6.16: Significance of the analysis of variance (F tests), variance components with their respective standard errors for Sampling intensity and Persistence in the New Zealand and Uruguay studies

Characters	Block	Population	Error a	Time	Pop.xTime	Error b
New Zealand						
Sampl.	-0.00^{NS}	-0.00^{NS}	0.00^{NS}	0.00**	-0.00^{NS}	0.00
Intensity	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Persistence	0.00^{NS}	0.02**	0.00*	0.01**	0.01**	0.00
	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)
Uruguay						
Sampl.	-0.00^{NS}	-0.02^{NS}	0.00^{NS}	0.00*	0.00^{NS}	0.00
Intensity	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Persistence	0.00^{NS}	0.02**	0.00**	0.06**	0.01**	0.00
	(0.00)	(0.01)	(0.00)	(0.05)	(0.00)	(0.00)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

Sampling intensity was only significant for Time effect at both sites. Persistence was significant for all effects except Block effect at both sites.

6.3.3.2 ANALYSIS OF MEANS

Means of sampling intensity per Population and Grazing Date are presented in Table 6.17 for New Zealand and Uruguay.

Table 6.17: Sampling intensity per Population and Grazing Date in New Zealand and Uruguay

Time	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	Mean
New Zealand										
19/01/95	99	100	100	99	99	96	98	99	100	98.8a[1]
13/02/95	83	90	94	81	89	93	88	82	96	88.4c
15/03/95	87	86	99	93	95	100	95	95	97	94.1b
19/04/95	97	95	100	100	98	100	100	99	99	98.7a
Mean	91.5	92.3	98.3	93.3	95.3	97.3	95.3	93.8	98.0	95.0
Uruguay										
13/11/95	99	100	100	100	100	100	100	100	100	99.9a[1]
13/12/95	99	100	100	100	99	100	99	99	100	99.6a
18/01/96	95	96	97	86	97	94	78	90	97	92.2b
21/02/96	100	98	100	100	100	100	97	100	100	99.4a
Mean	98.3	98.5	99.3	96.5	99.0	98.5	93.5	97.3	99.3	97.8

1 Values within the same column, followed by the same letter do not differ significantly ($P \geq 0.05$)

All populations were sampled with the same intensity for both sites according to the significance of the F tests (see Table 6.16). For New Zealand, sampling intensity was higher for the first and fourth grazings, and the least intensely sampled was the second grazing. For Uruguay, the third grazing was the only one sampled less intensively than the other three.

Means of persistence per Population and Grazing Date are presented in Table 6.18 for New Zealand and Uruguay.

Table 6.18: Persistence per Population and Grazing Date in New Zealand and Uruguay

Time	Turkish	Harnua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	Mean
New Zealand										
19/01/95	100	100	99.1	100	100	98.1	100	99.1	100	99.6a
13/02/95	100	100	94.4	100	100	82.4	100	95.4	100	96.9ab
15/03/95	99.1	100	90.7	100	100	73.1	100	91.7	98.1	94.7bc
19/04/95	97.2	99.1	89.8	100	100	67.6	100	84.3	97.2	92.8c
Mean	99.1a	99.8a	93.5b	100a	100a	80.4c	100a	92.6b	98.9a	96.0
Uruguay										
13/11/95	100	99.1	87.0	98.1	96.3	98.1	100	92.6	95.4	96.3a
13/12/95	97.2	91.7	83.3	84.3	95.4	82.4	89.8	88.9	75.0	87.6b
18/01/96	91.7	69.4	73.1	72.2	93.5	49.1	74.1	65.7	27.8	68.5c
21/02/96	82.4	54.6	64.8	60.2	90.7	40.7	58.3	58.3	15.7	58.4c
Mean	92.9a[1]	78.7b	77.1b	78.7b	94.0a	67.6bc	80.6ab	76.4b	53.5c	77.7

1 Values within the same row or column, followed by the same letter do not differ significantly ($P \geq 0.05$)

All populations had a very good persistence in New Zealand with almost 85% of the plants surviving to the fourth grazing, except E116 which at the last grazing had only 68% of the stand alive. There was a significant reduction between 2 and 3% of plants in each grazing. In Uruguay, Kenland and Turkish had very good persistence. Turoa's persistence was very poor followed by E116. An average of 8.7% of plants died from the first to the second grazing, 19.1% died from the second to the third and finally 10% died from the third to the last grazing.

6.3.4 BASE CONDITION OF THE POPULATIONS BEFORE GRAZING TREATMENTS WERE IMPOSED

Results were analysed before the first grazing and pooled across the two sites to evaluate pure plant properties without the effect of the grazing animals. Grazing animals increase variation due to their selective grazing, so by studying heritability values of plant properties with and without the grazing animal, the relative selection efficiencies could be determined.

6.3.4.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was performed. Results of the analyses of variance (F tests), variance components with their respective standard errors for all characters are presented in Table 6.19.

Table 6.19: Significance of the analysis of variance (F tests), variance components with their respective standard errors for all characters

Characters	Site	Block	Population	PopxSite	Error	Plant
HGT1	54.26** (46.45)	1.75** (1.04)	13.67^{NS} (11.00)	18.17** (8.20)	-1.51^{NS} (0.16)	23.60 (1.37)
SPR1	133.23** (113.58)	5.97** (3.61)	8.00^{NS} (13.77)	33.96** (15.56)	-1.67^{NS} (0.65)	49.77 (2.89)
LFN1	2.53* (2.84)	0.55** (0.38)	16.29* (8.89)	6.26** (2.96)	-0.59^{NS} (0.27)	19.45 (1.13)
DST	---	0.06** (0.04)	0.21** (0.10)	---	0.01^{NS} (0.01)	0.24 (0.02)
LSZ	0.42* (0.39)	0.06** (0.03)	0.21^{NS} (0.18)	0.30** (0.14)	-0.03^{NS} (0.01)	0.74 (0.04)
HBT	-0.01^{NS} (0.01)	0.00^{NS} (0.00)	0.42* (0.24)	0.20** (0.09)	-0.00^{NS} (0.01)	0.54 (0.03)
FLW	23.53** (20.47)	0.77** (0.48)	-0.00^{NS} (3.63)	11.32** (5.13)	-0.59^{NS} (0.13)	12.98 (0.75)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
--- Density was not measured before the first grazing in New Zealand

The Site, Block and PopxSite effects were significant for all characters measured pre-grazing except HBT for Site and Block. The Population effect was significant only for LFN1, DST and HBT. Error effect was not significant for any character.

6.3.4.2 HERITABILITIES

Heritability values (h^2) for plant characteristics measured before the first grazing provided a purely plant-focused indication of which characters will respond most to selection. Heritability values and their respective standard errors are presented in Table 6.20.

Table 6.20: Heritabilities and standard errors for all characters

Characters	Genetic fraction (g)[1]	Population Restricted[2]	Plant Restricted[3]	Overall Restricted[4]
HGT1	0.73	0.25^{NS}(0.17)	0.32** (0.07)	0.57** (0.14)
SPR1	0.54	0.09^{NS}(0.15)	0.30** (0.05)	0.39** (0.14)
LFN1	0.52	0.39**(0.14)	0.24** (0.05)	0.64**(0.10)
DST	0.78	0.46**(0.12)	0.41** (0.09)	0.87**(0.03)
LSZ	0.64	0.18^{NS}(0.13)	0.39** (0.06)	0.56** (0.11)
HBT	0.74	0.36**(0.14)	0.35** (0.07)	0.72** (0.09)
FLW	0.80	0.00^{NS}(0.15)	0.44**(0.07)	0.44**(0.17)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Equation 6.10
2...4 Equations 6.7...6.9 (Section 6.2.6)

All genetic fractions were greater than zero, therefore genetic variation at the Plant partition level was present.

Considering the overall restricted heritabilities, all the values of these pure plant properties, without the effect of the grazing animal, were medium to high (HGT1, LFN1, DST, LSZ and HBT) and medium (SPR1 and FLW). Plant and Population restricted heritabilities were significant for all characters, except HGT1, SPR1, LSZ and FLW for Population restricted heritability.

6.3.5 RESULTS FOR THE QUALITY ANALYSIS

Quality measurements (crude protein content and digestibility) were taken in the third grazing for both sites and the analysis was pooled for the two sites. As their model is different to that of the other characters, they have been presented separately.

6.3.5.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was performed. Results of the analyses of variance (F tests), variance components and their respective standard errors for protein and digestibility are presented in Table 6.21.

Table 6.21: Significance of the analysis of variance (F tests), variance components with their respective standard errors for protein and digestibility

Characters	Site	Block	Population	PopxSite	Error	Plant
PRT	1.06^{NS}	0.65**	1.00*	0.89**	-0.09^{NS}	3.42
	(1.14)	(0.39)	(0.68)	(0.43)	(0.05)	(0.21)
DGT	0.59^{NS}	0.41**	5.12*	4.06**	-0.22^{NS}	13.26
	(1.02)	(0.29)	(3.41)	(1.95)	(0.23)	(0.81)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

The effects of Site were not significant, so no analyses were performed by Site for PRT and DGT, except the presentation of the mean values. Block, Population and PopxSite were significant for both characters. The Error effect was not significant for any character.

6.3.5.2 ANALYSIS OF MEANS

Means for the quality characters are presented to describe the plant materials. Protein and digestibility grand means and means per Site with their respective standard errors are presented in Table 6.22.

Table 6.22: Grand means and means per Site with their respective standard errors for protein and digestibility

Characters	Grand means	New Zealand	Uruguay
PRT	16.29(0.10)	15.50(0.12)	17.17(0.15)
DGT	62.13(0.19)	61.35(0.20)	62.98(0.36)

Values of PRT and DGT were a little higher in Uruguay (but not significantly different) than in New Zealand and all values were in agreement with the expected values for Red clover in Summer.

Protein and digestibility means per Population with their respective standard errors are presented in Table 6.23.

Table 6.23: Means and standard errors per Population for protein and digestibility

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S.error
PRT	15.56e[1]	16.98bc	17.51ab	16.54cd	16.39d	16.59cd	13.56f	15.71e	17.71a	0.19
DGT	62.17cd	63.19bc	65.65a	61.60de	64.26b	60.87e	56.00f	60.72e	64.19b	0.41

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

The best PRT and DGT values were found in Turoa and Quiñiquelli while the lowest were found in Astred and F.2419.

Protein and digestibility means and standard errors per Site and Population are presented in Table 6.24.

Table 6.24: Means and standard errors per Site and Population for protein and digestibility

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa	S. error
New Zealand										
PRT	14.44ef[1]	16.23bc	15.90c	15.23d	14.81de	16.55b	14.00f	14.93de	17.40a	0.20
DGT	60.78de	62.28bc	63.42ab	61.82cd	62.03cd	59.78ef	58.47f	59.28f	64.21a	0.45
Uruguay										
PRT	16.72c	17.84b	19.16a	18.24ab	17.97b	16.60c	13.06d	16.63c	18.22ab	0.33
DGT	63.51b	64.22b	67.95a	61.37c	66.49a	62.27bc	53.11d	62.40bc	63.26b	0.67

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

A similar ranking was found for both sites in both characters. Turoa had the highest values of PRT and DGT in New Zealand and Quiñiquelli had the highest values of PRT and DGT in Uruguay. Astred had the lowest values for both sites.

6.3.5.3 HERITABILITIES

To consider the usefulness of the quality characters for selection purposes, heritabilities should be known. Heritability values of protein and digestibility with their respective standard errors are presented in Table 6.25.

Table 6.25: Heritabilities and standard errors for protein and digestibility

Characters	Genetic fraction (g)[1]	Population Restricted[2]	Plant Restricted[3]	Overall Restricted[4]
PRT	0.59	0.19^{NS}(0.11)	0.39** (0.05)	0.58** (0.08)
DGT	0.59	0.23^{NS}(0.13)	0.35**(0.05)	0.58**(0.09)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Equation 6.10
2...4 Equations 6.7...6.9 (Section 6.2.6)

PRT and DGT had medium to high overall restricted heritabilities. Population restricted heritabilities were not significant for both characters, while Plant restricted heritabilities were highly significant for PRT and DGT, meaning that there is possibility for selection inside populations for improving both quality characters.

6.3.5.4 CORRELATIONS AND REGRESSIONS

Phenotypic and genotypic correlations (see Section 6.2.9) among all characters with protein and digestibility were explored with the purpose of revealing relationships among themselves. As indicated in Section 6.2.9, the genetic fractions were needed for estimating the genetic correlations for the other characters other than only for PRT and DGT. The genetic fractions for the third grazing (when nutritional characters were measured) for HGT1, SPR1, LFN1, DST, LSZ, HBT, FLW, HGT2, SPR2, LFN2, DHGT, DSPR, DLFN and INTK were 0.66, 0.63, 0.47, 0.54, 0.62, 0.78, 0.44, 0.57, 0.58, 0.39, 0.17, 0.32, 0.33 and 0.30 respectively.

Phenotypic and genotypic correlations of protein and digestibility with all other characters are presented in Table 6.26.

Table 6.26: Phenotypic and genotypic correlations with protein and digestibility for all characters

	Phenotypic		Genotypic	
	PRT	DGT	PRT	DGT
HGT1	0.16**	0.40**	0.16**	0.43**
SPR1	-0.37**	-0.20**	-0.40**	-0.23**
LFN1	0.21**	0.16**	0.31**	0.22**
DST	-0.04^{NS}	0.03^{NS}	0.05^{NS}	0.05^{NS}
LSZ	0.25**	0.37**	0.27**	0.46**
HBT	-0.26**	-0.37**	-0.30**	-0.41**
FLW	-0.19**	-0.21**	-0.46**	-0.42**
HGT2	-0.05^{NS}	0.18**	0.06**	0.29**
SPR2	-0.44**	-0.28**	-0.45**	-0.33**
LFN2	-0.06^{NS}	-0.11**	-0.20**	-0.17**
DHGT	0.38**	0.48**	0.34**	0.56**
DSPR	0.22**	0.28**	0.17**	0.32**
DLFN	0.22**	0.23**	0.43**	0.33**
INTK	0.34**	0.46**	0.45**	0.57**
PRT	1.00**	0.60**	1.00**	0.64**
DGT	0.60**	1.00**	0.64**	1.00**

** Significantly different from zero at 0.01 level
NS Non significantly different from zero

Phenotypic and genotypic correlations for PRT and DGT were in quite good agreement, with a few exceptions (eg. FLW) where the genetic correlation is stronger than the phenotypic. The same was found for LFN2 and DLFN for PRT. The strongest phenotypic correlations for PRT were with SPR2, DHGT and INTK. PRT was genotypically correlated most strongly with SPR2, FLW, DLFN and INTK. The strongest phenotypic and genotypic correlations with DGT were DHGT and INTK (0.48; 0.46 (phenotypic) and 0.56; 0.57 (genotypic) respectively). The phenotypic and genotypic correlations between PRT and DGT were the strongest ones of 0.60 and 0.64 respectively.

To explain PRT and DGT by pre-grazing characters, post-grazing characters and differences pre- and post-grazing (Tables 6.27...6.32), all possible multiple regressions were performed. The standardised regression coefficients coming from these set of multiple regressions could be used as weighting factors for selection indices as in the example presented in Section 6.3.6.5. As stated in Section 6.2.7, the "best" models were chosen for regressions with low Cp values about equal to p (number of parameters including β_0).

Simple and multiple regressions with protein for characters measured pre-grazing are presented in Table 6.27.

Table 6.27: Simple and multiple regressions with protein for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	SPR1	2	157.77	0.15**	19.52**	-0.07**						
Best 2-variable model	SPR1 and LFN1	3	54.99	0.27**	15.18**	-0.09**	0.10**					
Best 3-variable model	LSZ, SPR1 and LFN1	4	3.91	0.33**	13.04**	0.57**	-0.09**	0.11**				
Best 4-variable model	LSZ, DST, SPR1 and LFN1	5	3.54	0.33**	13.32**	0.60**	0.27^{NS}	-0.09**	0.09**			
Best 5-variable model	HBT, LSZ, DST, SPR1 and LFN1	6	4.75	0.33**	13.04**	0.09^{NS}	0.66**	0.28^{NS}	-0.10**	0.09**		
Best 6-variable model	HBT, LSZ, DST, HGT1, SPR1 and LFN1	7	6.13	0.33**	12.62**	0.19^{NS}	0.63**	0.25^{NS}	0.02^{NS}	-0.11**	0.09**	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.33**	12.70**	0.19^{NS}	0.62**	-0.01^{NS}	0.26^{NS}	0.02^{NS}	-0.11**	0.09**

NS Non significant
** Significant at 0.01 level
1 Models rank
2 Mallow's Cp statistic

After the inclusion of three variables, the model was not improved further and only 33% of the variation could be explained by LSZ, SPR1 and LFN1. The closest Cp to the model's rank was at the 3 variable model (3.91). This equation is considered the best parsimonious solution with values of 0.25, -0.50 and 0.38 for the standardised betas of LSZ,

SPR1 and LFN1 respectively.

Simple and multiple regressions with digestibility for characters measured pre-grazing are presented in Table 6.28.

Table 6.28: Simple and multiple regressions with digestibility for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	HGT1	2	121.54	0.16**	57.26**	0.22**						
Best 2-variable model	FLW and HGT1	3	70.09	0.22**	58.44**	-0.26**	0.24**					
Best 3-variable model	HGT1, SPR1 and LFN1	4	38.38	0.26**	54.31**	0.23**	-0.10**	0.14**				
Best 4-variable model	FLW, HGT1, SPR1 and LFN1	5	18.51	0.29**	55.59**	-0.18**	0.24**	-0.09**	0.12**			
Best 5-variable model	LSZ, FLW, DST, HGT1 and SPR1	6	10.21	0.30**	58.13**	0.76**	-0.19**	1.66**	0.18**	-0.12**		
Best 6-variable model	LSZ, FLW, DST, HGT1, SPR1 and LFN1	7	6.28	0.31**	55.76**	0.72**	-0.16**	1.08**	0.18**	-0.11**	0.06*	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.31**	55.13**	0.16[NS]	0.73**	-0.16**	1.06**	0.20**	-0.12**	0.07*

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

The coefficient of determination continued to improve until the six variable model, but only 31% of the variation could be explained by pre-grazing measurements. The Mallow's Cp statistic was considered the best parsimonious solution at that same rank and the standardised betas 1 to 6 were 0.16, -0.16, 0.19, 0.32, -0.32 and 0.13 for LSZ, FLW, DST, HGT1, SPR1 and LFN1 respectively.

Simple and multiple regressions with protein for characters measured post-grazing are presented in Table 6.29.

Table 6.29: Simple and multiple regressions with protein for characters measured post-grazing

	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3
Best 1-variable model	SPR2	2	25.18	0.19**	19.28**	-0.08**		
Best 2-variable model	SPR2 and LFN2	3	4.26	0.22**	18.69**	-0.10**	0.05**	
3-variable model	HGT2, SPR2 and LFN2	4	4.00	0.23**	18.90**	-0.02^{NS}	-0.10**	0.05**

NS Non significant
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Only 23% of the variation in PRT was explained by the post-grazing measurements and no parsimonious solution was considered good. The standardised betas for the full rank model were -0.06, -0.54 and 0.23 for HGT2, SPR2 and LFN2 respectively.

Simple and multiple regressions with digestibility for characters measured post-grazing are presented in Table 6.30.

Table 6.30: Simple and multiple regressions with digestibility for characters measured post-grazing

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3
Best 1-variable model	SPR2	2	39.56	0.08**	65.82**	-0.10**		
Best 2-variable model	HGT2 and SPR2	3	5.17	0.13**	63.62**	0.17**	-0.11**	
3-variable model	HGT2, SPR2 and LFN2	4	4.00	0.14**	63.82**	0.19**	-0.10**	-0.04^{NS}

NS Non significant
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Only 14% of the variation in DGT was explained by the post-grazing measurements and no parsimonious solution was considered good. The standardised betas for the full rank

model were 0.27, -0.28 and -0.09 for HGT2, SPR2 and LFN2 respectively.

Simple and multiple regressions with protein for the three differences are presented in Table 6.31.

Table 6.31: Simple and multiple regressions with protein for the three differences

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3
Best 1-variable model	DHGT	2	29.94	0.15**	14.97**	0.21**		
Best 2-variable model	DHGT and DLFN	3	3.03	0.19**	13.95**	0.20**	0.04**	
3-variable model	DHGT, DSPR and DLFN	4	4.00	0.19**	13.88**	0.19**	0.02^{NS}	0.04**

NS Non significant
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Only 19% of the variation in PRT was explained by the three differences (DHGT, DSPR and DLFN). The best parsimonious solution was at the two variable model with standardised betas of 0.37 and 0.20 for DHGT and DLFN respectively.

Simple and multiple regressions with digestibility for the three differences are presented in Table 6.32.

Table 6.32: Simple and multiple regressions with digestibility for the three differences

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3
Best 1-variable model	DHGT	2	29.87	0.23**	58.97**	0.50**		
Best 2-variable model	DHGT and DLFN	3	4.72	0.28**	56.96**	0.45**	0.08**	
3-variable model	DHGT, DSPR and DLFN	4	4.00	0.28**	56.76**	0.46**	0.06^{NS}	0.08**

NS Non significant
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Only 28% of the variation in DGT was explained by the three differences (DHGT, DSPR and DLFN). No parsimonious solution was considered good. The standardised betas for the full rank model were 0.45, 0.06 and 0.19 for DHGT, DSPR and DLFN respectively.

The R^2 of intake with protein and digestibility was 0.11 and 0.21 respectively.

Canonical correlations of all characters in several sets with protein and digestibility as the other set of variables are presented in Table 6.33.

Table 6.33: Canonical correlations of all characters in several sets with protein and digestibility as the other set of variables

Dependent variables	Independent variables	Canonical correlation[1]
PRT and DGT	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	0.60**
PRT and DGT	HGT1, SPR1 and LFN1	0.56**
PRT and DGT	HGT2, SPR2 and LFN2	0.48**
PRT and DGT	DHGT, DSPR and DLFN	0.55**
PRT and DGT	INTK	0.46**

** Significant at 0.01 level

1 Only the first canonical correlation was considered for each correlation because they accounted for the 76%, 77%, 69%, 99% and 100% of the variation respectively.

The largest canonical correlation was obtained with all the pre-grazing measurements, followed by the three pre-grazing (HGT1, SPR1 and LFN1) and the three differences (DHGT, DSPR and DLFN).

6.3.6 RESULTS OF THE POOLED ANALYSIS

6.3.6.1 ANALYSIS OF VARIANCE

All results obtained in both sites were pooled and analysed together to obtain an overall picture of the plant materials behaviour regarding animal preference and their usefulness for breeding purposes.

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was performed. Results of the pooled analyses of variance (F tests), variance components with their respective standard errors for all characters are presented in Table 6.34.

Table 6.34: Significance of the pooled analysis of variance (F tests), variance components with their respective standard errors for all characters

Char.	Site	Blks	Pops	PxE	Error a	Plant	Time	TxP	TxE	TxPxE	Error b
HGT1	-1.11^{NS}	0.30**	19.19*	7.44**	-1.04^{NS}	14.57**	-2.73^{NS}	-0.54^{NS}	19.29**	5.03**	10.58
	(4.98)	(0.20)	(10.68)	(3.96)	(0.13)	(1.00)	(7.74)	(0.94)	(12.58)	(1.48)	(0.36)
SPR1	32.27*	2.95**	16.17^{NS}	17.53**	-3.38^{NS}	44.82**	22.87^{NS}	-1.21^{NS}	23.87**	14.08**	40.46
	(33.43)	(1.78)	(12.84)	(9.65)	(0.39)	(3.20)	(23.87)	(2.76)	(16.17)	(4.22)	(1.37)
LFN1	-2.56^{NS}	4.89**	10.24^{NS}	13.37**	-1.06^{NS}	12.44**	49.65^{NS}	-0.26^{NS}	22.03**	2.59**	34.64
	(5.18)	(2.87)	(8.37)	(6.38)	(0.20)	(1.26)	(39.13)	(0.65)	(14.19)	(0.99)	(1.17)
DST	-0.00^{NS}	0.02**	0.08*	0.04*	-0.00^{NS}	0.13**	0.09*	0.01^{NS}	0.01^{NS}	0.04**	0.21
	(0.01)	(0.01)	(0.05)	(0.03)	(0.00)	(0.01)	(0.06)	(0.01)	(0.01)	(0.02)	(0.01)
LSZ	0.27*	0.01**	0.41**	0.02^{NS}	-0.02^{NS}	0.31**	0.13^{NS}	-0.04^{NS}	0.08**	0.13**	0.34
	(0.25)	(0.01)	(0.19)	(0.03)	(0.00)	(0.02)	(0.11)	(0.02)	(0.06)	(0.04)	(0.01)
HBT	-0.01^{NS}	0.01**	0.50**	0.16**	-0.02^{NS}	0.37**	0.01^{NS}	-0.00^{NS}	0.01*	0.05**	0.18
	(0.01)	(0.05)	(0.27)	(0.08)	(0.00)	(0.02)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)
FLW	-5.73^{NS}	0.30**	2.22**	-0.95^{NS}	-0.19^{NS}	1.59**	-10.8^{NS}	-1.26^{NS}	23.47**	5.67**	9.39
	(3.82)	(0.18)	(1.00)	(0.47)	(0.04)	(0.24)	(7.67)	(0.95)	(15.26)	(1.65)	(0.32)
HGT2	19.60^{NS}	0.88**	9.29*	10.95**	-0.83^{NS}	11.64**	-3.18^{NS}	-0.44^{NS}	26.19**	3.95**	9.52
	(22.84)	(0.53)	(7.32)	(5.40)	(0.11)	(0.82)	(10.57)	(0.75)	(16.86)	(1.17)	(0.32)
SPR2	63.32*	6.37**	17.91*	19.48**	-3.01^{NS}	46.10**	30.75^{NS}	1.27^{NS}	54.26**	11.29**	33.52
	(65.45)	(3.78)	(14.22)	(10.24)	(0.46)	(3.17)	(41.08)	(2.66)	(35.18)	(3.39)	(1.14)
LFN2	14.61^{NS}	12.43**	16.07*	8.14**	-0.99^{NS}	18.35**	24.81^{NS}	0.33^{NS}	35.86**	4.01**	58.57
	(20.95)	(7.29)	(9.65)	(4.37)	(0.46)	(1.98)	(29.59)	(1.17)	(23.08)	(1.56)	(1.99)
DHGT	8.48**	0.51**	0.46^{NS}	3.26**	-0.16^{NS}	1.75**	0.37^{NS}	0.68*	0.46**	0.67**	10.38
	(7.34)	(0.31)	(1.32)	(1.57)	(0.05)	(0.27)	(0.50)	(0.35)	(0.36)	(0.27)	(0.35)
DSPR	3.75^{NS}	1.08**	0.79^{NS}	1.69**	-0.15^{NS}	1.02**	-0.90^{NS}	0.36^{NS}	9.28**	1.42**	21.40
	(5.37)	(0.65)	(1.01)	(0.98)	(0.10)	(0.41)	(3.89)	(0.47)	(6.01)	(0.56)	(0.73)
DLFN	31.22^{NS}	8.59**	9.53^{NS}	30.74**	-0.62^{NS}	15.57**	-3.22^{NS}	-0.24^{NS}	67.08**	6.56**	66.56
	(43.56)	(5.09)	(13.79)	(14.80)	(0.53)	(1.95)	(29.00)	(1.60)	(43.02)	(2.33)	(2.26)
INTK	-0.08^{NS}	0.21**	0.08^{NS}	0.68**	-0.02^{NS}	0.29**	-0.00^{NS}	-0.00^{NS}	-0.02^{NS}	0.18**	1.32
	(0.04)	(0.12)	(0.26)	(0.33)	(0.01)	(0.04)	(0.01)	(0.04)	(0.01)	(0.06)	(0.05)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

The Site effect was only significant for SPR1, SPR2, DHGT and LSZ. The Block, PopulationxSite, Plant, Time Site and TimexPopulationxSite effects were significant for almost all characters. Error a, Time and TimexPopulation effects were non significant for almost all characters. The Population effect was significant for all characters except SPR1, LFN1, DHGT, DSPR, DLFN and INTK.

6.3.6.2 ANALYSIS OF MEANS

Means are presented to describe the plant materials. Grand means, coefficients of variation and means per Site with their respective standard errors for all characters are presented in Table 6.35.

Table 6.35: Grand means, coefficients of variation and means per Site with their respective standard errors for all characters

Characters	Grand means	Coefficients of variation	New Zealand	Uruguay	S. error
HGT1	21.30 (0.11)[1]	39.3	22.50	19.95	0.12
SPR1	47.51 (0.24)	28.7	51.57a[2]	42.93b	0.25
LFN1	62.65 (0.16)	18.6	61.10	64.40	0.19
DST	2.72 (0.02)	31.4	2.61	2.77	0.02
LSZ	3.60 (0.02)	33.9	3.17b	4.08a	0.02
HBT	3.63 (0.02)	30.8	3.71	3.55	0.02
FLW	4.27 (0.06)	122.6	4.55	3.94	0.08
HGT2	16.09 (0.13)	53.7	19.42	12.33	0.14
SPR2	40.98 (0.31)	37.4	46.75a	34.47b	0.28
LFN2	31.61 (0.26)	41.4	34.68	28.15	0.30
DHGT	5.33 (0.10)	89.6	3.24b	7.69a	0.10
DSPR	6.76 (0.12)	89.9	5.09	8.65	0.14
DLFN	31.15 (0.29)	46.5	26.47	36.43	0.33
INTK	0.05 (0.03)	322.2	0.07	0.03	0.04

1 Standard errors of the grand means
2 Values within Sites, followed by different letter differ significantly ($P < 0.05$)

FLW, DHGT, DSPR and INTK had high coefficients of variation, while the other characters had medium values varying from 18% to 54%. HGT1, LFN1, DST, HBT, FLW, HGT2, LFN2, DSPR, DLFN and INTK were statistically the same for both sites, because they were not significant in the analysis of variance (Table 6.34). On average, plants in New

Zealand were grazed 8.5 cm larger in diameter and also remain larger post-grazing. Biggest leaves were found in Uruguay and also a larger DHGT.

