# A STUDY OF THE ACCEPTABILITY OF *HOLCUS* SPP. TO PERENDALE SHEEP

A Thesis presented in partial fulfilment of the requirements for the degree of

Master of Agricultural Science
in Plant Science

at
Massey University

Nicholas Evan Cameron
1979

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## ABSTRACT

Various characters are reputed to reduce the acceptability of Yorkshire fog grass *(Holcus lanatus)* to sheep. The relative importance of these characters in determining the acceptability of Yorkshire fog to sheep was investigated in summer, autumn, and early-winter of 1978, using standardised regression, and based upon a phenotypically diverse collection of spaced plants from fifty-three seed populations. A clump defoliation score was used to assess sheep preference.

Cluster analysis of ratios of the standardised partial regression coefficients from individual genotype populations generally confirmed the results obtained from the standardised partial regression coefficient ratios of pooled genotype populations.

Sheep rejected plants exhibiting a high proportion of inflorescences, dead leaf and sheath material and crown rust infection. The presence of inflorescences and crown rust were respectively 1.5 and 0.86 times as important as clump greenness over all genotype populations, in the summer period. Leaf pubescence was only 0.13 times as important as clump greenness and was therefore considered relatively unimportant in determining sheep preference. Leaf tensile strength, leaf width, clump height and diameter, clump erectness, leaf flavanol level and soluble sugar level, were also considered unimportant in this study, and ranged from 0.57 to 0.019 times as important as clump greenness in determining sheep preference. However only 20-25% of the variation in sheep preference was explained by the characters examined in the three seasons of this study. The unexplained variation may have been due to a high level of amongst sheep preference variance or to unassessed plant characters.

The phenotypic variation of each character was partitioned using a split-plot-in-time model. Broad-sense heritability estimates for all characters examined were low and ranged from 34% to 0.4%. It was suggested from these results that the acceptability of Yorkshire fog grass to sheep, by reduction of inflorescences and crown rust infection, and by removal of excessive dead leaf and sheath material, was largely under the control of grazing management (i.e. an aspect of the environment). However, some progress might be achieved by selection and breeding for genotypes with reduced levels of inflorescences ($\hat{h}^2$= 34%) and crown rust infection ($\hat{h}^2$= 29%).

ACKNOWLEDGEMENTS

I am particularly grateful to Dr I.L. Gordon of the Agronomy Department for his guidance and supervision throughout this study.

I wish to thank: Dr R.M. Haslemore of the Plant Physiology Division, D.S.I.R. for his thin-layer chromatography determinations and for providing random checks of samples for soluble sugar levels; Dr P.S. Evans of Grasslands Division, D.S.I.R. for the loan of his leaf strength machine; Dr N.C. Lambrechtsen of National Plant Materials Centre, M.O.W. for his Dutch-English translations; Mr Hugh Nielsen of the Horticulture Department for advice on photomicrography technique; Mr Terry Lynch of the Agronomy Department for assistance in weed spraying the collection and general sheep work; the post-graduate students of the Agronomy Department and others who assisted in the preliminary sheep sampling study; and Professor B.R. Watkin and other staff members of the Agronomy Department for useful discussions and their encouragement. Special thanks to Mrs J. Humphries for her typing of this thesis.

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# INTRODUCTION

Yorkshire fog grass *(Holcus lanatus)* is commonly found in many of the temperate pastoral grasslands of the world (Bocher and Larsen, 1958) and is widely distributed throughout New Zealand (Jacques and Munro, 1963). *Holcus mollis* is also found in New Zealand. Previous attempts to distinguish *H. lanatus*, *H. lanatus* x *mollis*, and *H. mollis* cytological races using morphological criteria have proved unsuccessful (Carroll and Jones, 1962). Chromosome counting appears to be the only positive means of identification at present (Jones, 1958). In much of the literature and in this study it is not certain which species are present, hence 'Yorkshire fog' or *Holcus* spp. have been prefered names in this thesis.

Many features of Yorkshire fog appear to recommend its use in New Zealand grasslands. Ecologically Yorkshire fog is exceptional for its almost complete lack of edaphic specialisation (Beddows, 1961a; Levy, 1970). It may be found growing on soils of low to high fertility and tolerates not only highly acidic soils but water-logged and wet soils as well as soils of average moisture content. In addition Yorkshire fog has a wide tolerance of temperature regimes (Mitchell and Lucanus, 1960).

Several agronomic advantages of Yorkshire fog are realised. Although suited to a lenient system of defoliation, Yorkshire fog also competes under close grazing (Watkin and Robinson, 1974) providing that the close defoliations are reasonably frequent and do not follow a period of lax grazing (Jacques and Munro, 1963). The seasonal production of *H. lanatus cv. 'Massey Basyn'* over a 2½ year period at Massey University was shown to compare favourably with the commonly used ryegrasses of New Zealand (Watkin and Robinson, 1974). A similar lack of disparity exists between Yorkshire fog and perennial ryegrass in terms of digestibility, average crude protein content and chemical composition (Bathhurst and Mitchell, 1958; Jacques, 1974; Watt, 1978). However, Yorkshire fog may be inferior because of low iodine content (Butler and Johnson, 1957) and the presence of flavanols (Gordon, unpubl.).

There are however other undesirable features including the presence of dead basal material, susceptibility to attack by crown rust *(Puccinia coronata)* and the putative low relative acceptability of Yorkshire fog at certain stages of growth. Jacques (1974 has suggested that improvement of the relative acceptability of Yorkshire fog by selection and breeding within the strain might be possible.

The primary objective of this study was to determine the importance of the characters putatively affecting the acceptability of Yorkshire fog to sheep, using a phenotypically diverse collection of *Holcus* plants over different seasons of the year. The plant characters were studied under a hard, monthly, grazing management system. A preliminary pilot study was carried out to determine the number of sheep and grazing time required for adequate sampling of all plants in the collection.

# CHAPTER 1. LITERATURE REVIEW

## 1.1 THE ACCEPTABILITY OF YORKSHIRE FOG TO RUMINANTS

Food selection by grazing ruminants is influenced by a complexity of plant, animal, and environmental factors. Previous attempts to review the process of food selection (Ivins, 1952; Jones, 1952; Garner, 1963; Heady, 1964; McBride *et al.*, 1967; Arnold and Hill, 1971) have shown that these factors may interact. Hence any assessment of the relative acceptability of one plant species with another is difficult.

Although it has been stated that Yorkshire fog is less acceptable to ruminants than other grasses in the sward (Davies, 1925; Stapledon and Milton, 1932; Milton, 1933; Ivins, 1952, 1964) such as perennial ryegrass *(Lolium perenne)*, cocksfoot *(Dactylis glomerata)* and timothy *(Phleum pratense)*, assessments have often been made when the grasses are at different stages of growth and the dietary history of the experimental animals not considered.

The effect of ruminant dietary history may be important in determining animal preference particularly if the grazing experience takes place in early life when imprinting occurs (Tribe and Gordon, 1950; Jones, 1952; Garner, 1963; Arnold, 1964b; Langlands, 1969; Arnold and Maller, 1977).

Watkin and Robinson (1974) observed that sheep coming off three ryegrass pastures and on to pure Yorkshire fog *(H. lanatus* cv. Massey Basyn*)* found it unacceptable. However, adequate defoliation of Yorkshire fog was achieved using a separate group of sheep which remained on this species throughout the year. Tribe and Gordon (1950) and Jones (1952) have suggested that although initially ignored, ruminants may acquire a taste for Yorkshire fog.

Another problem in assessing the relative acceptability of Yorkshire fog with other grass species to ruminants is that selectivity of grazing in the field may also be influenced by stocking rate, age, breed, and physiological condition of the animal in addition to social interactions with other animals. Consideration of the animal senses used in food selection allows some of these influences to be examined.

## 1.2 ANIMAL SENSES

In sheep, and probably for other ruminant species, the senses of touch, smell and taste are of greatest importance in food selection (Tribe, 1949; Arnold, 1966a). The role of sight is primarily one of orientation to the flock and to the vegetation when animals are grazing, but there is no conclusive evidence that sight is used in food selection (Tribe and Gordon, 1949; Arnold, 1966b). Surgically treated sheep having single and multiple sensory impairment (Arnold, 1966a) showed that touch (mental and infraorbital nerves), smell (olfactory lobes), and taste (lingual nerve, lingual branch of glossopharyngeal nerve) were all important in food selection. Marked changes in the relative acceptability of plant species or varieties occurred when each of these senses was impaired in turn. Arnold (1966a) considered that the selection of particular plants for food depended upon a combination of all the orosensory factors (taste, olfaction, and oral mechano-receptors) rather than to a single source of stimulus.

The current nutritional state or the presence of some metabolic disturbance in the animal may influence food selection. A hungry animal may lower either taste or smell rejection thresholds (Goatcher and Church, 1970a). Taste or smell rejection thresholds have not been reported for animals in pregnancy, lactation, or ill-health, although these conditions have been reported to influence food selection (Jones, 1952; Heady, 1964).

Differences in the taste responses of ruminant species, breeds and individuals have been demonstrated using the classical "two-choice" test method with ascending or descending concentrations of test solutions. Different ruminant species have been tested including goats (Bell, 1959; Goatcher and Church 1970 c,d), cattle (Stubbs and Kare, 1958; Bell and Williams, 1959; Mehren and Church, 1977), deer (Rice and Church, 1974) and sheep (Goatcher and Church, 1970 a,b,c,d; Arnold and Hill, 1971). Test solution intake as a proportion of total fluid intake has been assessed for acid, sweet, salty, or bitter tasting chemicals. Such assessments have been criticised on the grounds that the chemicals (or chemical form) used, such as quinine sulphate and acetic acid, are rarely found in plants. In addition the "taste" choice confronting the animal is not a simple choice situation in plants, but will involve levels of bitterness plus levels of acidity plus levels of sweetness plus levels of saltiness mixed together (Arnold and Hill, 1971).

Individual animal variation in food selection may be important. Taste (and smell) rejection thresholds for individuals within a species may vary (Goatcher and Church, 1970a). Even for a single animal the response to a particular food may vary with time (Arnold and Hill, 1971). Hence the response of a single individual of a species towards a food may not be a reliable guide to predicting that of another individual in the same population.

Despite the large variation between individuals and between groups of animals of a species, differences between breeds of animals in taste or smell responses may also occur. Significantly different taste (and smell) response curves for citric acid and acetic acid were shown for four sheep breeds of Australia (Arnold and Boundy - in Arnold and Hill, 1971).

The reaction of one ruminant species to a food may not be a reliable guide to predicting the reaction of another ruminant species (Goatcher and Church, 1970 c,d). The use of sheep to determine cattle preferences for six grass species in Rhodesia was shown to be totally misleading (Mills, 1977).

## 1.3 PLANT FACTORS INFLUENCING FOOD SELECTION

Assessment of characters determining the acceptability of a plant may be confounded by the availability of the herbage present and if the characters assessed are themselves highly corrrelated.

Grazing sheep move in the horizontal plane and select in the vertical plane (Arnold, 1964a). Hence highly preferred plants at the base of the sward may not be accessable until overlying herbage has been removed. Jacques (1974) suggested that Yorkshire fog plants having an extreme prostrate habit of growth may be unacceptable. However they may be simply unavailable to grazing stock.

Generally as plant availability decreases, often because of increased grazing pressure, so does selectivity and less acceptable forage must be eaten. However for highly unacceptable plant species, such as some *Phalaris arundinacea* and *P. tuberosa* x *arundinacea* strains (Roe and Mottershead, 1962; Marten and Jordan, 1974), the animal may considerably reduce its intake or even starve.

Interpretation of relationships between animal preference and herbage characters may be difficult if the measured characters are themselves highly correlated (Dudzinzki and Arnold, 1973). Correlation between a single character and preference is not proof that it is the main component influencing preference unless all other characters have been accounted for. There have been many attempts to relate ruminant preferences to the approximate composition of plants. Measurements such as nitrogen, "crude fibre", "energy", silica or "ash" will not be recognised by the animal since these fractions do not exist in this form at the molecular level in the plant (Arnold and Hill, 1971). Where correlations are found between approximate composition and animal preference, they must relate to specific compounds or some physical property of the plant. In the case of fibre, the ease of harvesting could be a significant factor (Evans, 1964; Evans, 1967b). Apparent preference for chemicals such as sulphur and phosphorus may occur if the animal selects for more green than dead herbage (Langlands and Sanson, 1976). Many investigations have demonstrated that sheep and cattle generally select leaf in preference to stem, and young leaves in preference to old leaves, particularly when the pasture has reached an advanced stage of maturity (Milton, 1953; Arnold, 1960a; Dudzinski and Arnold, 1973; McIvor and Watkin, 1973; Hunter *et al.*, 1976). The selected herbage is frequently higher in protein, phosphorus, soluble carbohydrates, digestibility and gross energy, and lower in lignin and structural carbohydrates than the pasture as a whole (Arnold, 1964b; McBride *et al.* 1967).

Langlands and Sanson (1976) investigated the diet selected by sheep and cattle on *Phalaris tuberosa* swards. Grazing sheep were shown to select a diet of higher digestibility and nitrogen content than cattle. However they also found that sheep consumed more green herbage than cattle, and the green material contained more nitrogen and was of higher digestibility than dead material. In recognising the problem of high herbage character correlations, Langlands and Sanson (1976) subjected their pasture measurements to a principal components analysis to create orthogonal variables before using multiple regression analysis to relate them to animal selection.

## 1.4 YORKSHIRE FOG CHARACTERS REPUTED TO DETERMINE ACCEPTABILITY

Several of the following characters of Yorkshire fog have been held responsible for its lack of acceptance by ruminants, and others are listed simply because of their uncertain importance in determining animal preference.

### 1.4.1 FLOWER AND SEED HEADS, CULMS AND LIGNIFICATION

It has been reported that with the onset of heading and in the presence of numerous flower and seed heads, a rapid decline in the acceptability of Yorkshire fog occurs (Stapledon, 1927; Cowlishaw and Alder, 1960; Garner, 1963; Jacques, 1974).

Lignification of the culms may also be important in determining sheep rejection. Culms of Yorkshire fog plants derived from commercial ryegrass seed cleanings were shown to be unacceptable to grazing animals (Stapledon and Milton, 1932). Watt (1978) suggests that lignification of the culm may be more important than pubescence on leaves and culms in determining rejection since aftermath growth, which is largely leaf material, is usually well grazed.

Lignification of leaf material is probably unimportant in influencing acceptability since the level of lignin in Yorkshire fog leaves appears to be of a similar level to that of other common festucoid grass species or varieties when grown under similar conditions. (Molloy and Richards, 1971; Harkin, 1973).

### 1.4.2 DEAD LEAF AND SHEATH MATERIAL

The accumulation of dead basal material in leniently grazed Yorkshire fog pastures is a serious problem (Jacques *et al.* 1974).

Cowlishaw and Alder (1960) found an inverse relationship between the percentage dead leaf and sheath of Yorkshire fog and preference ranking to bullocks. However where it is grazed alone and not allowed to become rank, sheep will consume Yorkshire fog readily throughout most of the year (Watkin, 1960).

Accumulation of dead basal material provides a medium for the growth of *Pithomyces chartarum*, the fungus causing facial eczema in grazing animals, encouraged by the lenient grazing of Yorkshire fog (Hartley, 1973).

### 1.4.3 PUBESCENCE

Many workers have commented upon the hairiness of the leaves and sheaths causing an unpleasant touch sensation to the animal mouth (Garner, 1963). Pubescence is one factor commonly listed as being partially responsible for the rejection of Yorkshire fog (Davies, 1925; Stapledon, 1927; Cowlishaw and Alder, 1960; Jacques and Munro, 1963; Watt, 1978).

Most of the aerial plant parts of Yorkshire fog, including the spikelets, culms, leaves, sheaths and ligules, have a covering of macro-hairs (Metcalfe, 1960) on their surfaces. However, considerable variation in the pubescence of Yorkshire fog both in terms of hair density and hair length has been observed although a completely glabrous plant has not yet been found (Beddows, 1961a).

Kruijne and de Vries (1968) mention the presence of two types of leaf hairs: "the ribs are covered with two types of hairs: one noticeably longer hair type on top of the ribs, and a much shorter hair type which is sometimes difficult to observe; the latter covers the whole rib." Klapp (1965) shows a cross-section of a *H. lanatus* blade with the longer hair type clearly shown on top of the ribs on both leaf surfaces, but the short hair type is not very evident. Generally the short hairs are more abundant between the veins (Metcalfe, 1960). Commonly both long and short hair types are found on both blade surfaces (Beddows, 1961a). The long hair type is found on the sheath (Beddows, 1961b); the hairs are usually reflexed. Long hairs are also found on the abaxial side of the ligule and surrounding the ligule's distinct irregularly notched fringe-like projections (Plates 1 and 2).

On the leaf and sheath of *H. lanatus* the long hair type appears to be dominant over the short hair type. The range in hair density and hair length suggests that for each type the inheritance is quantitative (Beddows, 1961b).

Plate 1. Long hair type on the abaxial surface of a non-peaked ligule of Yorkshire fog.

Plate 2. Long hair type on the abaxial surface of a peaked ligule of Yorkshire fog.

PLATE 1

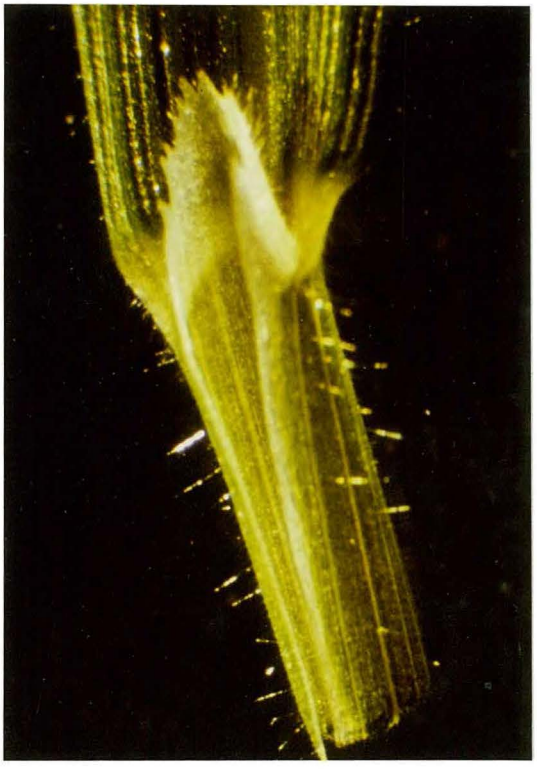


PLATE 2

### 1.4.4 CROWN RUST

The presence of brightly coloured orange crown rust *(Puccinia coronata)* pustules, more commonly found on the older leaves, over summer to early autumn are thought to reduce acceptability (Ivins, 1952; Corkill, 1956; Jacques and Munro, 1963). Jacques (1974) observed that a severe infection by crown rust on spaced plant material would lead to rejection by sheep.

### 1.4.5 FLAVANOLS

Condensed tannin precursors (flavanols) have been found in leaves of Yorkshire fog (Gordon, unpubl.) using Burn's spot test (Burns, 1963). The importance of flavanols in determining acceptability of Yorkshire fog to the grazing animal is not known. However the flavanols (Burns, 1966) flavan-3,4-diol and flavan-3-ol have been reported to influence the low acceptability of sericea lespedeza *(Lespedeza cuneata)* to sheep (Wilkins *et al.*, 1953) and cattle (Donnelly, 1954).

The astringency of condensed tannins is attributed to their ability to bind animal mouth protein and mucopolysaccharide thereby causing a contracting or drying 'sensation' in the mouth. This 'sensation' probably arises from the destruction of the lubricant property of the saliva and a contracting of the epithelial tissue of the tongue (Swain, 1962). It appears that maximum astringency is given by those molecules (M.W. 500-3000) which are sufficiently large to effectively cross-link proteins, but which are still readily extractable from the tissue (Haslam, 1975). Highly polymerised condensed tannins are of low astringency. Haslam (1977) has suggested that the loss of astringency in ripening fruit is due to increased polymerisation of condensed tannins. Similarly the low astringency of sainfoin *(Onobrychis viciifolia)* condensed tannin is attributed to its high proportion of prodelphinidin, a flavan-based polymer of very high molecular weight (17,000-28,000) (Jones *et al.* 1974). Hence although sainfoin contains high levels of condensed tannins it is nevertheless highly acceptable to ruminants (Reid *et al.*, 1974).

Condensed tannins are reputed to influence the digestibility of herbage and to prevent foam production of soluble leaf protein in the rumen, a causal factor of bloat. Soluble leaf protein-condensed tannin complexes form in the rumen at approximately pH 6.5 and dissociate in the duodenum at approximately pH 2.5 (Jones and Mangan, 1977).

Therefore it is suggested that more plant protein will reach the duodenum if condensed tannins are present. Jones and Mangan (1977) suggest that this process may allow more efficient N-digestion by the ruminant animal since N-digestion in the rumen by microbes is wasteful of N.

Methods of estimating the digestibility of forage containing high levels of flavanols have been re-examined recently (Cope and Burns, 1976). Digestibility estimates performed in vitro and based on dry matter disappearance (IVDMD) rely on rumen microflora to degrade the forage sample. Such bioassay methods provide considerably lower estimates of digestibility in the presence of a high flavanol concentration than alternative in vitro chemical methods which rely on detergents to fractionate the fibrous constituents of forages (Cope and Burns, 1976).

Tannins may play a role in protecting plant tissues against fungal attack through binding with fungal enzymes (Okasha *et al.* 1968). The presence of both hydrolysable and condensed tannins are reputed to inhibit degradation of plant debris (Basaraba and Starkey, 1966). However, the effect of endogenous tannins in determining the accumulation of dead leaf and sheath material in Yorkshire fog is unknown.

The production of flavanols and condensed tannins may occur only within certain plant parts and may be influenced by the growth stage of the plant or the season. These effects are not known for Yorkshire fog. However seasonal trends in flavanol levels have been reported for other herbage species. Low winter, but high summer flavanol content has been observed in many *Lotus* species populations (Ross and Jones, 1974). Similar seasonal trends occur in *Lespedeza cuneata* with a sharp rise in flavanol level with advancing summer and plant maturity, followed by a gradual reduction to low levels in early autumn (Cope *et al.*, 1971). The young leaves and flowers of *Lespedeza cuneata* generally contain a higher level (10-14%) of flavanols than the senescent leaves and seeds (4-6%) or the stem, roots and cotyledons (< 3%) (Burns, 1966).

### 1.4.5.1 THE CHEMISTRY OF CONDENSED TANNINS

The term 'condensed tannins' is synonymous with the terms 'flavolans', 'procyanidins' and 'phlobaphenes'. These are polymers (M.W. 500 - >28,000) of flavanols. Flavanols have in common the flavan nucleus (Figure 1) (Swain, 1965; Jones *et al.*, 1976).