6.3.6.3 HERITABILITIES

To consider the usefulness of the measured characters for selection purposes for both environments, pooled estimates of heritability should be known. Pooled heritabilities and standard errors for all characters are presented in Table 6.36.

Table 6.36: Pooled heritabilities and standard errors for all characters

Characters	Genetic fraction (g)[1]	Population Restricted[2]	Plant Restricted[3]	Overall Restricted[4]
HGT1	0.47	0.35**(0.13)	0.12** (0.02)	0.47** (0.11)
SPR1	0.36	0.13^{NS} (0.09)	0.13** (0.01)	0.25** (0.08)
LFN1	0.00	0.14^{NS} (0.11)	0.00^{NS} (0.00)	0.14^{NS} (0.11)
DST	0.00	0.16^{NS} (0.09)	0.00^{NS} (0.00)	0.16^{NS} (0.09)
LSZ	0.25	0.35**(0.11)	0.07** (0.01)	0.43** (0.10)
HBT	0.65	0.41**(0.13)	0.19** (0.04)	0.60** (0.10)
FLW	0.00	0.13* (0.06)	0.00^{NS}(0.00)	0.13* (0.06)
HGT2	0.32	0.21^{NS} (0.14)	0.08** (0.01)	0.30* (0.13)
SPR2	0.35	0.14^{NS} (0.10)	0.13** (0.02)	0.27** (0.09)
LFN2	0.00	0.15^{NS} (0.08)	0.00^{NS} (0.00)	0.15^{NS} (0.08)
DHGT	0.00	0.03^{NS}(0.08)	0.00^{NS} (0.00)	0.03^{NS}(0.08)
DSPR	0.00	0.03^{NS}(0.04)	0.00^{NS} (0.00)	0.03^{NS}(0.04)
DLFN	0.00	0.07^{NS} (0.10)	0.00^{NS} (0.00)	0.07^{NS} (0.10)
INTK	0.00	0.03^{NS}(0.10)	0.00^{NS} (0.00)	0.03^{NS}(0.10)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Equation 6.10
2...4 Equations 6.7...6.9 (Section 6.2.6)

LFN1, DST, FLW, LFN2, DHGT, DSPR, DLFN, and INTK had genetic fractions equal to zero, therefore, Plant restricted heritabilities were also equal to zero, due to no variation due to genetics at the Plant partition level.

The overall restricted heritabilities were medium to high for HBT, medium for LSZ and HGT1, medium to low for SPR1, HGT2, SPR2 and FLW, and not significantly different to zero for LFN1, DST, LFN2, DHGT, DSPR, DLFN and INTK. All those characters whose heritabilities were not significantly different from zero ($P \geq 0.05$) were not useful for breeding for both sites.

6.3.6.4 CORRELATIONS AND REGRESSIONS

Phenotypic and genotypic correlations (see Section 6.2.9) were explored among characters with the purpose of revealing relationships among themselves at a phenotypic and genotypic level. Phenotypic and genotypic correlations amongst all characters are presented in Table 6.37.

Table 6.37: Phenotypic and genotypic correlations amongst all characters[1]

	HGT1	SPR1	LFN1	DST	LSZ	HBT	FLW	HGT2	SPR2	LFN2	DHGT	DSPR	DLFN	INTK
HGT1	1	0.08**	-0.06**	-0.04*	0.53**	-0.68**	0.22**	0.79**	0.00^{NS}	0.08**	0.68**	0.18**	-0.12**	0.37**
SPR1	-0.25**	1	0.18**	0.54**	0.12**	0.46**	0.20**	0.16**	0.91**	0.41**	-0.07**	0.19**	-0.19**	-0.03^{NS}
LFN1	-0.16**	-0.10**	1	0.70**	0.30**	0.15**	-0.37**	-0.19**	0.15**	0.27**	0.11**	0.05*	0.61**	0.19**
DST	-0.40**	0.22**	0.88**	1	0.10**	0.37**	-0.11**	-0.03^{NS}	0.52**	0.43**	-0.05*	0.01^{NS}	0.23**	0.01^{NS}
LSZ	0.72**	-0.01^{NS}	-0.37**	-0.48**	1	-0.38**	-0.16**	0.27**	0.02^{NS}	0.09**	0.54**	0.22**	0.18**	0.29**
HBT	-0.86**	0.60**	0.16**	0.46**	-0.59**	1	0.02^{NS}	-0.55**	0.47**	0.17**	-0.45**	-0.03^{NS}	-0.02^{NS}	-0.26**
FLW	-0.14**	0.15**	-0.26**	-0.10**	-0.41**	0.14**	1	0.27**	0.21**	0.09**	0.03^{NS}	-0.03^{NS}	-0.39**	-0.18**
HGT2	0.71**	-0.14**	-0.04^{NS}	-0.20**	0.43**	-0.60**	0.04*	1	0.19**	0.28**	0.09**	-0.06**	-0.39**	-0.06**
SPR2	-0.27**	0.70**	0.00^{NS}	0.25**	-0.16**	0.50**	0.22**	-0.13**	1	0.55**	-0.22**	-0.23**	-0.33**	-0.33**
LFN2	-0.17**	0.20**	0.31**	0.41**	-0.27**	0.24**	0.28**	-0.03^{NS}	0.22**	1	-0.22**	-0.34**	-0.59**	-0.56**
DHGT	0.49**	-0.23**	-0.24**	-0.41**	0.52**	-0.48**	-0.29**	0.31**	-0.26**	-0.26**	1	0.36**	0.27**	0.68**
DSPR	0.25**	-0.05**	-0.27**	-0.31**	0.43**	-0.23**	-0.35**	0.10**	-0.14**	-0.26**	0.30**	1	0.32**	0.72**
DLFN	0.10**	-0.25**	0.18**	0.01^{NS}	0.09**	-0.16**	-0.41**	0.01^{NS}	-0.23**	-0.21**	0.15**	0.14**	1	0.62**
INTK	0.36**	-0.24**	-0.14**	-0.31**	0.45**	-0.38**	-0.44**	0.18**	-0.28**	-0.32**	0.38**	0.31**	0.26**	1

1 Phenotypic correlation (upper triangle); Genotypic correlation (lower triangle)
NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

Ninety one phenotypic and 91 genotypic correlations are detailed in Table 6.37. From the 91 pairs (one phenotypic and one genotypic of the same pair of characters), 16 showed a change in direction from a positive correlation to a negative correlation or vice-versa, 25 had an important change in magnitude but not in sign, and the rest were similar. One example of change in sign is LSZ with LFN1, being 0.30 for the phenotypic correlation and -0.37 for the genotypic correlation. An example for the change in magnitude is INTK with DSPR, being 0.72 for the phenotypic correlation and 0.31 for the genotypic correlation.

To explain INTK, DHGT, DSPR, DLFN, HGT2, SPR2 and LFN2 by pre-grazing measurements (Tables 6.38…6.44), all possible multiple regressions were performed. The standardised regression coefficients coming from these set of multiple regressions could be

used as weights for indices of selection as presented in the example in Section 6.3.6.5. As stated in Section 6.2.7 the "best" models were chosen for regressions with low Cp values about equal to p (number of parameters including β_0).

Simple and multiple regressions with Intake for characters measured pre-grazing are presented in Table 6.38.

Table 6.38: Simple and multiple regressions with Intake for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	HGT1	2	280.86	0.04**	-0.74**	0.04**						
Best 2-variable model	FLW and HGT1	3	106.97	0.11**	-0.71**	-0.09**	0.05**					
Best 3-variable model	FLW, HGT1 and LFN1	4	70.67	0.13**	-1.84**	-0.08**	0.05**	0.02**				
Best 4-variable model	FLW, DST, HGT1 and LFN1	5	30.44	0.25**	-3.15**	-0.07**	-0.38**	0.10**	0.04**			
Best 5-variable model	HBT, FLW, DST, HGT1 and LFN1	6	10.38	0.25**	-4.36**	0.25**	-0.08**	-0.54**	0.13**	0.04**		
Best 6-variable model	HBT, LSZ, FLW, DST, HGT1, and LFN1	7	6.13	0.26**	-4.41**	0.26**	-0.09*	-0.08**	-0.56**	0.14**	0.05**	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.26**	-4.42**	0.27**	-0.09*	-0.08**	-0.55**	0.14**	-0.00[NS]	0.05**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Almost all pre-grazing measurements were necessary to explain only 26% of the variation in INTK. The 6 variable model was considered the best parsimonious solution with standardised betas of 0.16, -0.07, -0.23, -0.28, 0.58 and 0.34 for HBT, LSZ, FLW, DST, HGT1 and LFN1 respectively.

Simple and multiple regressions with DHGT for characters measured pre-grazing are presented in Table 6.39.

Table 6.39: Simple and multiple regressions with difference in height for characters measured pre-grazing

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	HGT1	2	479.25	0.06**	2.48**	0.13**						
Best 2-variable model	LSZ and HGT1	3	292.94	0.17**	-0.74*	1.49**	0.03**					
Best 3-variable model	LSZ, HGT1 and SPR1	4	203.23	0.25**	3.68**	1.34**	0.08**	-0.10**				
Best 4-variable model	HBT, LSZ, HGT1 and SPR1	5	67.64	0.26**	-0.20^{NS}	1.04**	1.45**	0.18**	-0.16**			
Best 5-variable model	HBT, LSZ, HGT1 SPR1 and LFN1	6	37.41	0.27**	0.31^{NS}	1.08**	1.50**	0.18**	-0.15**	-0.01^{NS}		
Best 6-variable model	HBT, LSZ, DST, HGT1,SPR1 and LFN1	7	6.62	0.58**	-9.59**	1.47**	0.77**	-0.83**	0.52**	-0.10**	0.08**	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.58**	-9.55**	1.48**	0.76**	-0.01^{NS}	-0.83**	0.52**	-0.10**	0.07**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

With six variables, 58% of the variation in DHGT was explained. The six variable model was considered the best parsimonious solution with standardised betas of 0.36, 0.22, -0.16, 0.84, -0.28 and 0.21 for HBT, LSZ, DST, HGT1, SPR1 and LFN1 respectively.

Simple and multiple regressions with DSPR for characters measured pre-grazing are presented in Table 6.40.

Table 6.40: Simple and multiple regressions with difference in spread for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	LSZ	2	110.11	0.03**	3.65**	0.86**						
Best 2-variable model	LSZ and SPR1	3	56.61	0.04**	5.98**	0.87**	-0.04**					
Best 3-variable model	LSZ, DST and SPR1	4	31.03	0.09**	2.92**	0.82**	-0.83**	0.10**				
Best 4-variable model	LSZ, FLW, DST and SPR1	5	23.29	0.10**	3.21**	0.77**	-0.08**	-0.96**	0.12**			
Best 5-variable model	LSZ, FLW, DST, HGT1 and SPR1	6	12.90	0.10**	2.68**	0.54**	-0.11**	-0.93**	0.07**	0.12**		
Best 6-variable model	LSZ, FLW, DST, HGT1, SPR1 and LFN1	7	8.62	0.11**	1.14^{NS}	0.42**	-0.09**	-1.37**	0.08**	0.13**	0.04*	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.11**	-0.08^{NS}	0.33^{NS}	0.44**	-0.10**	-1.43**	0.11**	0.11**	0.04*

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Only 11% of the variation of DSPR was explained with the pre-grazing measurements. No parsimonious solution was considered good. The standardised betas for the full rank model were 0.07, 0.11, -0.10, -0.24, 0.16, 0.27 and 0.10 for HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1 respectively.

Simple and multiple regressions with DLFN for characters measured pre-grazing are presented in Table 6.41.

Table 6.41: Simple and multiple regressions with difference in leafiness for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	LFN1	2	407.06	0.26**	-8.95**	0.64**						
Best 2-variable model	SPR1 and LFN1	3	83.26	0.49**	8.11**	-0.51**	0.75**					
Best 3-variable model	DST, SPR1 and LFN1	4	27.13	0.49**	-3.81*	-3.75**	-0.26**	0.96**				
Best 4-variable model	FLW, DST, SPR1 and LFN1	5	8.97	0.49**	-1.31^{NS}	-0.26**	-3.59**	-0.23**	0.91**			
Best 5-variable model	FLW, DST, HGT1 SPR1 and LFN1	6	4.16	0.49**	0.08**	-0.23**	-3.68**	-0.09**	-0.23**	0.92**		
Best 6-variable model	HBT, FLW, DST, HGT1, SPR1 and LFN1	7	6.01	0.49**	-0.54^{NS}	0.17^{NS}	-0.23**	-3.72**	-0.07^{NS}	-0.23**	0.91**	
7-variable model	HBT, LSZ, FLW, DST,HGT1, SPR1 and LFN1	8	8.00	0.49**	-0.54^{NS}	0.17^{NS}	0.02^{NS}	-0.23**	-3.71**	-0.07^{NS}	-0.24**	0.91**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Two variables (SPR1 and LFN1) accounted for all the variation in DLFN that could be explained with all the pre-grazing measurements, but the best parsimonious solution according to the Cp statistic was the 5 variable model with standardised betas of -0.08, -0.22, -0.05, -0.20 and 0.77 for FLW, DST, HGT1, SPR1 and LFN1 respectively.

Simple and multiple regressions with HGT2 for characters measured pre-grazing are presented in Table 6.42.

Table 6.42: Simple and multiple regressions with post-grazing height for characters measured pre-grazing

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	HGT1	2	480.41	0.71**	-2.45**	0.87**						
Best 2-variable model	LSZ and HGT1	3	290.82	0.74**	0.79**	-1.50**	0.97**					
Best 3-variable model	LSZ, HGT1 and SPR1	4	214.63	0.77**	-3.49**	-1.35**	0.92**	0.10**				
Best 4-variable model	HBT, LSZ, HGT1 and SPR1	5	70.27	0.77**	0.60^{NS}	-1.10**	-1.47**	0.82**	0.16**			
Best 5-variable model	HBT, LSZ, HGT1 SPR1 and LFN1	6	32.85	0.77**	0.26^{NS}	-1.12**	-1.51**	0.82**	0.15**	0.01^{NS}		
Best 6-variable model	HBT, LSZ, DST, HGT1, SPR1 and LFN1	7	6.26	0.70**	10.08**	-1.51**	-0.77**	0.78**	0.48**	0.10**	-0.08**	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.70**	10.06**	-1.51**	-0.76**	0.01^{NS}	0.78**	0.48**	0.10**	-0.08**

NS Non significant
** Significant at 0.01 level

Three variables were enough to explain 77% of the variation in HGT2, but the best parsimonious solution according to the Cp statistic was the 6 variable equation with standardised betas of -0.31, -0.18, 0.13, 0.65, 0.24 and -0.18 for HBT, LSZ, DST, HGT1, SPR1 and LFN1 respectively.

Simple and multiple regressions with SPR2 for characters measured pre-grazing are presented in Table 6.43.

Table 6.43: Simple and multiple regressions with post-grazing spread for characters measured pre-grazing

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	SPR1	2	133.24	0.84**	-8.41**	1.04**						
Best 2-variable model	LSZ and SPR1	3	52.20	0.85**	-5.20**	-0.90**	1.04**					
Best 3-variable model	LSZ, DST and SPR1	4	34.87	0.84**	-2.11**	-0.84**	0.70**	0.89**				
Best 4-variable model	LSZ, DST SPR1 and LFN1	5	28.25	0.84**	-0.56^{NS}	-0.73**	1.22**	0.88**	-0.04**			
Best 5-variable model	LSZ, FLW, DST, HGT1 and SPR1	6	17.11	0.84**	-1.83**	-0.55**	0.11**	0.79**	-0.07**	0.88**		
Best 6-variable model	LSZ, FLW, DST, HGT1, SPR1 and LFN1	7	11.04	0.84**	-0.07^{NS}	-0.42**	0.09**	1.29**	-0.08**	0.87**	-0.05**	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.84**	1.63**	-0.47*	-0.44**	0.10**	1.37**	-0.13**	0.89**	-0.05**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

SPR1 explained almost all the variation in SPR2 that the pre-grazing measurements could explain. No parsimonious equation was considered good, and the full rank's standardised betas were -0.04, -0.05, 0.04, 0.10, -0.08, 0.89 and -0.05 for HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1 respectively.

Simple and multiple regressions with LFN2 for characters measured pre-grazing are presented in Table 6.44.

Table 6.44: Simple and multiple regressions with post-grazing leafiness for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7
Best 1-variable model	DST	2	125.83	0.19**	11.39**	5.94**						
Best 2-variable model	DST and SPR1	3	26.87	0.23**	5.82**	4.13**	0.24**					
Best 3-variable model	FLW, DST and SPR1	4	13.88	0.24**	5.50**	0.21**	4.48**	0.21**				
Best 4-variable model	FLW, DST, HGT1 and SPR1	5	8.27	0.24**	3.81**	0.18**	4.53**	0.09**	0.21**			
Best 5-variable model	FLW, DST, HGT1 SPR1 and LFN1	6	4.35	0.24**	0.57^{NS}	0.23**	3.68**	0.09**	0.22**	0.08*		
Best 6-variable model	HBT, FLW, DST, HGT1, SPR1 and LFN1	7	6.00	0.24**	1.52^{NS}	-0.26^{NS}	0.24**	3.73**	0.06^{NS}	0.23**	0.08*	
7-variable model	HBT, LSZ, FLW, DST, HGT1, SPR1 and LFN1	8	8.00	0.24**	1.52^{NS}	-0.26^{NS}	-0.01^{NS}	0.23**	3.72**	0.06^{NS}	0.23**	0.08*

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

LFN2 was poorly explained (only 24%) with all the pre-grazing characters. The best parsimonious equation was the 5 variable model with standardised betas of 0.09, 0.27, 0.06, 0.23 and 0.08 for FLW, DST, HGT1, SPR1 and LFN1 respectively.

Canonical correlations of all characters measured pre-grazing in several sets, with the 3 differences or 3 post-grazing measurements as another set, are presented in Table 6.45.

Table 6.45: Canonical correlations of all characters measured pre-grazing in several sets, with the 3 differences or 3 post-grazing measurements as another set

Dependent variables	Independent variables	Canonical correlation[1]
DHGT, DSPR and DLFN	HBT, LSZ, FLW and DST	0.63**
HGT2, SPR2 and LFN2	HBT, LSZ, FLW and DST	0.82**
HGT2, SPR2 and LFN2	HGT1, SPR1, LFN1, HBT, LSZ, FLW and DST	0.93**
HGT2, SPR2 and LFN2	HGT1, SPR1 and LFN1	0.92**
HGT2 and SPR2	HGT1 and SPR1	0.92**

1 Only the first canonical correlation was considered for each correlation because they accounted for the 70%, 82%, 68%, 67% and 68% of the variation respectively.

** Significant at 0.01 level

All canonical correlations were medium to high, being highest when HGT1, SPR1 and LFN1 were included to explain the post-grazing measurements.

6.3.6.5 SELECTION INDEX

A selection index was generated to explore the possibility of improving intake of spaced plants by considering 3 pre-grazing characters (HGT1, SPR1 and LFN1) and 3 post-grazing characters (HGT2, SPR2 and LFN2). The index used was considered by Baker (1986) as a Multiple trait selection-optimum selection indices (Section 6.2.10).

The phenotypic matrix (variances and covariances) is:

70.97	9.78	-6.11	62.28	0.001	9.34
9.78	210.42	31.55	21.72	221.38	82.45
-6.11	31.55	145.97	-21.48	30.39	45.22
62.28	21.72	-21.48	87.56	29.82	36.32
0.001	221.38	30.39	29.82	281.26	127.87
9.34	82.45	45.22	36.32	127.87	192.18

The genotypic matrix (variances and covariances) is:

26.04	-6.70	-2.62	13.19	-8.04	-3.42
-6.70	32.30	-1.67	-2.65	21.27	4.10
-2.62	-1.67	10.24	-0.46	0.002	3.89
13.19	-2.65	-0.46	13.02	-2.74	-0.43
-8.04	21.27	0.002	-2.74	34.05	5.01
-3.42	4.10	3.89	-0.43	5.01	16.07

Weights are the standardised regression coefficients of the multiple regression of intake with the 3 pre- and 3 post-grazing measurements all re-scaled to sum up to one.

The weighting vector is:

3.8
4.6
1.9
-2.3
-4.0
-3.0

The solutions vector is:

1.40495
2.25276
-0.26284
-0.81247
-2.05642
0.26788

The selection index (I) = 1.40495xHGT1 + 2.25276xSPR1 - 0.26284x LFN1 - 0.81247xHGT2 - 2.05642xSPR2 + 0.26788xLFN2 (6.21)

Equation 6.21 gives an index of selection to optimise genetic advance while improving INTK via HGT1, SPR1, LFN1, HGT2, SPR2 and LFN2.

6.4 DISCUSSION

6.4.1 GENETIC RATIOS AND HERITABILITIES

As plant breeders, one of the main interests is to study whether those characters were heritable and the relative magnitude of heritable variability (heritability).

Genetic partitions in the model were at two levels: Populations and Plants. The rest of the partitions were environmental effects. The Plant variance as explained in Section 6.2.6 is confounded between the effect of Plant genetics (meiotic segregation) and environment, which need to be separated. Clones were used to separate environment from genetic effects, because the differences between clones should be all environmental (including carry-over effects of cloning procedure). Surprisingly as shown in Table 6.15a; 6.15b; 6.25 and 6.36, many of the genetic ratios were zero (for LFN1, DST, FLW, LFN2, DHGT, DSPR, DLFN,

and INTK), meaning that the Plant variance was all environmental and that the only genetic source of variation was at the Population level for those characters. Possible explanations for this phenomenon are (a) that the piece of land was not as uniform as it was thought, (b) that the clones were not so uniform and that there was variation due to clones as stated by Mirzaie-Nodoushan and Gordon (1993) working with these same Red clover populations, (c) that the death of clones (persistence) reduced the three clones per Plant to one or two clones per Plant increasing the possible average variation, (d) that there was no additional genetic variation arising from Plant segregation, and that the variation was totally accounted for by the Population level, or (e) a combination of all the above.

Independently of the possible explanations for these results of genetic variances at the Plant partition level being equal to zero, the fact is that no genetic progress could be obtained by selecting for those characters without plant to plant variation. One possible alternative is to begin a crossing programme amongst populations, where the genetic results from an heterogeneous population might have a greater genetic variation at the Plant partition level.

The heritability of LFN2, DHGT, DSPR, DLFN and INTK per Site (Figure 6.1), and not the pooled estimates, are considered to show the relevance for breeding of these characters.

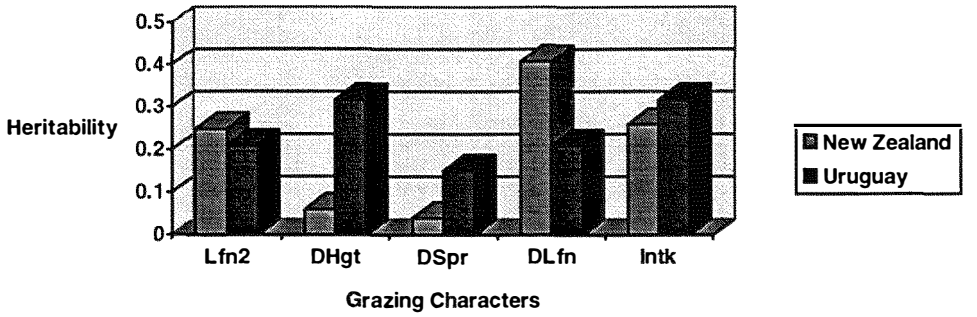

Figure 6.1: Heritability values of the five characters per Site

LFN2, DLFN and INTK are considered the best characters to use as breeding criteria to enhance animal preference because for both sites, the heritability values were "useful" (over 0.21). These values are intermediate to low values of heritability, but are, for example, in the same range of magnitude as the heritability of milk yield, protein yield and butterfat yield in Dairy Cattle breeding (Nicholas, 1987).

Crude protein content and digestibility had medium to high Overall restricted heritabilities as well as medium values for Plant heritabilities, making these characters very interesting for plant breeding.

6.4.2 GRAZING MANAGEMENT

The grazing management objectives for this experiment were: (1) that plants should be grazed when the semi-erect population reached on average 25 cm of height; (2) that animals should be removed from the plots to take the post-grazing measurements when leafiness was reduced to a level of 40%, and (3) that the sampling intensity should be at least 93.5%.

The overall average of the four grazings and two sites for HGT1 of the semi-erect populations was 23.25 cm, for all populations LFN2 was 32% and the sampling intensity was 96.4%. These values are close enough to the targets for the grazing management objectives were considered to have been achieved.

Grazing frequency was very similar in both sites, the interval between grazings being on average 30 days (26; 30 and 34 days between the first and second, second and third, and third and fourth grazings respectively) for New Zealand and 33 days (30; 36 and 33 days between the first and second, second and third, and third and fourth grazings respectively) for Uruguay.

6.4.3 GRAZING CHARACTERS

From all the characters measured, only the three measured post-grazing (HGT2, SPR2 and LFN2) or variables created from them such as DHGT, DSPR, DLFN and INTK, could be used as selection criteria to assess animal preference and tolerance to grazing for plant breeding purposes, because they were the only measurements that included the animal effects. The other characters (typical plant selection characters) were measured to have a detailed description of the plants, and for reference where, using the previous characters, it was found that animals were grazing some populations more than others.

From all the multiple regressions performed to assess the possibility of predicting grazing characters by the pre-grazing measurements, only HGT2 and SPR2 had R^2 greater than 0.7. No good prediction was found for the post-grazing characters, meaning that grazing could not be substituted or predicted by the pre-grazing characters and the grazing animals were needed to obtain such information.

To determine which of the post-grazing characters provided the most useful information (for not having to measure them all), the study of the relationship between pairs of characters of the same kind was made. From such study (HGT1 and HGT2 (R^2= 0.71), SPR1 and SPR2 (R^2= 0.84) and LFN1 and LFN2 (R^2= 0.10)), it was seen that HGT2 and SPR2 were highly associated with the conditions offered pre-grazing, and that LFN2 was not. The differences (DHGT, DSPR and DLFN) were not strongly associated with their respective pre-grazing measurements (R^2 = 0.06, 0.04 and 0.26 respectively).

An illustration of the problem of measuring HGT2 and SPR2 to determine animal influence, where the "skeleton" of the plants (prostrate and erect) post-grazing remained similar to pre-grazing even after the cleaning-off grazing is presented in Plate 6.5.

Plate 6.5: Illustration of close agreement between pre- and post-grazing Height and Spread for a prostrate and erect plant

LFN2, DHGT, DSPR, DLFN and INTK are better characters to observe the effect of selective grazing. The significance of the analysis of variance (F tests) for the Population and Plant effects that are the partitions containing the genetic information were presented in Tables 6.7 and 6.8. The five characters were significant at each site for the Population and Plant effect. The Population by Site interactions were studied, all of them being highly significant, meaning that the populations behaved differently for these characters at each site.

Means per character, Population and Site (Tables 6.9 and 6.12) are compared in Figures 6.2 to 6.6.

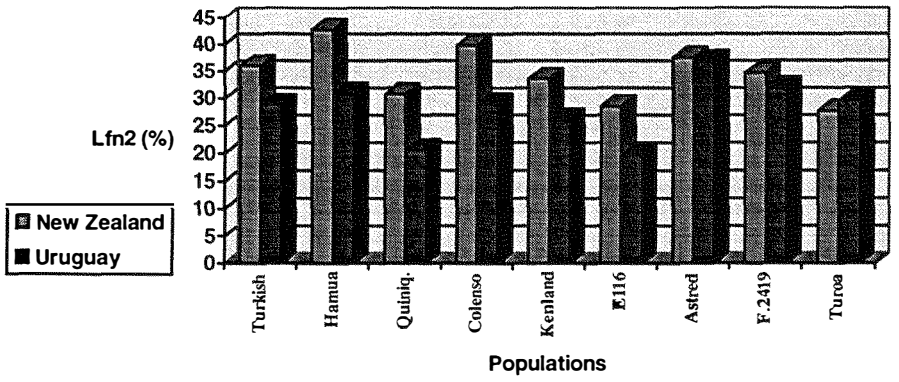

Figure 6.2: Means per Population and Site for post-grazing leafiness

Hamua and Colenso were the two populations with most leaves remaining after grazing in New Zealand while Astred and F.2419 were in Uruguay. Also, for all populations except Turoa, more leaves remained in the New Zealand study.

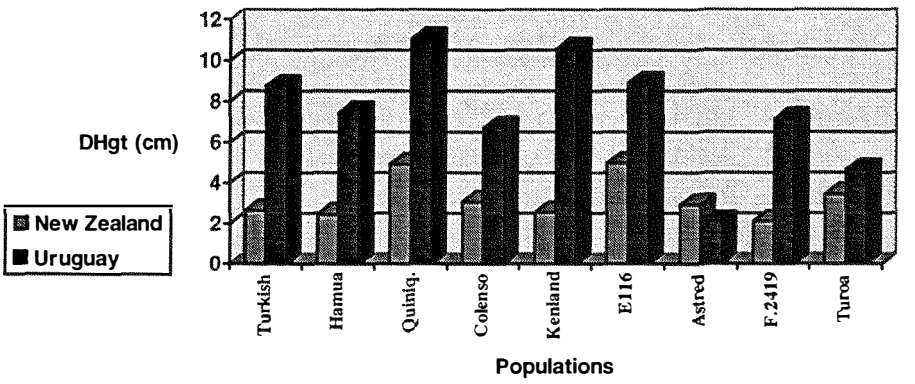

Figure 6.3: Means per Population and Site for difference in height

E116 and Quiñiquelli were the populations with the largest reductions in height in New Zealand while Quiñiquelli and Kenland were in Uruguay. For all populations except Astred, reductions in Height were greater in Uruguay than in New Zealand, meaning that grazings were on average more intense in Uruguay.

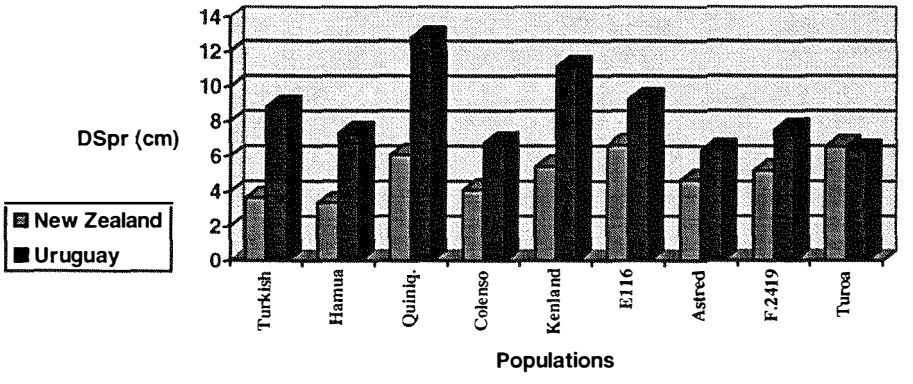

Figure 6.4: Means per Population and Site for difference in spread

Turoa and E116 were the populations with largest reductions in Spread in New Zealand while Quiñiquelli and Kenland were in Uruguay. For all populations except Turoa, largest reductions in Spread were found in Uruguay.

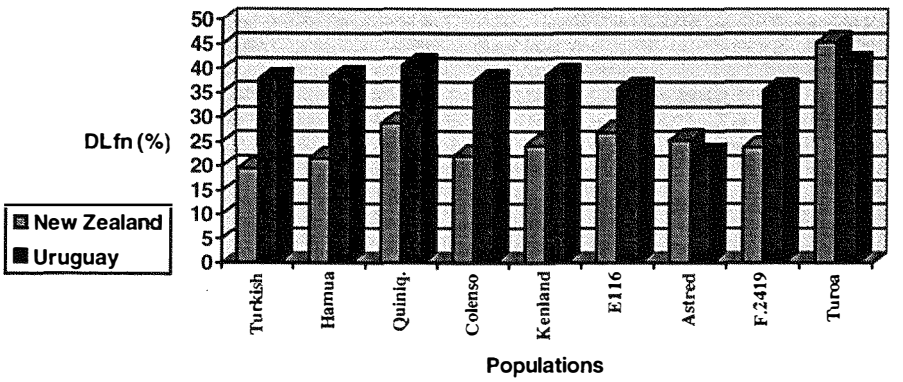

Figure 6.5: Means per Population and Site for difference in leafiness

Turoa, and Turoa and Quiñiquelli were the populations with largest reductions in leafiness in New Zealand and Uruguay respectively. For all populations except Astred and Turoa, largest reductions in leafiness were found in Uruguay.

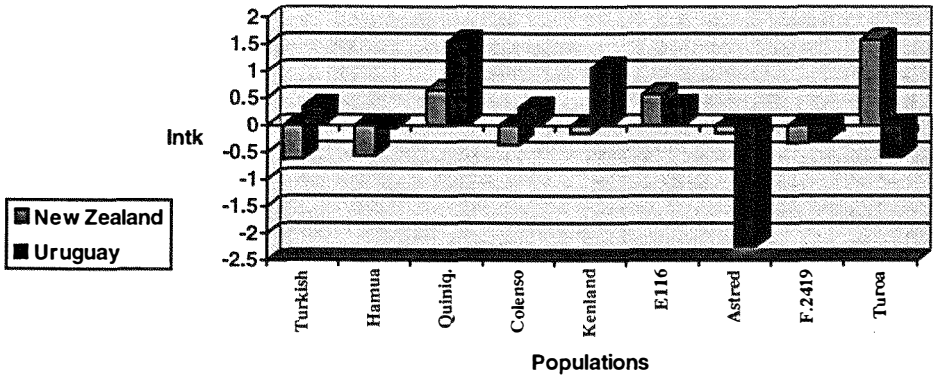

Figure 6.6: Means per Population and Site for intake

Turoa and Quiñiquelli were the populations with greatest intake in New Zealand while Quiñiquelli and Kenland were in Uruguay.

Discrepancies of results between sites for these grazing characters could be due to a different flowering behaviour (6.4.3.1), to a persistence or survival problem of the different populations (6.4.3.2), to a different grazing behaviour of the different sheep breeds used in each country (Perendale in New Zealand and Corriedale in Uruguay), to a combination of all of the above or to other factors not considered in the thesis.

6.4.3.1 FLOWERING BEHAVIOUR

The difference in flowering behaviour between sites could be almost entirely explained by planting and grazing dates. In New Zealand, the planting date was 8 Oct. and grazings were on the 19 Jan.; 13 Feb.; 15 Mar. and 19 Apr. In Uruguay, the planting date was 22 Aug. and grazings were on the 13 Nov.; 13 Dec.; 18 Jan. and 21 Feb..

This explains why the fourth grazing in April in New Zealand was in a completely vegetative state and the first grazing in November in Uruguay was also in a vegetative state.

All populations flowered at both sites with E116, Astred and Turkish being the ones that flowered most and Quiñiquelli and Turoa the ones that flowered least. It was not possible to observe indications of problems or differences in seed production because grazings were too frequent and severe after the cleaning-off grazing to make homogeneous the area.

6.4.3.2 PLANT PERSISTENCE

Plant survival or persistence was one of the main differences between sites. In New Zealand, at the fourth grazing 92.8% of the plants were still alive while only 58.4% were alive in Uruguay. This problem was aggravated, by the fact that death was not uniform amongst populations in both countries (Figure 6.7).

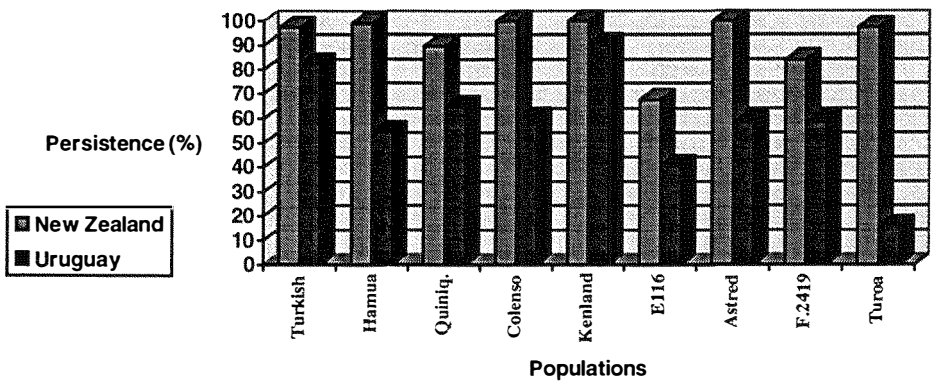

Figure 6.7: Persistence per Populations at the fourth grazing in each Site.

In New Zealand, a reduction of the stand of E116 to 67.6% was the main loss at the fourth grazing. In Uruguay, the extreme losses were suffered by Turoa with only 15.7% surviving at the fourth grazing, and 58.3%, 58.3%, 54.6%, 40.7% for Astred, F.2419, Hamua and E116 respectively.

6.4.4 MOST GRAZED POPULATIONS

As indicated in Table 6.34, there was genotype-environment interactions for all characters except LSZ and FLW. The two most grazed populations were Turoa in New Zealand and Quiñiquelli in Uruguay. What both populations have in common is that they are leafy plants (Turoa is a dense plant with a big proportion of leaves with respect to stems and Quiñiquelli has the biggest leaves). Also, neither of these populations flowered much during both experiments. Turoa had the highest values of PRT (17.4) and DGT (64.2) in New Zealand, and Quiñiquelli had the highest values of PRT (19.2) and DGT (68.0) in Uruguay. They did not have in common either height (one short and one tall), or habit (one prostrate and one erect). These characters are also highly phenotypically and genotypically correlated with PRT and DGT.

CHAPTER SEVEN
Sward-Animal Interaction Experiment

7.1 INTRODUCTION

Solid stands or sward conditions are rarely used for the early stages of a forage plant breeding programme because individuality is lost and the buffering effect of competition reduces the variability needed for selection. On the other hand, the final use of any forage species is under sward sowing conditions, therefore new cultivars should be bred to be winners under those circumstances. The objectives of this experiment were: (i) to test under sward conditions the same grazing method used in the spaced plant-animal interaction experiments, (ii) to determine the genetic correlation between the estimates of preference obtained in spaced plants and in swards, and (iii) to evaluate relative selection efficiencies by estimating the correlated genetic advance ratios.

7.2 MATERIALS AND METHODS

7.2.1 EXPERIMENTAL DETAILS

The experiment was conducted in Uruguay with a subset of six populations from the nine used in the previous experiments, because of limitations in the amount of seed available. They were: one erect (Hamua), three semi-erect (Estanzuela 116, Kenland and Colenso) and two prostrate (Turoa and Astred) (for a full description of the cultivars see Section 3.3.3). The sowing date was decided after analysing the sowing date and the date of the first cutting in eleven Red clover varietal trials in Uruguay from 1976 to 1991 (Pers. Comm. J. García and M. Rebuffo, 1995), to have the grazings synchronised with the spaced plant-animal interaction experiment. The management and experimental details are summarised in Table 7.1

Table 7.1: Management and experimental details

Management	Experimental Details
Site	La Estanzuela (34° 20'S, 57° 41'W)
Soil type	Planosoles[1]
Seed-bed preparation	Conventional ploughing procedure
Sowing method	Solid stand in monoculture (Sward)
Sowing date	8 June 1995
Sowing rate	9 kg/ha
Inoculation	Rhizobium leguminosarum-biovar trifolii was applied with the seed
Plot size	3.5 x 3.5 m
Treatments	6 populations
N° of blocks	3
Weed control	Round up[2] @ 3.5 l/ha before sowing and Basagran[3] @ 1.5 l/ha on 10 Sept. 1995
Insect control	Karate 50[4] @ 0.1 l/ha on 29 Aug. 1995
Fertilisation	60 kg P_2O_5/ha before sowing
Sampling for nutritional data	10 Jan. 1996
Sheep breed and class	Polworth ewes
Grazing dates	13 Nov. 1995 13 Dec. 1995 18 Jan. 1996 21 Feb. 1996

1 Víctora, 1985
2 Round up = glyphosate as active ingredient
3 Basagran 480 = Bentazone in the form of soluble concentrate
4 Karate 50 = lamda cialotrina

7.2.2 FIELD DESIGN

The six populations were sown in three blocks, each with three internal replicates. Each experimental unit was a square of 12.25 m^2 giving 36.75 m^2/population within a block, and 110.25 m^2/population overall. The experimental units were delimited by a single line of *Lolium multiflorum* lam. plants. The random location of the 3 internal replicates was to give a random "cafeteria" choice to the grazing animal and reduce the effect of external factors like location of the gate, fence proximity, people presence, etc. on diet selection. Also four internal sub-samples were taken on each experimental unit to estimate variation inside internal replicates.

The effect of sward location with reference to the electric fence was considered by setting up a concomitant dummy for covariance error adjustment similar in principle to the one used in the grazing management experiment (Section 5.2.2).

7.2.3 CHARACTERS RECORDED

The following pre-grazing measurements: height (HGT1), leaf size (LSZ), flowering (FLW), leafiness (LFN1) and plant density (DST) were obtained in the same way as in the grazing management experiment and spaced plant-animal interaction experiments (Sections 5.2.3 and 6.2.3). HGT1 and LSZ were considered as measurements of single plants in the sward and FLW, LFN1 and DST were considered as a pooled measure of three plants inside a quadrat of 4 dm^2.

Crude protein (PRT) and digestibility (DGT) were measured only once and in the same way as explained for the spaced plant-animal interaction experiment (Section 6.2.3), but for an estimate of four plants per bulked sample per plot.

The post-grazing measures, height (HGT2) and leafiness (LFN2) were measured in the same way as pre-grazing. Difference in height and leafiness pre- and post-grazing (DHGT

and DLFN) were also considered in the analysis, as well as intake (INTK).

$$\text{Intake} = [(\text{DHGT} + (\text{Mean}_{HGT2} - \text{Mean}_{HGT1}))/\text{s.e.of difference}] + [(\text{DLFN} + (\text{Mean}_{LFN2} - \text{Mean}_{LFN1}))/\text{s.e.of difference}] \quad (7.1)$$

$$\text{where standard error of the difference} = \sqrt{\left(\sigma^2_{Hgt1} + \sigma^2_{Hgt2} - 2\,\text{cov}_{Hgt1,Hgt2}\right)} \quad (7.2)$$

for height and the same for leafiness.

From now on, characters will be referred to with their abbreviation detailed in brackets with the description of each character.

No variables were transformed to improve normality because they were already normally distributed in the 504 cases studied (combination of Time, Block and Population for the 7 measured characters) (Appendix 7).

7.2.4 SELECTION OF GRAZING DATE AND NUMBER OF SHEEP

Grazings were synchronised with the spaced plant-animal interaction experiment, and the target HGT1 to graze the experiment was 25 cm for the semi-erect populations. The required number of animals was calculated considering the visual estimation of herbage offered and the herbage intake expected for sheep during one hour of grazing at that time.

From the grazing management experiment, it was decided that sheep were allowed to graze until LFN2 was on average approximately 40%. This criterion for taking animals out of the grazing areas was the one that gave consistency among grazings because the duration of grazing could not be used in a fixed way in such a short grazing of approximately four hours. The estimated herbage mass to be removed was approximately 2000 kg DM/ha, and as each plot had an effective area of 220.5 m^2, the DM available was 44.1 kg DM. For approximately four hours (beginning just after

sunrise) and using an area of 220.5 m^2 of solid stand, the stocking density used was 54 sheep to allow full exploration of the block without excessive defoliation. Calculations of herbage allowance were made for the first grazing only and these values were kept as fixed for the following three grazings.

Sheep were introduced again to defoliate to a uniform hard level in all plots after the post-grazing measurements were taken. No quantification was made of this post-grazing level, but the target was LFN2 of 20%.

A general view of the grazing animals and the state of the swards after grazing are shown in Plates 7.1 and 7.2.

Plate 7.1: General view of grazing animals

Plate 7.2: State of swards after grazing

7.2.5 STATISTICAL MODELS

Three statistical models were used to analyse the sward-animal interaction experiment. One was used to analyse the results obtained before the first grazing (7.2.5.1), another to analyse the nutritional characters (7.2.5.2) and the other to analyse the main results (7.2.5.3).

7.2.5.1 MODEL FOR ANALYSIS OF RESULTS OBTAINED BEFORE THE FIRST GRAZING

To characterise the populations prior to any effect of the grazing animals, only the data before the first grazing was considered. The experimental design was a randomised complete block (RCB) with two layers of nesting (Internal Repetitions and Samples). The model used to analyse the experiment was:

$$X_{ijkl} = \mu + B_i + P_j + \delta_{ij} + R_{(ij)k} + S_{(ijk)l} \qquad (7.3)$$

where X_{ijkl} = the phenotypic value of the l^{th} Sample ($l = 1...s$; $s = 4$) of the k^{th} Internal repetition ($k = 1...r$; $r = 3$) of the j^{th} Population ($j = 1...p$; $p = 6$) of the i^{th} Block ($i = 1...b$; $b = 3$), μ = the grand mean, B_i = the effect of the i^{th} Block, P_j = the effect of the j^{th} Population, δ_{ij} = random error associated with the Population level, $R_{(ij)k}$ = the effect of the k^{th} Internal repetition of the j^{th} Population of the i^{th} Block and $S_{(ijk)l}$ = the effect of the l^{th} Sample nested within the k^{th} Internal repetition of the j^{th} Population of the i^{th} Block.

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.* 1962; Searle, 1971). The E(MS) with infinite random inference are given in Table 7.2.

Table 7.2: Random inference of the expectations of mean squares

S.O.V.	D.F	M.S.	Expectation of M.S.	F.Test
Block	b-1	5	$\sigma^2_S+s\sigma^2_R+sr\sigma^2_\delta+srp\sigma^2_B$	5/3
Populations	p-1	4	$\sigma^2_S+s\sigma^2_R+sr\sigma^2_\delta+srb\sigma^2_P$	4/3
Error	(b-1)(p-1)	3	$\sigma^2_S+s\sigma^2_R+sr\sigma^2_\delta$	3/2
Internal Reps	bp(r-1)	2	$\sigma^2_S+s\sigma^2_R$	2/1
Samples	bpr(s-1)	1	σ^2_S	

In the table, σ^2_δ is the variance arising from δ_{ij}, σ^2_S from $S_{(ijk)l}$, σ^2_R from $R_{(ij)k}$, σ^2_P from P_j and σ^2_B from B_i.

All the F tests were recalculated from the SAS output, using the programme called THWAITE (Gordon, unpublished) to implement the Crump (1946, 1951) and Satterthwaite (1946) F tests and degrees of freedom. This programme also estimated the variance components and their standard errors.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS      Population Block Internal_Reps.;
MODEL...=  Block|Population Internal_Reps(Block Population) / SS2;
```

7.2.5.2 MODEL TO ANALYSE NUTRITIONAL DATA

Nutritional data was taken once during the experiment and a pooled sample was taken of each internal replicate. The experimental design was an RCB with one layer of nesting (Internal Repetitions). To analyse the nutritional data the following model was used:

$$X_{ijk} = \mu + B_i + P_j + \delta_{ij} + R_{(ij)k} \quad (7.4)$$

where X_{ijk} = the phenotypic value of the k^{th} Internal repetition (k = 1...r; r = 3) of the j^{th} Population (j = 1...p; p = 6) of the i^{th} Block (i = 1...b; b = 3), μ = the grand mean, B_i = the effect of the i^{th} Block, P_j = the effect of the j^{th} Population, δ_{ij} = random error associated with the Population level and $R_{(ij)k}$ = the effect of the k^{th} Internal repetition of the j^{th} Population of the i^{th} Block.

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.* 1962; Searle, 1971). The E(MS) with infinite random inference are given in Table 7.3.

Table 7.3: Random inference of the expectations of means squares

S.O.V.	D.F.	M.S.	Expectation of M.S.	F.Test
Block	b-1	4	$\sigma^2_R+r\sigma^2_\delta+rp\sigma^2_B$	4/2
Populations	p-1	3	$\sigma^2_R+r\sigma^2_\delta+rb\sigma^2_P$	3/2
Error	(b-1)(p-1)	2	$\sigma^2_R+r\sigma^2_\delta$	2/1
Internal Reps	bp(r-1)	1	σ^2_R	

In the table, σ^2_δ is the variance arising from δ_{ij}, σ^2_R from $R_{(ij)k}$, σ^2_P from P_j and σ^2_B from B_i.

All the F tests were recalculated from the SAS output as detailed in Section 7.2.5.1.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

PROC GLM;

CLASS Population Block Internal_Reps.;

MODEL...= Block|Population / SS2;

7.2.5.3 MODEL FOR ANALYSIS OF MAIN RESULTS

The experimental design was an RCB, with two layers of nesting ("cafeteria" repetitions (internal replicates), and samples), and split in time.

The model used to analyse the experiment was:

$$X_{ijklm} = \mu + B_i + P_j + \delta_{ij} + R_{(ij)k} + S_{(ijk)l} + T_m + TP_{mj} + \varepsilon_{ijklm} \quad (7.5)$$

where X_{ijklm} = the phenotypic value at the m^{th} Time (m = 1...t; t = 4) of the l^{th} Sample (l = 1...s; s = 4) of the k^{th} Internal repetition (k = 1...r; r = 3) of the j^{th} Population (j = 1...p; p = 6) of the i^{th} Block (i = 1...b; b = 3), μ = the grand mean, B_i = the effect of the i^{th} Block, P_j = the effect of the j^{th} Population, δ_{ij} = random error associated with the Population level, $R_{(ij)k}$ = the effect of the k^{th} Internal repetition of the j^{th} Population of the i^{th} Block, $S_{(ijk)l}$ = the effect of the l^{th} Sample nested within the k^{th} Internal repetition of the j^{th} Population of the i^{th} Block, T_m = the effect of the m^{th} Time, TP_{mj} = the effect of the mj^{th} interaction and ε_{ijklm} = random error $ijklm^{th}$

All effects in the model were considered to be infinite random (see Section 8.5), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946; 1951; Henderson, 1953; LeClerg *et al.* 1962; Searle, 1971). The E(MS) with infinite random inference are given in Table 7.4.

Table 7.4. Random inference of the expectations of mean squares

S.O.V.	D.F	M.S.	Expectation of M.S.	F.Test
Block	b-1	8	$\sigma^2_\varepsilon+t\sigma^2_S+st\sigma^2_R+rst\sigma^2_\delta+srpt\sigma^2_B$	8/6
Populations	p-1	7	$\sigma^2_\varepsilon+t\sigma^2_S+st\sigma^2_R+rst\sigma^2_\delta+brs\sigma^2_{TP}+srbt\sigma^2_P$	(7+1)/(6+2)
Error a	(b-1)(p-1)	6	$\sigma^2_\varepsilon+t\sigma^2_S+st\sigma^2_R+rst\sigma^2_\delta$	6/5
Internal Reps	bp(r-1)	5	$\sigma^2_\varepsilon+t\sigma^2_S+st\sigma^2_R$	5/4
Samples	bpr(s-1)	4	$\sigma^2_\varepsilon+t\sigma^2_S$	4/1
Time	t-1	3	$\sigma^2_\varepsilon+brs\sigma^2_{TP}+bprs\sigma^2_T$	3/2
Time x Pop.	(t-1)(p-1)	2	$\sigma^2_\varepsilon+brs\sigma^2_{TP}$	2/1
Error b	p(t-1)[(b-1)+b((r-1) + r(s-1))]	1	σ^2_ε	

In the table, σ^2_ε is the variance arising from ε_{ijklm}, σ^2_T from T_m, σ^2_{TP} from TP_{mj}, σ^2_δ is the variance arising from δ_{ij}, σ^2_S from $S_{(ijk)l}$, σ^2_R from $R_{(ij)k}$, σ^2_P from P_j and σ^2_B from B_i.

All the F tests were recalculated from the SAS output as detailed in Section 7.2.5.1.

The statistical computer package used to run all analyses was SAS (SAS Institute, 1988) and the procedure and model used were as follows.