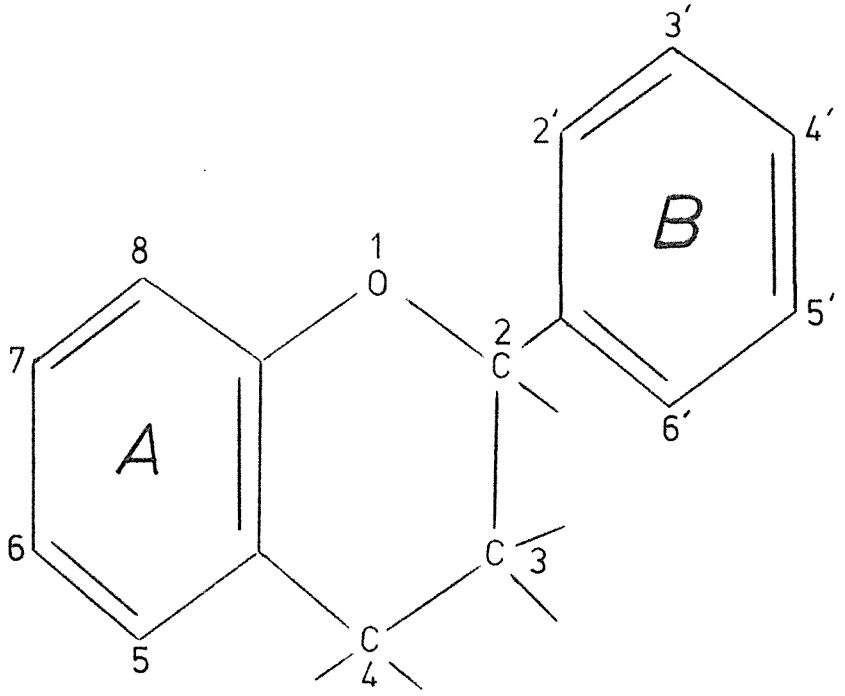



Figure 1 Structure and numbering system for the flavan nucleus

The flavanol flavan-3,4-diol is often called by the trivial name of "leucoanthocyanidin" because upon heating with acid it is converted partly into flavylium salts such as cyanidin and delphinidin (Figure 2). Hydroxylation of flavan-3,4-diol leads to the formation of flav-3-en-3-ol, which lacks an oxygen at C4 and has a double bond between C3 and C4. Oxidation of flav-3-en-3-ol yields a flavylium salt (synonym:anthocyanidin). Reduction of flav-3-en-3-ol leads to the formation of a flavan-3-ol. (Haslam, 1977).

Linkages between flavan units are mainly C4-C8 (Haslam, 1977). The restricted rotation around the interflavan bond partly determines the three dimensional structure of the polymer (Haslam, 1977). However, reaction of the C2 of flavan-3-ols, C4 of flavan-3,4-diols, or reduced flav-3-en-3-ol carbonium ion with the C6, C8, or alcoholic hydroxyl groups of other flavanols could result in C4-C6, C4-C8, C3-O-C7 and C3-O-C2 bonds, linking them in three dimensions (Ribèreau-Gayon, 1972; Wong, 1973; Haslam, 1977). Hence, the polymeric structures of condensed tannins may be complex.

For studies to have any biogenetic significance it is necessary to extract the condensed tannins as close to thechemical composition to those existing in the plant tissues. Factors such as high temperature and light appear to change the form of condensed tannins during extraction, hence fresh plant material (or freeze-dried) and cold solvents are generally used (Haslam, 1966; Wong, 1973; Broadhurst and Jones, 1978).

### 1.4.6 SOLUBLE SUGARS

Numerous workers have noted that soluble sugars in herbage may play a role in determining animal preference. Cattle in Finland are reported to show a high degree of acceptance for Yorkshire fog. This has been attributed to an abnormally high sugar content of plants grown under intense continuous sunlight(Jones, 1952). A significant positive correlation occurred between 'total soluble carbohydrates' and 'preference rating' to sheepin eleven cocksfoot *(Dactylis glomerata)* varieties (Saiga and Kawabata, 1975). Reid *et al.* (1966) and Reid *et al.* (1967) found significant positive correlations between preference rating and 'ethanol soluble carbohydrate' content with sheep on cocksfoot, but not with sheep on tall fescue (Reid and Jung, 1965). However,

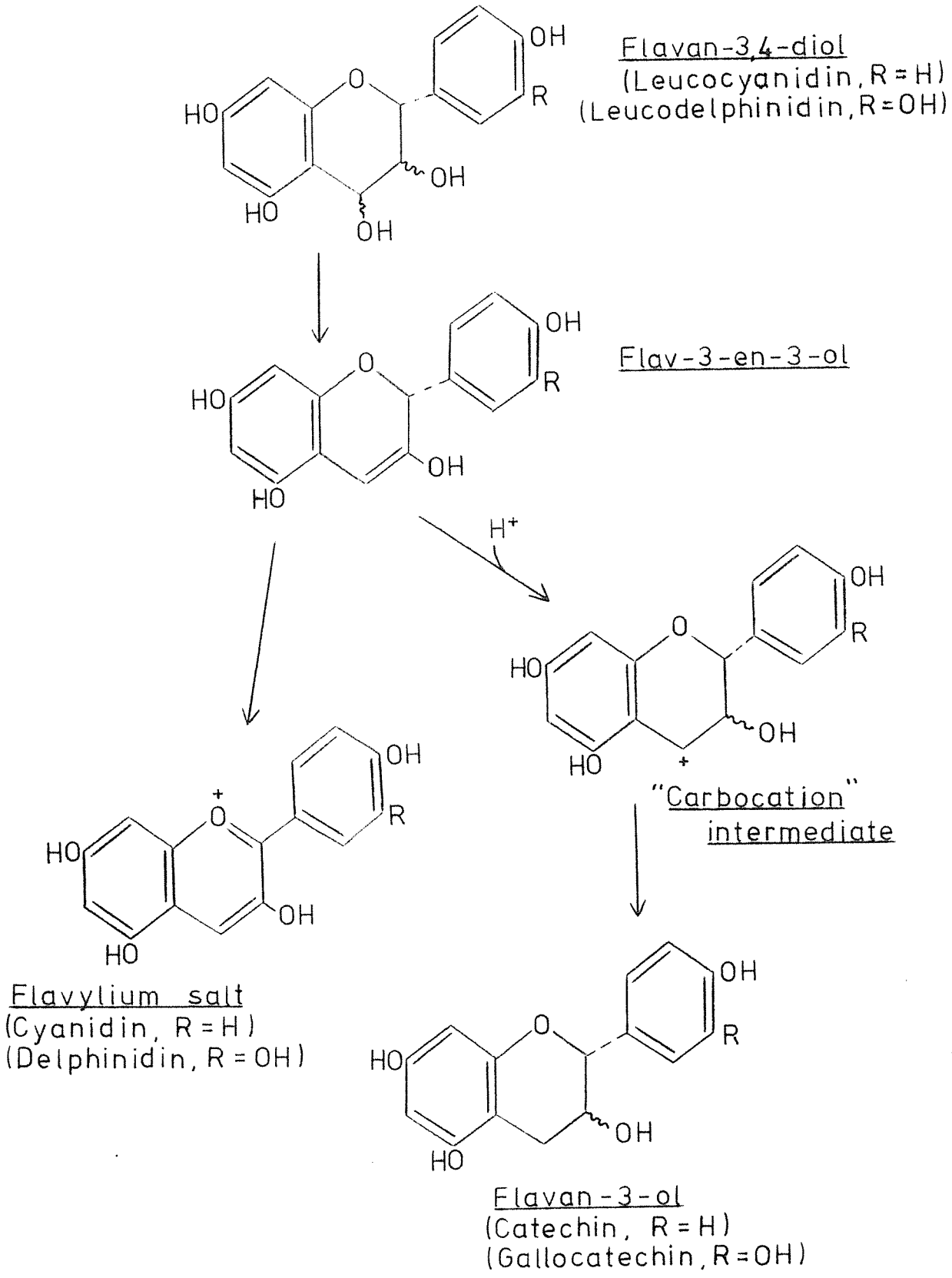



Figure 2. Structure and putative relationship of the flavanols - (from Haslam, 1977).

Simon (1974) found that 'total soluble carbohydrate' content had little effect in determining the voluntary intake of 5 grass and 4 legume species by wether sheep.

Bland and Dent (1964) compared the preferences of cattle amongst fourteen strains of cocksfoot at two locations at several times of the year. For early spring growth at one site, preferences were most closely correlated with percent "total sugars", with hexoses and with fructosans but not with sucrose content. At all other observations "total sugar" content and preferences were not related.

Interpretation of ruminant responses to plant carbohydrates is difficult. Variable extraction and determination procedures, variable amounts of mono-, di-, tri- or polysaccharides, glycosides or other derivatives each with a specific stereochemistry in the plant, along with the presence of other compounds in the plant perhaps reacting with them (Kalmus *et al.* 1977) are factors which add to this confusion.

Arnold and Hill (1971) suggest that it is highly unlikely that an animal could give an integrated response to "soluble carbohydrates". Different sugars and their stereo-isomers give a different sweetness response. For example, it has been shown that the sweetness response of sucrose to human subjects may be higher than for fructose and glucose, although at high concentrations sucrose becomes unacceptable (or unpleasant) to taste (Moskowitz, 1971). Hence, for sugars taste intensity (sweetness) may continue to rise whilst acceptability reaches a threshold level and then diminishes (Moskowitz and Klarman, 1975).

Taste thresholds of four ruminant species for sweet, sour, bitter and salty chemical solutions have been examined using the 'two choice preference test' (Goatcher and Church, 1970a,b,c,d). Animal preference was assessed, using ascending chemical concentrations, on the basis of test solution intake as a proportion of total fluid intake (% T.F.I.). The different thresholds (Figure 3) were arbitrarily set levels, at 20, 40, 60 and 80% T.F.I. Lactose, galactose and fructose solutions were tested at concentrations up to 1.94, 1.29, and 1.94% respectively using 30 different sheep. No discriminatry responses, i.e. outside the nondiscrimination zone, were observed. The sheep showed no preference and only weak rejection for a wide range of sucrose concentrations (0.08 to 20%) offerred. Goatcher and Church (1970 a,c) suggest

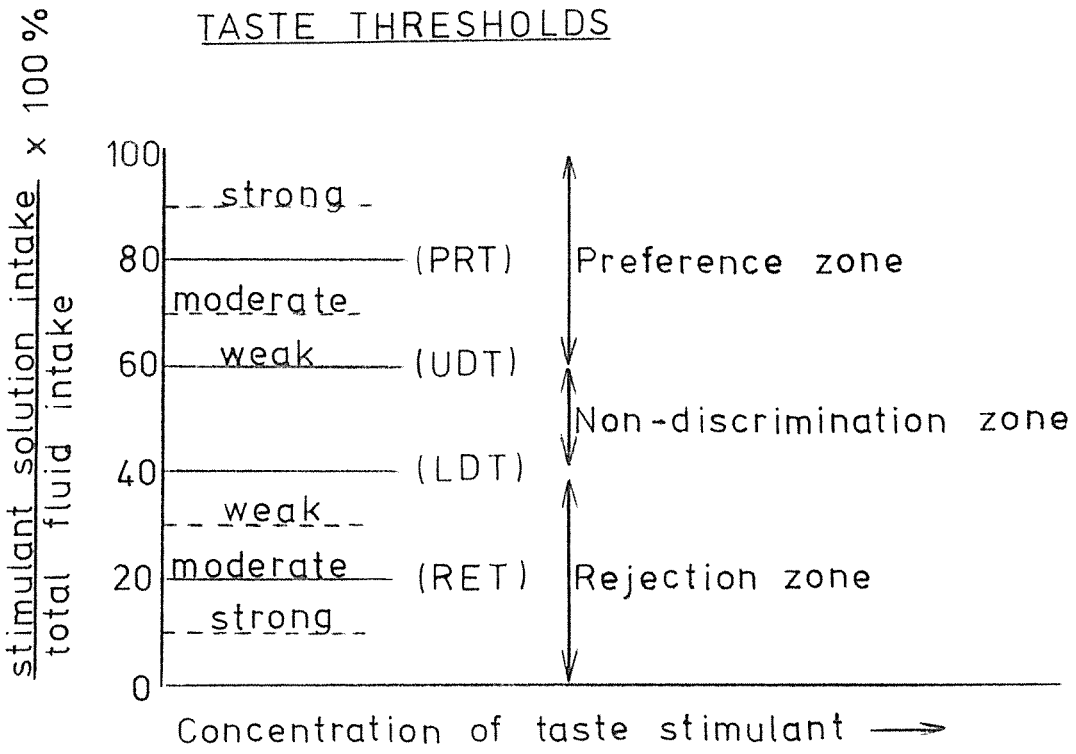



Figure 3 . Taste threshold levels most commonly used in classical two choice preference test experiments (from Goatcher and Church, 1970a).

that sheep are, on average, only weakly responsive to sweet tasting substances. Based upon the lowest sucrose concentration to be discriminated, the sensitivities of four ruminant species were in the order: cattle > normal goats > pygmy goats > sheep. Cattle showed a strong preference for sucrose at a relatively low test solution concentration (UDT ≃ 0.025M), although rejection occurred at higher concentrations (LDT ≃ 0.56M). (Goatcher and Church, 1970c).

#### 1.4.6.1 SOLUBLE SUGARS OF TEMPERATE GRASSES

Yorkshire fog *(H. lanatus)* is a member of the family Poaceae, subfamily Festucoideae and tribe Aveneae, and is of temperate origin. The disaccharide sucrose and polysaccharide fructosan are the predominant non-structural carbohydrates in temperate grasses (Smith, 1973a). Levans (β2-6 linked D-fructofuranose polymers) are the common form of fructosan in temperate grasses. Temperate origin grass species in the Aveneae tribe may accumulate fructosans in their stem bases. However, fructosan accumulation does not necessarily occur in other plant parts such as leaf blades (Smith, 1968).

Concentrations of total sugars and water soluble carbohydrates in temperate grass herbage appear to increase during morning hours until sometime in the afternoon and then decrease until daylight the following day (Greenfield and Smith, 1974). Considerable diurnal variation, particularly in sucrose concentration, in perennial ryegrass *(Lolium perenne)* aerial parts has been observed (Waite and Boyd, 1953).

Seasonal variations in nonstructural carbohydrate concentration occur particularly in the stem fructosan component of temperate grasses (Jung *et al.*, 1974). Higher concentrations of nonstructural carbohydrate in temperate grasses are usually found at mature rather than young growth stages, at cool than warm temperatures, and at low than high soil nitrogen levels (Smith, 1973a).

### 1.4.7 ORGANIC ACIDS, HCN, ALKALOIDS

Appreciable levels of trans-aconitic acid (0.36-1.69%) may accumulate in the leaves of *H. lanatus* cv. Massey Basyn (Molloy, 1969). The importance of trans-aconitic acid in Yorkshire fog in determining acceptability to ruminants is not known. However Arnold and Hill (1971)

have shown that the acceptability of aconitic acid offered to sheep in test solution, using the two choice preference test, rapidly declined at the higher concentrations (0.5 - 5.0%) offered.

The high level of HCN in Sudangrass and sorghum x Sudangrass hybrids, probably the most unacceptable character to grazing sheep and cattle in these species (Rabas *et al.*, 1970), is unlikely to effect rejection of Yorkshire fog. The level reported for *H. lanatus* on a total plant basis of 68 mg HCN. $kgm^{-1}$ F.Wt. (Devetak *et al.* 1971/72) is considerably lower than that reported for *Sorghum halepense* L. of 1500 mg HCN $Kgm^{-1}$ F.Wt. and well below the limit of ≃ 700 mg HCN $Kgm^{-1}$F.Wt. suggested for safety from cyanide toxicity in sheep (Coop and Blakely, 1950).

Bitter-tasting alkaloids (to humans) such as perloline in *Lolium perenne* were not detected in *H. lanatus* (White and Reifer, 1945).

## 1.5 TECHNIQUES USED TO ASSESS ANIMAL PREFERENCES

Methods of examining animal preference towards a particular herbage plant, cultivar or species usually vary according to the purpose of the study.

The purpose of many agronomic studies is often to examine the acceptability of one herbage species or cultivar in relation to other species or cultivars. With limited numbers of species or cultivars to be examined, the practical use of sward plot assessments and sophisticated animal measurements of plant material consumed (e.g. oesophageal fistulation) become feasible. Herbage dry matter assessments may be made by sub-plot pre-defoliation and whole-plot post-defoliation. From such measurements preference indices (Mills, 1977) may be calculated.

Plant breeding studies generally involve large numbers of genotypes, often within a single species, and measurements must be quickly and simply applied. For this reason visual scoring on spaced plant material based on residual forage remaining after grazing has been used to assess genotype acceptability to grazing animals. Defoliation scores have been used to assess the acceptability of *Phalaris arundinacea* (Barnes *et al.*, 1970; Simons and Marten, 1971), *Pennisetum flaccidum*

Burns *et al.*, 1978) and *Festuca arundinacea* (Ivins, 1955; Petersen *et al.*, 1958; Buckner and Burrus, 1962) genotypes to sheep and cattle at the initial stages of plant breeding programmes.

## 1.6 PHENOTYPIC PARTITIONING

Sprague and Federer (1951) showed how variance components could be used to partition out the effects of genotypes, environments and their interaction by equating the observed mean squares in the analysis of variance to their expectations in the random model. The components of variance corresponding to each effect may be extracted and used to obtain heritability estimates of the form: $h^2 = \sigma^2_G / \sigma^2_p$, where $\sigma^2_G$ = some appropriate "genetic" variance, and $\sigma^2_p$ = a model-oriented linear function of phenotypic variance components.

Some of the phenotypic partitioning models used by plant breeders include: a model for observations taken in single environments (Osborne and Paterson, 1952; Gordon *et al.*,1972); a model for observations taken over different environments (sites or years) of annual plants (Hanson, 1964; Gordon *et al.*,1972); and a model for serial observations of perennial plants (the split-plot-in-time model) (Steel and Torrie, 1960; Le Clerg *et al.*,1962; Gordon, 1979).

Gordon (1979) has defined several broadsense heritability definitions using variance components based on the split-plot-in-time model and pointed out possible limitations in its use. One particular problem in the split-plot-in-time analysis is that effects from different times (e.g. years) may not be independent because of physiological carry-over from one time to the next. This condition invalidates the assumption of no covariance among effects, leading to bias in variance component estimates, and in subsequent significance testing (Gordon, 1979). However, the split-plot-in-time model at least provides an estimate of genotype x time variance not provided by the non-pooled single environment model. The split-plot-in-time model has been used in this thesis and the appropriate estimators are presented and discussed in Section 3.3.

## 1.7 CLUSTER ANALYSIS

Cluster Analysis is a general term covering a wide range of numerical techniques used to sort a given set of individuals into meaningful patterns undefined *a priori* (Lance and Williams, 1967; Anderberg, 1973; Everitt, 1974; Clifford and Stephenson, 1975; Teow, 1978). Cluster analysis implies a numerical model and an interdependent strategy (or algorithm) whereby the model is implemented. The numerical model translates the concept of "similarity" into some measure which the strategy works upon.

A wide range of "similarity" or "dissimilarity" measures have been devised, although relatively few are in current use (Williams, 1971; Anderberg, 1973; Clifford and Stephenson, 1975). These measures may be size measures (e.g. distance measures) or shape measures (e.g. correlation measures). Size measures commonly used include the Euclidean distance measure, the first Minkowski metric, and the Shannon and Brillouin diversity indices. The definition, metric and additive nature, of these measures has been reviewed by Teow (1978). Another size measure which may become more commonly used is the dissimilatory index measure proposed by Lin and Thompson (1975) which uses test statistics for differences among regression lines. This dissimilarity index is not metric since triangular inequality does not hold in general.

The Euclidean distance measure, used in this study, is defined as:

$$d_{ij} = \left[ \sum_{k=1}^{n} W_k (X_{ik} - X_{jk})^2 \right]^{\frac{1}{2}} \quad ; \text{ where } d_{ij} =$$

Euclidean distance; $X_{ik}$ and $X_{jk}$ denote the value taken by two individuals (i) and (j), for the kth of n attributes; and $W_k$ is an optional weighting factor (set $W_k = 1$ if unweighted). The square of $d_{ij}$ is known as Squared Euclidean Distance (Anderberg, 1973).

If the original data matrix contains the attributes for each individual in rows then the distance measures are computed as differences between rows. Subsequent 'clustering of individuals' using a selected algorithm then follows. Subsequent 'clustering of attributes' requires that the original data matrix be transposed, and columns pre-standardised, prior to computation of the distance matrix (Veldman, 1967). Attributes

may be pre-standardised using z-scores (standardised normal deviates) to correct for scale, since attributes with small variance would contribute less to the distance measure than would attributes which were scaled with larger variances (Veldman, 1967).

### 1.7.1 CLUSTER STRATEGIES

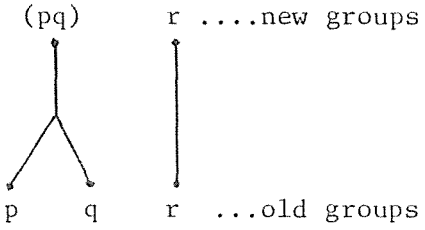
Only the cluster strategies used in this study are reviewed. Four hierarchical agglomerative clustering strategies are considered: Centroid Method; Median Method of Gower; Group Average Method; and Ward's Incremental Sum of Squares Method (Lance and Williams, 1967; Anderberg, 1973; Everitt,1974; Clifford and Stephenson, 1975). All of these methods are "combinatorial" (Lance and Williams, 1967). A "combinatorial" method implies reduction of the similarity matrix containing $d_{ij}$ by a row or column with each successive hierarchical fusion. The reduction process involves the fusion of pairs of rows (or columns) using one of the cluster algorithms. The individual similarity measures are discarded immediately a cluster is formed.

Combinatorial strategies have a computational advantage over non-combinatorial strategies such as Sokal and Michener's unweighted pair-group method (Lin and Thompson, 1975), since non-combinatorial strategies must retain the original inter-individual similarity measures for later calculations even though the individuals are already in a cluster.

Combinatorial strategies have the disadvantage that distances computed during clustering are not the same as the original inter-individual similarity measures and they may be difficult to interpret (Lance and Williams, 1967).

A recurrence formula may be used to relate the cluster strategies (Wishart, 1969):

$d_{r(pq)} = \alpha_p d_{rp} + \alpha_q d_{rq} + \beta d_{pq}$ ; where $d_{r(pq)}$ is the distance between a group r and a group (pq) formed by the fusion of groups p and q. Inter-group distances are $d_{rp}$, $d_{rq}$, and $d_{pq}$ and each group contains $M_r$, $M_p$ and $M_q$ individuals. In dendrogram form these groups may be visualised as:




The parameters $\alpha$ and $\beta$ for each strategy are:

Centroid: $\alpha_p = M_p/(M_p + M_q)$; $\alpha_q = M_q/(M_p + M_q)$; $\beta = -\alpha_p \alpha_q$.