```
PROC GLM;
CLASS     Population Block Internal_Reps. Sample Time;
MODEL...= Block|Population Time PopulationxTime
          Internal_Reps(Block Population)
          Sample(Block Population Internal_Reps.) / SS2;
```

7.2.6 HERITABILITY ESTIMATES

Heritabilities were calculated according to the appropriate model in a restricted definition (Allard, 1960; Gordon *et al.* 1972; Gordon, 1979) according to the following formula.

$$h^2 \text{ (Population, restricted)} = \sigma^2_P / \sigma^2_{\Sigma \text{ all variances containing population or plant effects}} \quad (7.6)$$

Heritabilities were calculated at single plant level for all measurements. The phenotypic variance obtained for the base analysis before the first grazing (Equation 7.3) was affected by multiplying Samples by three (number of plants assumed per quadrat) for LFN1 and DST. The phenotypic variance obtained for the analysis of main results (Equation 7.5) was affected by multiplying Error b by three (number of plants assumed per quadrat) for FLW, LFN1, DST, DLFN and INTK. For PRT and DGT, the phenotypic variance obtained for analysis of nutritional data (Equation 7.4) was affected by multiplying Internal Reps. by four (number of plants assumed per bulked sample/ plot). For all the characters not mentioned above, no adjustment was necessary because their measurements were already at a single plant level. Same Equation 7.6 was used to calculate the appropriate heritabilities.

Standard errors of heritabilities were obtained following Osborne and Paterson (1952) for each model (Appendix 8, 9 and 10). One tail t tests were performed for the heritabilities, dividing them by their respective standard error and using the degrees of freedom of the residual term.

7.2.7 ASSESSMENT OF PARSIMONIOUS MULTIPLE REGRESSIONS

As stated in Section 6.2.7, Mallow's Cp statistic was used to assess all possible multiple regressions to explore parsimonious equations to use instead of the full-rank model. The "best" model was chosen for a regression with a low C_P value about equal to p (number of parameters in the regression including β_0) (Draper and Smith, 1981).

7.2.8 TEST FOR TIME CORRELATION

The swards were harvested four times in successive periods, possibly causing failure of the assumption of independence of the error effects and biasing the expectation of the mean squares, the F tests, etc. The correlation across time was calculated according to the following formulae (developed from Gill, 1986; Gordon, 1994).

MS_δ = Error a = $\sigma^2_\varepsilon + t\sigma^2_S + st\sigma^2_R + rst\sigma^2_\delta$ (7.7)

MS_ε = Error b = σ^2_ε (7.8)

θ_δ = Time covariance (7.9)

$\sigma^2_\varepsilon = \sigma^2$ - Time covariance (7.10)

$\sigma^2 = \sigma^2_\varepsilon + \sigma^2_\delta$ (7.11)

Corr. Across Time = $\sigma^2_\delta / (\sigma^2_\varepsilon + \sigma^2_\delta)$ (7.12)

The t value to test the significance of the time correlation was calculated in the following way (Steel and Torrie, 1980).

$$t = \frac{r}{\sqrt{(1-r^2)/(n-2)}} \qquad (7.13)$$

where r = correlation across Time and n = total number of plots in each grazing period.

7.2.9 METHOD USED TO ESTIMATE CORRELATIONS

Multivariate analyses of variance (MANOVA) were performed to obtain the matrices of sums of squares (ss) and sums of cross products (sscp) at the Population partition level for: all morphological characters and nutritional characters (Section 7.3.3.4); all morphological characters (Section 7.3.4.4) and for characters measured as spaced plants and swards (Section 7.3.5.2.1). The following equation is an example of the latter case that was used to estimate each genetic correlation (r_g) (Gordon, 1994).

$$r_g = corr_{sp.plant,sward} = \frac{sscp_{sp.plant,sward}}{\sqrt{ss_{sp.plant} * ss_{sward}}} \qquad (7.14)$$

Phenotypic correlations were also calculated with Equation 7.14, but the total variance (phenotype) was used instead of a genetic partition.

7.3 RESULTS

7.3.1 BASE CONDITION OF THE POPULATIONS BEFORE GRAZING WAS IMPOSED

The base properties of the plant materials before grazing were described by five characters (HGT1, LFN1, DST, LSZ and FLW) with the purpose of comparing these same attributes after the grazing animal effects.

7.3.1.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was done. Results of the analyses of variance (F tests), variance components with their respective standard errors for all characters measured before the first grazing are presented in Table 7.5.

Table 7.5: Significance of the analysis of variance (F tests), variance components with their respective standard errors for all characters measured before the first grazing

Characters	Block	Population	Error	Internal Rep.	Samples
HGT1	0.06^{NS}	87.66**	-0.75^{NS}	2.13**	7.49
	(0.12)	(46.96)	(0.39)	(0.94)	(0.83)
LFN1	-0.06^{NS}	14.26**	0.15^{NS}	0.53**	0.77
	(0.03)	(7.69)	(0.17)	(0.17)	(0.09)
DST	-0.00^{NS}	0.20**	0.02^{NS}	0.01^{NS}	0.18
	(0.00)	(0.11)	(0.01)	(0.01)	(0.02)
LSZ	0.00^{NS}	0.84**	0.01^{NS}	0.07*	0.40
	(0.01)	(0.46)	(0.03)	(0.04)	(0.04)

NS Non significant
** Significant at 0.01 level

Population and Internal Repetitions effects were significant for all characters except DST for Internal Rep.. Block and Error effects were not significant for any character.

7.3.1.2 ANALYSIS OF MEANS

Population effect was highly significant for all characters measured pre-grazing, therefore mean separation was performed to describe the populations. Grand means and means per Population with their respective standard errors for all characters measured before the first grazing are presented in Table 7.6.

Table 7.6: Grand means and means per Population with their respective standard errors for all characters measured before the first grazing

Characters	Grand mean	Hamua	Colenso	Kenland	E116	Astred	Turoa	S. error
HGT1	23.44 (0.32)[1]	20.25d[2]	19.17d	22.33c	41.03a	24.42b	13.47e	0.44
LFN1	78.29 (0.25)	79.86a	79.86a	80.00a	70.56b	79.44a	80.00a	0.36
DST	3.57 (0.04)	3.79a	3.68a	3.82a	2.67b	3.63a	3.86a	0.10
LSZ	4.64 (0.07)	4.53c	4.25c	5.19b	5.67a	5.17b	3.06d	0.14
FLW	0.00 (0.00)	0.00	0.00	0.00	0.00	0.00	0.00	0.00

1 Standard error of the grand mean
2 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

E116 was the tallest population for the first grazing and Turoa on the other hand, was the shortest population. LFN1 and DST were very similar for all populations, E116 being the one with least leaves and density for this first grazing. The biggest leaves were found in E116, Kenland and Astred. All populations were in a vegetative state.

7.3.1.3 HERITABILITIES

Base heritability values and their respective standard errors for all characters measured before the first grazing are presented in Table 7.7.

Table 7.7: Base heritabilities and standard errors for all characters measured before the first grazing

Characters	Population Restricted[1]
HGT1	0.91** (0.05)
LFN1	0.83** (0.08)
DST	0.25** (0.11)
LSZ	0.64** (0.13)

** Significant at 0.01 level
1 Equations 7.6 (Section 7.2.6)

Heritability values for HGT1 and LFN1 were very high, and those for DST and LSZ were medium.

7.3.2 EVALUATION OF NEED FOR DATA ADJUSTMENT

Before beginning with the analysis of the main results, it was necessary to evaluate the necessity for adjustment for repeated measurements (7.3.2.1) and for location of the swards (internal replicates) with reference to the electric fence (7.3.2.2). Results for these issues are considered next.

7.3.2.1 REPEATED MEASUREMENTS ANALYSIS

The correlation across time (Equations 7.7...7.12) was calculated for each character (Table 7.8) to determine if there was any adjustment necessary for repeated measures effects (Section 7.2.8).

Table 7.8: Correlation across time (n=216[1])

Characters	Correlation across time
HGT1	-0.005^{NS}
LFN1	-0.038^{NS}
DST	0.045^{NS}
LSZ	0.020^{NS}
FLW	-0.013^{NS}
HGT2	0.003^{NS}
LFN2	-0.151*

1 Total number of plots in each grazing period
2 t value (Section 7.2.8)
NS Non significant
* Significant at 0.05 level

Correlation across time was significant for only one character (LFN2) and that correlation was very small (-0.15), so it was decided not to correct the data for such a small correlation.

7.3.2.2 SWARD LOCATION ANALYSIS

The significance of the F tests of sward location (concomitant dummy) with post-grazing (HGT2 and LFN2) and differences pre- and post-grazing measurements (DHGT and DLFN) at a time were studied (see Section 6.3.1.2). The location (concomitant dummy) was not significant for any post-grazing or differences pre- and post-grazing concomitant analysis (F tests).

Simple regressions between HGT2, LFN2, DHGT, and DLFN at a time with location were performed, all R^2 being not significant.

Considering the effect on Error partition (concomitant analysis) and the overall effect on the simple regressions, location was not considered further in the analysis.

7.3.3 RESULTS FOR NUTRITIONAL DATA

Quality measurements (crude protein content and digestibility) were taken with the purpose of studying their relation with animal preference.

7.3.3.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and the significance of them, an analysis of variance was performed. Results of the analysis of variance (F tests), variance components with their respective standard errors for protein and digestibility are presented in Table 7.9.

Table 7.9: Significance of the analysis of variance (F tests), variance components with their respective standard errors for protein and digestibility

Characters	Block	Population	Error	Internal Reps.
PRT	0.05^{NS}	0.59*	0.57*	1.49
	(0.18)	(0.53)	(0.45)	(0.35)
DGT	0.25^{NS}	3.07**	0.04^{NS}	2.15
	(0.27)	(1.78)	(0.35)	(0.51)

NS Non significant
** Significant at 0.01 level

The Population effect was significant for PRT and DGT. Block was not significant for PRT and DGT and Error was significant only for PRT.

7.3.3.2 ANALYSIS OF MEANS

The Population effect was significant for both quality characters, therefore mean separation was performed. Grand means and means per Population with their respective standard errors for protein and digestibility are presented in Table 7.10.

Table 7.10: Grand means and means per Population with their respective standard errors for protein and digestibility

Characters	Grand means	Hamua	Colenso	Kenland	E116	Astred	Turoa	S. error
PRT	19.59 (0.22)[1]	20.16a[2]	19.67a	20.60a	19.79a	17.76b	19.54a	0.60
DGT	68.89 (0.30)	68.61c	68.81bc	71.69a	70.05b	66.46d	67.69cd	0.50

1 Standard errors of the grand means
2 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

Astred had a lower crude protein level than all other populations. Kenland, followed by E116 had the highest values of DGT, while again Astred had the lowest.

7.3.3.3 HERITABILITIES

To evaluate the usefulness of protein and digestibility for plant breeding, the heritabilities were estimated at a single plant level (see Section 7.2.6). Heritability values and their respective standard errors are presented in Table 7.11.

Table 7.11: Heritabilities and standard errors for protein and digestibility

Characters	Population Restricted[1]
PRT	0.08 ^{NS} (0.07)
DGT	0.26** (0.11)

NS Non significant
** Significant at 0.01 level
1 Equations 7.6 (Section 7.2.6)

Heritability values for protein were medium to low but not significantly different from zero ($P \geq 0.05$). Estimates of heritability for digestibility were medium, meaning that digestibility would be easier to be improved by breeding than crude protein.

7.3.3.4 CORRELATIONS AND REGRESSIONS

Correlations between nutritional parameters (PRT and DGT) and morphological characteristics were explored with the purpose of revealing relationships at a phenotypic and genotypic level (Section 7.2.9). Phenotypic and genotypic correlations are presented in Table 7.12.

Table 7.12: Phenotypic and genotypic correlations with protein and digestibility for morphological characters

	Phenotypic		Genotypic	
	PRT	DGT	PRT	DGT
HGT1	0.16**	0.35**	0.23**	0.47**
LFN1	0.19**	0.05^{NS}	0.41**	0.13*
DST	0.35**	0.31**	0.60**	0.72**
LSZ	0.21**	0.40**	0.43**	0.69**
FLW	-0.27**	-0.29**	-0.48**	-0.38**
HGT2	0.11^{NS}	0.18**	-0.11^{NS}	0.26**
LFN2	0.23**	0.05^{NS}	0.23**	0.10^{NS}
DHGT	0.10^{NS}	0.25**	0.42**	0.46**
DLFN	-0.11^{NS}	-0.02^{NS}	0.34**	0.07^{NS}
INTK	-0.02^{NS}	0.16**	0.85**	0.67**
PRT	1.00**	0.62**	1.00**	0.83**
DGT	0.62**	1.00**	0.83**	1.00**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

Phenotypic correlations with protein and digestibility were not strong, 0.40 for LSZ-DGT being the highest correlation. Genotypic correlations were stronger in all cases than their respective phenotypic correlation. The strongest genotypic correlations were obtained between intake and protein (0.85**) and intake and digestibility (0.67**), meaning a gratifying close relationship between animal intake and quality. Also protein and digestibility had good phenotypic and genotypic correlations among themselves.

Multiple regressions of protein and digestibility with pre-grazing characters, differences pre- and post-grazing, post-grazing measurements or intake (Tables 7.13...7.18) were explored to assess the possibility of using those characters to predict quality and avoid measuring quality because of the high cost involved in obtaining the material, preparing the

material for chemical analyses and the chemical analyses *per se*. All possible regressions were assessed using Mallow's Cp statistic for the sake of parsimony. As stated in Section 7.2.7, the "best" models were chosen for regressions with low Cp values about equal to p (number of parameters including β_0).

Simple and multiple regressions with protein for characters measured pre-grazing are presented in Table 7.13.

Table 7.13: Simple and multiple regressions with protein for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2
Best 1-variable model	DST	2	2.92	0.13**
Best 2-variable model	FLW and HGT1	3	1.82	0.18**
Best 3-variable model	FLW, DST and HGT1	4	2.05	0.21**
Best 4-variable model	LSZ, FLW, DST and HGT1	5	4.03	0.21**
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.21**

** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Two variables (FLW and HGT1) were enough to explain almost all the variation in PRT that could be explained by the pre-grazing measurements. The two variable model was considered the "best" parsimonious equation according to Mallow's Cp statistic. The standardised betas for that equation were -0.43 and 0.36 for FLW and HGT1 respectively. None of the equations were considered good to predict protein.

Simple and multiple regressions with digestibility for characters measured pre-grazing are presented in Table 7.14.

Table 7.14: Simple and multiple regressions with digestibility for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2
Best 1-variable model	LSZ	2	17.53	0.16**
Best 2-variable model	FLW and HGT1	3	2.24	0.37**
Best 3-variable model	FLW, HGT1 and LFN1	4	3.02	0.39**
Best 4-variable model	LSZ, FLW, HGT1 and LFN1	5	4.07	0.40**
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.40**

** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

DGT was well explained (according to Mallow's Cp statistic) by the parsimonious equation of the two variable model (FLW and HGT1), almost as powerfully as if all pre-grazing measurements were used. The standardised betas for that equation were -0.56 and 0.60 for FLW and HGT1 respectively. None of the equations were considered good to predict digestibility.

Simple and multiple regressions with protein for characters measured post-grazing are presented in Table 7.15.

Table 7.15: Simple and multiple regressions with protein for characters measured post-grazing

Model	Variables	p[1]	Cp[2]	R^2
Best 1-variable model	LFN2	2	1.38	0.05^{NS}
Best 2-variable model	HGT2 and LFN2	3	3.00	0.06^{NS}

NS Non significant
1 Model's rank
2 Mallow's Cp statistic

PRT could not be explained by post-grazing measurements. The standardised betas for the best parsimonious equation (one variable) was 0.24 for LFN2.

Simple and multiple regressions with digestibility for characters measured post-grazing are presented in Table 7.16.

Table 7.16: Simple and multiple regressions with digestibility for characters measured post-grazing

Model	Variables	p^1	Cp^2	R^2
Best 1-variable model	HGT2	2	1.07	0.03^{NS}
Best 2-variable model	HGT2 and LFN2	3	3.00	0.03^{NS}

NS Non significant
1 Model's rank
2 Mallow's Cp statistic

DGT could not be explained by post-grazing measurements. The standardised betas for the best parsimonious equation (one variable) was 0.17 for HGT2.

Simple and multiple regressions with protein for differences pre- and post-grazing are presented in Table 7.17.

Table 7.17: Simple and multiple regressions with protein for differences pre- and post-grazing

Model	Variables	p^1	Cp^2	R^2
Best 1-variable model	DLFN	2	1.32	0.01^{NS}
Best 2-variable model	DHGT and DLFN	3	3.00	0.02^{NS}

NS Non significant
1 Model's rank
2 Mallow's Cp statistic

PRT could not be explained by differences pre- and post-grazing. The standardised betas for the best parsimonious equation (one variable) was -0.11 for DLFN.

Simple and multiple regressions with digestibility for differences pre- and post-grazing are presented in Table 7.18.

Table 7.18: Simple and multiple regressions with digestibility for differences pre- and post-grazing

Model	Variables	p[1]	Cp[2]	R^2
Best 1-variable model	DHGT	2	1.05	0.06^{NS}
Best 2-variable model	DHGT and DLFN	3	3.00	0.06^{NS}

NS Non significant
1 Model's rank
2 Mallow's Cp statistic

DGT could not be explained by post-grazing measurements. The standardised betas for the best parsimonious equation (one variable) was 0.25 for DHGT.

Simple regression of protein or digestibility with intake, were not significant with R^2 of 0.0005 and 0.03 for protein and digestibility respectively.

Canonical correlations between protein and digestibility with the pre-grazing characters, post-grazing measurements, differences pre- and post-grazing or all post-grazing, differences pre- and post-grazing and intake as another set are presented in Table 7.19.

Table 7.19: Canonical correlations between protein and digestibility with pre-grazing characters, post-grazing measurements, differences pre- and post-grazing or all post-grazing, differences pre- and post-grazing and intake

Dependent variables	Independent variables	Canonical correlation[1]
PRT and DGT	LSZ, FLW, DST, HGT1 and LFN1	0.63**
PRT and DGT	HGT2 and LFN2	0.26**
PRT and DGT	DHGT and DLFN	0.27**
PRT and DGT	HGT2, LFN2, DHGT, DLFN and INTK	0.41**

1 Only the first canonical correlation was considered for each correlation because they accounted for the 0.89%, 0.71%, 0.83% and 0.70% of the variation respectively.

** Significant at 0.01 level

These canonical correlations were performed with the purpose of revealing relationships among sets of characters. Canonical correlations of quality characters with pre-grazing characters were medium and medium to low with post-grazing, differences pre- and post-grazing or all post-grazing, differences pre- and post-grazing and intake, therefore not revealing strong links among any set of characters.

From all simple, multiple and canonical correlations performed it is concluded that protein and digestibility could not be predicted from any measured character in the field at the phenotypic level, emphasising the importance of measuring quality.

7.3.4 RESULTS FOR THE MAIN ANALYSIS

All results for all characters were analysed according to the design in Methods (Section 7.2.5.3, Equation 7.5) with the purpose of evaluating the behaviour of the plant material under a sward environment with reference to animal preference.

7.3.4.1 ANALYSIS OF VARIANCE

To study the relative importance of the different partitions in the model and their significance, an analysis of variance was performed. Results of the main analysis of variance (F tests), variance components and their respective standard errors are presented in Table 7.20.

Table 7.20: Significance of the analysis of variance (F tests), variance components with their respective standard errors for all characters

Characters	Blocks	Population	Error a	Inter.Reps.	Samples	Time	TimexPop.	Error b
HGT1	1.04**	28.64**	-0.08^{NS}	1.47**	-0.39^{NS}	8.92**	11.01**	15.70
	(0.82)	(16.94)	(0.34)	(0.55)	(0.45)	(6.88)	(3.92)	(0.88)
LFN1	1.06*	10.83**	-0.89^{NS}	4.46**	-1.55^{NS}	99.08**	4.75**	24.16
	(0.87)	(6.62)	(0.58)	(1.29)	(0.60)	(63.24)	(1.86)	(1.36)
DST	0.02*	-0.01^{NS}	0.01*	0.00^{NS}	0.01*	0.04*	0.12**	0.19
	(0.01)	(0.02)	(0.01)	(0.00)	(0.01)	(0.04)	(0.04)	(0.01)
LSZ	0.00^{NS}	0.53**	0.01^{NS}	0.03**	-0.00^{NS}	0.89**	0.09**	0.35
	(0.00)	(0.30)	(0.01)	(0.01)	(0.01)	(0.58)	(0.03)	(0.02)
FLW	0.09*	4.10**	-0.09^{NS}	0.36**	-0.32^{NS}	22.86**	4.08**	7.06
	(0.08)	(2.79)	(0.08)	(0.17)	(0.19)	(14.91)	(1.47)	(0.40)
HGT2	0.79*	6.89**	0.04^{NS}	2.50**	0.08^{NS}	2.41*	3.39**	11.72
	(0.69)	(4.35)	(0.52)	(0.75)	(0.37)	(1.93)	(1.27)	(0.66)
LFN2	13.47**	2.23^{NS}	-4.93^{NS}	12.26**	5.44**	30.05**	6.78**	37.64
	(9.57)	(2.27)	(1.23)	(3.69)	(1.72)	(19.83)	(2.68)	(2.12)
DHGT	0.35*	6.66**	-0.29^{NS}	1.05**	-0.94^{NS}	4.02*	5.41**	23.01
	(0.30)	(4.39)	(0.25)	(0.53)	(0.62)	(3.20)	(2.08)	(1.30)
DLFN	7.61**	3.93*	-5.48^{NS}	15.83**	-0.15^{NS}	65.94**	8.35**	55.34
	(5.49)	(3.49)	(1.52)	(4.43)	(1.70)	(42.76)	(3.39)	(3.12)
INTK	0.04**	0.02^{NS}	-0.03^{NS}	0.11**	-0.02^{NS}	0.59**	0.11**	0.58
	(0.03)	(0.03)	(0.01)	(0.03)	(0.02)	(0.39)	(0.04)	(0.03)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

Time and Time by Population effects were significant for all characters. Population, Block and Internal Repetitions effects were significant for all characters except DST, LFN2 and INTK for Population effect, LSZ for Block effect and DST for Internal Repetitions effect. Error a and Samples effects were not significant for any character except DST for Error a effect and DST and LFN2 for Samples effect.

7.3.4.2 ANALYSIS OF MEANS

Main effects (Population and Time) will be presented and commented on briefly because the interaction (Population by Time) was highly significant for all characters.

Grand means, coefficients of variation and means per Time with their respective standard errors for all characters are presented in Table 7.21.

Table 7.21: Grand means, coefficients of variation and means per Time with their respective standard errors for all characters

Characters	Grand means	Coefficients of variation	13/11/95	13/12/95	18/01/96	21/02/96	S. error
HGT1	23.18 (0.26)[1]	32.9	23.44b[2]	27.57a	21.95c	19.77d	0.27
LFN1	65.15 (0.32)	16.5	78.29a	67.59b	57.55c	57.18c	0.33
DST	3.21 (0.02)	18.8	3.57a	3.05c	3.13b	3.07bc	0.03
LSZ	4.47 (0.04)	28.2	4.64b	5.69a	4.11c	3.42d	0.04
FLW	5.72 (0.18)	98.3	0.00d	3.45c	9.01b	10.42a	0.18
HGT2	12.92 (0.17)	39.2	11.38c	15.39a	12.76b	12.16b	0.23
LFN2	21.66 (0.32)	43.9	28.22a	17.45c	24.40b	16.57c	0.42
DHGT	10.26 (0.21)	59.1	12.06a	12.18a	9.19b	7.61c	0.33
DLFN	43.49 (0.39)	26.4	50.07a	50.14a	33.15c	40.60b	0.51
INTK	8.31 (0.04)	13.4	8.96a	8.98a	7.47c	7.82b	0.05

1 Standard errors of the grand means

2 Values within grazing dates, followed by the same letter do not differ significantly ($P \geq 0.05$)

FLW had a high coefficient of variation, while the other characters had medium values varying from 13% to 60%. The second grazing was done with the tallest swards, while

the fourth grazing was done with the shortest ones. LFN1 decreased with each successive grazing, while FLW showed the opposite effect; at the first grazing plants were completely vegetative. Swards were denser for the first grazing than for later grazings. Biggest leaves were found in the second grazing and the smallest in the last grazing. After grazing, the tallest swards were in the second grazing and the most leafy ones were found in the first grazing. The largest differences in height, leafiness and INTK were obtained in the first and second, followed by the fourth and third grazings.

Means and standard errors per Population for all characters are presented in Table 7.22.

Table 7.22: Means and standard errors per Population for all characters

Characters	Hamua	Colenso	Kenland	E116	Astred	Turoa	S. error
HGT1	23.34b[1]	21.50c	24.28b	31.99a	23.54b	14.44d	0.48
LFN1	66.01bc	64.24c	66.81b	59.44d	64.34c	70.07a	0.57
DST	3.28	3.11	3.35	2.93	3.22	3.34	0.07
LSZ	4.64b	4.19c	5.13a	5.10a	4.62b	3.11d	0.09
FLW	5.45c	5.52c	3.72d	9.06a	7.53b	3.02d	0.22
HGT2	12.69b	12.08b	12.78b	17.49a	13.83b	8.67c	0.61
LFN2	22.43	19.65	21.81	18.85	23.89	23.33	0.36
DHGT	10.65bc	9.42d	11.50b	14.50a	9.72cd	5.77e	0.39
DLFN	43.58b	44.58b	45.00ab	40.59c	40.45c	46.74a	0.56
INTK	8.36	8.29	8.55	8.57	8.04	8.04	0.07

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

DST, LFN2 and INTK had no significant F tests, therefore means were statistically the same for those characters. The tallest population pre- and post-grazing was E116 and the shortest was Turoa. The opposite situation was found for leafiness pre- and post-grazing. Kenland and E116 had the biggest leaves and Turoa the smallest. E116 was the population that flowered most, and Kenland and Turoa least. The largest reductions in height during grazing were found in E116 and the smallest in Turoa. The situation was reversed for DLFN.

Means and standard errors per Population for each Grazing for all characters are presented in Table 7.23.

Table 7.23: Means and standard errors per Population for each Grazing for all characters

Characters	Hamua	Colenso	Kenland	E116	Astred	Turoa	S. error
13/11/95							
HGT1	20.25d[1]	19.17d	22.33c	41.03a	24.42b	13.47e	0.44
LFN1	79.86a	79.86a	80.00a	70.56b	79.44a	80.00a	0.36
DST	3.79a	3.68a	3.82a	2.67b	3.63a	3.86a	0.10
LSZ	4.53c	4.25c	5.19b	5.67a	5.17b	3.06d	0.14
FLW	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HGT2	10.31bc	8.75cd	10.19bc	20.50a	11.58b	6.94d	0.71
LFN2	30.28ab	26.94b	30.28ab	19.86c	33.06a	28.89ab	1.56
DHGT	9.94d	10.42cd	12.14bc	20.53a	12.83b	6.53e	0.57
DLFN	49.58ab	52.92a	49.72ab	50.69ab	46.39b	51.11ab	1.78
INTK	8.70bc	8.98b	8.95b	9.93a	8.79bc	8.43c	0.16
13/12/95							
HGT1	28.97bc	27.50c	31.83ab	32.94a	28.31bc	15.86d	1.17
LFN1	67.50b	66.53b	65.83b	62.22b	66.25b	72.22a	1.99
DST	3.03b	2.96b	2.75c	2.76c	2.99b	3.82a	0.05
LSZ	5.86a	5.94a	6.28a	5.97a	5.86a	4.19b	0.13
FLW	0.83c	1.81c	1.81c	9.86a	6.39b	0.00c	0.64
HGT2	16.33b	13.00bc	16.03b	20.81a	16.36b	9.83c	1.19
LFN2	17.22bc	15.83c	16.11c	18.89ab	20.00a	16.67bc	0.80
DHGT	12.64ab	14.50ab	15.81a	12.14b	11.94b	6.03c	1.02
DLFN	50.28bc	50.69b	49.72bc	43.33c	46.25bc	60.56a	2.28
INTK	9.04ab	9.27ab	9.35a	8.50b	8.68ab	9.04ab	0.26
18/01/96							
HGT1	23.14b[1]	20.36c	22.06bc	28.47a	20.78bc	16.89d	0.79
LFN1	59.72a	55.00b	61.67a	51.67c	55.00b	62.22a	0.99
DST	3.24b	2.94b	3.57a	3.04b	3.01b	3.00b	0.10
LSZ	4.58a	3.53b	4.94a	4.97a	3.83b	2.81c	0.14
FLW	9.44b	11.25a	5.00d	11.39a	10.97a	5.97c	0.30
HGT2	12.53a	13.94a	12.97a	14.64a	13.81a	8.67b	0.79
LFN2	24.72b	19.44c	26.39ab	23.89b	23.33b	28.61a	1.17
DHGT	10.61b	6.42c	9.08bc	13.83a	6.97c	8.22bc	1.02
DLFN	35.00ab	35.56a	35.28ab	27.78c	31.67b	33.61ab	1.15
INTK	7.75a	7.33ab	7.61a	7.60a	7.12b	7.40ab	0.14
21/02/96							
HGT1	21.00b	18.97c	20.89b	25.53a	20.67b	11.56d	0.34
LFN1	56.94bc	55.56c	59.72ab	53.33c	56.67bc	60.83a	1.15
DST	3.08ab	2.85bc	3.28a	3.26a	3.24a	2.69c	0.11
LSZ	3.58b	3.03c	4.11a	3.81ab	3.61b	2.39d	0.10
FLW	11.53b	9.03c	8.06c	15.00a	12.78b	6.11d	0.50
HGT2	11.58b	12.61ab	11.92b	14.03a	13.56a	9.25c	0.47
LFN2	17.50ab	16.39abc	14.44bc	12.78c	19.17a	19.17a	1.43
DHGT	9.42ab	6.36d	8.97bc	11.50a	7.11cd	2.31e	0.69
DLFN	39.44b	39.17b	45.28a	40.56ab	37.50b	41.67ab	1.68
INTK	7.93ab	7.58bc	8.29a	8.24a	7.55bc	7.31c	0.15

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

E116 was the tallest sward pre- and post-grazing for all four grazings. The shortest population was always Turoa. All populations showed similar LFN1 for the first grazing except E116 that was less leafy. For the second grazing only Turoa was more leafy than the rest. Turoa, Kenland and Hamua were the most leafy for the third grazing and Turoa and Kenland for the last grazing. E116 was the least dense population for the first three grazings, while Turoa was for the fourth. Kenland had the biggest leaves for grazings two and four, E116 for the first grazing and Hamua for the third grazing. Turoa was always the population with smallest leaves. E116 and Astred were the populations that flowered most in the second grazing, E116 and Colenso in the third one and E116 and Astred in the fourth grazing. Turoa was the lowest flowering population and also the only one not to begin the flowering period until the third grazing. The rest began flowering before the second grazing. E116 was the population with least leaves post-grazing for grazings one and four, while Colenso was for grazings two and three. The most leafy populations post-grazing were Astred for grazings one, two and four, and Turoa for the third grazing. DHGT was highest for E116 in grazings one, three and four, and Kenland for grazing two. Minimum DHGT were found in Turoa for grazings one, two and four and Colenso for grazing three. DLFN and INTK were very variable from grazing period to grazing period, greater values being found for E116 in the first grazing, Turoa in the second grazing, Colenso and Kenland in the third and Kenland in the fourth grazing.