Median: $\alpha_p = \alpha_q = \frac{1}{2}$ ; $\beta = -\frac{1}{4}$.

Group-Average: $\alpha_p = M_p/(M_p + M_q)$; $\alpha_q = M_q/(M_p + M_q)$; $\beta = 0$.

Ward's:
$$\alpha_p = (M_r + M_p)/(M_r + M_p + M_q);$$
$$\alpha_q = (M_r + M_q)/(M_r + M_p + M_q);$$
$$\beta = -M_r/(M_r + M_p + M_q).$$

Of these strategies, Ward's method is the only method which weights each distance measure proportionally by the number of individuals in the old and new groups. This effectively reduces the occurrence of "chaining." Chaining refers to the tendency to cluster together, at a relatively low level, individuals linked by chains of intermediates (Anderberg, 1973). Chaining is not always considered a defect (Everitt, 1974), but generally leads to problems of cluster identification and interpretation (Lance and Williams, 1967).

The Centroid and Median strategies may produce dendrograms with "reversals" because the distance function may decrease. A "reversal" is produced when fusion occurs at a lower distance than the original. Both the Group Average method and Ward's method are necessarily "monotonic" (i.e. do not produce reversals) because their distance functions are non-decreasing (since $\alpha_p + \alpha_q + \beta \geq 1$) (Lance and Williams, 1967).

# CHAPTER 2. PILOT STUDY

The purpose of this study was to examine the sampling intensity of sheep on Yorkshire fog spaced plants, in order to estimate the time required for adequate sampling of all plants for the main study.

## 2.1 EXPERIMENTAL METHOD

Thirty Perendale yearling wethers were used. Each sheep was identified with a spray-painted numeral on either side of its body. Because prior-diet and gut-fill affect both selectively and sampling intensity, sheep were yarded overnight prior to grazing the Yorkshire fog collection. The collection was laid out in a randomised complete block design (see Chapter 3). Each block was fenced off with wire mesh. Ten random sheep were introduced into a block of the collection and observed for 90 minutes. Five people were assigned to watch two sheep each, and the number of clumps sampled by each sheep over 90 minutes was counted. Tallies were recorded every 5 minutes. This procedure was repeated in the other two blocks using ten random sheep in each.

Each group of ten sheep in Blocks I, II, and III, had been pre-yarded (starved) for 19, 17, and 15 hours respectively.

### 2.1.1 REGRESSION ANALYSIS

Two-way plots of sheep sampling intensity (Y) against time (X) were made using the computer program SPSS/SCATTERGRAM (Nie *et al.* 1975) for individual sheep and for all sheep combined to check linearity. Simple linear regression analysis was used to relate sheep sampling intensity with time using the computer program SPSS/REGRESSION (Nie *et al.*, 1975). Regressions were performed for individual sheep, for each block, and for all sheep combined, using the equation $Y = \beta_0 + \beta_1 X$ where y = estimate of the number of clumps sampled over a five minute interval and X = time in minutes.

The standard error of $\beta_0$ (Draper and Smith, 1966) was obtained using the computer program REGSPS (Gordon, unpubl.). The $\sigma^2_{\beta_0}$ can be found from the **X'X** matrix (Draper and Smith, 1966). Output from SPSS/REGRESSION includes $\bar{X}$, $S_{\bar{X}}$, $\hat{\sigma}_{Y.X}$, and n from which the **X'X** matrix can be obtained:

$$\mathbf{X'X} = \begin{bmatrix} n & \Sigma X_i \\ \Sigma X_i & \Sigma X_i^2 \end{bmatrix}$$ , where the terms are

reconstituted in REGSPS as follows: $\Sigma X_i = n\bar{X}$ and $\Sigma X_i^2 = (n-1)S_X^{-2} + n\bar{X}^2$. The estimated s.e. $(\beta_i)$ is the square root of the $i^{th}$ diagonal term of the matrix $(\mathbf{X'X})^{-1} \hat{\sigma}_{Y.X}$ (Draper and Smith, 1966). Differences amongst blocks and amongst sheep were tested for significance by comparing the Y-intercepts ($\beta_0$) and the regression coefficients ($\beta_1$) using the pairwise 't test' (Steel and Torrie, 1960).

## 2.2 RESULTS

Regression statistics for each sheep are presented in APPENDIX I. Graphs of the regression equations for each sheep are shown in Figure 4. These graphs indicate that the sampling intensity of most of the sheep declined slightly during the 90 minute grazing period. Estimates of the variance about regression ($\hat{\sigma}^2_{Y.X}$) were extremely high for the sheep numbered 18, 23, 25 and 27 and reflected their abnormally fickle grazing behaviour. However differences between the actual functions of sheep 18, 23, 25 and 27 with all other sheep were statistically non-significant ($P>0.05$) (APPENDIX II). The lack of temporal consistency of sampling intensity for each sheep was apparent from the generally low values of $r^2$ (coefficient of determination) obtained.

Regressionsof sampling intensity against time for each block indicated that the sheep which had been pre-yarded for 17 and 19 hours sampled less consistently than those pre-yarded for 15 hours as shown by the $r^2$'s (Table 1). However differences between the actual functions of each block were statistically non-significant ($P>0.05$) (Table 2) hence a pooled regression analysis of all sheep over all blocks was carried out (Table 3). The pooled regression equation

TABLE 1 Regression data of sheep sampling intensity for each block in the pilot study

| Pre-starvation time (hours) | Block | Regression equation | se($\beta_0$) | se($\beta_1$) | F | $r^2$ |
|---|---|---|---|---|---|---|
| 19 | I | $\hat{Y}$=-0.19X+37.4 | 6.156 | 0.115 | NS | 0.015 |
| 17 | II | $\hat{Y}$=-0.21X+32.4 | 5.653 | 0.104 | * | 0.022 |
| 15 | III | $\hat{Y}$=-0.16X+30.5 | 1.378 | 0.025 | ** | 0.182 |

TABLE 2 Estimated t statistics for differences amongst pairs of $\beta_0$'s and $\beta_1$'s from the regression equations of sheep sampling intensity for each block in the pilot study

| $\beta_1$ differences / $\beta_0$ differences | | Blocks I | Blocks II | Blocks III |
|---|---|---|---|---|
| BLOCKS | I | - | 0.129 NS | 0.227 NS |
| | II | 0.603 NS | - | 0.433 NS |
| | III | 1.097 NS | 0.324 NS | - |

TABLE 3 Regression data of sheep sampling intensity for all sheep (pooled) in the pilot study

| Regression Equation | se ($\beta_0$) | se($\beta_1$) | F | $r^2$ |
|---|---|---|---|---|
| $\hat{Y}$ = -0.18X + 33.5 | 8.971 | 0.052 | ** | 0.022 |

SIGNIFICANCE SYMBOLS: NS = P >0.05

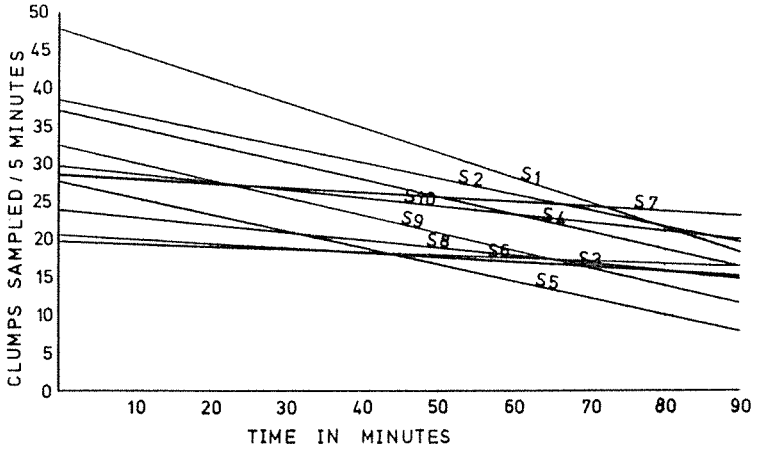
* = 0.05 $\geq$P >0.01

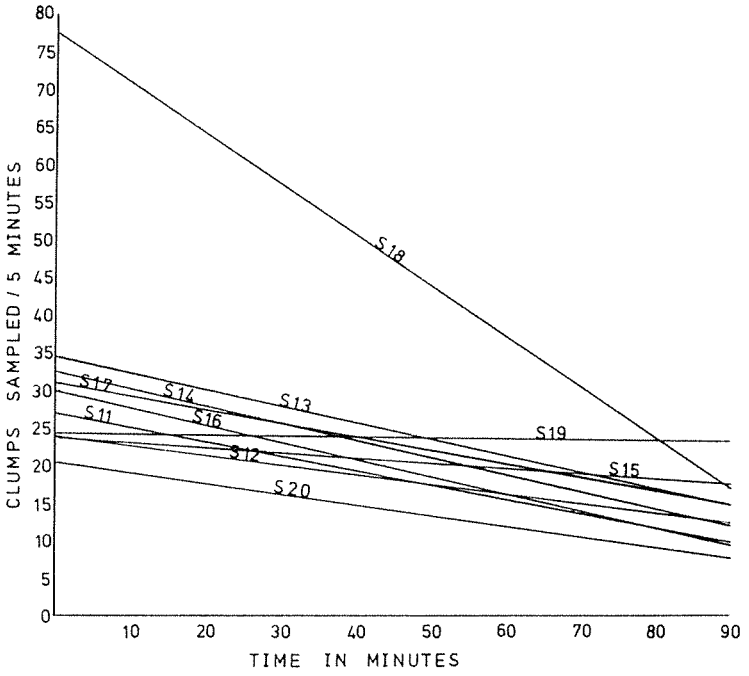
** = 0.01 $\geq$P

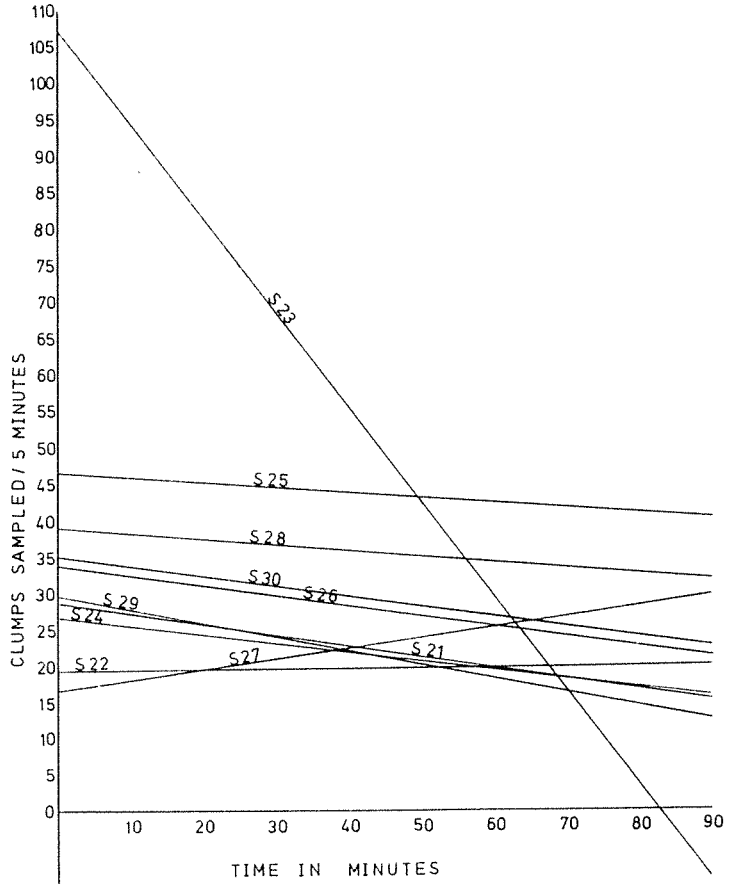
Figure 4. Graphs of sheep sampling intensity regressions for each sheep.

REGRESSIONS OF SHEEP SAMPLING INTENSITY

(S = Sheep identity)



was used to provide estimates of $\hat{Y}_k$ and $\Sigma\hat{Y}_k$, where $\Sigma\hat{Y}_k$ = the estimated total number of clumps sampled per sheep after k minutes (see Table 4). Therefore the use of 30 sheep over a 90 minute period, with 1280 clumps per block, provided potential sampling of each clump approximately 11 times. $\left[(30 \times 477.8)/1280 = 11.20,\ \Sigma\hat{Y}_{90} = 477.8\right]$.

## 2.3 DISCUSSION

The sheep which had been pre-yarded for a greater period of time exhibited less temporal consistency of sampling intensity. However, prevailing weather conditions changed from clear to cloudy sky and from no wind to a light breeze during the study and may have contributed to the more fickle grazing behaviour of these sheep. In any case, even the better sheep were quite inconsistent as judged from their low $r^2$ values.

Several sheep in the group exhibited abnormally inconsistent sampling over the 90 minute grazing period. However, exclusion of these 'abnormal' sheep from the pooled regression analysis was not done.

In this study one clump was recorded as being sampled even if a sheep sampled only a single bite from it. Detectable differences amongst plants sampled in such a manner are difficult to estimate using a visual clump defoliation score. The grazing of each block for 90 minutes with thirty hungry sheep in the subsequent main study provided sufficient removal to allow a clump defoliation score to be applied and additionally provided more than sufficient time to allow potential sampling of each clump.

TABLE 4 Estimates of clumps sampled per sheep for each 5 minute increment in time, and overall to 90 minutes. $\hat{Y} = -0.18X + 33.5$

| Time (k minutes) | $\hat{Y}_k$ | $\Sigma\hat{Y}_k$ |
|---|---|---|
| 0 | 33.5 | 33.5 |
| 5 | 32.5 | 66.0 |
| 10 | 31.6 | 97.6 |
| 15 | 30.7 | 128.3 |
| 20 | 29.8 | 158.1 |
| 25 | 28.8 | 186.9 |
| 30 | 27.9 | 214.8 |
| 35 | 27.0 | 241.8 |
| 40 | 26.1 | 267.9 |
| 45 | 25.1 | 293.0 |
| 50 | 24.2 | 317.2 |
| 55 | 23.3 | 340.5 |
| 60 | 22.4 | 362.9 |
| 65 | 21.5 | 384.4 |
| 70 | 20.5 | 404.9 |
| 75 | 19.6 | 424.5 |
| 80 | 18.7 | 443.2 |
| 85 | 17.8 | 461.0 |
| 90 | 16.8 | 477.8 |

# CHAPTER 3. MAIN STUDY - METHODS

## 3.1 EXPERIMENTAL METHOD AND DESIGN

A gene-pool collection of Yorkshire fog was used, which consisted of 160 seed populations established in 1972. The populations were collected from most regions of New Zealand (Teow, 1978). The collection was sited on an Ohakea silt loam at Massey University.

From this collection 53 genotype populations were selected for examination in this study. Selection of these 53 genotype populations was made from a cluster analysis based upon 8 agronomic characters (Teow, 1978), which yielded 44 clusters. One population was selected at random from each cluster. An additional population was selected at random from clusters containing more than three genotype populations (Figure 5). This procedure was carried out to obtain a representative sample of the total phenotypic variability occurring in the gene-pool collection.

The collection, laid out in a randomised complete block design, had three blocks. Each block consisted of 160 plots, each of which was made up of a single row of eight spaced plants. Plant spacing was 60 cm in either direction. The 53 selected genotype populations were identified with a numbered peg (Figure 6). Each block was fenced off with wire netting and fine-mesh, and a holding lane constructed to one side.

Thirty Perendale wether hoggets were used to provide the sheep preference measures in this study. The pilot study (see Chapter 2) revealed that the grazing of each block by 30 pre-yarded sheep for 90 minutes would provide adequate sampling of the collection. Three trial grazing periods were studied during the year, henceforth labelled 'Harvest 1', 'Harvest 2', 'Harvest 3' (see Table 5).

Prior to grazing each block, the 30 sheep were yarded for the previous evening. Grazing was carried out from 8.00 a.m. to 9.30 a.m. thereby achieving a "lax" defoliation of the spaced plants. Assessment of sheep preference followed. In the second and third harvests following the "lax" grazing and the assessment of sheep preference, the 30 sheep were re-yarded the same day in the eveining. The following

Figure 5. A cluster analysis of 160 genotype populations using Ward's method based upon 8 agronomic characters, truncated to produce 44 clusters (Teow, 1978), from which 53 populations were selected at random.

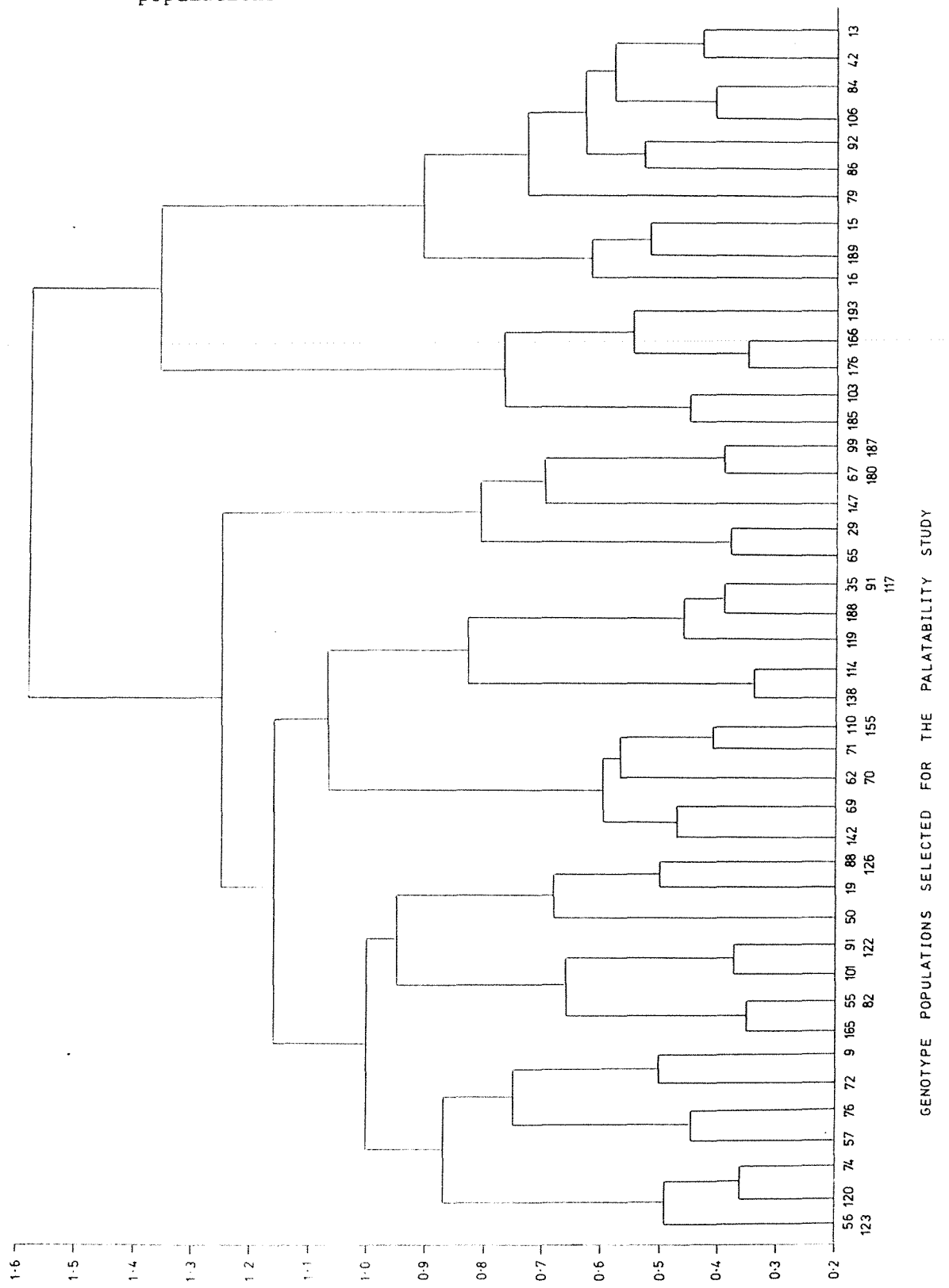


Figure 6. Field layout of the Yorkshire fog collection showing the 53 selected (S) populations and position of the sheep holding lane.

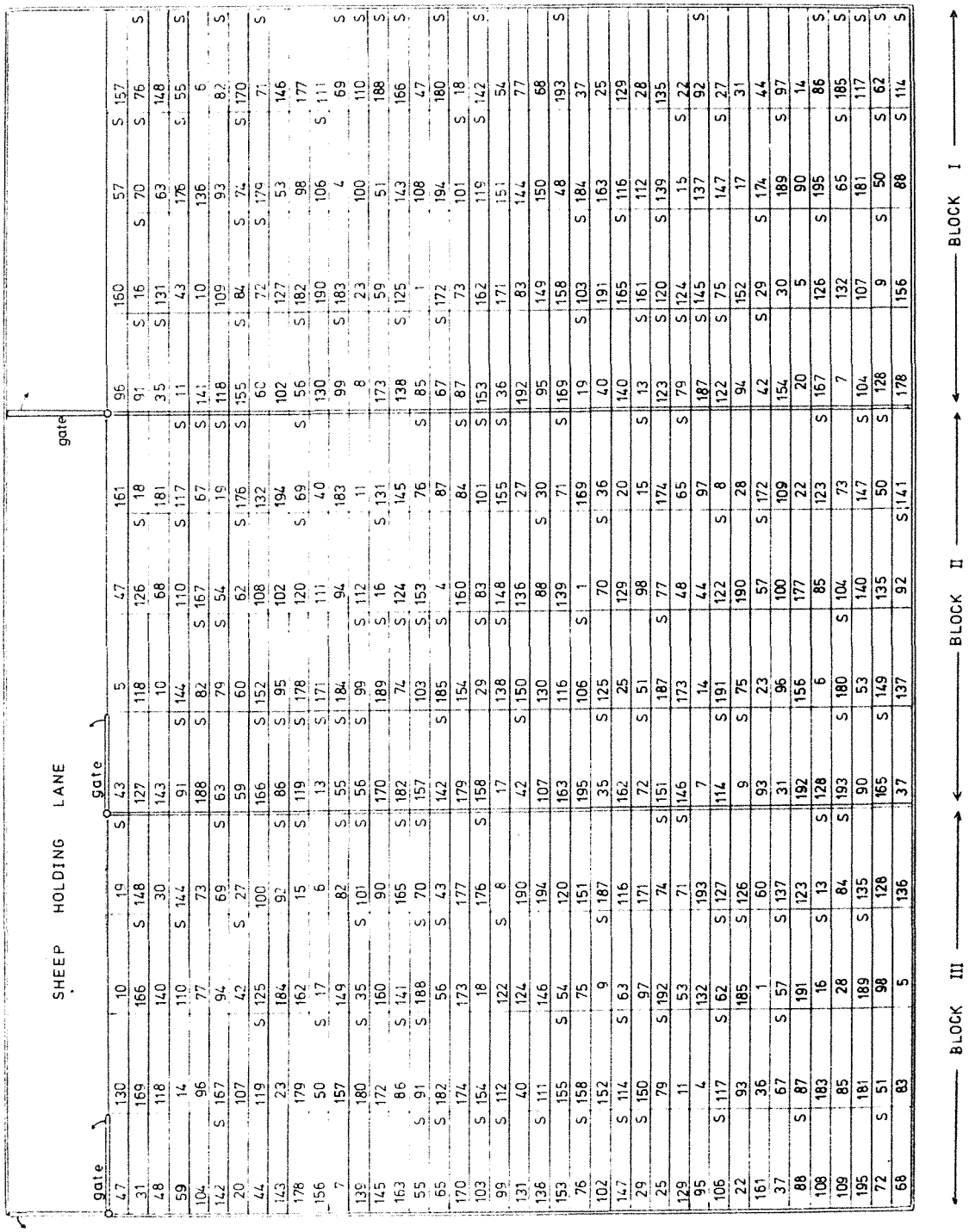


TABLE 5 - Experimental grazing procedure.

| | Date | Intensity of Defoliation |
|---|---|---|
| Pilot study | 13/12/77 | |
| | 17/12/77 to 21/12/77 | All plants defoliated to ≃3.0cm to bring all to a similar morphological state using a Scrub-saw |
| | 6/1/78 | Plants defoliated ≃1.5 - 3.0 cm to remove emerging inflorescences using 90 m.a. Romney ewes |
| Trial grazing Harvest 1 Summer | 10/2/78 Block I<br>12/2/78 Block II<br>14/2/78 Block III | "lax" grazing using 30 pre-yarded Perendale wethers for 90 minutes |
| | 15/2/78 to 20/2/78 | All plants defoliated to ≃ 2.5-3.5 cm resulting in removal of all flower and seed heads, using a scrub-saw |
| Trial grazing Harvest 2 Autumn | 23/3/78 | Block I "lax" grazing |
| | 24/3/78 | Block I "hard" grazing |
| | 26/3/78 | Block II "lax" grazing |
| | 27/3/78 | Block II "hard" grazing |
| | 28/3/78 | Block III "lax" grazing |
| | 29/3/78 | Block III "hard" grazing |
| | 30/3/78 | Plants defoliated to 2.0-3.0 cm using 90 m.a. Romney ewes |
| | 15/4/78 | Plants defoliated to 2.0-3.0 cm to remove leaf-tip burn which occurred following spraying between rows for weed control. |
| Trial grazing Harvest 3 Early-winter | 18/5/78 | Block I "lax" grazing |
| | 19/5/78 | Block I "hard" grazing |
| | 20/5/78 | Block II "lax" grazing |
| | 21/5/78 | Block II "hard" grazing |
| | 22/5/78 | Block III "lax" grazing |
| | 23/5/78 | Block III "hard" grazing |

morning re-grazing of the block was carried out for a further 90 minutes thus achieving a "hard" defoliation.

A relatively stable pre-experimental dietary background consisting largely of *Agrostis* spp., *Anthoxanthum odoratum*, *Cynosurus cristatus* and *Poa annua* was provided for the 30 wethers whilst not involved in the study area.

Following assessment after each harvest, the collection was defoliated to bring the plants to a similar morphological state, using either a large mob of mixed-age Romney ewes or a scrubsaw with a circular steel blade. Irrigation of the collection to field capacity was carried out immediately after the mowing or mob-stocking treatment during the dry summer period. *Rumex acetosella* initially a problem within the clumps, largely disappeared following application of lime (450 kg/ha) over the whole collection (Harris, 1971).

### 3.1.1 SHEEP PREFERENCE ASSESSMENT

Sheep preference was assessed on the basis of the plant material eaten from each clump after each 'lax' and 'hard' trial grazing. The presence of heteromorphological clumps and the number of measurements to be done prevented the use of quantitative measurements. It was also desirable to use procedures typical of early-generation plant breeding programmes (referred to in Section 1.5). A visual score of the percentage clump area defoliated was used:-

0 : no defoliation; 1 : <10% defoliation; 2 : 10-25%; 3 : 25-50%; 4 : 50-70%; 5 : >70%; A full unit difference in score was very distinct.

## 3.2 PLANT CHARACTERS EXAMINED

Many of the plant characters of *Holcus* spp. putatively influencing acceptability to sheep (see Chapter 1) were examined. Leaf tensile strength, leaf margin pubescence, leaf flavanol content, and soluble-sugar levels were assessed in one season only because of the length of time required to examine them. Characters such as the presence of flower and seed heads and crown rust infection could only be examined in the season in which they occurred.

### 3.2.1 CROWN RUST INFECTION

The degree of crown rust (*Puccinia coronata* var.*Holci*) infection was scored in the field once in summer and once in autumn. Blocks were scored one day before the trial grazing of each block. A graded score of 0-5 was used, increasing with density of pustules. These scores represented the percentage of clump leaf area infected with crown rust pustules;
0 : none; 1 : <10%; 2 : 10-25%; 3 : 25-30%; 4 : 50-70%;
5 : >70%.

### 3.2.2 PRESENCE OF FLOWER AND SEED HEADS

The percentage of tillers with flower and seed heads in each clump was scored once in summer one day before the trial grazing of each block. A graded score of 0-5 was used, increasing with the presence of flower and seed heads:- 0 : none; 1 : <10%; 2 : 10-25%; 3 : 25-50%; 4 : 50-70%; 5 : >70%.

### 3.2.3 LEAF WIDTH

Leaf width was scored immediately prior to each harvest with scores of 1-4, increasing with greater width. These scores represented leaf width measurements previously described in this collection (Teow, 1978); 1 : < 5mm; 2 : 5-8 mm; 3: 8-11 mm; 4 : 11-14 mm. These widths covered the range actually encountered in the collection. The assessment made was of the apparent average leaf width in each clump.

### 3.2.4 CLUMP ERECTNESS

Plant growth habit was scored immediately prior to each harvest. The average prostrateness or erectness of non-reproductive tillers in a clump was scored from 1-5, increasing with greater erectness; 1 : 0-15°; 2 : 15-30°; 3 : 30-45°; 4 : 45-68°; 5 : 68-90°.

### 3.2.5 PRESENCE OF GREEN LEAF AND SHEATH MATERIAL

The degree of green relative to dead leaf and sheath material in a clump was scored immediately before each harvest. Scoring was carried out one day before the trial grazing of each block. Scores were from 1-5, increasing with greater green leaf and sheath material; 1 : > 50% dead material; 2 : 20-50%; 3 : 10-20%; 4 : <10% dead material; 5 : all green.

### 3.2.6 CLUMP HEIGHT AND DIAMETER

The height of non-reproductive tillers in each clump and the diameter of each clump were measured immediately prior to each harvest, using 2.5 cm units.

### 3.2.7 LEAF TENSILE STRENGTH

Leaf strength and hair measurements were made concurrently prior to the first harvest.

Leaf tensile strength may vary with leaf maturity (Evans, 1967a; Theron and Booysen, 1968; Jacques, 1974). Therefore samples consisted of 3 'youngest-mature' leaves from vegetative tillers, from the centre of each clump. The 'youngest-mature' leaf of a tiller was defined as that leaf of which the ligule had most recently appeared.

Sampling was carried out in mid-morning and leaf strength and hair measurements were carried out on the same day.

Leaf tensile strength was measured on a machine, the use and construction of which has been described by Evans (Evans, 1964; Evans, 1967a). Basically the machine consists of an electric motor which applies a load to a steel beam through a system of gears and

a coiled spring. A 5 cm length leaf specimen is held between two clamps, one fixed to the beam and another fixed to the base of the apparatus. When the motor is switched on the spring is wound until the load applied to the beam is great enough to break the specimen. The beam then swings back against a stop which switches off the motor and operates a solenoid brake on the motor shaft. The degree of rotation of the motor shaft is measured on a turns-counting dial.

The machine was calibrated by hanging known weights on a string attached to the beam and passing over a pulley mounted on the front edge of the machine. Dial readings were expressed in terms of grams breaking load across the full range of dial readings measured for *Holcus* spp. in this experiment. The resulting calibration equation was $\hat{Y} = 5.02X - 81.8$, $r^2 = 0.995$, s.e. $(b_1) = 0.037$, s.e. $(b_0) = 4.158$, where $\hat{Y}$ = estimate of breaking load (gms), X = dial reading.

The dry weight (mg) of each 5 cm length sample was used to serve as an approximation of cross-sectional area, and used in an Index of strength equation:

$$\text{Index of Strength} = \frac{\text{breaking load (gms)}}{\text{dry weight (mg) of 5 cm length}} \quad \text{(Evans, 1964).}$$

Tensile strength is commonly expressed in Newtons. $M^{-2}$. However, leaf cross-sectional area ($M^{-2}$) is not used in this case since leaf width and leaf thickness may vary both within and amongst samples.

### 3.2.7.1 LEAF STRENGTH TEST PROCEDURE

Leaf tensile strength may vary from leaf tip to leaf base (Martens and Booysen, 1968; Connor and Bailey, 1972). Therefore each leaf was cut to a 5 cm length adjacent to the ligule to minimise within-leaf tensile strength variance. Leaf samples were brought to a standard moisture condition, since water content may affect leaf strength (Evans, 1967a). The procedure was as follows. One cut end of the sample was immersed in water in a glass vial within a desiccator. Vacuum was applied to the desiccator for 5 minutes bringing the leaf samples to a turgid state. Leaf samples then remained on water at atmospheric pressure until tested. Turgid leaves allowed for ease of handling, each sample being placed between

two clamps on the leaf strength machine. While clamped in this position, samples were assessed for pubescence, and then the previously described breaking load was measured. Broken specimens were oven-dried at 80°C, for 24 hours in a glass vial and the dry weight recorded.

### 3.2.8 LEAF PUBESCENCE

Two populations of leaf hairs were assessed under 30 x magnification. A binocular microscope was mounted on a rotatable stand directly above the leaf clamps of the leaf strength machine. This allowed both leaf margins of each sample to be scanned and scored for pubescence.

The pubescence score ranged from 1-5, increasing with greater overall pubescence. Photomicrographs were taken, at 30 x magnification, of representative pubescence scores (see Plates 3,4,5,6 and 7). As can be seen, a full unit difference in score was very distinct at this level of magnification. An *a posteriori* investigation of the pubescence scores was carried out (Table 6) simply to quantify the range in hair length for each hair population. The variation in density of the long hair population was also considered in the application of the pubescence score (see Plates 3-7).

### 3.2.9 LEAF FLAVANOLS

The concentration of the flavanoid precursors of condensed tannins was assessed using the vanillin-HCl method (Jones *et al.*,1973). It is speculatively assumed that this test shows similar specificity as for the related vanillin - $H_2SO_4$ test (Swain and Hillis, 1959). The vanillin-$H_2SO_4$ test is specific for flavanoids with a single bond at the C2-C3 position of the 'A' ring (see Figure 1) and free meta-orientated hydroxyl groups on the 'B' ring, viz: flavan-3-ols; flavan-3,4-diols; dihydrochalcones; and anthocyanins (Sakar and Howarth, 1976).

Sampling and testing of each clump was carried out in March prior to Harvest 2, over a period of 15 days, 5 days for each block. Sampling was carried out to minimise putative within-plant variation. Samples consisted of 3 or 4 'youngest-mature' (previously defined) leaves from vegetative tillers from the centre of each clump.

Leaf Pubescence Score = 5

Leaf Pubescence Score = 4

Leaf Pubescence Score = 3

Leaf Pubescence Score = 2

Leaf Pubescence Score = 1

PLATE 3

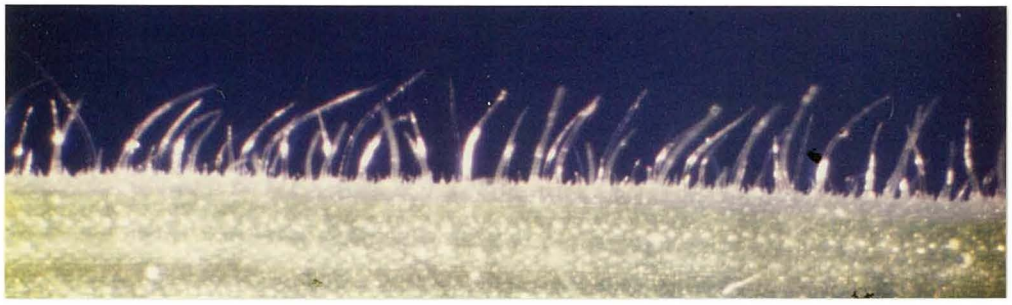


PLATE 4

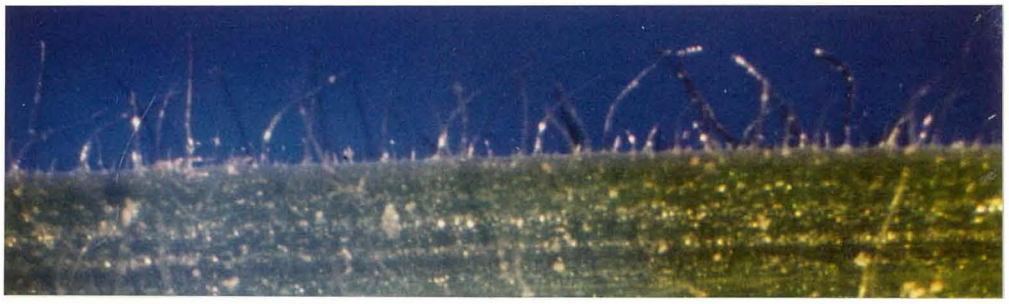


PLATE 5

PLATE 6

PLATE 7

Since inter-conversions of condensed tannins are likely thermodynamically, and possibly enzymatically, (Haslam, 1977) controlled, samples were stored at 2-5$^{o}$C prior to testing later in the same day.

Whole leaves (0.5 - 1.0 gm F.Wt) were homogenised in 5 cm$^{3}$ of chilled 10% (v/v) aqueous methanol for 60 seconds in an ice-cooled high speed blender. Extracts were spotted onto Whatman No. 3 filter paper ( ≈10 µl extract/spot) and respotted with chilled vanillin reagent consisting of 10% (w/v) vanillin in methanol and an equal volume of concentrated hydrochloric acid. A duplicate procedure was run in Block I, with each spot being treated with the same reagent minus the vanillin to discount the possible presence of dihydrochalcones and anthocyanins (Sakar and Howarth, 1976). The modification of this test was suggested by Sakar and Howarth (1976), who used the vanillin/HCl test in conjunction with chromatographic analyses to show that a positive, but false, reaction for flavanols in lucerne cultivars could occur in the presence of anthocyanins. The spots were placed under hydrochloric acid fumes for 10 minutes to allow full colour development. Colour intensity was scored in daylight, using a graded score from 1-5, increasing with greater flavanol concentration (see Table 7).

### 3.2.10 SOLUBLE SUGAR LEVEL

Soluble sugar levels were estimated by the phenol-sulphuric acid test of Dubois *et al.*, (1956) using essentially the same procedure as Haslemore and Roughan (1977). Sampling of each clump was carried out in May prior to Harvest 3 over a period of 6 days, 2 days for each block. A plucked sample of leaves from each clump was collected during mid-afternoon to minimise diurnal variance in soluble sugars (Waite and Boyd, 1953; Greenfield and Smith, 1974) and dried at 2.0 mm Hg; 40$^{o}$C for 24 hours. Interconversion of carbohydrates was minimised by grinding dried samples (0.5 mm screen) and storing them in sealed vials at -3$^{o}$C prior to testing (Nelson and Smith, 1972).

Dried samples (40-60 mg) were extracted with 10 cm$^{3}$ of 62.5% (v/v) aq. methanol at 55$^{o}$C for 15 minutes. After cooling, a 4 cm$^{3}$ aliquot of the extract was taken, from which non-carbohydrate, interfering materials (pigments, phenols) were precipitated by the addition

of 0.1 $cm^3$ saturated lead acetate. Lipids were removed by shaking with 5 $cm^3$ chloroform on a mixing platform. Soluble sugars were retained within an upper aqueous methanol layer.

A 100 µl aliquot was taken from the upper layer and added to 1 $cm^3$ of 5% (v/v) aq. phenol in a thick-walled test tube. Tubes were placed in ice-water and 4 $cm^3$ of 98% sulphuric acid was added to each. Samples were removed from ice-water, stood to cool to room temperature and absorbances read at 490 nm on a 'Bausch and Lomb Spectronic 20'.

Duplicate sucrose standards were prepared as recommended (Haslemore and Roughan, 1977) to give the equivalent of 1, 2, 3, 4, 5, 6, and 10% soluble sugars on a dry weight basis. The standard curve estimate was: $\hat{Y} = 6.33X - 1.36$, s.e. $(b_0) = 0.256$, s.e. $(b_1) = 0.266$, $r^2 = 0.979$; where $\hat{Y}$ = estimate of % soluble-sugars.$gm^{-1}$ D.Wt.; and X = absorbance reading at 490 nm. Soluble sugar levels were expressed as percent of dry weight of plant material.

In this extraction some short-chain fructosans would have been included (Haslemore and Roughan, 1977), and, since Yorkshire fog is included in the temperate grass group, their presence may have led to inflated estimates of soluble sugar levels (Smith, 1973a). Therefore a random group of 10 ground samples from Block II were examined using thin-layer chromatography (Haslemore, *pers.comm.*). Qualitative estimates of the soluble sugar extracts in all 10 samples provided similar results: 60 - 70% sucrose, 10 - 20% glucose and fructose, and ≃ 10% oligosaccharide (mixture of tri-, tetra- and pentasaccharides) (Haslemore, *pers.comm.*).

TABLE 6 Leaf Margin Pubescence Scores

| | Long-hair population | | Short-hair population |
|---|---|---|---|
| Pubescence Score | Length (μ) | Density | Length(μ) |
| 1 | 220-400 | low | ≈ 10 |
| 2 | 220-400 | low | 20 - 40 |
| 3 | 220-400 | medium | 20 - 40 |
| 4 | 220-400 | high | 20 - 40 |
| 5 | 220-560 | high | 20 - 40 |

TABLE 7 Leaf Flavanol Scores

| Score | Colour |
|---|---|
| 1 | Blue green |
| 2 | Trace pink |
| 3 | Light pink over green chlorophyll colouration |
| 4 | Light red with a trace of green |
| 5 | Red |

## 3.3 PHENOTYPIC ANALYSIS

The phenotypic analysis was carried out using the computer programme PHANIE, (Gordon, unpubl.). Characters scored 0-5 were re-coded from 1-6 prior to analysis.

Characters assessed in a single harvest were analysed using the usual random effects model for randomised complete block experiments: SINGLE HARVEST MODEL: $X_{ij} = \mu + \gamma_i + \beta_j + \varepsilon_{ij}$, where $X_{ij}$ = $ij^{th}$ phenotypic variate, $\gamma_i$ = the $i^{th}$ genotype population effect; $\beta_j$ = the $j^{th}$ block effect, $\varepsilon_{ij}$ = residual error, i = 1,..,g genotype populations, j = 1,..., b blocks, and $\mu$ = the harvest mean. Residual error has also been partitioned further giving a within-plot effect and plot error. All effects are assumed random, independent, $\mathcal{N}(0, \sigma^2)$. The expectations of the mean squares are presented in Table 8.

Characters assessed in all harvests were analysed using a random effects split-plot-in-time model (Steel and Torrie, 1960; Gordon, 1979): POOLED HARVESTS MODEL: $X_{ijk} = \mu + \gamma_i + \beta_j + \delta_{ij} + \tau_k + \gamma\tau_{ik} + \varepsilon_{ijk}$, where $X_{ijk}$ = $ijk^{th}$ phenotypic variate, $\mu$ = pooled harvest mean, $\gamma_i$ = $i^{th}$ genotype population effect, $\beta_j$ = $j^{th}$ block effect, $\delta_{ij}$ = ijth genotype population x block interaction effect (Error A), $\tau_k$ = $k^{th}$ harvest effect, $\gamma\tau_{ik}$ = $ik^{th}$ genotype population x harvest interaction effect, and $\varepsilon_{ijk}$ = $ijk^{th}$ error effect (Harvest Error) (Gordon, 1979). In addition, $\varepsilon_{ijk}$ has been partitioned further in two ways: into a block-harvest interaction effect with its associated residual error (Error C); and a within-plot effect and plot error; where i = 1,..., g genotype populations, j = 1,..,b blocks, k = 1,...,p harvests, and s = number of observations per plot. All effects are assumed random, independent, $\mathcal{N}(0, \sigma^2)$. The expectations of the mean squares are presented in Table 9.

The validity of pooling in each character was examined by testing the homogeneity of the error variances across the three harvests. Bartlett's chi-square test was used to test error variance homogeneity following the procedure of Steel and Torrie (1960) (after Bartlett, 1937).

### 3.3.1 HERITABILITY ESTIMATION

Two forms of broad-sense heritability are estimated (Gordon *et al.*, 1972); a full or complete phenotypic variance definition ($h^2$), and a

restricted phenotypic variance definition ($h^{2\prime}$).

In the pooled harvests model each form of heritability was defined as: $\hat{h}^2 = Z_1 = x/y_1$; $\hat{h}^{2\prime} = z_2 = x/y_2$;

where $x = \hat{\sigma}_G^2$

$$y_1 = \hat{\sigma}_e^2 + \hat{\sigma}_{GH}^2 + \hat{\sigma}_H^2 + \hat{\sigma}_{GB}^2 + \hat{\sigma}_G^2 + \hat{\sigma}_B^2,$$

$$y_2 = \hat{\sigma}_e^2 + \hat{\sigma}_{GH}^2 + \hat{\sigma}_{GB}^2 + \hat{\sigma}_G^2, \quad \text{(Gordon, 1979).}$$

The coefficient of variation, C.V.$(\hat{h}^2)$ = standard error $(\hat{h}^2)/\hat{h}^2$, provided an estimate of the relative precision of $\hat{h}^2$. The square root of heritability variance provided $\hat{\sigma}_{(\hat{h}^2)}$

TABLE 8: Expectations of mean squares for the single harvest model

| Source of variation | d.f. | MS | E(MS) | F |
|---|---|---|---|---|
| Blocks | b-1 | MS3 | $\sigma^2_e + g\,\sigma^2_B$ | MS3/MS1 |
| Genotype populations | g-1 | MS2 | $\sigma^2_e + b\,\sigma^2_G$ | MS2/MS1 |
| Experimental error A* | (b-1)(g-1) | MS1 | $\sigma^2_e = (\sigma^2_\psi + \sigma^2_W)$ | |
| Within-plots | bg(s-1) | | $\sigma^2_\psi + s\,\sigma^2_W$ | |
| Plot error | g(b-1(s-1) | | $\sigma^2_\psi$ | |
| Total | bgs - 1 | | | |

* 'A' links this model with the corresponding 'Error A' in the pooled model.