7.3.4.3 HERITABILITIES

To assess the usefulness of the measured characters from a plant breeder's point of view, heritabilities should be known. Heritabilities were estimated as detailed in Section 7.2.6. Heritabilities and standard errors for all characters are presented in Table 7.24.

Table 7.24: Heritabilities and standard errors for all characters

Characters	Population Restricted[1]
HGT1	0.51** (0.15)
LFN1	0.12* (0.06)
DST	0.00^{NS} (0.03)
LSZ	0.53** (0.14)
FLW	0.14^{NS} (0.08)
HGT2	0.28** (0.13)
LFN2	0.02^{NS} (0.02)
DHGT	0.19^{NS} (0.10)
DLFN	0.02^{NS} (0.02)
INTK	0.01^{NS} (0.02)

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Equations 7.6 (Section 7.2.6)

Heritability for HGT1 and LSZ were medium to high. LFN1 and HGT2 were medium to low values, and other heritabilities were not significantly different from zero. From the results of this experiment, genetic progress in characters considering the animal effects under sward conditions could only be obtained by selection in post-grazing height.

7.3.4.4 CORRELATIONS AND REGRESSIONS

Correlations were explored among characters with the purpose of revealing relationships among them at a phenotypic and genotypic level (Section 7.2.9). Phenotypic and genotypic correlations amongst all characters are presented in Table 7.25.

Table 7.25: Phenotypic and genotypic correlations amongst all sward characters[1]

	HGT1	LFN1	DST	LSZ	FLW	HGT2	LFN2	DHGT	DLFN	INTK
HGT1	1	-0.03 ^{NS}	-0.18**	0.65**	0.02 ^{NS}	0.61**	-0.03 ^{NS}	0.75**	-0.01 ^{NS}	0.44**
LFN1	-0.91**	1	0.43**	0.21**	-0.71**	-0.20**	0.36**	0.13**	0.63**	0.54**
DST	-0.72**	0.92**	1	-0.00 ^{NS}	-0.21**	-0.22**	0.34**	-0.04 ^{NS}	0.12**	0.06 ^{NS}
LSZ	0.89**	-0.68**	-0.36**	1	-0.25**	0.43**	0.03 ^{NS}	0.46**	0.17**	0.40**
FLW	0.81**	-0.93**	-0.85**	0.56**	1	0.20**	-0.18**	-0.14**	-0.52**	-0.46**
HGT2	0.99**	-0.95**	-0.77**	0.83**	0.89**	1	-0.01 ^{NS}	-0.06 ^{NS}	-0.18**	-0.17**
LFN2	-0.62**	0.71**	0.79**	-0.39**	-0.43**	-0.57**	1	-0.03 ^{NS}	-0.49**	-0.37**
DHGT	0.99**	-0.85**	-0.64**	0.92**	0.70**	0.94**	-0.64**	1	0.15**	0.70**
DLFN	-0.78**	0.83**	0.65**	-0.63**	-0.95**	-0.86**	0.19**	-0.67**	1	0.81**
INTK	0.73**	-0.51**	-0.36**	0.75**	0.22**	0.61**	-0.71**	0.83**	-0.15**	1

1 Phenotypic correlations upper triangle; Genotypic correlations lower triangle
NS Non significant
** Significant at 0.01 level

From the 45 pairs (one phenotypic and one genotypic correlation of the same pair of characters), 11 showed a change in direction from a positive correlation to a negative correlation or vise-versa, 15 had a change in magnitude greater than 0.5, and the rest were of the same sign and of a similar magnitude. One example of change in direction (sign) was DHGT with LSZ, the phenotypic correlation being 0.21 and the genotypic correlation -0.85. An example of change in magnitude was LFN1 with HGT1, the phenotypic correlation being -0.03 and the genotypic correlation -0.91.

Multiple regressions of the pre-grazing characters with intake, differences pre- and post-grazing or post-grazing measurements (Tables 7.26...7.30) were explored to assess the possibility of using those characters to predict the results obtained post-grazing. All possible regressions were assessed using Mallow's Cp statistic for the sake of parsimony. As stated in Section 7.2.7, the "best" models were chosen for regressions with low Cp values about equal to p (number of parameters including β_0).

Simple and multiple regressions with intake for characters measured pre-grazing are presented in Table 7.26.

Table 7.26: Simple and multiple regressions with Intake for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5
Best 1-variable model	LFN1	2	415.59	0.29**	4.69**	0.06**				
Best 2-variable model	HGT1 and LFN1	3	38.61	0.50**	3.05**	0.07**	0.06**			
Best 3-variable model	FLW, HGT1 and LFN1	4	19.41	0.51**	3.96**	-0.03**	0.07**	0.05**		
Best 4-variable model	FLW, DST, HGT1 and LFN1	5	7.42	0.52**	4.22**	-0.03**	-0.19**	0.06**	0.05**	
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.52**	4.29**	-0.05^{NS}	-0.03**	-0.18**	0.07**	0.05**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

Two variables (HGT1 and LFN1) were enough to explain almost all the variation in INTK that could be explained by the pre-grazing measurements. No parsimonious equation was considered good according to Mallow's Cp statistic. The standardised betas for the full rank model were -0.06, -0.15, -0.10, 0.48 and 0.50 for LSZ, FLW, DST, HGT1 and LFN1 respectively.

Simple and multiple regressions with difference in height for characters measured pre-grazing are presented in Table 7.27.

Table 7.27: Simple and multiple regressions with difference in height for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5
Best 1-variable model	HGT1	2	86.87	0.56**	-3.55**	0.60**				
Best 2-variable model	FLW and HGT1	3	35.52	0.59**	-2.64**	-0.17**	0.60**			
Best 3-variable model	LSZ, FLW and HGT1	4	17.59	0.60**	-1.16*	-0.65**	-0.21**	0.67**		
Best 4-variable model	LSZ, FLW, DST and HGT1	5	8.10	0.60**	-3.86**	-0.69**	-0.19**	0.77**	0.68**	
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.60**	-5.90**	-0.71**	-0.15**	0.56*	0.68**	0.04*

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

DHGT could be explained by HGT1 almost as powerfully as if all pre-grazing measurements were used. No parsimonious equation was considered good according to Mallow's Cp statistic. The standardised betas for the full rank model were -0.15, -0.14, -0.06, 0.86 and 0.07 for LSZ, FLW, DST, HGT1 and LFN1 respectively.

Simple and multiple regressions with difference in leafiness for characters measured pre-grazing are presented in Table 7.28.

Table 7.28: Simple and multiple regressions with difference in leafiness for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5
Best 1-variable model	LFN1	2	51.37	0.40**	-0.74^{NS}	0.68**				
Best 2-variable model	DST and LFN1	3	9.08	0.43**	5.14*	-3.60**	0.77**			
Best 3-variable model	FLW, DST and LFN1	4	3.36	0.44**	11.02**	-0.21**	-3.37**	0.68**		
Best 4-variable model	FLW, DST, HGT1 and LFN1	5	4.99	0.44**	11.67**	-0.21**	-3.44**	-0.02^{NS}	0.68**	
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.44**	11.21**	0.33^{NS}	-0.19*	-3.47**	-0.06^{NS}	0.68**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

DLFN could be explained by LFN1 almost as powerfully as using all pre-grazing variables. The 3 variable model was considered the best parsimonious solution with standardised betas of -0.10, -0.18 and 0.64 for FLW, DST and LFN1 respectively.

Simple and multiple regressions with height measured post-grazing for characters measured pre-grazing are presented in Table 7.29.

Table 7.29: Simple and multiple regressions with height measured post-grazing for characters measured pre-grazing

Model	Variables	p[1]	Cp[2]	R^2	β_0	β_1	β_2	β_3	β_4	β_5
Best 1-variable model	HGT1	2	86.87	0.37**	3.55**	0.40**				
Best 2-variable model	FLW and HGT1	3	35.52	0.41**	2.64**	0.17**	0.40**			
Best 3-variable model	LSZ, FLW and HGT1	4	17.59	0.42**	1.16*	0.65**	0.21**	0.33**		
Best 4-variable model	LSZ, FLW, DST and HGT1	5	8.10	0.43**	3.86**	0.69**	0.19**	-0.77**	0.32**	
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.43**	5.90**	0.71**	0.15**	-0.56*	0.32**	-0.04*

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

HGT2 could be explained only using HGT1 and other pre-grazing measurements were redundant. No parsimonious equation was considered good according to Mallow's Cp statistic. The standardised betas for the full rank model were -0.18, 0.16, -0.07, 0.48 and -0.08 for LSZ, FLW, DST, HGT1 and LFN1 respectively.

Simple and multiple regressions with leafiness measured post-grazing for characters measured pre-grazing are presented in Table 7.30.

Table 7.30: Simple and multiple regressions with leafiness measured post-grazing for characters measured pre-grazing

Model	Variables	p^1	Cp^2	R^2	β_0	β_1	β_2	β_3	β_4	β_5
Best 1-variable model	LFN1	2	51.37	0.13**	0.74^{NS}	0.32**				
Best 2-variable model	DST and LFN1	3	9.09	0.17**	-5.14**	3.60**	0.23**			
Best 3-variable model	FLW, DST and LFN1	4	3.36	0.18**	-11.02**	0.21**	3.37**	0.32**		
Best 4-variable model	FLW, DST, HGT1 and LFN1	5	4.99	0.18**	-11.67**	0.21**	3.44**	0.02^{NS}	0.32**	
5-variable model	LSZ, FLW, DST, HGT1 and LFN1	6	6.00	0.18**	-11.21**	-0.33^{NS}	0.19*	3.47**	0.06^{NS}	0.32**

NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level
1 Model's rank
2 Mallow's Cp statistic

LFN2 was not well explained (only 18% of the variation) even with all the pre-grazing variables included in the model. The 3 variable model was considered the best parsimonious solution with standardised betas of 0.12, 0.21 and 0.36 for FLW, DST and LFN1 respectively.

Canonical correlations between all characters measured pre-grazing, and differences between pre- and post-grazing measurements or the post-grazing measurements as another set are presented in Table 7.31. These two canonical regressions were chosen with the purpose of revealing the relationship among all pre-grazing characters with the characters influenced by animal effects.

Table 7.31: Canonical correlations of characters measured pre-grazing, and the differences pre- and post-grazing or post-grazing measurements

Dependent variables	Independent variables	Canonical correlation[1]
DHGT and DLFN	LSZ, FLW, DST, HGT1 and LFN1	0.78**
HGT2 and LFN2	LSZ, FLW, DST, HGT1 and LFN1	0.67**

1 Only the first canonical correlation was considered for each correlation because they accounted for the 0.67% and 0.82% of the variation respectively.
** Significant at 0.01 level

All canonical correlations were medium, not revealing strong links among any set of characters.

7.3.5 COMPARISON OF SPACED PLANT AND SWARD RESULTS

Results obtained under a spaced plant environment presented in Chapter 6 for the spaced plant-animal interaction experiments and sward environment presented in Section 7.3.4.3 were compared for the purposes of evaluating and understanding the behaviour of plant materials under those environments, from a plant breeder's point of view.

7.3.5.1 HERITABILITIES ESTIMATED UNDER SPACED PLANT AND SWARD ENVIRONMENTS

Calculation of the heritabilities estimates are needed before any decision can be made about the best environment in which to select breeding material. Heritabilities are shown in Table 7.32 for estimates obtained under a spaced plant environment from Chapter 6 (Section 6.3.2.3) and for estimates obtained under sward conditions (Section 7.3.4.3).

Table 7.32: Heritability estimates for spaced plants and swards

Characters	h^2 for spaced plants	h^2 for swards
HGT1	0.53a[1] (0.11)	0.51a (0.15)
LFN1	0.26a (0.10)	0.12a (0.06)
DST	0.20a (0.07)	0.00b (0.03)
LSZ	0.41a (0.12)	0.53a (0.14)
FLW	0.09a (0.06)	0.14a (0.08)
HGT2	0.26a (0.10)	0.28a (0.13)
LFN2	0.21a (0.10)	0.02b (0.02)
DHGT	0.32a (0.12)	0.19b (0.10)
DLFN	0.21a (0.09)	0.02b (0.02)
INTK	0.32a (0.12)	0.01b (0.02)

1 Values within the same row, followed by the same letter do not differ significantly ($P \geq 0.05$)

Heritability estimates for HGT1, LFN1, LSZ, FLW and HGT2 were statistically the same for the two estimates, while DST, LFN2, DHGT, DLFN and INTK had significantly higher values for estimates under spaced plant conditions.

7.3.5.2 CORRELATIONS AND REGRESSIONS

7.3.5.2.1 PHENOTYPIC AND GENOTYPIC CORRELATIONS

Phenotypic and genotypic correlations for individual characters measured under a spaced plant environment and sward environment are presented in Table 7.33. Genotypic and phenotypic correlations were obtained as detailed in Section 7.2.9.

Table 7.33: Phenotypic and genotypic correlations for individual characters measured under a spaced plant environment and a sward environment

Characters	Phenotypic correlation	Genotypic correlation
SP[1]-HGT1 vs Sw[2]-HGT1	0.24**	0.39**
SP-LFN1 vs Sw-LFN1	0.61**	0.82**
SP-DST vs Sw-DST	0.21**	0.87**
SP-LSZ vs Sw-LSZ	0.47**	0.66**
SP-FLW vs Sw-FLW	0.65**	0.96**
SP-HGT2 vs Sw-HGT2	0.07^{NS}	0.18**
SP-LFN2 vs Sw-LFN2	-0.04^{NS}	0.80**
SP-DHGT vs Sw-DHGT	0.23**	0.57**
SP-DLFN vs Sw-DLFN	0.31**	0.68**
SP-INTK vs Sw-INTK	0.13*	0.89**

1 Spaced Plant environment
2 Sward environment
NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

All genotypic correlations were stronger than their respective phenotypic correlations. One extreme example is LFN2 where the genotypic correlation was 0.80 and the phenotypic correlation was -0.04. The best correlations were the genotypic correlations for FLW, INTK and DST.

7.3.5.2.2 PHENOTYPIC AND GENOTYPIC CORRELATIONS BY TIME

The phenotypic and genotypic correlations for individual characters measured under spaced plant and sward environments (Section 7.2.9) at corresponding times are presented in Table 7.34. The purpose of this study was to explore if there was a different correlation pattern arising from Grazing Times.

Table 7.34: Phenotypic and genotypic correlations between individual characters measured under spaced plant and sward environments at corresponding Time

Grazing Dates	13/11/1995		13/12/1995		18/01/1996		21/02/1996	
Characters	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic
SP[1]-HGT1 vs Sw[2]-HGT1	0.44**	0.63**	0.31**	0.55**	0.22**	0.37**	0.14**	0.24**
SP-LFN1 vs Sw-LFN1	0.54**	0.80**	0.24**	0.46**	0.17**	0.74**	0.03 NS	0.81**
SP-DST vs Sw-DST	0.43**	0.91**	-0.07 NS	-0.05 NS	-0.01 NS	0.12 NS	-0.01 NS	0.15**
SP-LSZ vs Sw-LSZ	0.45**	0.88**	0.34**	0.56**	0.30**	0.60**	0.33**	0.63**
SP-FLW vs Sw-FLW	—	—	0.46**	0.97**	0.23**	0.74**	0.14**	0.86**
SP-HGT2 vs Sw-HGT2	0.13 NS	0.39**	0.14**	0.47**	-0.07 NS	-0.11 NS	-0.10 NS	-0.28**
SP-LFN2 vs Sw-LFN2	0.18**	0.91**	-0.16**	0.23**	-0.23**	-0.58**	0.01 NS	0.79**
SP-DHGT vs Sw-DHGT	0.44**	0.78**	0.22**	0.58**	0.17**	0.53**	0.19**	0.46**
SP-DLFN vs Sw-DLFN	0.08 NS	0.88**	0.02 NS	0.29**	-0.09 NS	0.31**	0.07 NS	0.61**
SP-INTK vs Sw-INTK	0.24**	0.70**	0.05 NS	0.47**	-0.01 NS	0.93**	0.22**	0.79**

1 Spaced Plant environment
2 Sward environment
NS Non significant
* Significant at 0.05 level
** Significant at 0.01 level

For the four grazings and all characters, the genotypic correlations were always stronger than their respective phenotypic correlations, with no change in direction. One exception was with LFN2, where the phenotypic correlation was -0.16 and the genotypic correlation was 0.23. There were 14 phenotypic correlations not significantly different from zero and three genotypic correlations not significantly different from zero.

All correlations behaved similarly for each Grazing Time except DST and LFN2. Strong correlations were obtained for DST only for the first grazing but not for the rest. LFN2 had, for example, positive and strong genotypic correlation for the first grazing, but medium and negative for the third.

7.4 DISCUSSION

7.4.1 GRAZING MANAGEMENT

The grazing management objectives for this experiment were: (1) that swards should be grazed when the semi-erect populations reached an average of 25 cm for HGT1; (2) that animals should be removed from the plots to take the post-grazing measurements when LFN2 was reduced to a level of 40%.

The overall average of the four grazings for HGT1 of the semi-erect populations was 25.9 cm, and for all populations LFN2 was 22%. The value of HGT1 was close enough to target, but LFN2 was not so close, meaning that the swards were grazed more intensely than the expected intensity.

7.4.2 HERITABILITIES WITH AND WITHOUT GRAZING INFLUENCE

Heritability values for characters measured before the first grazing (HGT1, LFN1, DST and LSZ) were compared with the same characters for all grazing periods, including the later influence of the grazing animal (Figure 7.1).

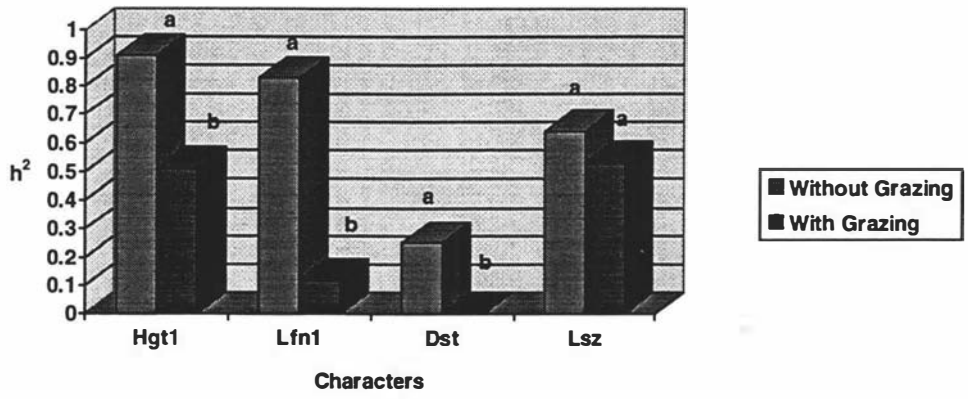

Figure 7.1: Heritability values with and without the influence of the grazing animal for pre-grazing height and leafiness, density and leaf size (bars of the same character followed by the same letter do not differ significantly ($P \geq 0.05$))

The inclusion of the grazing animal added a new source of variation into the system, reducing the heritability values on average by 0.41. All characters except LSZ suffered significant reductions in heritability values. These reductions of heritability values is the price that breeders have to pay for including the grazing animal in forage breeding, and must be balanced against the value of animal based information.

7.4.3 GRAZING CHARACTERS

From the seven measured characters, HGT2 and LFN2 were the only ones measuring the effect of the grazing animal. Variables created from them (DHGT, DLFN and INTK) were also considered to determine which were the characters useful for measuring animal effects for breeding purposes.

Only HGT2 had a heritability value significantly different from zero (0.28), identifying this as the only character likely to lead to progress by selection under sward conditions.

7.4.4 MOST GRAZED POPULATIONS

Grazing is measured better by DHGT, DLFN and INTK, so they were considered to detect the most grazed populations (Figures 7.2, 7.3 and 7.4)

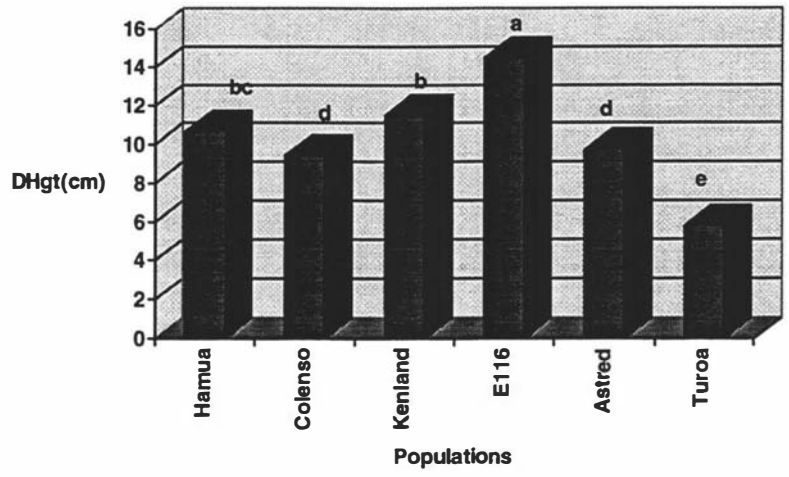

Figure 7.2: Means per Population for differences in height (bars followed by the same letter do not differ significantly ($P \geq 0.05$))

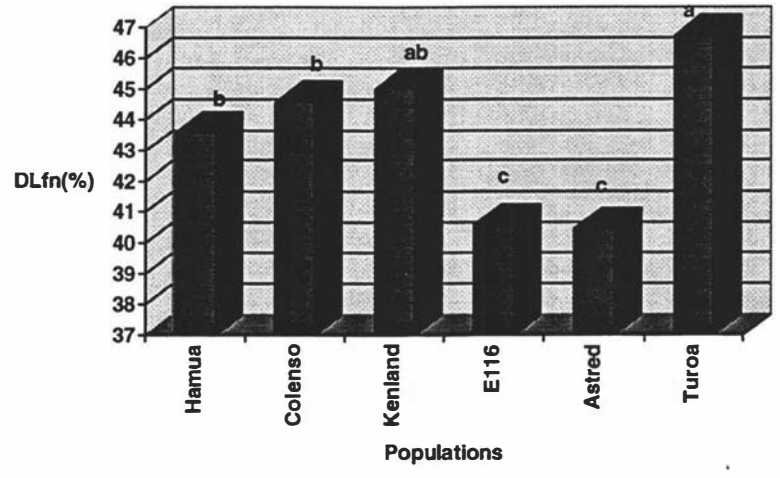

Figure 7.3: Means per Population for difference in leafiness (bars followed by the same letter do not differ significantly ($P \geq 0.05$))

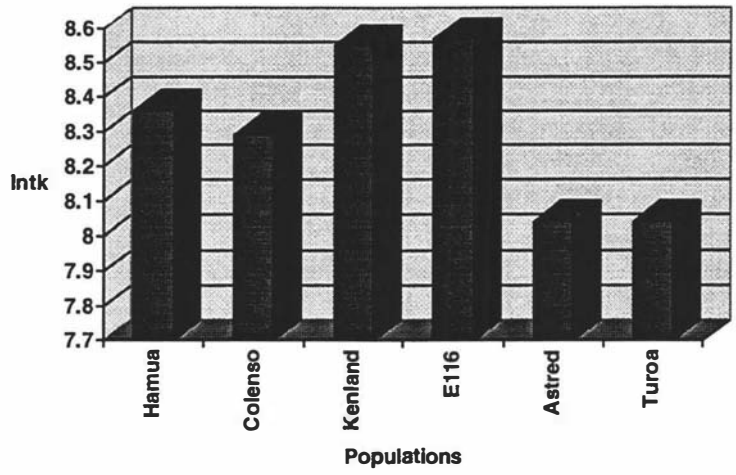

Figure 7.4: Means per Population for intake

Considering these three characters, E116 and Kenland were the most grazed populations even though differences in INTK were not significantly different. This tendency agreed with the nutritional characters, the most grazed populations being the ones with better quality characteristics (E116: PRT = 19.79 and DGT = 70.05; Kenland: PRT = 20.60 and DGT = 71.69). E116 and Kenland were also the two tallest swards and had larger leaves.

All simple, multiple and canonical regressions performed between protein and/or digestibility with other characters showed a poor relationship. This emphasised the independence and importance of quality determination to help breeders make good choices for breeding, to increase animal performance from pastures.

7.4.5 RELATIVE SELECTION EFFICIENCIES FOR SPACED PLANTS AND SWARDS

The relative selection efficiencies under a sward environment and a spaced plant environment were studied (Table 7.35) to determine the sowing method that is best to obtain genetic gains under a sward environment, which is the final use of any new cultivar. For this

reason, direct genetic advance was considered for swards and correlated genetic advance for spaced plant environments. If the ratio of the correlated response to the direct response is greater than one, it means that it is better to select in spaced plants to obtain greater genetic advance under sward conditions (Lerner and Cruden, 1948; Gordon, 1994).

For sward direct genetic advance, 5 selection intensities (i = 0.8, 0.95, 1.15, 1.4 and 1.8 for 50%, 40%, 30%, 20% and 10% respectively) were assessed in conjunction with 3 selection intensities for the correlated spaced plant genetic advance (i = 1.4, 1.8 and 2.0 for 20%, 10% and 5% respectively).

Table 7.35: Genetic advance ratios for direct sward genetic advance under 5 selection intensities with 3 selection intensities for correlated spaced plant genetic advance

Characters	i_{SW}=0.8 (50%)	i_{SW}=0.95 (40%)	i_{SW}=1.15 (30%)	i_{SW}=1.4 (20%)	i_{SW}=1.8 (10%)
i_{SP}=1.4 (20%)					
HGT1	0.70	0.59	0.49	0.40	0.31
LFN1	2.09	1.76	1.45	1.19	0.93
DST	>>1	>>1	>>1	>>1	>>1
LSZ	1.01	0.85	0.70	0.58	0.45
FLW	1.36	1.15	0.95	0.78	0.61
HGT2	0.30	0.26	0.21	0.17	0.13
LFN2	4.60	3.87	3.20	2.63	2.04
DHGT	1.29	1.09	0.90	0.74	0.57
DLFN	3.91	3.29	2.72	2.23	1.74
INTK	8.88	7.48	6.18	5.07	3.95
i_{SP}=1.8 (10%)					
HGT1	0.90	0.76	0.63	0.52	0.40
LFN1	2.69	2.26	1.87	1.54	1.19
DST	>>1	>>1	>>1	>>1	>>1
LSZ	1.30	1.10	0.91	0.74	0.58
FLW	1.75	1.47	1.22	1.00	0.78
HGT2	0.39	0.33	0.27	0.22	0.17
LFN2	5.91	4.98	4.11	3.38	2.63
DHGT	1.66	1.40	1.16	0.95	0.74
DLFN	5.03	4.23	3.50	2.87	2.23
INTK	11.41	9.61	7.94	6.52	5.07
i_{SP}=2.0 (5%)					
HGT1	0.99	0.84	0.70	0.57	0.45
LFN1	2.99	2.52	2.08	1.71	1.33
DST	>>1	>>1	>>1	>>1	>>1
LSZ	1.45	1.22	1.01	0.83	0.64
FLW	1.95	1.64	1.35	1.11	0.86
HGT2	0.43	0.36	0.30	0.25	0.19
LFN2	6.57	5.53	4.57	3.76	2.92
DHGT	1.85	1.55	1.28	1.05	0.82
DLFN	5.59	4.70	3.89	3.19	2.48
INTK	12.68	10.68	8.82	7.25	5.64

For all selection intensities considered in the sward environment and all intensities of selection considered as spaced plants, DST, LFN2, DLFN and INTK achieved greater genetic advance for sward conditions if selection was made as spaced plants, while for HGT1 and HGT2 the best genetic advance was achieved if selection was made under sward conditions.

LFN1 was only best selected directly under sward conditions when the intensity of selection in swards was 10% and 20% for spaced plants.

LSZ, FLW and DHGT were the three characters that could be selected under both environments and best results depended on the selection intensities applied for spaced plants or swards.

No general conclusion could be obtained from these analyses and each character should be studied in detail to determine the best environment under which selection should be performed.

CHAPTER EIGHT
General Discussion

8.1 INTRODUCTION

The main objectives of this research were:

(1) to define procedures for including grazing animal effects in the early stages of a forage breeding programme by testing novel field and statistical designs;
(2) to investigate the influence of selective grazing on genetic evaluation;
(3) to evaluate the significance of spaced plant/sward relationships to methodology; and
(4) to establish the importance of plant growth habit/morphology in influencing selection in Red clover.

These four objectives are viewed here from the perspectives of plant breeding and grazing ecology, with the objective of developing evaluation procedures which meet the needs of both sets of interests.

All experiments were carried out with Red clover, so the following discussion will apply to that species in particular, but the proposed experimental and analytical methodology is intended to be extrapolated to any forage species. From the review of literature, and current thinking in pasture breeding, it was considered to be important to include the grazing animal from the early stages of a forage breeding programme. Because a suitable methodology was lacking, as well as an indication of what characters a plant breeder should measure to assess animal preference and/or tolerance to grazing, this research was done. For animal based evaluations, final animal product per unit of area is usually used, but this is not a suitable variable when only a few selected plants are available for evaluation and breeding purposes. Other means of measuring animal effects should be developed, suitable for the early stages of a breeding programme and that measure the animal effect directly on the plants and not from animal performance.

The general discussion will concentrate on the following topics:

(8.2) plant materials, sampling and cloning procedures,
(8.3) field design,
(8.4) grazing management proposed,
(8.5) statistical designs,
(8.6) characters useful to evaluate animal effects for forage breeding,
(8.7) heritabilities and genetic advance,
(8.8) heritabilities and genetic advance without and with grazing animals,
(8.9) genetic correlations and genetic advance under a spaced plant or sward environment,
(8.10) genetic advance and experimental noise, and
(8.11) characterisation of populations across experiments.

8.2 PLANT MATERIALS, SAMPLING AND CLONING PROCEDURES

There is always the problem of limited time and resources when planning and conducting any research, and this research is no exception, therefore only a limited number of populations and plants were used for the experiments reported here.

Nine populations were carefully chosen for contrasting growth habit (three erect, three semi-erect and three prostrate), same ploidy level (2n = 14) and to provide representatives from the main temperate regions of the world (see Table 3.2) to reduce bias that might arise from such a small sample. As Red clover is a cross-pollinated species and all nine populations were field collections, open-pollinated composites or synthetic cultivars, great plant to plant variation was expected. This heterogeneous plant material contributed to the objective of offering the grazing animals a complete spectrum in growth habit and therefore in plant height. Prostrate plants were shorter than erect plants, as confirmed in Table

6.37 where the phenotypic and genotypic correlations of growth habit and plant height were -0.68** and -0.86** respectively. These two characters were reported in the literature to affect animal response (see Section 2.3.5.1), but this research did not confirm those results for Red clover.

To overcome the restriction of the limited number of genotypes used per population, a sampling method using parsimonious principal component analyses (see Section 4.2.3.2.1) was utilised for seedling discrimination in order to effect unbiased stratified randomisation of the plant populations, based on multivariate consideration of the five recorded characters. The objective was to represent as much of the original population variability as possible in the sub-sample of 12 plants from each population of 100 seedlings, which themselves were from a random seed sample. The principal component defines linear orthogonal functions of these attributes (up to five in this case), each of which provides a "local optimum" discriminator amongst the plants by maximising the principal-score variance amongst plants. This property is analogous to maximum discrimination among plants. The first (most discriminatory) component explains the highest percentage of the total original variance, the second accounts for the second highest (and is orthogonal to the first), and so on (Morrison, 1990). In the interests of parsimony, it is common practice to use only those components which cumulatively reach an arbitrary total of the explained variance (Morrison, 1990). A level of 70% was adopted here, which led to the first two components being used. From the plot of the first factor against the second, for each population, plants were chosen from the periphery and the centre to maximise representation of the original variability in the relatively small sample of twelve plants. The same philosophy is applied in germplasm banks to select a core (or working) collection to be multiplied and distributed upon request (Crossa *et al.*, 1993; Holbrook *et al.*, 1993; Spagnoletti-Zeuli and Qualset, 1993; Diwan *et al.*, 1994). The main objective in both cases is to keep as much variation as possible to assure representativeness and minimum bias.

Means and variances of the selected 12 plants were compared to means and variances of the 100 seedlings (Tables 4.6 and 4.7). All sample means were similar to population means.

Sample variances were compared at an infinite plant variance level (corrected by Church equation (Equation 4.2)) to eliminate the effect of sample size (12 plants vs. 100 plants). All variances were the same or slightly bigger in the sample, indicating no artificial restriction in sample variation. Another method of clustered full principal components (see Section 4.2.3.2.2) was also used afterwards to compare both sampling methods. Both sampling methods were considered equally satisfactory (see Section 4.4.3), but the clustered full principal components was considered best to be used in any situation for the following reasons. The method of parsimonious principal component analyses is satisfactory when the two factors account for at least an arbitrary figure of 70% of the variation. The only way to improve this method is to plot three factors in a three dimensions plot. However, even with three factors in some cases a small proportion of the variation might be explained, therefore the parsimonious solution might be considered not good enough. The second method proposed is to use cluster analysis (Ward's minimum variance method) after the principal component analysis, where 100% of the variation (all factors) could easily be used, and the number of factors involved is immaterial. This last method is considered best to be used in any situation as a sampling procedure.

Twelve plants x 9 populations (108 genets) were used to create the necessary material by cloning to begin the spaced plant-animal interaction experiments reported in Chapter 6. Clones were used to provide genetically identical replicates inside each block. The variation among those clones would be due to environment (except for mutation), so this is a direct measure of an environment effect. Mirzaie-Nodoushan and Gordon (1993) reported phenotypic variation among clones of Red clover due to cloning of different portions of the plants. The authors worked with basal, mid and tip cuttings. In this work, the leaf-bud propagule methodology suggested by Cumming and Steppler (1961) was used to avoid that source of variation, and also because they reported that the leaf-bud propagules produce plants similar to those coming from seedlings. This latter conclusion was considered important because the clones were used under defoliation, therefore after a hard grazing it was essential that plants had a functional crown to be able to regrow from buds located under the defoliation level. If plants were not able to develop a crown, a hard grazing could easily