Unbiased estimators: - $\hat{\sigma}^2_e$ = MS1, $\hat{\sigma}^2_G$ = (MS2 - MS1)/b,

$\hat{\sigma}^2_B$ = (MS3-MS1)/g.

TABLE 9: Expectations of mean squares for the pooled harvests model

| Source of variation | d.f. | MS | E(MS) | F |
|---|---|---|---|---|
| Blocks (B) | b-1 | MS6 | $\sigma^2_e + p\sigma^2_{GB} + g\sigma^2_B$ | MS6/MS4 |
| Genotype populations (G) | g-1 | MS5 | $\sigma^2_e + p\sigma^2_{GB} + b\sigma^2_{GH} + bp\sigma^2_G$ | $\frac{(MS5 + MS1)}{(MS4 + MS2)}$ |
| Error A (B x G) | (b-1)(g-1) | MS4 | $\sigma^2_e + p\sigma^2_{GB}$ | MS4/MS1 |
| Harvests (H) | p - 1 | MS3 | $\sigma^2_e + b\sigma^2_{GH} + gb\sigma^2_H$ | MS3/MS2 |
| G x H | (g-1)(p-1) | MS2 | $\sigma^2_e + b\sigma^2_{GH}$ | MS2/MS1 |
| Harvest error | g(b-1)(p-1) | MS1 | $\sigma^2_e$ $(=\sigma^2_c + \sigma^2_{BH})$ | |
| B x H | (b-1)(p-1) | MS10 | $\sigma^2_c + g\sigma^2_{BH}$ | MS10/MS9 |
| Error C | (g-1)(b-1)(p-1) | MS9 | $\sigma^2_c$ | |
| Within-plots | bgp (s-1) | MS8 | $\sigma^2_\psi + s\sigma^2_W$ | MS8/MS7 |
| Plot error | g(b-1)(p-1)(s-1) | MS7 | $\sigma^2_\psi$ | |
| Total | bgsp-1 | | | |

Unbiased estimators: - $\hat{\sigma}^2_e$ = MS1; $\hat{\sigma}^2_{GH}$ = (MS2-MS1)/b;

$\hat{\sigma}^2_H$ = (MS3-MS2)/gb; $\hat{\sigma}^2_{GB}$ = (MS4 - MS1)/p;

$\hat{\sigma}^2_G$ = (MS5 - MS4 - MS2 + MS1)/bp; $\hat{\sigma}^2_B$ = (MS6-MS4)/g.

### 3.3.2 VARIANCES OF HERITABILITIES

Variances of each form of heritability may be obtained following the method outlined by Gordon (1979). The partitions of Harvest Error Mean Square were not considered in the estimation of heritability, hence development of heritability variances considers only MS1-MS6 of Table 9.

For the pooled-harvest model unbiased variance estimates ($\hat{\sigma}_t^2$) were obtained from the linear functions of mean squares (Table 9). The variance of the unbiased estimates var($\hat{\sigma}_t^2$) may be found using the procedure outlined by Crump (1951). Re-defining $\hat{\sigma}_t^2$ as t, and var ($\hat{\sigma}_t^2$) as $V_t$:-

if $t = a_1MS_1 + a_2MS_2 + \ldots + a_k\ MS_k$, where $MS_i$ $(i = 1,2,\ldots,k)$ is a mean square based on $f_i$ degrees of freedom;

then $$V_t = \sum_{i=1}^{k} \left[ \frac{2a_i^2 \left[E(MS_i)\right]^2}{f_i} \right]$$

Variance of z can be obtained approximately from:

$$\sigma_z^2 = \left[\mu_y^2\ \sigma_x^2 + \mu_x^2\ \sigma_y^2 - 2\ \mu_x\mu_y\ \mathrm{cov}(x,y)\right]/\mu_y^4,$$

where $\mu_y = E(y)$, for each of the definitions of y and $\mu_x = G$ (Gordon, 1979). The estimators for $\sigma_x^2$, $\sigma_y^2$ and cov(x,y) appropriate to $z_1$ and $z_2$ are:

$$\sigma_x^2 = V_G;$$

$$\sigma_{y_1}^2 = V_e + V_{GH} + V_H + V_{GB} + V_G + V_B +$$

$$2\ \{\left[(b^2 + p^2 + 2bgp - b - p - bgp^2 - b^2gp)/b^2g^2p^2\right]V_e +$$

$$\left[(1 - p - g)/gp\right]\ V_{GH} + \left[(1 - g - b)/bg\right]\ V_{GB}\}\ ;$$

$$\sigma_{y_2}^2 = V_e + V_{GH} + V_{GB} + V_G + 2\ \{\left[(2-p-b)/bp\right]\ V_e - V_{GH}/p - V_{GB}/b\}\ ;$$

$$\mathrm{cov}\ (x,y_1) = V_G + \left[(bgp - b - p)/b^2g\ p^2\right]V_e + \left[(1 - g)/gp\right]\ V_{GH} + \left|(1 - g)/bg\right]V_{GB};$$

$$\mathrm{cov}\ (x,y_2) = V_G + V_e/bp - V_{GH}/p - V_{GB}/b.$$

## 3.4 MULTIPLE REGRESSION ANALYSIS

Multiple regression analysis was used to assess which characters were more important in determining sheep preference. The computer programme SPSS/REGRESSION was used (Nie *et al.*, 1975).

The sheep preference assessment under 'LAX grazing' (Y variate) was regressed against the plant characters (X variates), for each harvest separately both for all genotype populations combined and for each genotype population separately. Plots were made of Y against each X for each genotype population separately and for all genotype populations combined, using SPSS/SCATTERGRAM (Nie *et al.*, 1975). This was done to check linearity and hence whether transformations were required. Characters which had been scored from 0-5 were re-coded from 1-6.

Standardised partial regression coefficients ($b'$) were used to determine the relative importance of each X variate in determining sheep preference. The standardised regression equation, $\hat{Y}' = b'_1X'_1 + b'_2 X'_2 + \ldots + b'_nX'_n$, where n = number of variables, with $b' = b(s.e._X/s.e._Y)$, $X' = (X - \hat{\mu})/\hat{\sigma}$, examines each variable in standard measure, making each $b'$ independent of the original units of measurement (scale free) and adjusted for variance heterogeneity (Steel and Torrie, 1960). Therefore, a comparison of the $b'$'s indicates the relative contribution of the independent variables in determining the dependent variable (in this case sheep preference). To facilitate comparisons, the ratio $z = b'_j/b'_c$ was estimated, where $b'_j$ = the standardised partial regression coefficient of each of the X variates, and $b'_c$ = the standardised partial regression coefficient of clump greenness. Clump greenness was chosen as the base character for comparison, since it was assessed in all harvests and appeared to be relatively important in the pooled genotype population analyses.

## 3.5 CLUSTER ANALYSIS

Cluster analysis was performed on the $z$'s across genotype populations for each harvest in an attempt to group genotype populations with similar $z$'s configurations.

The $z$'s for each harvest were converted to a 53 x 53 dissimilarity matrix using the computer programme SIMMAT (Teow, 1978). The dissimilarity measure used was based on the Euclidean distance D:

$$D_{kk'} = \left[ \sum_{j=1}^{n} (z_{jk} - z_{jk'})^2 \right]^{\frac{1}{2}}, \text{ for } j \neq j' \text{ where } n = \text{number of}$$

X variates, and $k \neq k'$, where $k = 1,.....,g$ (g = number of genotype populations). In this case the use of standardised variables (i.e. $z$'s) avoided the need for pre-weighting variables. The distance D was used rather than $D^2$, because $D^2$ has the often undesirable property that single large differences may dominate smaller differences (Clifford and Stephenson, 1975).

Four hierarchical agglomerative clustering methods were used and compared: Centroid method; Median method of Gower; Group Average method; and Ward's Incremental Sums of Squares Method. (Anderberg, 1973; Everitt, 1974; Clifford and Stephenson, 1975).

The cluster methods were executed using the computer subroutines CNTRL, CLSTR, MTXIN, LFIND, METHOD and TREE of Anderberg (1973), (Teow, 1978). At this stage of the analysis it was decided to examine further only the results produced using Ward's method, and abandon the other three methods.

Truncation of the dendrograms for each harvest was carried out. The cut-off point was subjectively based, the decision being assisted partially by examining over-laid plots of the $z$'s configurations for each genotype population. Hence the cut-off point produced clusters which appeared to have similar within-cluster $z$ configurations.

Post cluster analyses were performed on the clusters produced from Ward's method. Differences between the means for each character's 'observed value' and '$z$ value' across clusters were tested using Duncan's new multiple range test (Steel and Torrie, 1960), and executed using the programme POSTCA (Gordon, unpubl. - see Teow, 1978).

# CHAPTER 4 RESULTS AND ASSOCIATED DISCUSSION

## 4.1 PHENOTYPIC ANALYSIS

Means data for each genotype population in each harvest are presented in Appendix III. Analysis of variance results including the overall mean, standard error and coefficient of variation, for each character in each harvest are presented in Tables 10, 11 and 12. Prior to pooling analyses for the three harvests, a chi-square test for homogeneity of error variances was carried out. Heterogeneous error variances were obtained for clump height, clump green material, leaf width, and sheep preference (Table 13). Where the error variances are heterogeneous, the calculated F-value is likely to be over-estimated with the result that significance is obtained more often than it should. However, the pooled analyses for these characters probably provides the best overall variance estimates, in spite of the presence of error variance heterogeneity (Cochran, 1947).

Within-plot variation was consistently about half the variance due to experimental error for most of the characters. This suggests that micro-environmental differences within plots were large. Potential genotypic variation also existed within plots, since the 24 plants making up each population were produced from a locally cross-pollinated seed population uncloned across blocks.

Results of the pooled analyses, for characters assessed in all harvests, are presented in Table 14. Environmental variances are likely inflated by pooling across seasons, rather than pooling across years as is traditionally done. Nevertheless, the results indicate that for all of the characters assessed, environmental variances were much larger than genetic variances. This is exemplifed by the low broadsense heritability estimates obtained (Tables 15, 16). The relative precision of heritability estimation for most of the characters was generally very low, with coefficients of variation of the heritability estimates often above 100%.

The level of genetic variation for these characters, assessed across a reasonably representative sample of Yorkshire fog material, suggests that the level of genetic diversity of Yorkshire fog in New Zealand might not be as great as was previously thought (Jacques, 1974).

TABLE 10: Anova of characters measured in harvest one

(1) Presence of flower and seed heads (ORIG: 0-5) (TRANSF: 1 - 6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.4304 | NS | 0.0025 | 0.0058 | 0.01 |
| G. Populat-ions | 52 | 0.7563 | **** | 0.1536 | 0.0504 | 0.52 |
| Error | 104 | 0.2955 | | 0.2955 | 0.0406 | |
| Within-plots | 1108 | 1.4499 | | 0.1821 | 0.0077 | 0.62 |
| Plot error | | | | 0.1134 | 0.0477 | 0.38 |
| Grand mean = 3.5293 | | | | Coefficient of variation = 15.4% | | |
| s.e. grand mean =0.0431 | | | | | | |

(2) Clump rust (ORIG: 0 - 5) (TRANSF: 1 - 6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 1.1786 | ** | 0.0177 | 0.0157 | 0.07 |
| G. Populat-ions | 52 | 0.3274 | NS | 0.0293 | 0.0237 | 0.12 |
| Error | 104 | 0.2395 | | 0.2395 | 0.0329 | |
| Within-plots | 1108 | 1.1147 | | 0.1404 | 0.0060 | 0.59 |
| Plot error | | | | 0.0991 | 0.0381 | 0.41 |
| Grand mean = 2.2857 | | | | Coefficient of variation = 21.4% | | |
| s.e. grand mean =0.0388 | | | | | | |

(3) Presence of clump green leaf and sheath material (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.7823 | **** | 0.0133 | 0.0104 | 0.17 |
| G. Populat-ions | 52 | 0.1469 | *** | 0.0229 | 0.0101 | 0.29 |
| Error | 104 | 0.0784 | | 0.0784 | 0.0108 | |
| Within-plots | 1108 | 0.3366 | | 0.0424 | 0.0018 | 0.54 |
| Plot error | | | | 0.0360 | 0.0122 | 0.46 |
| Grand mean = 2.9979 | | | | Coefficient of variation = 9.3% | | |
| s.e. grand mean =0.0222 | | | | | | |

(4) Leaf tensile strength (gms/mgm. 5 cm. leaf)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 253.9446 | **** | 4.2059 | 3.3890 | 0.14 |
| G. Populations | 52 | 26.6600 | NS | 1.4569 | 2.2234 | 0.05 |
| Error | 104 | 31.0306 | | 31.0306 | 4.2624 | |
| Within-plots | 1105 | 158.2469 | | 19.9230 | 0.8468 | 0.64 |
| Plot error | | | | 11.1076 | 5.0653 | 0.36 |

Grand mean = 45.0628 Coefficient of variation = 12.4%

s.e. grand mean = 0.4418

(5) Leaf pubescence (3-15)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 1.7843 | * | 0.0257 | 0.0238 | 0.06 |
| G. populations | 52 | 0.7600 | ** | 0.1126 | 0.0524 | 0.27 |
| Error | 104 | 0.4222 | | 0.4222 | 0.0580 | |
| Within-plots | 1105 | 2.4944 | | 0.3140 | 0.0133 | 0.74 |
| Plot error | | | | 0.1082 | 0.0723 | 0.26 |

Grand mean = 10.5722 Coefficient of variation = 6.1%

s.e. grand mean =0.0515

(6) Leaf width (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.3090 | * | 0.0042 | 0.0041 | 0.05 |
| G. populations | 52 | 0.1836 | *** | 0.0317 | 0.0125 | 0.36 |
| Error | 104 | 0.0885 | | 0.0885 | 0.0122 | |
| Within-plots | 1105 | 0.3925 | | 0.0494 | 0.0021 | 0.56 |
| Plot error | | | | 0.0391 | 0.0139 | 0.44 |

Grand mean = 2.1248 Coefficient of variation = 14.0%

s.e. grand mean =0.0236

(7) Clump erectness (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.9826 | *** | 0.0156 | 0.0131 | 0.10 |
| G. populations | 52 | 0.1915 | NS | 0.0122 | 0.0142 | 0.08 |
| Error | 104 | 0.1549 | | 0.1549 | 0.0213 | |
| Within-plots | 1108 | 0.5337 | | 0.0674 | 0.0029 | 0.44 |
| Plot error | | | | 0.0875 | 0.0232 | 0.56 |

Grand mean = 2.7847 Coefficient of variation = 14.1%

s.e. grand mean =0.0312

(8) Clump height (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 17.2061 | **** | 0.3141 | 0.2296 | 0.56 |
| G. populations | 52 | 0.6541 | NS | 0.0314 | 0.0492 | 0.06 |
| Error | 104 | 0.5598 | | 0.5598 | 0.0769 | |
| Within-plots | 1108 | 1.6038 | | 0.2030 | 0.0086 | 0.36 |
| Plot error | | | | 0.3568 | 0.0818 | 0.64 |

Grand mean = 6.1278 Coefficient of variation = 12.2%

s.e. grand mean =0.0593

(9) Clump diameter (2.5cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 205.8921 | **** | 3.8584 | 2.7469 | 2.76 |
| G. populations | 52 | 1.7487 | NS | 0.1165 | 0.1292 | 0.08 |
| Error | 104 | 1.3992 | | 1.3992 | 0.1922 | |
| Within-plots | 1108 | 5.6523 | | 0.7150 | 0.0304 | 0.51 |
| Plot error | | | | 0.6842 | 0.2158 | 0.49 |

Grand mean = 14.2494 Coefficient of variation = 8.3%

s.e. grand mean = 0.0938

(10) Sheep preference assessment (ORIG: 0-5) (TRANSF: 1-6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 1.8712 | *** | 0.0304 | 0.0250 | 0.12 |
| G. Populations | 52 | 0.3117 | NS | 0.0178 | 0.0232 | 0.07 |
| Error | 104 | 0.2582 | | 0.2582 | 0.0355 | |
| Within-plots | 1108 | 1.0918 | | 0.1377 | 0.0059 | 0.53 |
| Plot error | | | | 0.1206 | 0.0402 | 0.47 |

Grand mean = 2.2985 Coefficient of variation = 22.1%

s.e. grand mean =0.0403

Significance symbols:

N.S., not significant = $P > 0.10$

(N.S.) = $0.10 \geq P > 0.05$

* = $0.05 \geq P > 0.01$

** = $0.01 \geq P > 0.005$

*** = $0.005 \geq P > 0.001$

**** = $0.001 \geq P$

TABLE 11: Anova of characters measured in harvest two.

(1) Clump rust (ORIG: 0-5) (TRANSF: 1-6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 1.1229 | *** | 0.0180 | 0.0150 | 0.11 |
| G. populations | 52 | 0.3963 | **** | 0.0759 | 0.0266 | 0.45 |
| Error | 104 | 0.1687 | | 0.1687 | 0.0232 | |
| Within-plots | 1086 | | | 0.0886 | 0.0038 | 0.52 |
| Plot error | | | | 0.0802 | 0.0262 | 0.48 |

Grand mean = 1.9671 Coefficient of Variation = 20.9%

s.e. grand mean = 0.0326

(2) Presence of clump green leaf and sheath material (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 3.1570 | **** | 0.0557 | 0.0421 | 0.27 |
| G. populations | 52 | 0.2031 | NS | 0.0012 | 0.0161 | 0.01 |
| Error | 104 | 0.2066 | | 0.2066 | 0.0284 | |
| Within-plots | 1086 | 0.7159 | | 0.0921 | 0.0040 | 0.45 |
| Plot error | | | | 0.1145 | 0.0311 | 0.55 |

Grand mean = 4.0327 Coefficient of variation = 11.3%

s.e. grand mean = 0.0360

(3) Leaf width (1-4)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.0595 | N S | 0.0008 | 0.0008 | 0.01 |
| G. populations | 52 | 0.1375 | N S | 0.0114 | 0.0100 | 0.11 |
| Error | 104 | 0.1035 | | 0.1035 | 0.0142 | |
| Within-plots | 1086 | 0.3429 | | 0.0439 | 0.0019 | 0.42 |
| Plot error | | | | 0.0596 | 0.0154 | 0.58 |

Grand mean = 1.8477 Coefficient of variation = 17.4%

s.e. grand mean = 0.0255

(4) Clump erectness (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 1.6359 | **** | 0.0276 | 0.0218 | 0.16 |
| G. populations | 52 | 0.2982 | ** | 0.0416 | 0.0207 | 0.24 |
| Error | 104 | 0.1735 | | 0.1735 | 0.0238 | |
| Within-plots | 1086 | 0.4589 | | 0.0589 | 0.0025 | 0.34 |
| Plot error | | | | 0.1146 | 0.0252 | 0.66 |

Grand mean = 2.6679 Coefficient of variation = 15.6%

s.e. grand mean = 0.0330

(5) Clump height (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 22.1477 | **** | 0.4115 | 0.2955 | 1.21 |
| G. populations | 52 | 0.2927 | NS | 0.0159 | 0.0244 | 0.05 |
| Error | 104 | 0.3404 | | 0.3404 | 0.0468 | |
| Within-plots | 1086 | 0.9405 | | 0.1207 | 0.0052 | 0.35 |
| Plot error | | | | 0.2197 | 0.0496 | 0.65 |

Grand mean = 4.0896 Coefficient of variation = 14.3%

s.e. grand mean = 0.0463

(6) Clump diameter (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 10.6755 | **** | 0.1802 | 0.1425 | 0.16 |
| G. populations | 52 | 1.2718 | NS | 0.0495 | 0.0964 | 0.04 |
| Error | 104 | 1.1232 | | 1.1232 | 0.1543 | |
| Within-plots | 1086 | 3.7768 | | 0.4875 | 0.0210 | 0.43 |
| Plot error | | | | 0.6357 | 0.1682 | 0.57 |

Grand mean = 12.8418 Coefficient of variation = 8.3%

s.e. grand mean = 0.0840

(7) Leaf flavanol score (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.5004 | N S | 0.0037 | 0.0067 | 0.01 |
| G. populations | 52 | 0.3162 | N S | 0.0034 | 0.0247 | 0.01 |
| Error | 104 | 0.3059 | | 0.3059 | 0.0420 | |
| Within-plots | 1086 | 1.2895 | | 0.1645 | 0.0070 | 0.54 |
| Plot error | | | | 0.1414 | 0.0047 | 0.46 |

Grand mean = 2.3791 Coefficient of variation = 23.4%

s.e. grand mean = 0.0439

(8) Sheep preference assessment (ORIG: 0-5) (TRANSF: 1-6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 6.8119 | **** | 0.1167 | 0.0909 | 0.19 |
| G. populations | 52 | 0.6122 | NS | 0.0046 | 0.0486 | 0.01 |
| Error | 104 | 0.6260 | | 0.6260 | 0.0860 | |
| Within-plots | 1086 | 1.5290 | | 0.1950 | 0.0083 | 0.31 |
| Plot error | | | | 0.4310 | 0.0901 | 0.69 |

Grand error = 2.8429 Coefficient of variation = 27.8%

s.e. grand mean = 0.0627

SIGNIFICANCE SYMBOLS:

N S not significant = $P > 0.10$

* = $0.05 \geq P > 0.01$

** = $0.01 \geq P > 0.005$

*** = $0.005 \geq P > 0.001$

**** = $0.001 \geq P$

TABLE 12: Anova of characters measured in harvest three

(1) Presence of clump green leaf and sheath material (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.0702 | N S | 0.0021 | 0.0010 | 0.01 |
| G. populations | 52 | 0.1608 | N S | 0.0066 | 0.0132 | 0.04 |
| Error | 104 | 0.1808 | | 0.1808 | 0.0248 | |
| Within-plots | 1069 | 0.7908 | | 0.1031 | 0.0045 | 0.57 |
| Plot error | | | | 0.0776 | 0.0286 | 0.43 |

Grand mean = 4.0515 Coefficient of variation = 10.5%

s.e. grand mean = 0.0337

(2) Leaf width (1-4)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 2.9184 | **** | 0.0540 | 0.0389 | 0.94 |
| G. populations | 52 | 0.0606 | NS | 0.0010 | 0.0047 | 0.02 |
| Error | 104 | 0.0575 | | 0.0575 | 0.0079 | |
| Within-plots | 1069 | 0.2862 | | 0.0373 | 0.0016 | 0.65 |
| Plot error | | | | 0.0202 | 0.0094 | 0.35 |

Grand mean = 1.6377 Coefficient of variation = 14.6%

s.e. grand mean = 0.0190

(3) Clump erectness (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.5882 | * | 0.0087 | 0.0079 | 0.07 |
| G. populations | 52 | 0.1791 | (NS) | 0.0168 | 0.0129 | 0.13 |
| Error | 104 | 0.1287 | | 0.1287 | 0.0177 | |
| Within-plots | 1069 | 0.5048 | | 0.0657 | 0.0028 | 0.51 |
| Plot error | | | | 0.0630 | 0.0199 | 0.49 |