kill them, because they would not have buds remaining, and not because that particular clone (ramet), plant (genet) or population (genotype-population) was not suitable for grazing purposes. Based on all considerations, it was decided to perform the leaf-bud propagule cloning procedure. Measures of persistence or plant survival (Table 6.18) show that plants were able to regrow after the hard grazings, with more than 93% of average persistence between sites at the second grazing. **IT IS RECOMMENDED THAT IN GRAZING STUDIES OF THIS KIND A MEASUREMENT OF CROWN PRESENCE SHOULD BE RECORDED FOR EACH SPACED PLANT TO ASSESS ITS INFLUENCE ON PLANT PERSISTENCE.**

There is no report of phenotypic variation from clones produced by the leaf-bud propagule procedure in Red clover, but the high variation found among clones in this study suggests that such variation exists. **IT IS SUGGESTED THAT DETAILED STUDIES OF CLONAL VARIATION SHOULD BE CARRIED OUT USING THE CLONING METHOD OF LEAF-BUD PROPAGULE SUGGESTED BY CUMMING AND STEPPLER (1961).**

Another source of variation for all cloning methods is that clones cannot possibly be created all at the same time, therefore a carry-on effect of clone size could be extended to field conditions. Different initial growth could make clones morphologically different for the rest of the experimental period. It is very difficult to quantify this effect, because even clones of similar size could have different early development. **IT IS SUGGESTED THAT MEASUREMENTS OF CLONE SIZE AND VIGOUR SHOULD BE MADE JUST AFTER TRANSPLANTING, AND THAT THIS INFORMATION SHOULD BE USED IN THE SUBSEQUENT ANALYSIS FOR DATA ADJUSTMENT IF IT IS FOUND TO BE A SIGNIFICANT FACTOR.**

Two other methods (Residual variance and Block variance) of calculating the environmental effect were explored during data analysis. These methods were not based on biological units, but were based on statistical analyses to estimate environmental effects by

exploiting the Harvey Smith equations (Smith, 1938) on field heterogeneity.

Residual variance (σ^2_ε) was considered an unbiased estimate of background residual. As each plant was an aggregate of t grazing times, σ^2_ε was divided by t^b, where b = coefficient of homoscedascity (Smith, 1938) to estimate the residual variance. In the absence of a local estimate of the coefficient of homoscedascity, the value 0.414 was used (the average of 45 world-wide experiments reviewed by Smith (1938)).

Block variance (σ^2_B) was also considered an unbiased estimate of meso-environmental variation. As each block had 324 plants, the same Harvey Smith equation could be used by multiplying the σ^2_B by n^b where n = number of plants per block and b = coefficient of homoscedascity (Smith, 1938) to estimate the residual variance. Again, the value 0.414 was used as the coefficient of homoscedascity. This method does ignore the presence of any clinal variance in the field, for which blocks are utilised. Therefore, this method is the most dubious.

The three environmental estimates: clones, residual variance and block variance, were compared by estimating a genetic fraction of Plant variance by subtracting the residual estimate from the Plant variance, and dividing it by the whole Plant variance. The estimates obtained by the clone procedure are reported in Table 6.15a, 6.15b, 6.25 and 6.36. The estimates obtained by using the error variance and block variance were mainly all zeros (all variation is environmental), and ones (all variation is genetic) respectively. It is biologically difficult to agree with values of 0% or 100% genetic variation. The main reason for obtaining these non-real results was that no local b (coefficient of homoscedascity) was estimated, and a pooled estimate (b = 0.414) from several world experiments was used, as well as no consideration of clinal variation. After this preliminary/exploratory statistical exercise, it was decided not to proceed further with these two methods and that all analyses would be based on the clonal estimates, given the limitations mentioned above.

8.3 FIELD DESIGN

Three kinds of field experiments were conducted: the grazing management experiment (GME) (reported in Chapter 5), the spaced plant-animal interaction experiments (SPAIE) (reported in Chapter 6) and the sward-animal interaction experiment (SAIE) (reported in Chapter 7), all of which were developed for this thesis because none of them were readily available in the literature. All of them had the distinct characteristic that all spaced plants (or swards, depending on the kind of experiment) from all populations were assigned to the field location completely at random and the location of each spaced plant/sward was recorded in a field map. The random location of spaced plants/swards was considered extremely important to evaluate animal defoliation discrimination. If spaced plants/swards were not located at random, and were located in groups of spaced plants in the same row/s (as is the usual way in experiments testing animal preference called "cafeteria trials" (Lascano *et al.*, 1988; Petersen *et al.*, 1989; Schultze-Kraft *et al.*, 1989; McGraw *et al.*, 1989; Davis, 1993), or without internal replicates for the sward-animal interaction experiment, several factors could influence grazing of specific field areas and could not be easily separated by sub-sequence analysis. For example, the presence of people may lead animals to concentrate grazing on areas as far as possible from the interference. The presence of fences may also influence grazing behaviour; plants close to the electric fences may be avoided, but those close to conventional fences may be preferentially grazed (Pers. Comm. C. Matthew, 1994). To minimise this problem, because electric fences were used, an extra distance of 1 m from the outer layer of plants to the electric fence was left without any vegetation. Gate location could be another possible factor that might affect plants, because of overgrazing and treading of the areas next to the gate.

The methodology developed is not intended to be used as a strict recipe, and the same principles could be applied to a larger area to either test more plants or to test them with different animal species such as cattle. When the area is increased, the random location of the plants is even more important because more factors (such as shade areas, closeness to sources of water, etc.) may influence heterogeneity of defoliation.

To investigate this fence problem, a concomitant dummy was taken for plants (for experiments reported in Chapters 5 and 6) or swards (for the experiment reported in Chapter 7) to define proximity to the electric fence (see Section 5.2.2). The studies showed that plant or sward location had no significant effect on the grazing animal either when it was considered as a covariance error adjustment or when the overall influence was analysed by regression analysis (see Sections 5.3.2.2; 6.3.1.2 and 7.3.2.2). These results indicate clearly that precautions (inherent in the designs) over arrangement and animal observations were successful in minimising extraneous effects in grazing behaviour.

Under these conditions, it is reasonable to conclude that population differences in defoliation were not due to chance but were a consequence of the effects of specific characteristics of the population on sheep behaviour. As reported in Section 5.3.3.1, leafiness pre-grazing was not significant in the analysis of variance for the Population and Plant effects, but was significant for the post-grazing leafiness. In Section 6.3.2.1, all differences between pre- and post-grazing height, spread and leafiness were significant in the analysis of variance for the Population and Plant effects. In Section 6.3.6.1, the analysis of variance was not significant for leafiness pre-grazing, but was significant for leafiness post-grazing in the pooled analysis of variance for the Population effect and also was significant for the differences (pre- and post-grazing in height, spread and leafiness) and intake for the Plant effect. In Section 7.3.4.1, the differences in height and leafiness between pre- and post-grazing measurements were significant in the analysis of variance for the Population effect. These results clearly show that grazers were not defoliating at random in any of the trials.

It was also considered important to explore if animals were defoliating all populations in the same way across grazings and across experiments, to see if a consistent behaviour was detected. Mean separations were performed for each grazing time of the grazing management experiment (Table 5.13), spaced plant-animal interaction experiments (Tables 6.11a, b (for New Zealand) and Tables 6.14a, b (for Uruguay)), and sward-animal interaction experiment (Table 7.23). Population-by-Time effects were significant for the grazing management experiment, and for the spaced plant-animal interaction experiment for each site, but were not

significant for the pooled across sites spaced plant-animal interaction experiments. For the grazing management experiment, E116 was the most grazed population for the first grazing and Turoa for the last two grazings. For the spaced plant-animal interaction experiment in New Zealand, the most grazed population was Turoa for all grazings. In Uruguay the most grazed populations were E116 for the first grazing, Quiñiquelli for the second and third grazings and Kenland for the last grazing. Turoa was not very well ranked in the Uruguayan experiments because of a serious problem of persistence (Table 6.18), probably due to susceptibility to soil fungus diseases (Pers. Comm. N. Altier, 1995). For the sward-animal interaction experiment, E116 was the most grazed population for the first grazing, Kenland for the second grazing, Hamua, Kenland and E116 for the third grazing and Kenland and E116 for the fourth grazing. It is important to note here, that this experiment was also conducted in Uruguay and that Turoa suffered the same diseases as suffered in the spaced plant-animal interaction experiment, and that Quiñiquelli, Turkish and F.2419 were not sown.

Even though Red clover is considered a short term component of perennial pastures because it generally disappears after 2-3 years (Lancashire, 1984), persistence was not considered a problem in New Zealand for this short experimental period. Only E116 suffered a 30% reduction in stand at the end of the experimental period. Red clover in Uruguay is considered to have a persistence of only 1-2 years (Pers. Comm. J. García and M. Rebuffo), and this was confirmed by these results (Table 6.18), where the mean persistence at the fourth grazing was 58.4% for the spaced plant-animal interaction experiment.

As indicated in the overall ranking reported in Section 8.11, Quiñiquelli, E116, Kenland and Turoa were the four most grazed populations and results were considered consistent across grazings and experiments. The least grazed populations were Astred and Turkish.

These consistent results were obtained from 63072 measurements (grazing management experiment (720 spaced plants x 3 grazings x 6 measurements (pre- and post-grazing height, spread and leafiness), spaced plant-animal interaction experiment (2 sites x

972 spaced plants x 4 grazings x 6 measurements) (pre- and post-grazing height, spread and leafiness), and sward-animal interaction experiment (216 samples x 4 grazings x 4 measurements)).

Secondary compounds were not included in the plant analyses because there is no particular secondary compound identified in Red clover that affects preference. To measure some of them as an exploratory exercise was not considered to be useful. It would be preferable to identify first extreme plant materials with reference to grazing preference before embarking on exploratory secondary compound analysis. **IT IS PROPOSED THAT, NOW THAT THE EXTREME POPULATIONS IN PREFERENCE ARE IDENTIFIED (MOST GRAZED VS. LEAST GRAZED), IT WOULD BE APPROPRIATE TO TEST FOR DIFFERENCES IN AS MANY SECONDARY COMPOUNDS AS POSSIBLE, BEGINNING WITH FORMONONETIN AND ALKALOIDS, AND TO PERFORM FURTHER DETAILED STUDIES ON THEM TO VERIFY IF THEY ARE AFFECTING THE PREFERENCE/DISCRIMINATION SHOWN BY GRAZERS.**

8.4 GRAZING MANAGEMENT PROPOSED

The experiment reported in Chapter 5 was performed to determine the optimum choice of Stocking Density and Time of Day to enable the best discrimination among plants for preference. If grazing was too lax, many plants would not be grazed because animals would not have time to graze them all: and if grazing was too hard, no variation would be available to work with for selection. Forage nurseries are not usually grazed, or if they are, they are grazed to a hard level, testing mainly survival and persistence under grazing, rather than preference followed by resistance to grazing as is proposed in this thesis. For the usual case, there is no need for information on the optimum time for taking the animals out of the grazing area, but it is crucial if a measure of preference is required.

The proposed grazing management (see Section 5.4.1) was to graze when semi-erect populations of Red clover reached an average height of approximately 25 cm. Several factors such as weather, sheep's hunger, etc., could affect a short period of grazing, so it was recommended that animals should be removed from the grazing area when leafiness reached on average 40% of total plant material and to be flexible with length of the grazing period. This criterion to remove the grazing animals appears to give consistency among grazings and/or experiments.

The Stocking Density obtained from the grazing management experiment (Chapter 5) recommended to graze to an average 40% residual leaf was equivalent to 5 sheep/18 m^2 (2778 sheep/ha) for one hour, to be able to obtain a sampling intensity of at least 94% (Table 5.17) at either morning or evening. Grazings were done just after sunrise and before sunset to take advantage of the normal grazing pattern of sheep (Hodgson, 1990a), in order to minimise interference with their normal selectivity or ability to discriminate. After the post-grazing measurements were taken, sheep were introduced again (preferably hungry) to defoliate quickly all plants to a uniform level to provide plant materials for the following grazing without the carry over effect of the previous grazing.

Sampling intensity was determined using the differences between pre- and post-grazing measurements of height, spread and leafiness. If any plant had a value ≥ zero in any of these parameters, that plant was considered grazed (see Section 5.2.3). The zero value was also included because one or two days of active growth usually occurred between the pre-grazing and post-grazing measurements, therefore remaining at the same height, spread or leafiness meant that the equivalent to two days of growth was grazed. The four Stocking Densities used in the experiment reported in Chapter 5 gave a sampling intensity of 69.3%, 81.9%, 93.5% and 98% for 2, 3, 5 and 9 sheep/plot respectively (Table 5.17). A sampling intensity of 94% was selected because almost all plants were sampled, but the risk of overgrazing due to an accidental delay in taking animals out of the grazing area was controlled. For example, for the highest Stocking Density, a delay of only ten minutes, assuming that animals graze at a constant rate during grazing, could cause a complete loss in

remaining plant variability. The target of 94% sampling intensity was used for the spaced plant-animal interaction experiments, and a level of 95.0% and 97.8% was actually achieved for New Zealand and Uruguay respectively (Table 6.17). Sampling intensity was not significant for the Population effect for any site, therefore all populations were sampled at the same rate.

This proposed grazing method for spaced plant breeding nurseries is similar to the mob-grazing technique of Mislevy *et al.* (1982), but overcomes the problem pointed out by these authors of losing the most preferred materials due to overgrazing. Measuring the plants before overgrazing occurs adds a further level of complexity to the grazing evaluation scheme by grazing in two stages for each animal evaluation period, but the duration of the first grazing is crucial to obtain the expected results of preferential grazing. It also adds the cafeteria opportunity for animals to show preferential grazing as previously discussed in Section 8.3. Smith *et al.* (1989), Brummer and Bouton (1991), Smith and Bouton (1993) and Bouton *et al.* (1993) detailed a method for holding alfalfa under continuous close grazing for several years and selecting high yielding and persistent plants after that period. Comparing the proposed method with this last one, the proposed method has a measure of palatability or plant preference/discrimination and the most preferred materials would not be lost by overgrazing. The generation interval for the latter method is at least three years, whereas cycles of selection could be done annually with the proposed method, thus reducing the generation interval by three and therefore incrementing the annual genetic advance.

To consider the effect of the grazing animals via preference or selective grazing, there is no need to apply any special breeding method, but to graze the nursery instead of cutting, with the recommended management, measure LFN2 after removing the animals for the first time and then allowing grazers to defoliate all the nursery until a uniform defoliation is achieved. This last grazing should be for a short period of time so that no regrowth be grazed to overcome the problem of overgrazing the most preferred materials. Plants or swards should be randomly assigned to the grazing unit to allow identification of selective grazing. The inclusion of the grazing animal is not the final objective of the breeder, but a routine

method to graze the nurseries, with the bonus being information regarding grazing preference, and the disadvantage being reduction in heritability values and rates of genetic advance (see Section 8.8).

TO EVALUATE GRAZING TOLERANCE, IT IS RECOMMENDED THAT EXPERIMENTS SHOULD BE CONTINUED FOR AT LEAST 2 YEARS AND THAT DEATH OF THE PLANTS SHOULD BE MONITORED TO DETERMINE IF THE CAUSE OF DEATH WAS BECAUSE OF INTOLERANCE TO GRAZING OR ANY OTHER REASON.

8.5 STATISTICAL DESIGNS

Ten experimental models were used to analyse all the data for the different experiments, and seven of them were developed as part of this thesis, because they were not readily available in the literature (see definitions and Model Equations 4.1 for the Preliminary glasshouse experiment; Equations 5.1 and 5.2 for the Grazing management experiment; Equations 6.3, 6.4, 6.5 and 6.6 for the Spaced plant-animal interaction experiments and Equations 7.3, 7.4 and 7.5 for the Sward-animal interaction experiment).

Personal computers were sufficient to run all models except for the models used for the spaced plant-animal interaction experiments. Main-frame computers at both Massey University (128M of RAM) and INIA had insufficient memory to handle the full model for the spaced plant-animal interaction experiments, so the model was reduced by one layer (clones) to be able to run on the available computers. The clone-sums-of-squares was obtained by using another programme called REAL4 (developed by I.L. Gordon) and a new data file was generated by averaging clones within plants (see Section 6.2.5). This model was able to run, but about 10 hours were necessary for each run on Massey University's main-frame UNIX.

All effects in the models were considered to be infinite random, (see later discussion), normal, independent deviates with expectations equal to zero, and generating variances of corresponding designations. The variance components arising from such random effect designs may be found by equating the mean square estimates to their expectations, and solving the resultant linear functions (Crump, 1946;1951; LeClerg *et al.*, 1962; Searle, 1971).

As stated before, all effects were considered random (Site, Population, Plant, Blocks, Grazing Date (Time), Stocking Density, Time of Day, Internal Repetitions and Samples). Sites were considered random effects because these sites were located at different parts of the world, both in typical temperate regions representing the regions where results of these work might be used. Populations were also not selected at random, but were carefully selected to have broad genetic material representing temperate regions of the world (to reduce bias as much as possible) with all the variations possible in growth habit, and were also selected to reduce the probability that the materials were related by descent (see Section 3.3.3). The main reason for regarding this factor as random was to be able to draw conclusions about Red clover in general rather than just the experimental material. The same argument was considered for the Stocking Density effect. Plants were also not selected at random, but were selected from large populations to assure representativeness and reduce bias (see Section 4.2.3.2). Any plant was as important as any other for comparisons among plants. The same case applied for Samples and Internal Repetitions in the sward-animal interaction experiment. Plant materials were assigned at random to each block, and blocks were also randomised. Grazing Date (Time effect) was also considered a random effect, because there was no pre-planning of Grazing Dates and grazings were done when plants were considered ready for grazing (see Section 6.2.4). Times of day were pre-planned, but not at a fixed time, depending on sun-rise and sun-set, and therefore were also considered random effects.

Random F tests are (always) more conservative than those from fixed effect models. For example (see Table 6.5), if Site effects were considered fixed, instead of having 8 variance components in the expectation of mean squares, all the interactions with Site would not be included in the Site effect and only 4 variance components would be part of the

expectations of mean squares. Therefore, this effect with only 4 variance components would be declared significant more often than if the effect was considered random. The same statement applies to all random effects in the model.

Clones from individual plants and plants from the same population were located at random and not next to each other as is the usual case. In this study, treatments were dispersed all over each block as discussed in Section 8.3.

For both sites, the experimental area (0.016 ha for the largest case) historically had been under the same management, and it was not considered a serious violation of any experimental/statistical assumption to have the plants dispersed over each block for these experiments. When this area is increased (as stated before, for testing other species, more plants or with a different animal species such as cattle), the problem is more serious and data adjustment should be considered. **IT IS RECOMMENDED TO WRITE A COMPUTER PROGRAMME THAT SUBTRACT FROM EACH OBSERVATION ALL EFFECTS STATED IN THE MODEL, LEAVING THE RESIDUAL FOR EACH OBSERVATION. A FIELD MAP OF THE RESIDUALS COULD BE USED TO EXPLORE ANY PLANT LOCATION AND COMPARE THE RESULTS BY ESTIMATING THE BIAS ARISING FROM NON-CONTIGUOUS PLANTS IN COMPARISON WITH CONTIGUOUS PLANTS PER TREATMENT BY EXPLOITING THE HARVEY SMITH EQUATIONS (SMITH, 1938) ON FIELD HETEROGENEITY.**

8.6 CHARACTERS USEFUL TO EVALUATE ANIMAL EFFECTS FOR FORAGE BREEDING

To characterise the populations and to quantify defoliation, plant height, plant spread, and leafiness pre- and post-grazing were measured. Morphological measurements were taken to characterise the populations according to plant density, leaf size, growth habit and

flowering state. Laboratory measurements were done for two main reasons: (1) to quantify the existing variation in crude protein content and in digestibility present in Red clover, and (2) to check the quality of the most-grazed materials because preference will not necessary help animal performance unless it is linked to an advantage in nutrient intake (Chapters 5, 6 and 7).

As reported in Sections 6.4.4, 7.4.4, 8.3 and 8.11, the most grazed populations were Quiñiquelli, E116, Kenland and Turoa. These **four most intensively grazed populations** represented extremes in morphology (one erect, two semi-erect and one prostrate genotype); leaf size; plant density; plant height; leafiness and flowering state, indicating that these variables were not influencing animal preference in any simple sense, as might have been expected by reviewing the literature (see Section 2.3.5.1). However, the four populations have in common their "leafy appearance". For example, Quiñiquelli having the biggest leaves, Turoa being very dense and with many small leaves, while Kenland and E116 were intermediate, but this "leafy appearance" was very difficult to quantify. Therefore, none of the **pre-grazing** morphological characters measured were useful to detect or predict the defoliation level (Sections 6.3.6.4 and 7.3.4.4).

Based on the previous considerations, only measurements which quantify defoliation (HGT2, SPR2, LFN2, DHGT, DSPR, DLFN and INTK) might be useful for breeding purposes, depending on their heritability values. **IT IS RECOMMENDED NOT TO BASE A SELECTION PROGRAMME ON SIMPLE MORPHOLOGICAL CHARACTERS, BECAUSE GRAZING AND PREFERENTIAL DEFOLIATION IS A COMPLEX PHENOMENON IN WHICH ALL FACTORS INVOLVED AND THEIR INTERACTIONS ARE NOT ENTIRELY UNDERSTOOD.**

From previous discussions (Sections 6.4.3 and 7.4.3), it was concluded that LFN2, DHGT, DSPR, DLFN and INTK were the most appropriate characters to measure animal effects, while HGT2 and SPR2 were discarded (according to data summarised in Table 8.1) because of their respective high coefficients of determination and overall genetic correlation with their respective pre-grazing measurements (HGT1 and SPR1).

Table 8.1: Coefficients of determination and overall genetic correlations between pre- and post-grazing height, spread and leafiness

	SPAIE[1]		SAIE[2]	
	R^2	r_g	R^2	r_g
HGT2 and HGT1	0.71	0.71	0.37	0.99
SPR2 and SPR1	0.84	0.70	---	---
LFN2 and LFN1	0.10	0.31	0.13	0.71

--- Not applicable
1 Spaced plant-animal interaction experiments
2 Sward-animal interaction experiment

High coefficients of determination, as well as high overall genetic correlations, meant that for HGT2 and SPR2 the pre-grazing state of the plant materials was more influential than the effect achieved by the grazing animals on those measurements.

The usefulness of the remaining characters (LFN2, DHGT, DSPR, DLFN, INTK, PRT and DGT) is discussed based on their heritabilities and genetic advance.

8.7 HERITABILITIES AND GENETIC ADVANCE

Heritability estimates provide information about the usefulness of the attributes from a genetical point of view, indicating to which extent genetic progress could be made through selection. Estimation of genetic advance gives breeders the necessary information to predict the final output of breeding programmes under different alternatives of selection strategies and intensities of selection.

Three definitions of heritabilities were used, depending on the numerator used. The numerator was the variance component for Population, Plant or both (Overall) and the denominator was a restricted phenotype (Allard, 1960; Gordon et al., 1972; Gordon, 1979; Singh *et al.*, 1993). Each of these definitions have their particular advantages or usefulness. The Population heritabilities are the most suitable for cultivar evaluation. The Plant heritabilities are the most suitable for plant breeding, when variation from plant to plant is of primary interest, and the Overall heritabilities are the best to evaluate overall usefulness of characters. Therefore, main emphasis was given to the Plant and Overall restricted definitions.

All three definitions are broad-sense heritabilities, including all types of genetic variance (additive and non-additive variances). The method of measuring heritability by separating the genetic effect from the environment only provides broad-sense information for the cross-pollinated populations of Red clover. To obtain narrow-sense (additive variance) heritabilities, several methods are available, but all need some planned crossing scheme, which was not performed as part of this thesis.

All estimates of variance components were used as obtained (including negative estimates) to estimate heritabilities, in order to avoid the positive bias arising from equating negative estimates to zero.