Grand mean = 2.4373 Coefficient of variation = 14.7%

s.e. grand mean = 0.0285

(4) Clump height (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 0.8482 | * | 0.0120 | 0.0113 | 0.06 |
| G. populations | 52 | 0.2538 | N S | 0.0144 | 0.0189 | 0.07 |
| Error | 104 | 0.2106 | | 0.2106 | 0.0289 | |
| Within-plots | 1069 | 0.7529 | | 0.0978 | 0.0042 | 0.46 |
| Plot error | | | | 0.1128 | 0.0319 | 0.54 |

Grand mean = 2.8144 Coefficient of variation = 16.3%

s.e. grand mean = 0.0364

(5) Clump diameter (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 42.8018 | **** | 0.7779 | 0.5711 | 0.49 |
| G. populations | 52 | 1.6512 | NS | 0.0259 | 0.1281 | 0.02 |
| Error | 104 | 1.5734 | | 1.5734 | 0.2161 | |
| Within-plots | 1069 | 4.3565 | | 0.5686 | 0.0246 | 0.36 |
| Plot error | | | | 1.0049 | 0.2298 | 0.64 |

Grand mean = 12.1927 Coefficient of variation = 10.3%

s.e. grand mean = 0.0995

(6) Soluble sugar level (% sugar $gm^{-1}$ D.Wt)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 219.0933 | **** | 4.0622 | 2.9231 | 1.07 |
| G. populations | 52 | 5.0253 | NS | 0.4091 | 0.3663 | 0.11 |
| Error | 104 | 3.7981 | | 3.7981 | 0.5217 | |
| Within-plots | 1060 | 4.9599 | | 0.6572 | 0.0286 | 0.17 |
| Plot error | | | | 3.1409 | 0.5295 | 0.83 |

Grand mean = 13.034 Coefficient of variation = 15.0%

s.e. grand mean = 0.1546

(7) Sheep preference assessment (ORIG: 0-5) (TRANSF: 1-6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks | 2 | 6.0376 | **** | 0.1074 | 0.0806 | 0.31 |
| G. populations | 52 | 0.2809 | NS | 0.0209 | 0.0239 | 0.06 |
| Error | 104 | 0.3436 | | 0.3436 | 0.0472 | |
| Within-plots | 1069 | 0.8146 | | 0.1055 | 0.0045 | 0.31 |
| Plot error | | | | 0.2381 | 0.0494 | 0.69 |

Grand mean = 4.1617 Coefficient of variation = 14.1%

s.e. grand mean = 0.0465

SIGNIFICANCE SYMBOLS:

N S ,not significant = P >0.10

(N S ) = 0.10 $\geq$ P >0.05

* = 0.05 $\geq$ P >0.01

** = 0.01 $\geq$ P >0.005

*** = 0.005$\geq$ P> 0.001

**** = 0.001 $\geq$ P

TABLE 13: Homogeneity of error variances

| Character | df | $Chi^2$ | Probability |
|---|---|---|---|
| Sheep preference assessment | 312 | 21.890 | 0.000 |
| Leaf width | 312 | 9.153 | 0.011 |
| Clump green material | 312 | 25.542 | 0.000 |
| Clump erectness | 312 | 2.330 | 0.312 NS |
| Clump height | 312 | 24.455 | 0.000 |
| Clump diameter | 312 | 2.988 | 0.223 NS |

Probabilities of > 0.05 = NS, indicate that harvest error variances are homogeneous

TABLE 14: Anova for pooled harvests

(1) Sheep preference assessment (ORIG: 0-5) (TRANSF: 1-6)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error B |
|---|---|---|---|---|---|---|
| Blocks (B) | 2 | 0.5457 | N S | 0.0001 | 0.0025 | 0.00 |
| G. populations (G) | 52 | 0.5513 | N S | 0.0135 | 0.0162 | 0.03 |
| Error A (GxB) | 104 | 0.5629 | N S | 0.0343 | 0.0297 | 0.07 |
| Harvests (H) | 2 | 146.4515 | **** | 0.9190 | 0.6513 | 2.00 |
| (G x H) | 104 | 0.3268 | N S | 0.0444 | 0.0211 | 0.10 |
| Error B | 212 | 0.4599 | | 0.4599 | 0.0445 | |
| (B x H) | 4 | 7.0875 | **** | 0.1275 | 0.0773 | 0.28 |
| Error C | 208 | 0.3325 | | 0.3325 | 0.0324 | |
| Within-plots | 3260 | 1.1465 | | 0.1464 | 0.0036 | 0.32 |
| Plot error | | | | 0.3135 | 0.0460 | 0.68 |

Grand pooled mean = 3.1020 Coefficient of variation (Error B) = 21.9%

s.e. grand pooled mean = 0.0322 Coefficient of variation (Error A) = 24.2%

(2) Leaf width (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error B |
|---|---|---|---|---|---|---|
| Blocks (B) | 2 | 0.6684 | * | 0.0033 | 0.0030 | 0.04 |
| G. populations (G) | 52 | 0.2513 | ** | 0.0129 | 0.0060 | 0.17 |
| Error A (G x B) | 104 | 0.1457 | **** | 0.0233 | 0.0071 | 0.31 |
| Harvests (H) | 2 | 9.4899 | **** | 0.0593 | 0.0422 | 0.78 |
| (G x H) | 104 | 0.0652 | NS | 0.0035 | 0.0039 | 0.05 |
| Error B | 212 | 0.0756 | | 0.0756 | 0.0073 | |
| (B x H) | 4 | 1.3093 | **** | 0.0237 | 0.0143 | 0.31 |
| Error C | 208 | 0.0519 | | 0.0519 | 0.0051 | |
| Within-plots | 3260 | 0.3411 | | 0.0437 | 0.0011 | 0.58 |
| Plot error | | | | 0.0319 | 0.0081 | 0.42 |

Grand pooled mean = 1.8701 Coefficient of variation (Error B) = 14.7%

s.e. grand pooled mean = 0.0144 Coefficient of variation (Error A) = 20.4%

(3) Presence of clump green leaf and sheath material (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks (B) | 2 | 0.5625 | (NS) | 0.0021 | 0.0025 | 0.01 |
| G. Populations | | | | | | |
| (G) | 52 | 0.2670 | NS | 0.0081 | 0.0071 | 0.05 |
| Error A (G x B) | 104 | 0.2233 | ** | 0.0239 | 0.0113 | 0.16 |
| Harvests (H) | 2 | 57.8051 | **** | 0.3628 | 0.2571 | 2.39 |
| (G x H) | 104 | 0.1219 | NS | 0.0098 | 0.0074 | 0.07 |
| Error B | 212 | 0.1515 | | 0.1515 | 0.0146 | |
| (B x H) | 4 | 1.7235 | **** | 0.0302 | 0.0188 | 0.20 |
| Error C | 208 | 0.1213 | | 0.1213 | 0.0118 | |
| Within-plots | 3260 | 0.6117 | | 0.0785 | 0.0019 | 0.52 |
| Plot error | | | | 0.0730 | 0.0159 | 0.48 |

Grand pooled mean = 3.6940 Coefficient of variation (Error B) = 10.5%

s.e. grand pooled mean = 0.0192 Coefficient of variation (Error A) = 12.8%

(4) Clump erectness (1-5)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error |
|---|---|---|---|---|---|---|
| Blocks (B) | 2 | 1.4566 | ** | 0.0074 | 0.0065 | 0.07 |
| G. populations | | | | | | |
| (G) | 52 | 0.4781 | * | 0.0218 | 0.0113 | 0.22 |
| Error A (G x B) | 104 | 0.2865 | **** | 0.0621 | 0.0135 | 0.62 |
| Harvests (H) | 2 | 4.9694 | **** | 0.0307 | 0.0221 | 0.31 |
| (G x H) | 104 | 0.0953 | NS | 0.0016 | 0.0054 | 0.02 |
| Error B | 212 | 0.1002 | | 0.1002 | 0.0097 | |
| (B x H) | 4 | 0.8750 | **** | 0.0149 | 0.0096 | 0.15 |
| Error C | 208 | 0.0853 | | 0.0853 | 0.0083 | |
| Within-plots | 3260 | 0.4993 | | 0.0640 | 0.0016 | 0.64 |
| Plot error | | | | 0.0362 | 0.0110 | 0.36 |

Grand pooled mean = 2.6300 Coefficient of variation (Error B) = 12.0%

s.e. grand pooled mean = 0.0184 Coefficient of variation (Error A) =20.4%

(5) Clump height (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error B |
|---|---|---|---|---|---|---|
| Blocks (B) | 2 | 20.9445 | **** | 0.1274 | 0.0931 | 0.33 |
| G. populations | | | | | | |
| (G) | 52 | 0.6847 | NS | 0.0135 | 0.0189 | 0.03 |
| Error A (G x B) | 104 | 0.6923 | **** | 0.1018 | 0.0341 | 0.26 |
| Harvests (H) | 2 | 444.1335 | **** | 2.7917 | 1.9752 | 7.21 |
| (G x H) | 104 | 0.2579 | NS | 0.0430 | 0.0172 | 0.11 |
| Error B | 212 | 0.3870 | | 0.3870 | 0.0374 | |
| (B x H) | 4 | 9.6288 | **** | 0.1777 | 0.1049 | 0.46 |
| Error C | 208 | 0.2092 | | 0.2092 | 0.0204 | |
| Within-plots | 3260 | 1.1028 | | 0.1415 | 0.0035 | 0.37 |
| Plot error | | | | 0.2455 | 0.0391 | 0.63 |

Grand pooled mean = 4.3449 Coefficient of variation (Error B) = 14.3%

s.e. grand pooled mean = 0.0320 Coefficient of variation (Error A) = 19.2%

(6) Clump diameter (2.5 cm units)

| Source | DF | MS | SIGNIF | $\hat{\sigma}^2$ | s.e. | Ratio to Error B |
|---|---|---|---|---|---|---|
| Blocks (B) | 2 | 36.9206 | **** | 0.2138 | 0.1642 | 0.08 |
| G. populations | | | | | | |
| (G) | 52 | 3.1172 | *** | 0.2318 | 0.0860 | 0.09 |
| Error A (G x B) | 104 | 2.9263 | NS | 0.0846 | 0.1593 | 0.03 |
| Harvests (H) | 2 | 175.7648 | **** | 1.1006 | 0.7817 | 0.41 |
| (G x H) | 104 | 0.7773 | NS | 0.6317 | 0.0932 | 0.24 |
| Error B | 212 | 2.6723 | | 2.6723 | 0.2583 | |
| (B x H) | 4 | 111.2244 | **** | 2.0875 | 1.2116 | 0.78 |
| Error C | 208 | 0.5848 | | 0.5848 | 0.0571 | |
| Within-plots | 3260 | 4.6032 | | 0.5924 | 0.0147 | 0.22 |
| Plot error | | | | 2.0799 | 0.2626 | 0.78 |

Grand pooled mean = 13.0946 Coefficient of variation (Error B) = 12.5%

s.e. grand pooled mean = 0.0760 Coefficient of variation (ErrorA) = 13.1%

SIGNIFICANCE SYMBOLS:

NS = $P > 0.10$

(NS) = $0.10 \geq P > 0.05$

* = $0.05 \geq P > 0.01$

** = $0.01 \geq P > 0.005$

*** = $0.005 \geq P > 0.001$

**** = $0.001 \geq P$

TABLE 15: Estimates of heritability and associated coefficients of variation for characters assessed only in a single harvest

| Character | | Heritability estimate | s.e. ($\hat{h}^2$) | C.V. ($\hat{h}^2$) |
|---|---|---|---|---|
| Leaf tensile strength | $Z_1$ | 0.043 | 0.065 | 151% |
| | $Z_2$ | 0.049 | 0.075 | 153% |
| Leaf pubescence | $Z_1$ | 0.201 | 0.084 | 42% |
| | $Z_2$ | 0.211 | 0.092 | 44% |
| Inflorescences | $Z_1$ | 0.340 | 0.087 | 26% |
| | $Z_2$ | 0.340 | 0.094 | 28% |
| Clump rust Harvest 1 | $Z_1$ | 0.102 | 0.080 | 79% |
| | $Z_2$ | 0.109 | 0.087 | 80% |
| Clump rust Harvest 2 | $Z_1$ | 0.289 | 0.084 | 29% |
| | $Z_2$ | 0.310 | 0.094 | 30% |
| Leaf flavanols | $Z_1$ | 0.011 | 0.079 | 718% |
| | $Z_2$ | 0.011 | 0.080 | 727% |
| Soluble sugars | $Z_1$ | 0.049 | 0.047 | 95% |
| | $Z_2$ | 0.097 | 0.086 | 88% |

TABLE 16: Estimates of heritability and associated coefficients of variation for characters assessed in all harvests (pooled)

| Character | | Heritability Estimate | s.e. ($\hat{h}^2$) | C.V. ($\hat{h}^2$) |
|---|---|---|---|---|
| Sheep | $Z_1$ | 0.010 | 0.013 | 128% |
| preference | $Z_2$ | 0.030 | 0.036 | 121% |
| Leaf width | $Z_1$ | 0.075 | 0.039 | 51% |
| | $Z_2$ | 0.119 | 9.052 | 44% |
| Green | $Z_1$ | 0.015 | 0.015 | 100% |
| material | $Z_2$ | 0.047 | 0.041 | 87% |
| Clump | $Z_1$ | 0.099 | 0.050 | 50% |
| erectness | $Z_2$ | 0.120 | 0.053 | 58% |
| Clump | $Z_1$ | 0.004 | 0.006 | 152% |
| height | $Z_2$ | 0.029 | 0.041 | 141% |
| Clump | $Z_1$ | 0.063 | 0.027 | 429% |
| diameter | $Z_2$ | 0.098 | 0.037 | 371% |

## 4.2 MULTIPLE REGRESSION ANALYSIS

Multiple regression analysis was carried out on untransformed data, following a check on the linearity between Y and each X variate (see Section 3.4). Correlations between the X variates were low, and therefore the X variates may be regarded as independent. Correlation matrices for pooled genotype populations are presented in Appendix V.

Characters in the pooled genotype population multiple regression analyses have been listed in order of estimated relative importance in determining sheep preference, for each harvest (see Tables 17, 18 and 19). The sign of the standardised partial regression coefficient ($b'$) indicates the direction in which sheep preference was exhibited. Ratios of the standardised partial regression coefficients ($z = b_j'/b_c'$), using clump green material as the basis of comparison amongst the plant characters (refer to Section 3.4), provided information as to the relative importance of each character in determining sheep preference. The pooled analysis provided an "average" result. In order to examine possible genotype population x sheep preference interactions, each genotype population was also regressed separately, the $z$'s obtained, and subjected to cluster analysis (Section 4.3).

Results of the pooled regressions (Tables 17, 18, 19) suggest that sheep rejected clumps exhibiting a high proportion of inflorescences, dead leaf and sheath material and crown rust infection. The other characters appeared to be relatively unimportant in determining sheep preference, across the three harvests.

In each harvest the coefficient of multiple determination, $R^2$, (Steel and Torrie, 1960; Draper and Smith, 1966) indicated that approximately 20-25% of the total variation in Y (sheep preference) was described by the X variates entered into the equation. Hence 75-80% of the variation in Y has not been explained by these variables. The variation in Y not explained may have been due to unassessed X variates or due simply to sheep fickleness of grazing. Unassessed X variates might include those characters measured in a single harvest only, or perhaps presently unrecognised characters important in determining sheep preference. Trans-aconitate level may perhaps be important in determining the acceptability of Yorkshire fog? (See Section 1.4.7). It is unlikely that leaf pubescence, leaf tensile strength, leaf flavanol

TABLE 17: Multiple Regression for Pooled Genotype Populations

Harvest One

$\hat{Y}$ = estimate of sheep preference

| X variates | $b'_j$ | F(1,1244) | $z$ |
|---|---|---|---|
| Flower + seed heads | -0.316 | ** | -1.533 |
| Clump green material | 0.206 | ** | +1.0 |
| Clump rust | -0.177 | ** | -0.859 |
| Leaf width | 0.119 | ** | +0.577 |
| Clump height | 0.110 | ** | +0.534 |
| Clump diameter | -0.093 | ** | -0.451 |
| Clump erectness | -0.035 | NS | -0.170 |
| Leaf pubescence | -0.027 | NS | -0.131 |
| Leaf strength | 0.004 | NS | +0.019 |

$R^2 = 0.2167$ $\sigma^2_{y.x} = 0.9837$

$b'$ = standardised partial regression coefficient

$z = b'_j / b'$clump green material

SIGNIFICANCE SYMBOLS

NS, not significant = $P > 0.10$

** = $P < 0.01$

TABLE 18: Multiple Regression for Pooled Genotype Populations

Harvest two

$\hat{Y}$ = estimate of sheep preference

| X variates | b´ | F(1,1217) | z |
|---|---|---|---|
| Clump green material | 0.446 | ** | + 1.0 |
| Clump rust | -0.242 | ** | - 0.543 |
| Clump erectness | -0.089 | ** | - 0.199 |
| Flavanol level | -0.081 | ** | - 0.182 |
| Leaf width | 0.057 | ** | + 0.128 |
| Clump diameter | -0.032 | NS | - 0.072 |
| Clump height | 0.027 | NS | + 0.061 |

$R^2$ = 0.2496 $\sigma^2_{y.x}$ = 1.4567

b´ = standardised partial regression coefficient

z = b´/b´clump green material.

SIGNIFICANCE SYMBOLS

NS, not significant = P >0.10

** = P <0.01

TABLE 19: Multiple regression for pooled genotype populations

Harvest three

$\hat{Y}$ = estimate of sheep preference

| X variates | b´ | F(1,1194) | z |
|---|---|---|---|
| Clump green material | 0.420 | ** | +1.0 |
| Clump diameter | -0.140 | ** | -0.333 |
| Clump erectness | 0.092 | ** | +0.219 |
| Leaf width | -0.080 | ** | -0.190 |
| Clump height | 0.069 | ** | +0.164 |
| Soluble sugar level | -0.021 | NS | -0.050 |

$R^2$ = 0.2057 $\sigma^2_{y.x}$ = 0.8153

b´ = standardised partial regression coefficient

z = b´/b´clump green material

SIGNIFICANCE SYMBOLS

NS, not significant = P >0.10

** = P <0.01

level and soluble sugar level would account for the unexplained variation in Y for the harvests in which these characters were not considered, since they were relatively unimportant in the harvests for which they were assessed.

## 4.3 CLUSTER ANALYSIS

Dendrograms produced using the Centroid method (Figure 7), Median method (Figure 8) and Group Average method (Figure 9) have been presented for the first harvest only, to allow a comparison of the clustering strategies. Dendrograms produced using Ward's method are presented for each harvest (Figures 10, 11, 12).

### 4.3.1 Cluster strategy comparison

Both the Centroid and Median methods produced dendrograms with reversals, i.e. fusion at a lower distance than the original, making clear separation of the clusters difficult in two dimensional space, (See Figures 7,8). Reversals often occur in using the Centroid and Median methods because their distance functions may decrease (refer to Section 3.5). Reversals are conceptually difficult to interpret, and for this reason the Centroid and Median methods are often avoided in favour of "monotonic" strategies, i.e. those in which reversals do not occur (Anderberg, 1973; Clifford and Stephenson, 1975).

The Centroid (Figure 7), Median (Figure 8), and Group Average (Figure 9) strategies all produced dendrograms in which "space-distortion" (i.e. chaining) was evident. Earlier work using the Group Average method had suggested that this method was "space-conserving", i.e. did not have a tendency to chain (Lance and Williams, 1967). However, results from this study using the Euclidean distance D, and results from another study using $D^2$ (Teow, 1978) suggest that this strategy does have a tendency to distort space.

Ward's method, a monotonic strategy, was the only method used which was space-conserving (see Figures 10, 11, 12). The fairly even distribution of cluster size allowed clear separation of the clusters in two dimensional space.

4.3.2 Post cluster analyses

Clusters produced using Ward's method are shown in Figures 10, 11 and 12. Results of the post-cluster analyses have been tabulated for each harvest in Tables 20, 21 and 22. These results indicated that for the larger cluster groups in the three harvests, the presence of inflorescences, clump green material and clump rust infection were the most important characters, of those assessed, determining sheep preference. The small cluster groups in each harvest, containing six or less genotype populations in each cluster had different $z$'s configurations to those of the larger cluster groups. For example, in the cluster groups numbered 5,6 and 7 in the first harvest (Table 20) leaf pubescence appeared to be relatively important. Similarly, in the third harvest for the small cluster groups numbered 4, 5, 6, 7 and 8 (Table 22) clump erectness appeared to be relatively important. Post cluster analyses of each characters mean value did not help to explain the different $z$'s configurations of the smaller cluster groups in each of the harvests (see Tables 20, 21, 22). These different $z$ configurations may simply reflect sheep fickleness of grazing i.e. represent sheep preference x genotype population interaction. The small cluster groups may be regarded as 'outliers' in the pooled regression analysis (Draper and Smith, 1966).

The results of the cluster analyses generally confirmed the results obtained from the pooled multiple regression analyses.