Standard errors of heritabilities were estimated for seven of the models presented in Chapters 5, 6 and 7 and are presented in Appendices 2, 3, 5, 6, 8, 9 and 10, because none of

them were available in the literature. Although the general method for arriving at these standard errors of heritability was available (Osborne and Paterson, 1952; Gordon *et al.*, 1972; Gordon, 1979), tedious work was required to obtain them. For example, for one of the estimations more than 190 covariances were involved that had to be estimated first. Considering the remaining three models, only one had the equations to estimate the standard errors of heritabilities available (Gordon, 1994) and for the other two, estimation of heritabilities were not necessary, therefore the equations for estimating the standard errors of heritabilities were not worked out. **THE FORMULAE FOR THE STANDARD ERROR OF HERITABILITIES FOR THESE NEW MODELS ARE NOW AVAILABLE AND COULD BE WIDELY USED IN PASTURE BREEDING AND EVALUATION WORK.**

The usefulness of the characters is summarised in the heritability values for post-grazing leafiness (LFN2), differences between pre- and post-grazing (DHGT, DSPR and DLFN) and INTK for the two experiments (SPAIE and SAIE). These are presented in Table 8.2.

Table 8.2: Overall restricted heritability values for post-grazing leafiness, differences between pre- and post-grazing and Intake for the SPAIE and SAIE

Characters	SPAIE (NZ)	SPAIE (ROU)	SAIE
LFN2	0.25**	0.21*	0.02^{NS}
DHGT	0.06^{NS}	0.32**	0.19^{NS}
DSPR	0.04^{NS}	0.15*	---
DLFN	0.41**	0.21*	0.02^{NS}
INTK	0.26*	0.32**	0.01^{NS}

--- Not applicable
NS Not significant
* Significant at 0.05 level
** Significant at 0.01 level

Heritability estimates for DHGT and DSPR were not significantly different from zero for the experiment conducted in New Zealand (SPAIE), therefore these characters were also not considered further due to its inconsistent behaviour across sites. The differences which result in heritability estimations for the same characters with the same populations emphasise the importance of the reported experimental methodology to obtain the larger heritability estimators by controlling all other possible sources of variation, to determine the genetic value of the plant materials as accurately as possible.

Use of LFN2 had the great advantage that only one measurement is necessary, while DLFN requires two measurements (LFN1 and LFN2) and INTK requires six measurements (HGT1, HGT2, SPR1, SPR2, LFN1 and LFN2). For example, in each of the (SPAIE), 3888 measurements of LFN2 were taken, while to obtain DLFN, 7776 were required and to obtain INTK, 23328 measurements were required. These are very important differences in labour required, making the measurement of LFN2 the best option. Also LFN2 was the character with the smallest coefficient of variation of the three characters (LFN2, DLFN and INTK) (Tables 5.9, 6.35 and 7.21). Its heritability values of 0.25 and 0.21 for SPAIE (NZ) and SPAIE (ROU) respectively, make this a useful character for plant breeding. Values are medium to low, but they are similar to the values for heritability of milk yield, protein yield and butterfat yield for dairy cattle breeding (Nicholas, 1987), on which most dairy breeding programmes are based.

It is important to note that for all these heritabilities, the only genetic input was at the Population variance component level, because the Plant variance component was not significantly different from zero. This phenomenon could be explained from a Plant variance and a Clone variance perspective. From a Plant variance perspective, it might be that there was no plant to plant variation for those characters, because there was no additional genetic variation arising from plant segregation, and that the variation was totally accounted for at the Population level. From a Clone variance perspective, it might be that the clones were not so uniform as assumed, having greater variation than expected from environmental factors, that the death of clones (persistence) reduced the three clones per plant to two or one clone per

plant, increasing the possible average variation, or a combination of all of the above. **FOR FUTURE BREEDING PROGRAMMES, IT IS SUGGESTED TO DO A COMPLEX-CROSS EXERCISE OF THE BEST POPULATIONS FOLLOWED BY SELECTION (LINE BREEDING) WHERE PLANT VARIANCES MIGHT BE DIFFERENT FROM ZERO.**

The usefulness of crude protein content and digestibility were also assessed by their heritability estimates. Heritability values were 0.58** for both protein content and digestibility, both highly significantly different from zero for the spaced plant-animal interaction experiments (Table 6.25), and 0.08^{NS} and 0.26** for protein content and digestibility respectively for the sward-animal interaction experiment (Table 7.11). These values are in the same range as heritability values reported by Clements (1973), Oram *et al.* (1974), Dennis and Frandsen (1986), Minson (1990), Broderick and Buxton (1991), Mousset-Declas *et al.* (1993) and Vogel and Sleper (1994) for several forage species. These heritabilities show that there is enough variation to make genetic progress under both sowing methods for digestibility and under a spaced plant environment for protein content.

Phenotypic and genotypic correlations for crude protein content and digestibility were 0.60** and 0.64** respectively for the SPAIE and 0.62** and 0.83** respectively for the SAIE. All phenotypic or genotypic correlations with the rest of the characters were medium and medium to low for the SPAIE, none of them being higher than 0.6 (Table 6.26) and high, medium and low for the SAIE (Table 7.12), intake being the strongest genotypic correlation with protein (0.85**) and with digestibility (0.67**). None of the simple and multiple regressions of characters measured pre-grazing, post-grazing or differences between pre- and post-grazing with protein or digestibility were strong (Tables 6.27 to 6.32 for the SPAIE and Tables 7.13 to 7.18 for the SAIE), 0.40 being the maximum R^2 for all pre-grazing characters with digestibility for the SAIE. The best canonical correlation was also between pre-grazing characters with protein and digestibility of 0.60** and 0.63** for the SPAIE and SAIE, respectively, also not being considered strong. From all the correlations (phenotypic and genotypic), and regression studies, it is concluded that protein content and digestibility were

quite independent from the morphological characters measured, emphasising the importance of measuring quality characters as well as morphological characters.

HERITABILITY ESTIMATES INDICATE THAT IT MAY BE POSSIBLE TO SELECT GENOTYPES THAT COULD BE GRAZED MORE AND THUS OBTAIN GREATER ANIMAL PRODUCTION, CONSIDERING ALSO THAT THE MOST GRAZED POPULATIONS WERE ALSO THOSE SHOWING BEST QUALITY CHARACTERISTICS (see Section 6.4.4).

As detailed in Section 6.2.3, crude protein content and digestibility were measured from samples including stems and leaves. The quality results were a compound result from the stem quality and the leaf quality. **MEASUREMENTS OF LEAF REMOVAL WERE CONSIDERED AMONGST THE MOST IMPORTANT CHARACTERS, THEREFORE IT IS SUGGESTED THAT QUALITY ASSESSMENT FOR FUTURE WORK SHOULD BE MADE IN LEAVES AND NOT IN TOTAL PLANT FOR RED CLOVER.**

The best values for protein and digestibility were found in Turoa and Quiñiquelli in New Zealand and in Quiñiquelli, Kenland and Turoa in Uruguay for the spaced plant animal interaction experiment (Table 6.24); and Kenland (Table 7.10) for the sward animal interaction experiment (it is important to remember here that Quiñiquelli was not sown in the sward-animal interaction experiment). The best quality of Turoa might be partially explained by the high leaf/stem ratio as indicated by LFN1, but this explanation is not suitable for the high quality characteristics of Quiñiquelli or Kenland because their leaf/stem ratio (LFN1) was not high (sixth and seventh in an overall ranking for LFN1: see Section 8.11).

For all populations, the overall phenotypic and genotypic correlations between LFN1 and PRT or DGT for the SPAIE or the SAIE were less than 0.4, meaning that the association was not strong and that not always the plants with more leaves had the best quality.

For the SAIE, heritabilities were calculated for the Population partition. Characters were measured inside a quadrat of 4 dm^2, except height which was measured with the sward stick (Hodgson, 1990a) on a surface approximately 2 cm^2 and leaf size which was measured as detailed in Section 5.2.3. The last two characters were considered measurements on single plants in swards. In the space-planted experiments, plants always exceeded 4 dm^2. Plants were not counted inside quadrats for the sward environment where individual plants never reach a large size due to plant competition, so three plants per quadrat were considered for the heritability estimations for those characters. **IT IS RECOMMENDED FOR FUTURE STUDIES TO COUNT PLANTS INSIDE MEASURING UNITS (QUADRATS), TO ENABLE ACCURATE ESTIMATION OF HERITABILITIES UNDER A SWARD ENVIRONMENT AT AN INDIVIDUAL PLANT LEVEL.**

8.8 HERITABILITIES AND GENETIC ADVANCE WITHOUT AND WITH GRAZING ANIMALS

This section is presented to compare heritabilities and genetic advances without and with the grazing animal. The eight characters presented (HGT1, SPR1, LFN1, VOL, DST, LSZ, HBT and FLW) were all the characters that were not directly measuring animal effects like the post-grazing characters (HGT2, SPR2 and LFN2) or equations created using them (DHGT, DSPR, DLFN and INTK), because grazing animals were needed to obtain them, and no measurement could be taken without the animal influence.

The reductions in heritability values between the measurements without grazing animals (before the first grazing) and with the grazing animal influence (all grazings considered) for the GME (Tables 5.6 and 5.16), for the SPAIE (Tables 6.20 and 6.36) and for the SAIE (Tables 7.7 and 7.24) are summarised in Table 8.3.

Table 8.3: **Reductions** in heritability values between characters without and with the grazing animal effects for three types of experiments (GME, SPAIE and SAIE)

Characters	GME	SPAIE (pooled)	SAIE
HGT1	0.33	0.10	0.40
SPR1	0.29	0.14	---
LFN1	0.53	0.50	0.71
VOL	0.41	---	---
DST	---	0.71	0.25
LSZ	0.26	0.13	0.11
HBT	0.25	0.12	---
FLW	0.30	0.31	---

--- Not applicable

There is an overall average mean reduction of 0.33 units of heritability for the inclusion of the grazing animal, from an overall average heritability of 0.64 for characters measured before the first grazing to an overall average of 0.31 when all grazings were considered.

SPR1, LSZ and HBT were the characters in which heritabilities were least affected by the inclusion of the grazing animal and LFN1 was the most affected character.

If the intention of the experiment or the breeding objective is to select any of the pre-grazing characters, then it is better to select directly on them and not to use the grazing animal. If the intention of the breeding programme is to include the effect of the grazing animal by measuring post-grazing characters, then reductions in heritabilities are inevitable for the pre-grazing characters and slower and lower genetic progress should be expected, but fortunately in the right direction towards an improved cultivar under farm conditions.

Genetic advance without and with the grazing animal effects for the GME, SPAIE-NZ, SPAIE-ROU and SAIE are presented in Table 8.4 with the assumption that selection is done in both sexes, that individual selection strategy is applied, and that the same intensity of selection is used.

Table 8.4: Genetic advance (ΔG^1) without ($\Delta G1$) and with ($\Delta G2$) grazing animal effects for GME, SPAIE (Pooled), and SAIE

Characters	GME			SPAIE (Pooled)			SAIE		
	$\Delta G1$	$\Delta G2$	$\%^2$	$\Delta G1$	$\Delta G2$	$\%^2$	$\Delta G1$	$\Delta G2$	$\%^2$
HGT1	9.14	8.40	9	9.18	7.07	30	14.86	7.02	112
SPR1	16.26	9.66	68	8.85	6.14	44	---	---	---
LFN1	13.17	0.02	65750	7.38	2.93	152	5.98	5.02	19
VOL	0.62	0.20	210	---	---	---	---	---	---
DST	---	---		1.08	0.24	350	0.53	0.001	52900
LSZ	1.50	0.89	69	1.21	0.94	29	1.24	1.20	3
HBT	1.60	1.31	22	1.36	1.21	12	---	---	---
FLW	0.17	0.13	31	4.72	1.22	287	---	2.73	---

1 $\Delta G = ih^2\sigma_p$ where i = 1.8 for a 10% selection intensity for all characters with or without grazing effect for a large population (Falconer, 1960).
2 Percentage of increase in ΔG for not including the grazing animals
--- Not applicable

Selection advances are always greater when grazing animals are excluded, being very much greater for LFN1 under the GME and DST under the SAIE, and only HGT1 (8.8%) under the GME, HBT (12.4%) under the SPAIE (Pooled), LFN1 (19.1%) and LSZ (3.3%) under the SAIE had smaller increases in genetic advance for omitting the animal effects. These results confirm the comments that if there is not a need for the inclusion of the grazing animal, from a genetic advance point of view, it is better not to include them.

8.9 GENETIC CORRELATIONS AND GENETIC ADVANCE UNDER SPACED PLANT OR SWARD ENVIRONMENT

Genetic correlations between spaced plant and sward test conditions for each character were presented in Table 7.33. All genetic correlations were positive and significantly different from zero, therefore the study of the correlated genetic advance was justified. The genetic advance ratios are defined here as the ratios of the correlated genetic advance of each character when selection is done under spaced plants conditions to obtain results under sward conditions (which is the final use for any forage cultivar), to the direct genetic advance under sward conditions. To perform the ratios of correlated genetic response to direct genetic advance, five selection intensities were used for selection under sward conditions (50%, 40%, 30%, 20% and 10%) in all possible combinations with three selection intensities for the spaced plant environment (20%, 10% and 5%) and results were presented in Table 7.35.

GREATER SELECTION GAINS COULD BE OBTAINED IN DST, LFN2, DLFN AND INTK IF SELECTION IS DONE ON SPACED PLANTS IN COMPARISON WITH A SWARD ENVIRONMENT, WHEN THE INTENDED FINAL USE IS UNDER SWARD CONDITIONS, FOR ALL POSSIBLE COMBINATIONS OF SELECTION INTENSITIES. WITHIN THESE FOUR CHARACTERS ARE INCLUDED THE THREE (LFN2, DLFN AND INTK) MOST USEFUL ONES FOR MEASURING AND SELECTING FOR ANIMAL PREFERENCE.

For the other characters, greater selection advances could be achieved if selection is done under sward conditions, for use under sward conditions, when the same selection intensities are applied to spaced plants and swards environments. If selection intensity is greater under spaced plant conditions (that is the most likely situation), this latter sowing

method becomes the most suitable for most characters except HGT1 and HGT2, which are almost always best selected under sward conditions. For example, to obtain the same genetic advance in HGT2 under spaced plant conditions (for final use in swards) than direct selection in swards, when for example 20% intensity of selection was used for swards, an intensity of selection of 8.08 should at least be used for spaced plants. This value of selection intensity is much greater than values used in plant breeding under field conditions where an intensity of selection of 3.37 is equivalent to 1/1000 (McWilliam and Latter, 1970). From these results, it is concluded that Height was always best selected directly under sward environments for final use under sward conditions.

As stated before, for most characters, best genetic progress depended on the intensity of selection used. **AS GREATER GENETIC PROGRESS COULD BE ACHIEVED BY BREEDING IN A SPACED PLANT ENVIRONMENT FOR SOME CHARACTERS AND IN A SWARD ENVIRONMENT FOR OTHERS, FOR A BREEDING PROGRAMME IN RED CLOVER IT IS RECOMMENDED TO USED BOTH ENVIRONMENTS TO COMBINE THE VIRTUES OF BOTH TECHNIQUES.** McWilliam and Latter (1970), reached a similar conclusion from their results for breeding *Phalaris tuberosa* L., when comparing two characters (autumn growth and winter growth for the second year) in spaced plants and swards. However, the genetic correlation was significantly different from zero ($P < 0.05$) for one character but not for the other, making it difficult to draw any objective conclusion. The same authors also suggested the possibility of achieving significant progress through intensive selection for a correlated response like seedling growth for their studies which is easily measured and can be subjected to a very high selection pressure.

To evaluate plant material as spaced plants in a sward of another species also has its limitations because different results have been reported due to differences in sward species and cultivar within species (Brink and Rowe, 1993). Therefore, no method is regarded as entirely satisfactory because no cultivars are released to be sown with only one species or one cultivar within species.

To select under sward conditions, only bulk harvesting could be used, or other methods that do not rely on individual plants, because individual plants could not be harvested as individuals in a sward environment, and this method of selection is one of the most inefficient.

The concluding comments of this section, considering the results obtained from the selection advance under spaced plant and sward conditions and combining two phases of selection, are that individual selection for a spaced plant phase and amongst-line selection for a sward phase is a desirable condition. Only 4 out of 10 characters obtained better selection advance under spaced plant conditions when their final use was in swards if the same selection intensities were used: if greater selection intensity is applied to spaced plants, almost all characters are best selected as spaced plants except HGT1 and HGT2. Another option is to sow the spaced plants in a pasture of another species, allowing the competition effect while retaining individuality (Van Dijk and Winkelhorst, 1978; Caradus, 1991; Brink and Rowe, 1993) for the spaced plant phase, and using combined selection strategy (best plants of best lines) that is more efficient, allowing greater genetic advance. The sward phase could also be used as a progeny test if characters with very low heritability are being considered (Latter, 1964).

8.10 GENETIC ADVANCE AND EXPERIMENTAL NOISE

Genetic advance values should be compared with the square root of the residual or error term of the corresponding model for each experiment and character to evaluate if progress could be made, or if in fact the experimental noise was too high to make genetic progress (Table 8.5).

Table 8.5: Genetic advance and square root of the experimental residual term

Character	GME		SPAIE (NZ)		SPAIE (ROU)		SAIE	
	ΔG	σ_e	ΔG	σ_e	ΔG	σ_e	ΔG	σ_e
HGT1	8.40	0.78	11.24	0.50	6.36	0.51	7.02	0.88
SPR1	9.66	2.50	10.11	1.50	8.03	2.49	---	---
LFN1	0.02	3.33	7.49	1.54	5.81	1.81	5.02	1.36
VOL	0.20	0.01	---	---	---	---	---	---
DST	---	---	0.57	0.01	0.30	0.01	0.01	0.01
LSZ	0.89	0.03	0.98	0.02	0.85	0.02	1.20	0.02
HBT	1.31	0.02	1.61	0.01	1.30	0.02	---	---
FLW	0.13	0.18	0.55	0.41	0.87	0.49	2.73	0.40
HGT2	6.48	1.08	11.46	0.57	1.92	0.30	2.55	0.66
SPR2	11.39	2.92	12.31	1.42	8.78	1.84	---	---
LFN2	2.65	5.14	5.90	2.14	4.59	3.66	0.68	2.12
DHGT	0.11	1.07	0.42	0.51	2.65	0.48	2.08	1.30
DSPR	0.02	2.29	0.45	1.09	1.43	0.94	---	---
DLFN	0.04	8.43	9.32	2.53	5.53	4.03	1.03	3.12
INTK	---	---	0.64	0.05	1.07	0.08	0.06	0.03
PRT	---	---	2.61[1]	0.05[1]	---	---	0.64	0.45
DGT	---	---	4.98[1]	0.22[1]	---	---	2.33	0.35

1 Pooled result for New Zealand and Uruguay. No separate analyses were performed by site because the Site effect was not significant in the analysis of variance (see Table 6.21)

--- Not applicable

No genetic advance could have been achieved for LFN1, LFN2, DHGT, DSPR and DLFN for the grazing management experiment, DHGT and DSPR for the spaced plant-animal interaction experiment conducted in New Zealand, and DST, LFN2 and DLFN for the sward-animal interaction experiment. That is, in 10 out of 55 characters measured in the four experiments reported in Table 8.5, the experimental noise was greater than the theoretical expected genetic advance. For all other characters, genetic advance was theoretically possible under the existing experimental conditions. It is also important to note that for all characters it was possible to obtain genetic advance within the experimental conditions under spaced plant or sward conditions.

To increase ΔG, the most efficient breeding strategy and method should be applied, considering that diploid Red clover is a highly self-incompatible species (Pandey, 1956; Meglic and Smith, 1992), so selfing is not an easy option. The closest inbreeding method is full-sib mating, to allow dispersion to occur, but high levels of inbreeding depression have been reported in the literature (Williams and Silow, 1933; Williams and Williams, 1947a; 1947b; Taylor and Anderson, 1980). Also, the best experimental design should be applied followed by complex statistical analysis to reduce the size of the residual or error term, such as the ones proposed in this work.

8.11 CHARACTERISATION OF POPULATIONS ACROSS EXPERIMENTS

Six experiments were conducted in New Zealand (NZ) and Uruguay (ROU) and nineteen attributes were measured which were used to characterise the nine populations over all experiments.

From all measurements, only the ones used in more than one kind of experiment were considered. With this constraint, Hairiness and Leaf number of the Preliminary Glasshouse Experiments and Volume of the Grazing Management Experiment are not considered further

because their results were shown in their respective chapters. Rankings among population for all other measurement (Tables 8.6, 8.7 and 8.8) are considered here in order to evaluate their relative magnitudes: absolute values for individual variables were discussed for each experiment in their respective chapters. Only 6 populations (Hamua, Colenso, Kenland, E116, Astred and Turoa) were used in the Sward-Animal Interaction Experiment (SAIE) so rankings will be from 1 to 6. Weighted averages were calculated to obtain an overall ranking of the populations across experiments. Because the rankings of each character for each experiment correspond to different absolute values and statistical analyses were performed in each corresponding Chapter, no statistical analysis was done on these overall weighted rankings.

Table 8.6: Ranking among populations for HGT1, SPR1, LFN1, DST and LSZ

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HGT1[1]									
PGE(NZ)	3	6	1	5	4	2	8	7	9
PGE(ROU)	3	6	1	8	5	2	4	7	9
GME(NZ)	4	7	2	5	3	1	9	6	8
SPAIE(NZ)	1	3	5	2	4	6	9	7	8
SPAIE(ROU)	2	5	3	7	1	4	9	6	8
SAIE(ROU)	---	4	---	5	2	1	3	---	6
Weighted average[6]	2.3	5.2	2.1	5.4	3.2	2.8	7.2	5.8	8.1
SPR1[2]									
GME(NZ)	8	7	2	6	4	3	1	5	9
SPAIE(NZ)	7	4	6	3	2	9	1	5	8
SPAIE(ROU)	4	7	5	9	2	3	1	6	8
Weighted average[6]	6.3	6.0	4.3	6.0	2.7	5.0	1.0	5.3	8.3
LFN1[3]									
GME(NZ)	9	3	4	6	8	5	1	7	2
SPAIE(NZ)	8	2	5	4	7	9	3	6	1
SPAIE(ROU)	4	2	7	5	6	9	8	3	1
SAIE(ROU)	---	3	---	5	2	6	4	---	1
Weighted average[6]	7.0	2.5	5.3	5.0	6.1	7.4	4.0	5.3	1.3
DST[4]									
SPAIE(NZ)	8	3	7	4	6	9	2	5	1
SPAIE(ROU)	4	3	8	7	6	9	2	5	1
SAIE(ROU)	---	3	---	5	1	6	4	---	2
Weighted average[6]	6.0	3.0	7.5	5.4	4.8	8.3	2.5	5.0	1.3
LSZ[5]									
PGE(NZ)	5	8	1	6	3	2	4	7	9
PGE(ROU)	3	5	2	7	4	1	6	8	9
GME(NZ)	3	5	1	6	2	4	8	7	9
SPAIE(NZ)	4	5	1	3	2	6	9	8	7
SPAIE(ROU)	5	4	1	7	2	3	9	6	8
SAIE(ROU)	---	3	---	5	1	2	4	---	6
Weighted average[6]	4.0	5.1	1.2	5.7	2.4	3.1	6.8	7.2	8.1

1 1 tallest…9 shortest
2 1 largest diameter…9 smallest diameter
3 1 most leafy…9 least leafy
4 1 most dense…9 least dense
5 1 biggest leaves…9 smallest leaves
6 Weighted average of all experiments detailed above
--- not applicable

HGT1 was one of the two characters measured in all experiments (Table 8.6). Results (rankings) for the two glasshouse experiments and the three spaced plant experiments (GME (NZ) and SPAIE (NZ) and (ROU)) were very similar. The shortest populations were Turoa, Astred and F.2419 in these five experiments. For the sixth experiment (SAIE), Turoa was still the shortest, but Astred was the third tallest. This result was in agreement with field observations, which indicate that Astred under solid stand sowing conditions tends to be much more erect than under a spaced plant environment. The rankings for the tallest populations were not so consistent among experiments, but Turkish, Quiñiquelli, Kenland and E116 were almost always the populations in the first four places of the ranking.

SPR1 was only measured for spaced plants (Table 8.6), because it was not possible to measure it under sward conditions or glasshouse pots. Astred was always the population with largest diameter and Turoa the one with smallest diameter, for all spaced-planted experiments.

LFN1 was a character that behaved consistently among experiments (Table 8.6), Turoa almost always being the most leafy population and E116 almost always the least leafy population pre-grazing. Kenland was one of the populations that was more leafy under a sward environment than as a spaced plant environment.

DST was also consistent among experiments (Table 8.6), Turoa, Astred, Hamua and Kenland being the most dense populations and E116 and Quiñiquelli being the least dense populations.

LSZ was the second of the two characters measured in all experiments (Table 8.6). LSZ is usually used to characterise populations, and these results confirm that use because of the consistent results obtained across experiments. For example, Turoa followed by F.2419 and Astred almost always had the smallest leaves and Quiñiquelli followed by Kenland and E116 the biggest leaves. The smallest leaves were found in the prostrate growth habit and that result was confirmed by the phenotypic and genotypic correlations of -0.38 and -0.59

respectively reported in Table 6.37 for the spaced plant-animal interaction experiments.

Table 8.7: Ranking among populations for HBT, FLW, HGT2, SPR2 and LFN2

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
HBT[1]									
PGE(NZ)	6	3	9	5	8	7	4	1	2
PGE(ROU)	7	4	9	6	8	5	3	2	1
GME(NZ)	6	4	8	5	7	9	1	3	2
SPAIE(NZ)	9	6	4	8	5	7	1	2	3
SPAIE(ROU)	8	5	9	4	7	6	1	3	2
Weighted average[6]	7.2	4.4	7.8	5.6	7.0	6.8	2.0	2.2	2.0
FLW[2]									
GME(NZ)	2	7	8	3	6	1	4	5	9
SPAIE(NZ)	2	5	8	7	6	1	3	4	9
SPAIE(ROU)	3	6	8	4	5	1	2	7	9
SAIE(ROU)	---	4	---	3	5	1	2	---	6
Weighted average[6]	2.3	5.6	8.0	4.4	5.5	1.0	2.8	5.3	8.5
HGT2[3]									
GME(NZ)	3	7	4	5	1	2	9	6	8
SPAIE(NZ)	1	2	5	3	4	6	7	9	8
SPAIE(ROU)	1	4	6	7	2	3	9	5	8
SAIE(ROU)	---	4	---	5	3	1	2	---	6
Weighted average[6]	1.7	4.3	5.0	5.0	2.5	3.2	7.2	6.7	7.6
SPR2[4]									
GME(NZ)	8	7	2	5	6	3	1	4	9
SPAIE(NZ)	7	3	6	2	4	9	1	5	8
SPAIE(ROU)	4	6	8	9	3	2	1	5	7
Weighted average[6]	6.3	5.3	5.3	5.3	4.3	4.7	1.0	4.7	8.0
LFN2[5]									
GME(NZ)	6	3	8	4	7	9	1	5	2
SPAIE(NZ)	4	1	7	2	6	8	3	5	9
SPAIE(ROU)	6	3	8	5	7	9	1	2	4
SAIE(ROU)	---	3	---	5	4	6	1	---	2
Weighted average[6]	5.3	2.5	7.7	3.9	6.2	8.2	1.5	4.0	4.5

1 1 prostrate...9 erect
2 1 flower most...9 flower less
3 1 tallest...9 shortest
4 1 largest diameter...9 smallest diameter
5 1 most leafy...9 least leafy
6 Weighted average of all experiments detailed above
--- not applicable

HBT is also a character used to classify populations, but results did not confirm this use (Table 8.7). The three prostrate populations were almost always the three most prostrate, but the erect and semi-erect populations were not so easy to separate as groups. Even though HBT could not be measured under sward conditions, the behaviour of Astred for HGT1 (higher in ranking for the sward than for the spaced plant environment; see Table 8.6), showed that under these conditions it was turning its habit into a semi-erect or erect category. Results for Quiñiquelli, Hamua, E116 and Turoa were in agreement with those reported by Claydon and Rumball (1982).

FLW was another character that was very consistent among experiments (Table 8.7), E116 and Turkish always being the populations that flowered most and Turoa and Quiñiquelli least.

HGT2 was similar between the spaced plant experiments (Table 8.7), but some differences were found for the sward environment with Astred. The shortest populations for the spaced plant experiments were Astred and Turoa, and the tallest were Turkish and Kenland. For the sward experiments the shortest population was Turoa and the tallest ones were E116 and Astred. This same inconsistency of Astred's behaviour according to sowing method was also reported for pre-grazing height.

SPR2 was also only measured in the spaced-planted experiments (Table 8.7) for the same reasons reported for the pre-grazing spread and was on average across experiments always largest for Astred and smallest for Turoa. The other populations were very similar in post-grazing spread with a range of weighted average from 4.3 to 6.3.

LFN2 (Table 8.7) was greatest in Astred and Hamua (more leaves remaining) and least leafy in E116 and Quiñiquelli. This character was also fairly consistent among experiments.

Table 8.8: Ranking among populations for DHGT, DSPR, DLFN, INTK, PRT and DGT