Figure 7. Dendrogram of Harvest 1 standardised partial regression coefficient ratios clustered by the Centroid Method (Y axis = clustering stage)

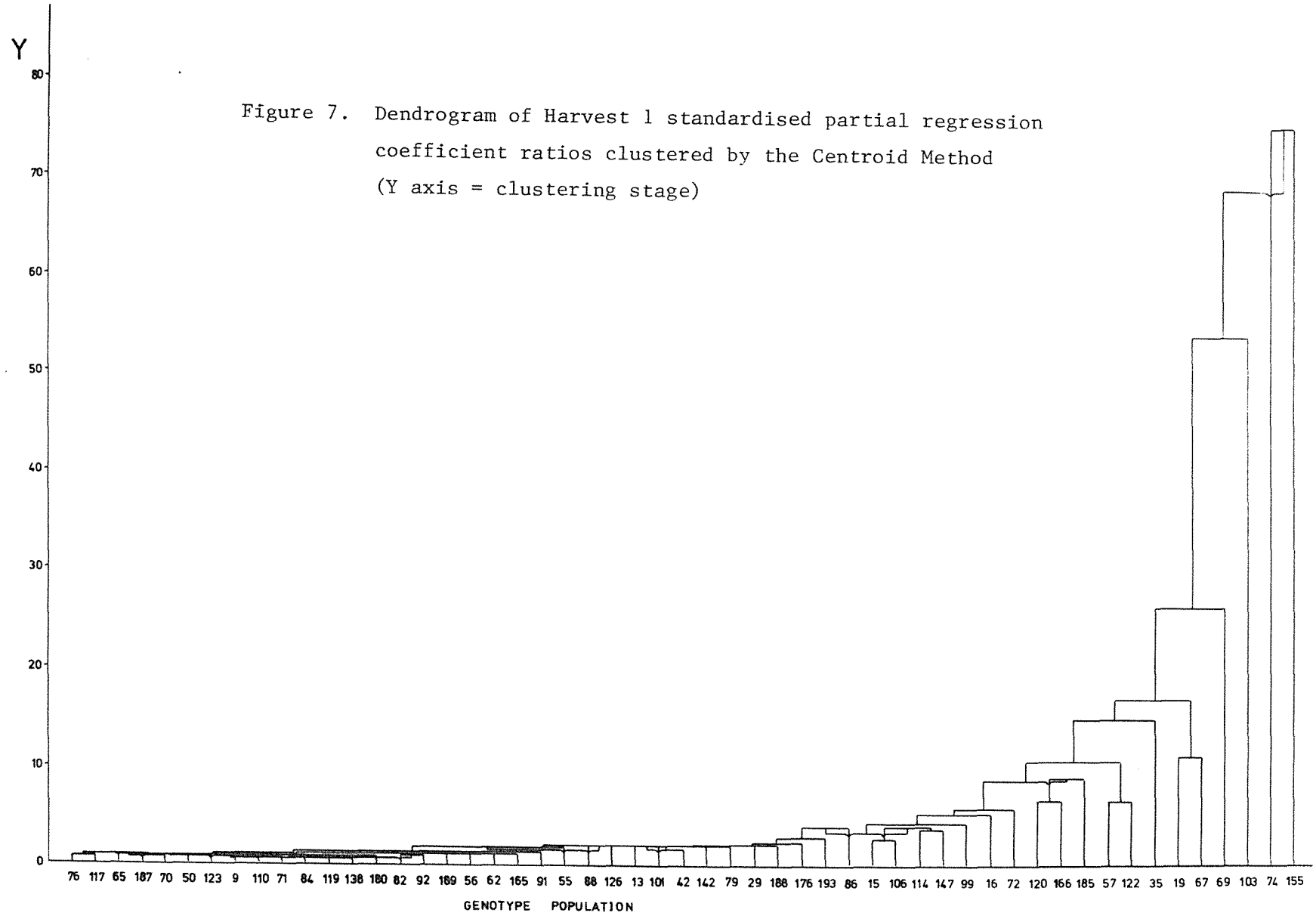


Figure 8. Dendrogram of Harvest 1 standardised partial regression coefficient ratios clustered by the Median Method.
(Y axis = clustering stage)

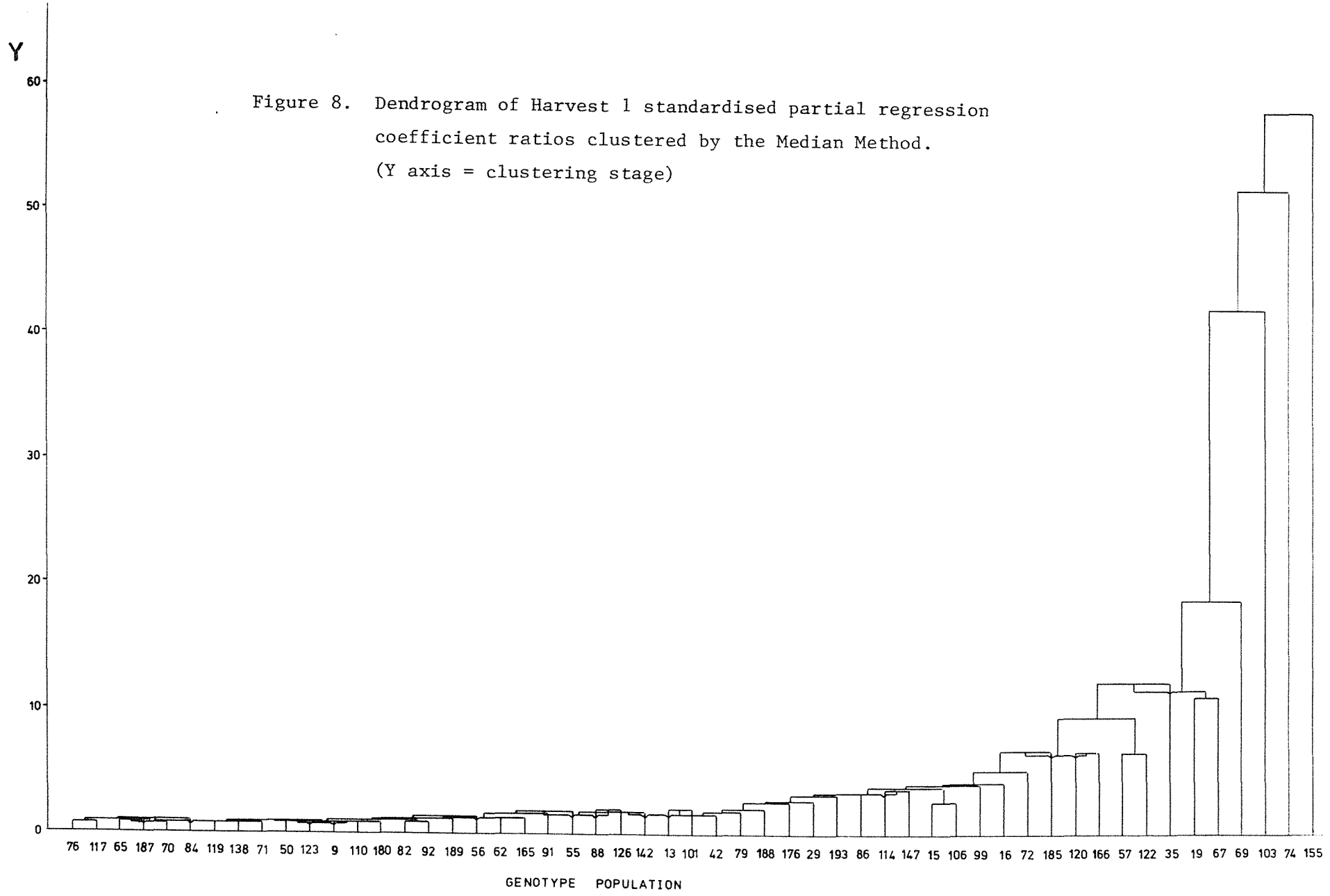


Figure 9. Dendrogram of Harvest 1 standardised partial regression coefficient ratios clustered by the Group Average Method. (Y axis = clustering stage)

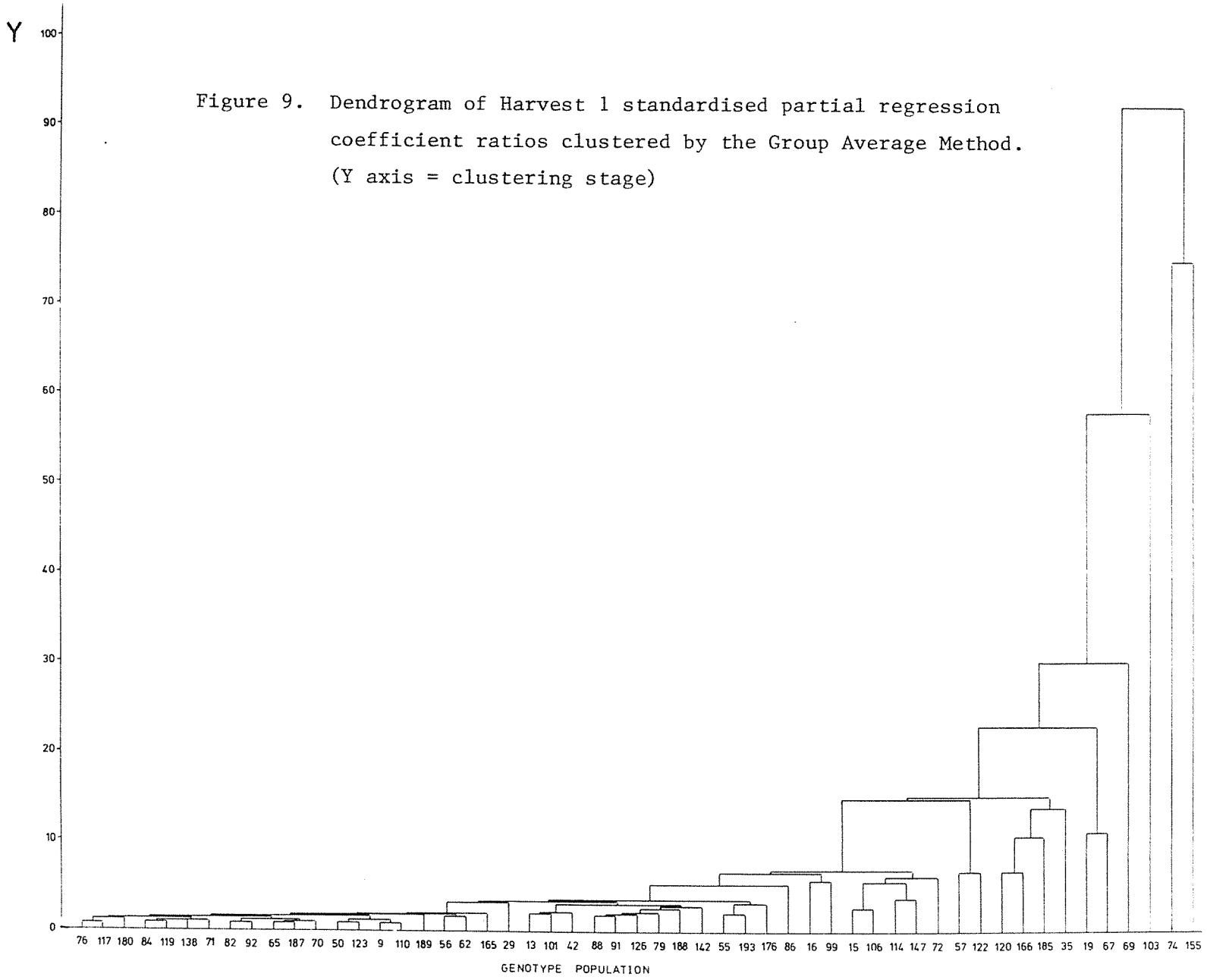


Figure 10. Dendrogram of Harvest 1 standardised partial regression coefficient ratios clustered by Ward's Method.
(Y axis = clustering stage)

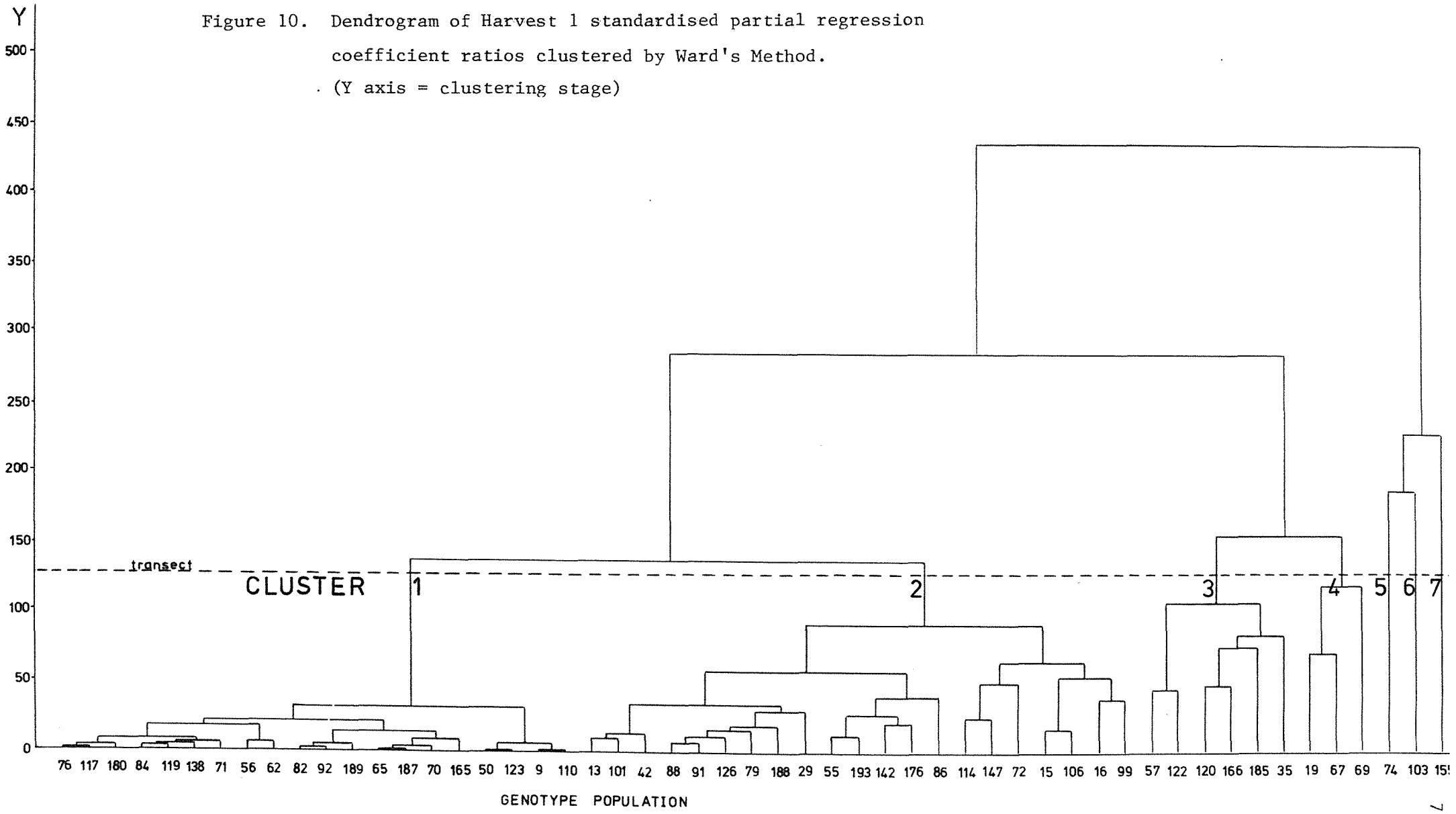


Figure 11. Dendrogram of Harvest 2 standardised partial regression coefficient ratios clustered by Ward's Method. (Y axis = clustering stage).

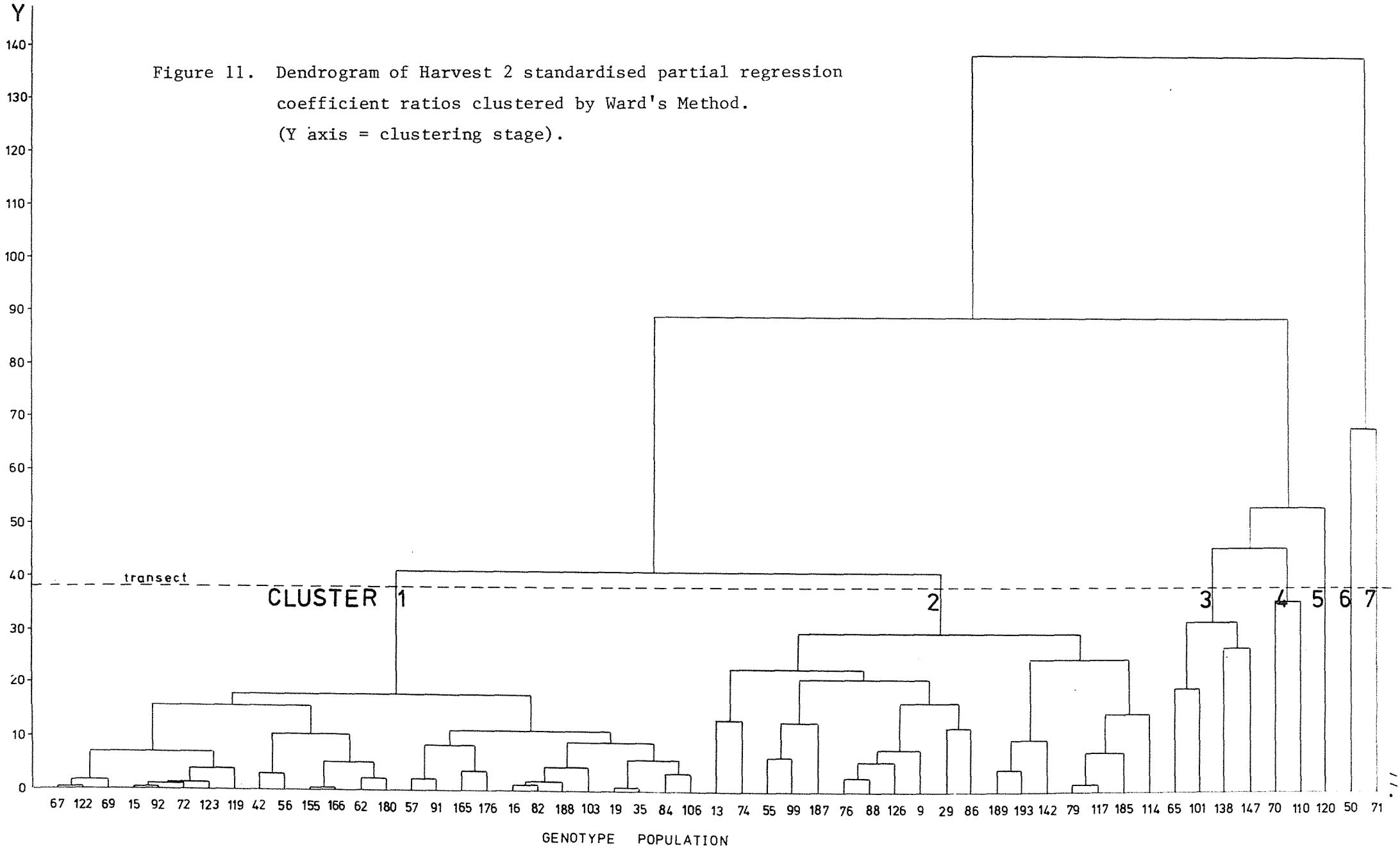


Figure 12. Dendrogram of Harvest 3 standardised partial regression coefficient ratios clustered by Ward's Method. (Y axis = clustering stage)

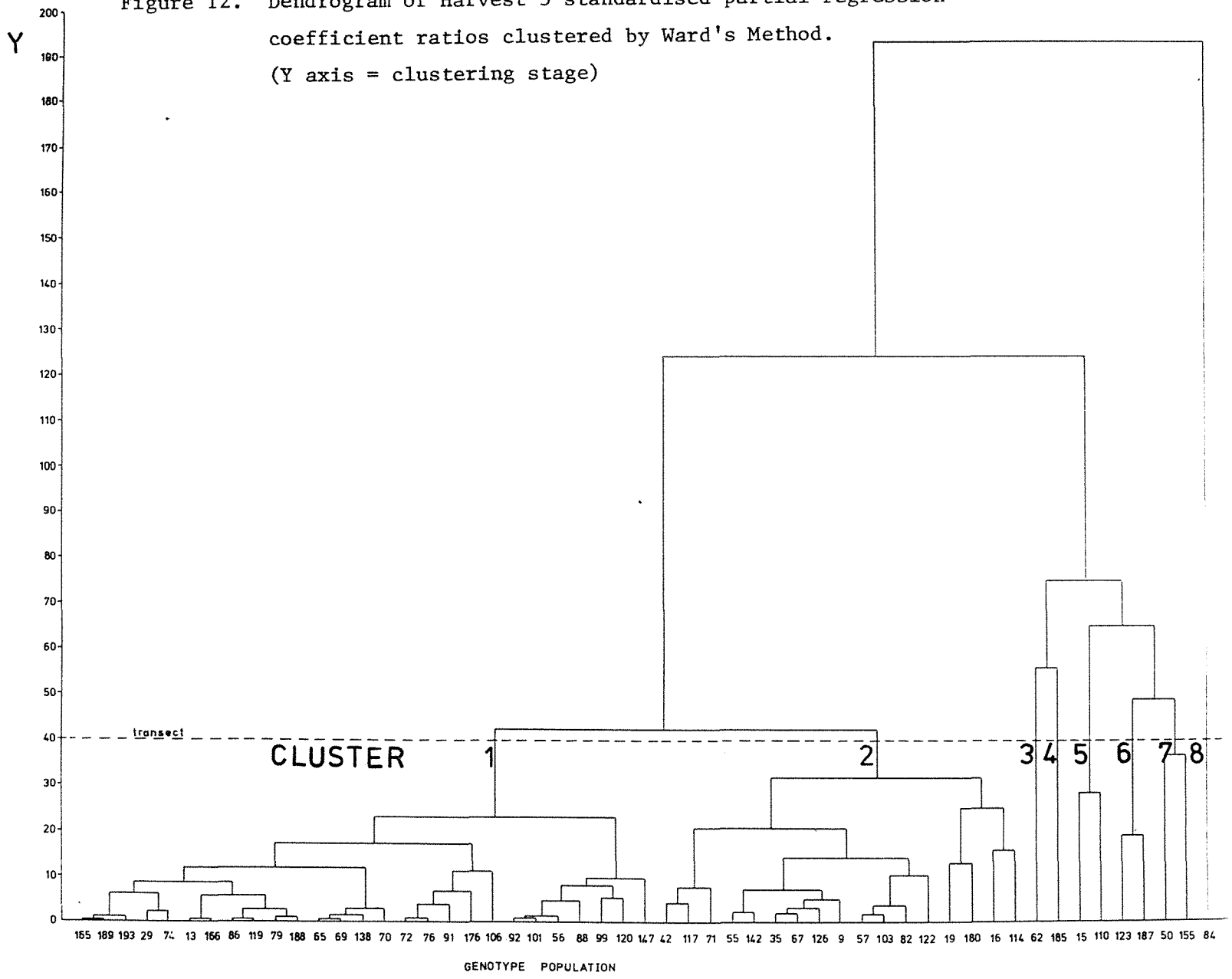


TABLE 20: Post-cluster analyses on Ward's method for harvest one.

| Cluster | No. of genotype populations/ cluster | Character | Mean $\bar{z}$ | Character mean |
|---|---|---|---|---|
| 1 | 20 | Clump green material | 1.0 | 2.94 b |
| | | Flower + seed heads | -0.743 | 3.26 |
| | | Clump rust | -0.613 | 2.29 |
| | | Clump height | +0.268 | 5.88 |
| | | Clump diameter | -0.247 | 14.13 a |
| | | Leaf tensile strength | +0.232 | 43.89 |
| | | Leaf width | +0.195 | 2.07 |
| | | Leaf pubescence | -0.179 | 10.34 |
| | | Clump erectness | -0.091 | 2.70 |
| 2 | 21 | Flower + seed heads | -2.609 | 3.72 |
| | | Clump rust | -1.012 | 2.39 |
| | | Clump green material | +1.0 | 3.01 b |
| | | Clump height | +0.753 | 6.24 |
| | | Clump diameter | -0.611 | 14.31 a |
| | | Leaf width | +0.563 | 2.19 |
| | | Leaf pubescence | +0.422 | 10.69 |
| | | Leaf tensile strength | +0.389 | 46.34 |
| | | Clump erectness | -0.220 | 2.80 |
| 3 | 6 | Flower + seed heads | -5.735 | 3.72 |
| | | Leaf width | +3.107 | 2.07 |
| | | Clump rust | -1.875 | 2.02 |
| | | Clump green material | 1.0 | 3.01 b |
| | | Clump erectness | -0.853 | 2.87 |
| | | Clump diameter | 0.803 | 14.00 a |
| | | Leaf pubescence | 0.488 | 11.00 |
| | | Leaf tensile strength | -0.331 | 44.67 |
| | | Clump height | -0.266 | 6.13 |
| 4 | 3 | Leaf tensile strength | -11.01 | 46.07 |
| | | Leaf width | +9.413 | 2.23 |
| | | Clump height | +9.283 | 6.83 |
| | | Clump erectness | -9.200 | 2.92 |
| | | Clump rust | -3.227 | 2.15 |
| | | Flower + seed heads | -2.571 | 3.78 |
| | | Clump green material | 1.0 | 3.10 a |
| | | Clump diameter | -0.873 | 14.83 a |
| | | Leaf pubescence | -0.116 | 10.56 |

| | | | | |
|---|---|---|---|---|
| 5 | 1 | Leaf pubescence | -62.11 | 10.00 |
| | | Clump diameter | -29.36 | 13.67 b |
| | | Leaf tensile strength | -29.34 | 46.49 |
| | | Clump rust | -29.28 | 2.54 |
| | | Clump height | 19.83 | 6.46 |
| | | Clump erectness | 19.44 | 2.92 |
| | | Flower + seed heads | -13.38 | 3.33 |
| | | Leaf width | 5.004 | 2.00 |
| | | Clump green material | 1.0 | 2.83 b |
| 6 | 1 | Clump height | 45.984 | 5.92 |
| | | Flower + seed heads | -27.85 | 3.13 |
| | | Clump diameter | -19.807 | 14.54 a |
| | | Leaf pubescence | 19.079 | 10.79 |
| | | Clump erectness | -10.07 | 2.92 |
| | | Clump rust | -9.041 | 2.17 |
| | | Leaf width | 4.343 | 1.92 |
| | | Leaf tensile strength | 1.741 | 42.52 |
| | | Clump green material | 1.0 | 3.21 a |
| 7 | 1 | Flower + seed heads | -76.369 | 3.54 |
| | | Clump erectness | 45.845 | 3.04 |
| | | Leaf pubescence | 44.926 | 10.46 |
| | | Clump diameter | -26.298 | 15.42 a |
| | | Clump height | 23.768 | 6.67 |
| | | Clump rust | -15.586 | 2.04 |
| | | Leaf width | 11.369 | 2.13 |
| | | Leaf tensile strength | -10.487 | 42.14 |
| | | Clump green material | 1.0 | 3.50 a |

SIGNIFICANCE SYMBOLS: Significance groups of means tested at P = 0.05 using Duncan's new multiple range test are indicated by lower case letters.