Character	Turkish	Hamua	Quiñiq.	Colenso	Kenland	E116	Astred	F.2419	Turoa
DHGT[1]									
GME(NZ)	8	3	2	7	9	1	6	4	5
SPAIE(NZ)	6	8	2	4	7	1	5	9	3
SPAIE(ROU)	4	5	1	7	2	3	9	6	8
SAIE(ROU)	---	3	---	5	2	1	4	---	6
Weighted average[7]	6.0	4.9	1.7	5.8	5.3	1.5	6.2	6.3	5.5
DSPR[2]									
GME(NZ)	5	9	1	8	2	6	3	7	4
SPAIE(NZ)	8	9	3	7	4	1	6	5	2
SPAIE(ROU)	4	6	1	7	2	3	8	5	9
Weighted average[7]	5.7	8.0	1.7	7.3	2.7	3.3	5.7	5.7	5.0
DLFN[3]									
GME(NZ)	7	6	2	8	3	1	9	5	4
SPAIE(NZ)	9	8	2	7	5	3	4	6	1
SPAIE(ROU)	5	4	2	6	3	7	9	8	1
SAIE(ROU)	---	4	---	3	2	5	6	---	1
Weighted average[7]	7.0	5.6	2.0	6.3	3.4	3.9	7.1	6.3	1.8
INTK[4]									
SPAIE(NZ)	9	8	2	7	4	3	5	6	1
SPAIE(ROU)	4	5	1	7	2	3	9	6	8
SAIE(ROU)	---	3	---	4	2	1	6	---	5
Weighted average[7]	6.5	5.6	1.5	6.3	2.8	2.5	6.8	6.0	4.7
PRT[5]									
SPAIE(NZ)	8	3	4	5	7	2	9	6	1
SPAIE(ROU)	6	5	1	2	4	8	9	7	3
SAIE(ROU)	---	2	---	4	1	3	6	---	5
Weighted average[7]	7.0	3.5	2.5	3.7	4.4	4.5	8.3	6.5	2.8
DGT[6]									
SPAIE(NZ)	6	3	2	5	4	7	9	8	1
SPAIE(ROU)	4	3	1	8	2	7	9	6	5
SAIE(ROU)	---	4	---	3	1	2	6	---	5
Weighted average[7]	5.0	3.3	1.5	5.6	2.5	5.8	8.3	7.0	3.5

1 1 largest DHGT...9 smallest DHGT
2 1 largest DSPR...9 smallest DSPR
3 1 largest DLFN...9 smallest DLFN
4 1 largest INTK...9 smallest INTK
5 1 largest PRT values...9 smallest PRT values
6 1 largest DGT values...9 smallest DGT values
7 Weighted average of all experiments detailed above
--- not applicable

Quiñiquelli and E116 had the largest reductions in height during grazing (Table 8.8), while Turkish, Astred and F.2419 had the smallest ones across experiments.

DSPR (Table 8.8) was also only calculated for the spaced-planted experiments where Quiñiquelli was the population with largest reductions in diameter and Harnua and Colenso showed the smallest reductions across experiments.

Turoa and Quiñiquelli were the populations with largest reductions in leafiness post-grazing (Table 8.8) while Astred and Turkish were the ones with least reduction in leafiness post-grazing.

INTK (Table 8.8) was largest for Quiñiquelli, Kenland, E116 and Turoa and smallest for Astred and Turkish, results being in good agreement across experiments.

PRT and DGT (Table 8.8) behaved in a similar way, Astred always being the population with lowest crude protein content and lowest digestibility, while Quiñiquelli, Kenland, Harnua and Turoa had the best values for both characters.

Combining results for groups of characters provides an overall description of the populations with respect to:

(a) population size before grazing (HGT1, SPR1 and HBT); where Turkish, Quiñiquelli, Kenland, E116 and Astred were the biggest populations pre-grazing.

(b) population size after grazing (HGT2 and SPR2);where Turkish, Kenland, E116 and Astred were the biggest populations post-grazing.

(c) most leafy and dense populations before grazing (LFN1, LSZ and DST); where Turoa and Quiñiquelli were the most leafy populations.

(d) quality and state of growth (PRT, DGT and FLW); where Quiñiquelli, Turoa, Harnua and Kenland had the best protein and digestibility values, and also showed least flowering.

(e) most grazed populations (LFN2, DHGT, DSPR, DLFN and INTK); were Quiñiquelli, Kenland, E116 and Turoa as reported in Section 8.3 and 8.6.

Combining further the results, Quiñiquelli and Kenland were the most grazed and had the best quality characteristics of all populations.

CONCLUDING COMMENTS

The four main objectives defined in Section 8.1 were considered achieved. Novel field and statistical designs were presented (Chapters 5, 6 and 7) where the grazing animal effects were accurately measured and genetic advance could be obtained under the experimental conditions.

Low genetic progress has been a common feature amongst open-pollinated forage species since the beginning of pasture breeding in the early nineteen-hundreds. This research work revealed a major reason for this, namely low heritability estimates which were obtained for most characters. These low heritabilities were even lower when estimation was done according to the response of a second species (grazers). The variation due to animal preferential defoliation on top of the intrinsic plant variation resulted in heritability estimates at the lower limits for being useful. These adverse breeding circumstances are the ones that forage breeders have to work with. This necessitates the use of sophisticated field design and subsequent analyses (such as the ones proposed in this thesis) in order to succeed in developing better forage cultivars. The main innovation of the experimental design was the use of non-contiguous plots. This is essential to provide random grazing choices. An optimum allocation study could be performed to assess the best combination of clones and plants to be used.

The importance of grazing preference as a way of increasing animal production, when pastures are offered as a sole choice, is debated in the literature. On the other hand, there is consensus that animals do not graze randomly, and that when offered a mixed sward strong

preferences are shown (Provenza and Balph, 1990; Black, 1990; Newman *et al.*, 1992). Therefore, the diet balance could be drastically changed by differences in relative preference of pasture components. The present method allows selection for grazing preference in both directions: to improve, or decrease, relative preference. Plant selection to decrease relative grazing preference might be useful for over-sowing situations where, for example, the main objective might be to increase soil fertility by the introduction of legumes and they should persist to achieve their objective.

With the novel field design in this work, it was possible to detect differential defoliation at the plant and population level. Differential defoliation at the plant level was only detected due to the inclusion of clones in the experiment which allowed genetically identical plants to be offered to the grazers to effect their selective grazing. For future breeding work, where differences might be sought at a dispersion line level, the possibility of increasing the number of breeding materials by excluding the clonal replicates could be considered. The exclusion of the clonal replicates brings further advantages, such as a reduction in time necessary for each selection cycle, and **the use of plants coming from seedlings**. These plants allow grazing managements closer to farmers' practice. With clones these practices were not possible due to the absence of tap roots and the high risk of killing the clones due to overgrazing. For other species rather than the tap-rooted ones, this criticism should not be a problem.

Plant breeders have historically paid more attention to grazing tolerance than to grazing preference because the former is very much involved in pasture persistence. Grazing preference is becoming an important trait when high yielding and persistent materials are available. Grazing tolerance could be measured from plant vigour from one grazing to the next, but a few months of experimental period in the first year of the plants life is too short a period to detect grazing tolerance differences. Grazing tolerance should be measured for longer periods of at least two years and the cause of plant death should be assessed to confirm if intolerance to grazing was the death cause. Other methods such as the mob-grazing technique (Mislevy *et al.*, 1982) and selection under continuos close grazing (Smith *et al.*,

1989) are available to select for grazing tolerance.

Similar results to McWilliam and Latter (1970) were found where for some characters direct selection under sward conditions was more effective than "indirect" selection under spaced plant conditions. Conversely, for other characters "indirect" selection proved to be an effective way of improving sward performance for defoliation rate. This was shown by their heritability estimates and their genetic correlations. The best environment to perform selection as shown by the results of this thesis is dependent on the characters considered; and most probably the results are species specific, which might explain the absence of an agreement in the literature of the best environment under which to conduct pasture breeding. Also, most comparisons in the literature were done at a phenotypic level, which hardly yields useful results for breeders, who ultimately manipulate genes.

Plant growth habit/morphology seemed less important in influencing selection in Red clover, and a direct measurement of forage removal was considered a better selection criterion. Post-grazing leafiness was found to be the best estimate of forage removal for spaced plant nurseries because it gave useful information with minimum use of time and resources. The Delphi survey conducted by Wheeler and Corbett (1989) from a panel of 19 ruminant nutritionists showed digestibility and crude protein content as consensus characters to improve feeding value. This research also confirms the usefulness of these characters for breeding purposes due to their plant heritability values and for their medium to high genetic correlation with the removal characters.

Conclusions

1. According to the analyses of variance reported in Chapters 5, 6 and 7 it was concluded that grazing was not at random, but was consistently affected by specific plant characteristics.

2. The four most intensively grazed populations (Quiñiquelli, Kenland, E116 and Turoa), represented extremes in morphology (one erect, two semi-erect and one prostrate genotype), leaf size, density, plant height, leafiness and flowering behaviour, indicating that these variables were not influencing animal preference as was expected.

3. The four populations have in common their leafy appearance, for example Quiñiquelli having the biggest leaves, Turoa being very dense and with many small leaves, while Kenland and E116 are intermediate, and with high nutritive value as determined by crude protein content and digestibility.

4. Post-grazing leafiness, difference between pre- and post-grazing leafiness, and index of intake were considered the most appropriate characters to measure animal effects because they were highly significant in the analyses of variance (F tests) for population and plant effects.

5. Overall restricted heritabilities in a broad sense for post-grazing leafiness were 0.25 and 0.21 for the spaced plant - animal interaction experiments in New Zealand and Uruguay respectively. These values were considered good enough to obtain genetic progress.

6. The assessment of post-grazing leafiness values was less laborious than alternative variables (difference between pre- and post-grazing leafiness, and index of intake).

7. It is recommended that a selection programme should not be based on simple morphological characters because grazing and preferential defoliation are complex phenomena in which all factors involved and their interactions are not entirely understood.

Following the proposed grazing methodology, preferential defoliation could be measured accurately, and breeding using those measurements could be done.

8. Phenotypic and genotypic correlations between crude protein content or digestibility and all other characters emphasised the importance of measuring both nutritional and morphological characters because they provide complementary information.

9. Crude protein content (0.58**) and digestibility (0.58**) had medium to high values of overall restricted heritability, and were considered useful for genetic advance in plant breeding.

10. For the characters plant density, post-grazing leafiness, difference between pre- and post-grazing leafiness and index of intake, spaced plants were the best environment to select for animal preference when applied to sward conditions.

11. The greatest genetic advance in pre- and post-grazing height was obtained when selection was done directly under sward conditions when its final use was also as swards.

12. For the characters pre-grazing leafiness, leaf size, flowering, and difference between pre- and post-grazing height, the best selection environment to obtain greater genetic advance when the final use was in sward conditions, depended on the intensity of selection that could be achieved under each environment.

13. The proposed new designs and analyses worked well.

14. It is concluded from Sections 8.10 and 8.11 that under the experimental conditions for either spaced plants or swards, genetic progress could be obtained for all characters.

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APPENDICES

APPENDIX 1 NORMALITY TESTS (432) FOR EACH CHARACTER IN THE GRAZING MANAGEMENT EXPERIMENT

Table A1.1: Normality test for each combinations of Time, Block, Population and Grazing management for each character

Characters	Percentage of Populations non-normally distributed
Habit	0%
Flower	<10%
Leaf Size	0%
Volume	<5%
Height 1	0%
Spread 1	0%
Leafiness 1	0%
Height 2	0%
Spread 2	0%
Leafiness 2	0%

APPENDIX 2 STANDARD ERROR OF HERITABILITY FOR THE MODEL FOR THE FIRST GRAZING FOR THE GRAZING MANAGEMENT EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P$,

$x_2 = S\,g$, (g = genetic fraction)

$x_3 = P + Sg$,

$y = S + \varepsilon + P$,

$\sigma^2_{x_1} = V_P$

$\sigma^2_{x_2} = V_S\,g^2$

$\sigma^2_{x_3} = V_P + V_S\,g^2$

$\sigma^2_y = V_S + V_\varepsilon + V_P + 2\,\{(-V_S / s) + ((V_S - s^2V_\varepsilon) / rbs^2)\}$

$\operatorname{cov}(x_1,y) = V_P + ((V_S - s^2V_\varepsilon) / rbs^2)$

$\operatorname{cov}(x_2,y) = g\{(-V_S / s)\}$

$\operatorname{cov}(x_3, y) = \operatorname{cov}(x_1, y) + \operatorname{cov}(x_2, y)$

All covariances are presented in Table A2.1.

Table A2.1: Covariances

	ε	P	R	B
S	$-V_S/s$	0	0	0
ε	---	$(V_S-s^2V_\varepsilon)/s^2rb$	$(V_S-s^2V_\varepsilon)/s^2p$	0
P	---	---	$(-V_S+s^2V_\varepsilon)/s^2rbp$	0
R	---	---	---	$(-V_S+s^2V_\varepsilon-s^2p^2V_R)/s^2p^2r$

APPENDIX 3 STANDARD ERROR OF HERITABILITY FOR THE MAIN MODEL OF THE GRAZING MANAGEMENT EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P,$

$x_2 = S\ g,$ (g = genetic fraction)

$x_3 = P + Sg,$

$y = \varepsilon + TPUM + TPM + TPU + TP + S + \delta + PUM + PM + PU + P,$

$\sigma^2_{x_1} = V_P$

$\sigma^2_{x_2} = V_S\ g^2$

$\sigma^2_{x_3} = V_P + V_S\ g^2$

$$\sigma^2_y = V_\varepsilon + V_{TPUM} + V_{TPM} + V_{TPU} + V_{TP} + V_S + V_\delta + V_{PUM} + V_{PM} + V_{PU} + V_P +$$
$$2\{((V_\varepsilon(t(-t - bs + 2) - 1)) / bst^2) + ((V_{TPM}(-t - m + 2)) / tm) +$$
$$((V_{TPU}(-t - u + 2)) / tu) + (-V_{TPUM}/t) + (-V_{TP} / t) + (-V_{PM} / m) + (-V_{PU} / u) +$$
$$(((V_\varepsilon - b^2s^2V_{TPUM})(t(m + u) - 2(t + m + u) + 4)) / b^2s^2mut) + ((V_\varepsilon - t^2V_S) / st^2) +$$
$$((V_\varepsilon - t^2(V_S - s^2V_\delta)) / bs^2t^2) +$$
$$(((2V_\varepsilon - t^2(V_S - s^2(V_\delta - b^2V_{PUM})))(m + u - 2)) / b^2s^2t^2mup)\}$$

$$\operatorname{cov}(x_1,y) = V_P + (V_{TPM} / tm) + (V_{TPU} / tu) + (-V_{TP} / t) + (-V_{PM} / m) + (-V_{PU} / u) +$$
$$((V_\varepsilon - b^2s^2V_{TPUM}) / b^2s^2mut) +$$
$$((-2V_\varepsilon + t^2(V_S - s^2(V_\delta - b^2V_{PUM}))) / b^2s^2t^2mu)$$

$$\text{cov}(x_2,y) = g\{V_S + ((V_\varepsilon(t\,(-bs + 1)\,-1)) / bst^2) + ((V_\varepsilon - t^2V_S) / st^2)\}$$

$$\text{cov}(x_3, y) = \text{cov}(x_1, y) + \text{cov}(x_2, y)$$

All covariances are presented in Table A3.1a, b, c and d.

Table A3.1a: Covariances

	TPUM	TPM	TPU	TP	TUM	TM	TU	T	S	δ
ε	$\frac{-V\varepsilon}{sb}$	0	0	0	0	0	0	0	$\frac{-V\varepsilon}{t}$	0
TPUM	---	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2u}$	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2m}$	$\frac{-V\varepsilon + s^2b^2V_{TPUM}}{s^2b^2mu}$	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2p}$	$\frac{-V\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pu}$	$\frac{-V\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pm}$	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2pmu}$	$\frac{V\varepsilon}{sbt}$	0
TPM	---	---	$\frac{-V\varepsilon + s^2b^2V_{TPUM}}{s^2b^2um}$	$\frac{-V_{TPM}}{m}$	$\frac{-V\varepsilon + s^2b^2V_{TPUM}}{s^2b^2up}$	$\frac{-V_{TPM}}{p}$	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2pmu}$	$\frac{V_{TPM}}{pm}$	0	0
TPU	---	---	---	$\frac{-V_{TPU}}{u}$	$\frac{-V\varepsilon + s^2b^2V_{TPUM}}{s^2b^2mp}$	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2pmu}$	$\frac{-V_{TPU}}{p}$	$\frac{V_{TPU}}{pu}$	0	0
TP	---	---	---	---	$\frac{V\varepsilon - s^2b^2V_{TPUM}}{s^2b^2pmu}$	$\frac{V_{TPM}}{pm}$	$\frac{V_{TPU}}{pu}$	$\frac{-V_{TP}}{p}$	0	0
TUM	---	---	---	---	---	$\frac{-V_{TUM}}{u}$	$\frac{-V_{TUM}}{m}$	$\frac{V_{TUM}}{mu}$	0	0
TM	---	---	---	---	---	---	$\frac{V_{TUM}}{um}$	$\frac{-V_{TM}}{m}$	0	0
TU	---	---	---	---	---	---	---	$\frac{-V_{TU}}{u}$	0	0
T	---	---	---	---	---	---	---	---	0	0
S	---	---	---	---	---	---	---	---	---	$\frac{V\varepsilon - t^2V_S}{st^2}$

Table A3.1b: Covariances

	PUM	PM	PU	P	τ	UM	M	U	B
ε	$\frac{V_\varepsilon}{stb}$	0	0	0	0	0	0	0	0
TPUM	$\frac{-V_{TPUM}}{t}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2ut}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2mt}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2utm}$	0	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pt}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2utp}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2tmp}$	0
TPM	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2ut}$	$\frac{-V_{TPM}}{t}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2utm}$	$\frac{V_{TPM}}{tm}$	0	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2utp}$	$\frac{V_{TPM}}{pt}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2utmp}$	0
TPU	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2mt}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2mut}$	$\frac{-V_{TPU}}{t}$	$\frac{V_{TPU}}{ut}$	0	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2mpt}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2utmp}$	$\frac{V_{TPU}}{pt}$	0
TP	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2mut}$	$\frac{V_{TPM}}{mt}$	$\frac{V_{TPU}}{tu}$	$\frac{-V_{TP}}{t}$	0	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2utmp}$	$\frac{-V_{TPM}}{mpt}$	$\frac{-V_{TPU}}{ptu}$	0
TUM	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pt}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2put}$	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2mpt}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pmut}$	0	$\frac{-V_{TUM}}{t}$	$\frac{V_{TUM}}{ut}$	$\frac{V_{TUM}}{mt}$	0
TM	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2put}$	$\frac{V_{TPM}}{pt}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pmut}$	$\frac{-V_{TPM}}{tpm}$	0	$\frac{V_{TUM}}{ut}$	$\frac{-V_{TM}}{t}$	$\frac{-V_{TUM}}{umt}$	0
TU	$\frac{V_\varepsilon - s^2b^2V_{TPUM}}{s^2b^2pmt}$	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pmut}$	$\frac{V_{TPU}}{tp}$	$\frac{-V_{TPU}}{tpu}$	0	$\frac{V_{TUM}}{mt}$	$\frac{-V_{TUM}}{tmu}$	$\frac{-V_{TU}}{t}$	0
T	$\frac{-V_\varepsilon + s^2b^2V_{TPUM}}{s^2b^2pmut}$	$\frac{-V_{TPM}}{pmt}$	$\frac{-V_{TPU}}{tpu}$	$\frac{V_{TP}}{tp}$	0	$\frac{-V_{TUM}}{mut}$	$\frac{V_{TM}}{mt}$	$\frac{V_{TU}}{tu}$	0
S	$\frac{-V_\varepsilon}{st^2b}$	0	0	0	0	0	0	0	0

Table A3.1c: Covariances

	PUM	PM	PU	P	τ
δ	$\frac{-V_\varepsilon + t^2(V_S - s^2 V_\delta)}{s^2 t^2 b}$	0	0	0	$\frac{-V_\varepsilon + t^2(V_S - s^2 V_\delta)}{s^2 t^2 p}$
PUM	---	$\frac{2V_\varepsilon - t^2(V_S - s^2(V_\delta - b^2 V_{PUM}))}{s^2 t^2 b^2 u}$	$\frac{2V_\varepsilon - t^2(V_S - s^2(V_\delta - b^2 V_{PUM}))}{s^2 t^2 b^2 m}$	$\frac{-2V_\varepsilon + t^2(V_S - s^2(V_\delta - b^2 V_{PUM}))}{s^2 t^2 b^2 mu}$	$\frac{V_\varepsilon - t^2(V_S - s^2 V_\delta)}{s^2 t^2 bp}$
PM	---	---	$\frac{-2V_\varepsilon + t^2(V_S - s^2(V_\delta - b^2 V_{PUM}))}{s^2 t^2 b^2 mu}$	$\frac{-V_{PM}}{m}$	0
PU	---	---	---	$\frac{-V_{PU}}{u}$	0
P	---	---	---	---	0

Table A3.1d: Covariances

	UM	M	U	B
δ	$\frac{V_\varepsilon - t^2\left(V_S - s^2 V_\delta\right)}{s^2 t^2 bp}$	0	0	0
PUM	$\frac{V_\varepsilon - s^2 b^2 t^2 V_{PUM}}{s^2 t^2 b^2 p}$	$\frac{-2V_\varepsilon + t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 up}$	$\frac{-2V_\varepsilon + t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 mp}$	0
PM	$\frac{-2V_\varepsilon + t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 up}$	$\frac{-V_{PM}}{p}$	$\frac{2V_\varepsilon - t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 mpu}$	0
PU	$\frac{-2V_\varepsilon + t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 mp}$	$\frac{2V_\varepsilon - t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 mpu}$	$\frac{-V_{PU}}{p}$	0
P	$\frac{2V_\varepsilon - t^2\left(V_S - s^2\left(V_\delta - b^2 V_{PUM}\right)\right)}{s^2 t^2 b^2 mpu}$	$\frac{V_{PM}}{mp}$	$\frac{V_{PU}}{up}$	0
τ	$\frac{-V_\tau}{b}$	0	0	$\frac{V_\varepsilon - t^2\left(V_S - s^2\left(V_\delta - p^2 V\tau\right)\right)}{s^2 t^2 p^2 mu}$
UM	---	$\frac{s^2 t^2 p^2\left(V_\tau - b^2 V_{UM}\right)}{s^2 b^2 t^2 p^2 u}$	$\frac{s^2 t^2 p^2\left(V_\tau - b^2 V_{UM}\right)}{s^2 b^2 t^2 p^2 m}$	$\frac{-V_\varepsilon + t^2\left(V_S - s^2\left(V_\delta - p^2 V\tau\right)\right)}{s^2 t^2 p^2 mub}$
M	---	---	$\frac{s^2 t^2 p^2\left(-V_\tau + b^2 V_{UM}\right)}{s^2 b^2 t^2 p^2 mu}$	0
U	---	---	---	0

APPENDIX 4 NORMALITY TESTS (216) FOR EACH CHARACTER IN THE SPACED PLANT-ANIMAL INTERACTION EXPERIMENTS

Table A4.1: Normality test for each combinations of Time, Block and Population for each character

Characters	Percentage of Populations non-normally distributed
Habit	<11%
Flower	<36%
Leaf Size	<5%
Density	<3%
Height 1	<3%
Spread 1	<3%
Leafiness 1	<8%
Height 2	<5%
Spread 2	0%
Leafiness 2	0%

APPENDIX 5 STANDARD ERROR OF HERITABILITY FOR FIRST GRAZING AND QUALITY CHARACTERS FOR THE SPACED PLANT-ANIMAL INTERACTION EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P$

$x_2 = S\ g$ (being g the genetic fraction)

$x_3 = P + S\ g$

$y = S + \varepsilon + PE + P$

$\sigma^2_{x1} = V_P$

$\sigma^2_{x2} = V_S\ g^2$

$\sigma^2_{x3} = V_P + V_S\ g^2 + 2g\ \operatorname{cov}(P,S)$

$\sigma^2_y = S + \varepsilon + PE + P + 2\ [(-V_S/s) + (((V_S - s^2\ V_\varepsilon)(p + b - 1)) / s^2bp) +$

$((-V_S + s^2\ V_\varepsilon - s^2\ b^2\ V_{PE}) / s^2b^2e)]$

$\operatorname{cov}(x_1, y) = ((-V_S + s^2\ V_\varepsilon - s^2\ b^2\ V_{PE}) / s^2b^2e) + V_P$

$\operatorname{cov}(x_2, y) = g[V_S - V_S/s]$

$\operatorname{cov}(x_3, y) = \operatorname{cov}(x_1, y) + \operatorname{cov}(x_2, y)$

All covariances are presented in Table A5.1.

Table A5.1: Covariances

	ε	PE	P	B	E
S	$-V_S/s$	0	0	0	0
ε	---	$(V_S-s^2V_\varepsilon)/s^2b$	0	$(V_S-s^2V_\varepsilon)/s^2p$	$(-V_S+s^2V_\varepsilon)/s^2bp$
PE	---	---	$(-V_S+s^2V_\varepsilon-s^2b^2V_{PE})/s^2b^2e$	$(-V_S+s^2V_\varepsilon)/s^2bp$	$-V_{PE}/p$
P	---	---	---	0	$(V_S-s^2V_\varepsilon+s^2b^2V_{PE})/s^2b^2ep$
B	---	---	---	---	$-V_B/b$

APPENDIX 6 STANDARD ERROR OF HERITABILITY FOR MAIN MODEL OF THE SPACED PLANT-ANIMAL INTERACTION EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P$,

$x_2 = Sg$, (g = genetic fraction)

$x_3 = P + S\,g$,

$y = \varepsilon + TPE + TP + S + \delta + PE + P$,

$\sigma^2_{x_1} = V_P$

$\sigma^2_{x_2} = V_S\,g^2$

$\sigma^2_{x_3} = V_P + V_S\,g^2$

$$\sigma^2_y = V_\varepsilon + V_{TPE} + V_{TP} + V_S + V_\delta + V_{PE} + V_P +$$
$$2\{((V_\varepsilon(t(-t - bs + 2) - 1) / bst^2)) + (((V_\varepsilon - b^2s^2V_{TPE})(-t - 2p)) / b^2s^2pet) +$$
$$(-V_{TP} / t) + ((-V_\varepsilon + t^2(V_S - s^2V_\delta)) / bs^2t^2) + ((V_\varepsilon - t^2V_S) / st^2) +$$
$$((2V_\varepsilon - t^2(V_S - s^2(V_\delta - b^2V_{PE}))) / b^2s^2et^2) + (-V_{TPE}/t)\}$$

$$\operatorname{cov}(x_1,y) = ((-V_\varepsilon + b^2s^2V_{TPE}) / b^2s^2et) + (-V_{TP} / t) +$$
$$((2V_\varepsilon - t^2(V_S - s^2(V_\delta - b^2V_{PE}))) / b^2s^2et^2) + V_P$$

$$\operatorname{cov}(x_2,y) = g\{((V_\varepsilon(t(-bs + 1) - 1)) / bst^2) + ((V_\varepsilon - t^2V_S) / st^2) + V_S\}$$

$$\operatorname{cov}(x_3,y) = \operatorname{cov}(x_1,y) + \operatorname{cov}(x_2,y)$$

All covariances are presented in Table A6.1.

Table A6.1: Covariances

	TPE	TE	TP	T	S	δ	PE	P	B	E
ε	$\frac{-V_\varepsilon}{bs}$	0	0	0	$\frac{-V_\varepsilon}{t}$	0	$\frac{V_\varepsilon}{bst}$	0	0	0
TPE	---	$\frac{V_\varepsilon - b^2s^2V_{TPE}}{b^2s^2p}$	$\frac{V_\varepsilon - b^2s^2V_{TPE}}{b^2s^2e}$	$\frac{-V_\varepsilon + b^2s^2V_{TPE}}{b^2s^2pe}$	$\frac{V_\varepsilon}{bst}$	0	$\frac{-V_{TPE}}{t}$	$\frac{-V_\varepsilon + b^2s^2V_{TPE}}{b^2s^2et}$	0	$\frac{-V_\varepsilon + b^2s^2V_{TPE}}{b^2s^2pt}$
TE	---	---	$\frac{-V_\varepsilon + b^2s^2V_{TPE}}{b^2s^2pe}$	$\frac{-V_{TE}}{e}$	0	0	$\frac{-V_\varepsilon + b^2s^2V_{TPE}}{b^2s^2pt}$	$\frac{V_\varepsilon - b^2s^2V_{TPE}}{b^2s^2pet}$	0	$\frac{-V_{TE}}{t}$
TP	---	---	---	$\frac{-V_{TP}}{p}$	0	0	$\frac{-V_\varepsilon + b^2s^2V_{TPE}}{b^2s^2et}$	$\frac{-V_{TP}}{t}$	0	$\frac{V_\varepsilon - b^2s^2V_{TPE}}{b^2s^2pet}$
T	---	---	---	---	0	0	$\frac{V_\varepsilon - b^2s^2V_{TPE}}{b^2s^2pet}$	$\frac{V_{TP}}{pt}$	0	$\frac{V_{TE}}{et}$
S	---	---	---	---	---	$\frac{V_\varepsilon - t^2V_S}{st^2}$	$\frac{-V_\varepsilon}{bst^2}$	0	0	0
δ	---	---	---	---	---	---	$\frac{-V_\varepsilon + t^2(V_S - s^2V_\delta)}{s^2t^2b}$	0	$\frac{-V_\varepsilon + t^2(V_S - s^2V_\delta)}{s^2t^2p}$	$\frac{V_\varepsilon - t^2(V_S - s^2V_\delta)}{s^2t^2bp}$
PE	---	---	---	---	---	---	---	$\frac{2V_\varepsilon - t^2(V_S - s^2(V_\delta - b^2V_{PE})}{s^2t^2b^2e}$	$\frac{V_\varepsilon - t^2(V_S - s^2V_\delta)}{s^2t^2bp}$	$\frac{V_\varepsilon - b^2s^2t^2V_{PE}}{s^2t^2b^2p}$
P	---	---	---	---	---	---	---	---	0	$\frac{-V_\varepsilon + b^2s^2t^2V_{PE}}{s^2t^2b^2ep}$
B	---	---	---	---	---	---	---	---	---	$\frac{-V_B}{b}$

APPENDIX 7 NORMALITY TESTS (72) FOR EACH CHARACTER IN THE SWARD-ANIMAL INTERACTION EXPERIMENT

Table A7.1: Normality test for each combinations of Site, Time, Block and Population for each character

Characters	Percentage of Populations non-normally distributed
Flower	<15%
Leaf Size	<15%
Density	<10%
Height 1	0%
Leafiness 1	0%
Height 2	0%
Leafiness 2	0%

APPENDIX 8 STANDARD ERROR OF HERITABILITY FOR FIRST GRAZING FOR THE SWARD-ANIMAL INTERACTION EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P$

$y = S + \varepsilon + PE + P$

$\sigma^2_{x1} = V_P$

$\sigma^2_y = S + R + \varepsilon + P + 2[(-V_S/s) + ((V_S - s^2 V_R) / s^2 r) + ((-V_S + s^2 V_R - s^2 r^2 V_\varepsilon) / s^2 r^2 b)]$

$\operatorname{cov}(x_1, y) = ((-V_S + s^2 V_R - s^2 r^2 V_\varepsilon) / s^2 r^2 b) + V_P$

All covariances are presented in Table A8.1.

Table A8.1: Covariances

	R	ε	P	B
S	$-V_S/s$	0	0	0
R	---	$(V_S-s^2V_R)/s^2r$	0	0
ε	---	---	$(-V_S+s^2V_R-s^2r^2V_\varepsilon)/s^2r^2b$	$(-V_S+s^2V_R-s^2r^2V_\varepsilon)/s^2r^2p$
P	---	---	---	$(V_S-s^2V_R+s^2r^2V_\varepsilon)/s^2r^2bp$

APPENDIX 9 STANDARD ERROR OF HERITABILITY FOR NUTRITIONAL DATA FOR THE SWARD-ANIMAL INTERACTION EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P$

$y = \varepsilon + PE + P$

$\sigma^2_{x1} = V_P$

$\sigma^2_y = R + \varepsilon + P + 2[(-V_R/r) + ((V_R - r^2 V_\varepsilon) / r^2 b)]$

$\operatorname{cov}(x_1, y) = ((V_R - r^2 V_\varepsilon) / r^2 b)] + V_P$

All covariances are presented in Table A9.1.

Table A9.1: Covariances

	ε	P	B
R	$-V_R/r$	0	0
ε	---	$(V_R-r^2V_\varepsilon)/r^2b$	$(V_R-r^2V_\varepsilon)/r^2p$
P	---	---	$(-V_R+r^2V_\varepsilon)/r^2bp$

APPENDIX 10 STANDARD ERROR OF HERITABILITY FOR THE MAIN MODEL OF THE SWARD-ANIMAL INTERACTION EXPERIMENT

The variance of the heritability (h^2) was calculated following Osborne and Paterson (1952), being $\sigma^2_{h^2} = \left[\mu_y^2\sigma_x^2 + \mu_x^2\sigma_y^2 - 2\mu_x\mu_y \operatorname{cov}(x,y)\right] / \mu_y^4$.

$x_1 = P$,

$x_2 = S\ g$, (g = genetic fraction)

$x_3 = P + Sg$,

$y = \varepsilon + TP + S + R + \delta + P$,

$\sigma^2_{x_1} = V_P$

$\sigma^2_{x_2} = V_S\ g^2$

$\sigma^2_{x_3} = V_P + V_S\ g^2 - 2V_\varepsilon / brst^2$

$\sigma^2_y = V_\varepsilon + V_{TP} + V_S + V_R + V_\delta + V_P + 2\ \{((V_\varepsilon(t(-t - brs + 2) - 1) / brst^2)) +$

$(-V_{TP}/t) + ((V_\varepsilon - t^2V_S) / st^2) + ((-V_\varepsilon + t^2(V_S - s^2V_R)) / rs^2t^2) +$

$((V_\varepsilon - t^2(V_S + s^2(V_R - r^2V_\delta))) / br^2s^2t^2)\}$

$\operatorname{cov}(x_1,y) = ((V_\varepsilon(t - 1)) / brst^2) + (-V_{TP}/t) + ((-V_\varepsilon - t^2(V_S + s^2(V_R - r^2V_\delta))) / br^2s^2pt^2) +$

V_P

$\operatorname{cov}(x_2,y) = g\{((V_\varepsilon(t\ (-brs + 1) - 1)) / brst^2) + V_S + ((V_\varepsilon - t^2V_S) / st^2)\}$

$\operatorname{cov}(x_3, y) = \operatorname{cov}(x_1, y) + \operatorname{cov}(x_2, y)$

All covariances are presented in Table A10.1.

Table A10.1: Covariances

	TP	T	S	R	δ	P	B
ε	$\frac{-V_\varepsilon}{brs}$	0	$\frac{-V_\varepsilon}{t}$	0	0	$\frac{V_\varepsilon}{brst}$	0
TP	---	$\frac{V_\varepsilon - b^2r^2s^2V_{TP}}{b^2r^2s^2p}$	$\frac{V_\varepsilon}{brst}$	0	0	$\frac{-V_{TP}}{t}$	0
T	---	---	0	0	0	$\frac{-V_\varepsilon + b^2r^2s^2V_{TP}}{b^2r^2s^2pt}$	0
S	---	---	---	$\frac{V_\varepsilon - t^2V_S}{st^2}$	0	$\frac{-V_\varepsilon}{brst^2}$	0
R	---	---	---	---	$\frac{-V_\varepsilon + t^2(V_S - s^2V_R)}{rs^2t^2}$	0	0
δ	---	---	---	---	---	$\frac{V_\varepsilon - t^2(V_S - s^2(V_R - r^2V_\delta))}{br^2s^2t^2}$	$\frac{V_\varepsilon - t^2(V_S - s^2(V_R - r^2V_\delta))}{pr^2s^2t^2}$
P	---	---	---	---	---	---	$\frac{-V_\varepsilon + t^2(V_S - s^2(V_R - r^2V_\delta))}{pbr^2s^2t^2}$