TABLE 21: Post-cluster analyses on Ward's method for harvest two

| Cluster | No. of Genotype populations/ cluster | Character | Mean $\bar{z}$ | Character Mean |
|---|---|---|---|---|
| 1 | 26 | Clump green material | + 1.0 | 3.99 |
| | | Clump rust | - 0.258 | 1.80 |
| | | Clump diameter | - 0.234 | 12.79 |
| | | Leaf flavanol level | - 0.210 | 2.45 |
| | | Leaf width | + 0.087 | 1.83 a |
| | | Clump height | - 0.083 | 4.07 |
| | | Clump erectness | - 0.081 | 2.71 |
| 2 | 18 | Clump green material | + 1.0 | 4.03 |
| | | Clump rust | - 0.947 | 2.14 |
| | | Leaf width | + 0.302 | 1.84 a |
| | | Leaf flavanol level | - 0.283 | 2.38 |
| | | Clump diameter | + 0.198 | 12.86 |
| | | Clump erectness | - 0.136 | 2.60 |
| | | Clump height | + 0.118 | 4.07 |
| 3 | 4 | Clump diameter | + 2.726 | 12.79 |
| | | Clump rust | - 1.636 | 2.30 |
| | | Leaf flavanol level | - 1.391 | 2.11 |
| | | Clump height | + 1.358 | 4.26 |
| | | Clump erectness | - 1.319 | 2.77 |
| | | Clump green material | + 1.0 | 4.02 |
| | | Leaf width | + 0.324 | 1.91 a |
| 4 | 2 | Clump diameter | + 7.826 | 13.02 |
| | | Clump rust | - 5.116 | 2.08 |
| | | Clump erectness | - 3.996 | 2.46 |
| | | Clump height | + 1.947 | 3.83 |
| | | Leaf width | + 1.876 | 2.06 |
| | | Clump green material | + 1.0 | 3.92 |
| | | Leaf flavanol level | - 0.650 | 2.46 |
| 5 | 1 | Clump rust | -11.587 | 1.79 |
| | | Clump height | + 4.935 | 4.49 |
| | | Leaf flavanol level | - 2.813 | 2.46 |
| | | Clump diameter | + 1.088 | 13.45 |
| | | Clump green material | + 1.0 | 4.35 |
| | | Clump erectness | + 0.892 | 2.88 |
| | | Leaf width | + 0.045 | 2.17 a |

| | | | | |
|---|---|---|---|---|
| 6 | 1 | Clump rust | -17.686 | 2.11 |
| | | Clump erectness | -15.482 | 2.75 |
| | | Clump diameter | -11.165 | 13.54 |
| | | Clump height | + 9.225 | 4.39 |
| | | Leaf width | + 8.348 | 1.62 b |
| | | Leaf flavanol level | + 1.683 | 1.96 |
| | | Clump green material | + 1.0 | 4.18 |
| 7 | 1 | Clumpheight | +28.251 | 4.00 |
| | | Clump rust | -21.663 | 1.71 |
| | | Leaf flavanol level | +18.821 | 1.96 |
| | | Clump diameter | -14.509 | 12.58 |
| | | Leaf width | + 8.854 | 1.67 b |
| | | Clump erectness | - 1.004 | 2.58 |
| | | Clump green material | + 1.0 | 4.00 |

SIGNIFICANCE SYMBOLS: Significance groups of means tested at P = 0.05 using Duncan's new multiple range test are indicated by lower case letters.

TABLE 22: Post-cluster analyses on Ward's method for harvest three

| Cluster | No. of Genotype Populations/ cluster | Character | Mean $\bar{x}$ | Character mean |
|---|---|---|---|---|
| 1 | 27 | Clump green material | +1.0 | 3.97 |
| | | Leaf width | -0.204 | 1.63 |
| | | Soluble sugar level | -0.124 | 12.93 |
| | | Clump erectness | +0.097 | 2.42 |
| | | Clump diameter | -0.054 | 12.06 |
| | | Clump height | +0.034 | 2.76 a |
| 2 | 17 | Clump green material | +1.0 | 4.11 |
| | | Clump height | +0.713 | 2.81 a |
| | | Clump diameter | -0.682 | 12.38 |
| | | Leaf width | -0.316 | 1.64 |
| | | Soluble sugar level | -0.124 | 13.06 |
| | | Clump erectness | +0.094 | 2.41 |
| 3 | 1 | Soluble sugar level | -10.950 | 12.90 |
| | | Clump erectness | +7.269 | 2.58 |
| | | Clump diameter | -2.474 | 12.71 |
| | | Clump green material | +1.0 | 4.17 |
| | | Leaf width | +0.662 | 1.63 |
| | | Clump height | +0.362 | 3.11 a |
| 4 | 1 | Clump erectness | +17.568 | 2.39 |
| | | Soluble sugar level | -7.298 | 11.39 |
| | | Clump height | +6.790 | 2.63 b |
| | | Leaf width | +5.107 | 1.65 |
| | | Clump diameter | +4.246 | 11.79 |
| | | Clump green material | +1.0 | 4.21 |
| 5 | 2 | Clump erectness | +6.341 | 2.67 |
| | | Clump diameter | -5.780 | 12.49 |
| | | Leaf width | -1.603 | 1.78 |
| | | Soluble sugar level | +1.118 | 12.95 |
| | | Clump green material | +1.0 | 4.20 |
| | | Clump height | +0.038 | 3.09 a |
| 6 | 2 | Clump erectness | +4.824 | 2.35 |
| | | Clump height | +3.221 | 2.58 c |
| | | Leaf width | -1.754 | 1.50 |
| | | Clump green material | +1.0 | 4.08 |
| | | Soluble sugar level | -0.368 | 13.55 |
| | | Clump diameter | -0.135 | 11.49 |

| | | | | |
|---|---|---|---|---|
| 7 | 2 | Clump diameter | -11.18 | 12.54 |
| | | Clump erectness | + 9.584 | 2.69 |
| | | Leaf width | - 3.945 | 1.58 |
| | | Clump height | + 2.284 | 3.29 a |
| | | Clump green material | + 1.0 | 4.44 |
| | | Soluble sugar level | - 0.268 | 14.09 |
| 8 | 1 | Clump erectness | +52.96 | 2.73 |
| | | Clump diameter | -41.04 | 12.65 |
| | | Soluble sugar level | +23.48 | 14.23 |
| | | Leaf width | -22.88 | 1.81 |
| | | Clump height | +10.87 | 3.34 a |
| | | Clump green material | + 1.0 | 3.90 |

SIGNIFICANCE SYMBOLS: Significance groups of means tested at P = 0.05 using Duncan's new multiple range test are indicated by lower case letters.

# CHAPTER 5. GENERAL DISCUSSION

## 5.1 THE SHEEP PREFERENCE ASSESSMENT

The method of assessing sheep preference was based on residual forage remaining after controlled grazing. The acceptability of *Phalaris arundinacea* spaced plant material to sheep has been assessed successfully, using similar defoliation scores and an almost identical grazing procedure to that used in this study (Barnes *et al.*, 1970). Such defoliation scores could be criticised on the grounds that they do not take into account directly the amount of herbage initially on offer. However, such a consideration is probably more important for inter-species comparisons where differences in growth rate may be vast (Mills, 1977). In any case, the hard grazing management imposed on the collection at the start of each regrowth cycle ensured that the clump sizes were not vastly different at the time of assessment of sheep preference.

There was little genotypic variation for sheep preference. Most of the variance in sheep preference was due to environmental effects (see Table 14). Environmental variance was inflated by the variation due to sheep. This variation due to sheep may have arisen from differences in individual sheep preferences (Arnold and Hill, 1971) and a lack of temporal consistency of sampling intensity for individual sheep (refer to Chapter 2).

The possibility that sheep may have acquired a 'taste' for Yorkshire fog (Watkin and Robinson, 1974) as the grazing trials progressed across harvests was indirectly examinable from the results of this study. If the sheep did acquire a 'taste' for Yorkshire fog then a reduction in sheep preference variance might be expected. However, this did not occur between the first and second harvests. Sheep preference variance ($\sigma^2_{y.x}$) increased between the first and second harvests (Tables 17, 18) suggesting the possibility that monthly grazing may have minimised the chance of sheep becoming accustomed to the 'taste' of Yorkshire fog.

## 5.2 PLANT CHARACTERS EXAMINED

### 5.2.1 Presence of flower and seed heads

Of the characters assessed in this study, the sheep most strongly rejected clumpscontaining a high proportion of inflorescences. This result confirms the suggestion of earlier workers (see section 1.4.1) that the presence of numerous flower and seed heads reduces the acceptability of Yorkshire fog. The presence of lignified culms, perhaps causing an unpleasant touch sensation, may have been a factor influencing sheep rejection of clumps containing numerous inflorescences. It is unlikely that the pubescence of the culms or spikelets determined rejection by sheep, since leaf pubescence was relatively unimportant in determining sheep preference in this study (Table 17).

Differences between genotype populations were highly significant ($P < 0.005$), although the greatest source of variation arose from within the plots, i.e. amongst the 24 plants making up each genotype population (Table 10). Potential genotypic variation existed within each population, however, much of this within-plot variation likely arose from micro-environmental differences and from previous grazing management. Heteromorphological clumps did not allow a precise defoliation intensity at the start of the regrowth cycle and may have led to differences in the number of floral initials being removed at this time. Close defoliation of grasses following floral initiation can have a major influence on the number of flowering to vegetative tillers produced (Davies *et al.*, 1971). The hard grazing treatment applied one month before assessment likely removed a large number of potential inflorescences since, overall, only 20% (approx.) of tillers of each clump had inflorescences at the time of assessment (Table 10).

### 5.2.2 Clump green material

Following the removal of all culms and inflorescences, the presence of clump green material was the most important character determining sheep preference (Tables 18, 19). Sheep selected for green rather than dead leaf and sheath material.

Little genotypic variation for the presence of green material was found (Table 14). However, under the hard grazing management applied, excessive accumulation of dead basal tissue did not occur and therefore full expression of this character was not realised.

Condensed tannins are reputed to inhibit decay through binding with fungal enzymes (Basaraba and Starkey, 1966; Okasha *et al.*, 1968). Perhaps endogenous condensed tannins of Yorkshire fog might inhibit leaf decay, thereby allowing accumulation of basal dead tissue? In this study, the grazing procedure carried out allowed little opportunity for dead material to accumulate. Hence an assessment of leaf decay inhibition by flavanols or condensed tannins was, in this study, not possible.

### 5.2.3 Crown rust infection

Results obtained in this study support the observation that severe infection by crown rust may lead to rejection by sheep (Jacques 1974). Whether rejection is due to an unpleasant taste, touch, smell or appearance, of crown rust pustules to the sheep is not known. However, the bright orange colour of the pustules is probably unlikely to effect rejection by sheep, since sheep are reputedly unable to discern between green and orange colours (Tribe and Gordon, 1949).

Crown rust infection occurred over the summer and autumn period. By late-autumn, following two hard grazing treatments spaced two weeks apart, all visual evidence of crown rust infection had disappeared. Besides grazing management, air temperature and humidity, and the distribution of spores probably contributed to the large environmental variance recorded for crown rust infection.

The possible presence of other orange-coloured leaf fungi found on *Holcus lanatus* in New Zealand needs mention. *Ramulaspera holci-lanati*, the most common leaf spot fungus on *H. lanatus*, is found on this grass throughout the year but does not appear to have any pronounced seasonal peak of infection (Latch,1964).

### 5.2.4 Clump erectness

Jacques (1974) suggested that an extreme prostrate habit of growth might influence low acceptability of Yorkshire fog. However in the current study sheep showed little preference for or against plants with a prostrate habit of growth. In relation to the other characters examined clump erectness was unimportant in determining sheep preference.

### 5.2.5 Leaf pubescence

Leaf pubescence was relatively unimportant in determining sheep preference in this study, negating previously held views to the contrary (see section 1.4.3). This result was not totally unexpected, since leaf hairs are found on several other common herbage species. For example, sheep do not find red clover *(Trifolium pratense)* unacceptable yet this plant has particularly long hairs up to 1500μ on both leaf surfaces.

Perhaps removal of leaf hairs could prove disadvantageous. The presence of hairs and an ability to roll its leaf under low atmospheric moisture conditions (Arber, 1965) may provide Yorkshire fog with a sensitive method of conserving moisture through exposing less surface area to sunlight and air movement, thereby retaining moisture droplets physically on the leaf surface with leaf hairs.

Considerable variation in hair density and length was observed although almost glabrous types were rare. The frequency distribution for the total pubescence score was skewed slightly towards greater pubescence (Appendix IV).

### 5.2.6 Leaf tensile strength

Leaf tensile strength was the most unimportant character, of those assessed, in determining sheep preference in the summer harvest (Table 17). The leaf tensile strength of Yorkshire fog is unlikely to cause harvesting difficulties to the animal since this grass has a relatively lower leaf tensile strength than other common temperate grasses (Evans, 1967b; Jacques, 1974; Clements and Easton, 1974). In an inter-species comparison the mean leaf tensile strengths in

gm LOAD. $mgm^{-1}$D.Wt. 5 cm. leaf, over 15 sampling dates, were: *Lolium perenne*, 112.8; *Dactylis glomerata*, 105.4; *Phleum pratense*, 84.0; and *Holcus lanatus*, 50.8 (Evans, 1967b).

The range of values which occurred across the genotype populations in this study was 25-63, with an overall mean of 45.0, gm LOAD. $mgm^{-1}$ D.Wt. 5 cm leaf and followed a nearly normal distribution (Appendix IV). This range of values fell within the range of values recorded for Yorkshire fog by other workers (Evans, 1967b; Clements and Easton, 1974).

A shortcoming of the sampling procedure was that only 'youngest-mature' leaves were tested for strength and these may not necessarily be representative of the whole clump on which the selection choice by the sheep was based. Nevertheless this sampling procedure did allow valid comparisons amongst the clumps.

### 5.2.7 Leaf width

Leaf width was relatively unimportant in determining sheep preference in this study (see Tables 17, 18, 19).

Differences between genotype populations for leaf width were highly significant in the summer harvest, but were not significant for the subsequent harvest periods. The overall average leaf width, assessed for each clump, decreased with successive harvests (see Tables 10, 11, 12 and 14). This highly significant shift is probably an annual occurrence.

### 5.2.8 Clump height and diameter

Under the grazing management applied, clump height and diameter were relatively unimportant in determining the acceptability of *Holcus* spp. to sheep in this study. (Tables 17, 18, 19).

Work carried out on *Dactylis glomerata* and *Lolium perenne* in the sward condition has demonstrated that sheep tend to graze the largest tillers at any one time, and that younger leaves on any tiller are more likely to be removed by grazing than older leaves (Hodgson,

1966; McIvor and Watkin, 1973). However, in using swards it is difficult to relate linear leaf measurements to the sward height, and it may be that larger tillers and younger leaves are simply more 'accessible' in the sward condition to the grazing animal. The use of spaced plants, in the current study, largely reduced this problem of distinguishing between 'accessibility' and 'acceptability' whilst still permitting assessment under field conditions.

Grand means for clump height and diameter, in each harvest, considered together indicate that less total plant material was available with successive harvests for each trial grazing (Tables 10, 11, 12, 14).

### 5.2.9 Leaf flavanols

In this study mainly the monomeric flavanols, i.e. flavan-3-ols and flavan-3,4-diols, were assessed. Leaf flavanol level was unimportant in determining sheep preference in the autumn harvest (see Table 18). However, most of the variation in leaf flavanol levels arose from a high error variance (Table 11). This probably reflects the lack of knowledge about flavanols in *Holcus* spp. Further research is required to investigate the effects of season, temperature, light intensity, and plant maturity on flavanol and condensed tannin levels in Yorkshire fog so that variance due to sampling is minimised. Since this study was carried out, a re-assessment of the acidified vanillin method has been made, and a modified test procedure developed (Broadhurst and Jones, 1978). The new test procedure of Broadhurst and Jones (1978) should overcome some of the lack of sensitivity and reproducibility, apparent in previous versions of this test (Burns, 1963; Jones *et al.*, 1973).

Future research should consider the astringency of *Holcus* spp. oligoflavalans to sheep. It has been noted that the flavanols (monomers) themselves, although readily soluble, are not as markedly astringent as the extractable polymers (oligoflavalans) (Swain, 1962). The haemanalysis technique (Bate-Smith, 1973) provides a measure of astringency by precipitating out the flavanols/oligoflavalans which are able to bind with human blood protein. Perhaps an alternative test could be devised using cattle or sheep mouth glyco-protein, instead of human blood protein, since it is possible that changes in

protein structure and size could change astringency rating. Both the protein component and the flavanol/oligoflavalan components removed from solution should be assessed. Future research should investigate the molecular weight range and structure of *Holcus* spp. condensed tannins.

### 5.2.10 Soluble sugars

Since diurnal fluctuations in soluble sugar levels were likely to be important (Haslemore, *pers. comm.* ), sampling was carried out during the afternoon aimed at obtaining the peak diurnal level. The soluble sugar levels recorded for leaf tissue of genotype populations in this study were high in relation to the level reported for the leaf tissue of *Lolium perenne* of 8.5% $gm^{-1}$D.Wt (Haslemore and Roughan, 1976). In this study, genotype population mean soluble sugar levels ranged from 10.75 - 15.64%, with an overall mean of 13.03% $gm^{-1}$D.Wt. leaf (see Table 12). Differences between the genotype populations in soluble sugar level were not significant. However, differences in soluble sugar level between the blocks were highly significant (Table 12). Block differences may have arisen since each block was sampled on different days.

Results of thin-layer chromatography determinations on Yorkshire fog extracts indicated that the soluble sugars contained mainly sucrose (Haslemore, *pers. comm*). Soluble sugars were unimportant in determining sheep preference in late-autumn in this study (see Table 19). This result was not unexpected in view of the results obtained using sucrose solutions in two-choice preference tests (Goatcher and Church, 1970a). Of all the chemicals tested sucrose was the least discriminated by sheep, and of four ruminant species tested (cattle, pygmy goats, normal goats and sheep), sheep were the least sensitive to sucrose over a wide range of test concentrations (Goatcher and Church, 1970a).

## 5.3 PLANT BREEDING PROSPECTS

Jacques (1974) suggested that improvement of the relative acceptability of Yorkshire fog by selection and breeding within the

species might be possible. However, the rate of improvement by selection will be dependent upon the intensity of selection applied and the level of predictive heritability of the characters important in determining acceptability. In general terms, the expected genetic advance, $\Delta G$, may be expressed as:-

$$\Delta G = i.\sigma_p h^2$$

where i is the standardised selection differential, $\sigma_p$ is the phenotypic standard deviation, and $h^2$ is a predictive form of heritability estimate for a particular selection system (Falconer, 1975). Expected genetic advance formulae for different selection procedures have been presented by Shelbourne (1969) and Falconer (1975). For characters with low *predictive* heritability (i.e. includes additive and additive x additive gene action in the genetic variance component) the genetic advance under selection will be slow. With the presence of non-additive gene action (i.e. dominance and various types of epistasis) progress under selection is likely to be further restricted. Estimates of heritability of the broad-sense form contain both additive and non-additive gene effects in the genetic variance component.

Since Yorkshire fog is a cross-pollinating species, shown to be highly self-incompatible (Beddows, 1961b) many of the characters *may* be due not only to additive gene action, but also to dominance and perhaps epistatic gene action as well. Hence the heritability estimates obtained in this study are perhaps best described as "descriptive" rather than "predictive" as they are likely to be of the broadsense form. Genetic partitioning experiments (Griffing, 1956; Hayman, 1958) could be set up to investigate the nature of gene action of these characters.

The relative efficiencies of four methods of selection have been compared using appropriate equations of expected genetic advance for each (Falconer, 1975). For characters with low heritabilities, such as for the characters in the present study (Tables 15, 16), line (syn. family) selection of half- or full-sib lines is likely to be of greatest practical use whilst providing a reasonable level of expected genetic advance (Falconer, 1975). The efficacy of line selection rests on the fact that the environmental deviations of the

individuals tend to cancel each other out in the mean value of the line (Falconer, 1975).

In the application of line selection during the breeding programme it would be desirable to simultaneously select for all of the characters considered important in determining sheep preference. Of three simultaneous selection procedures examined by Hazel and Lush (1942) the total score method (selection index) was the most efficient. A selection index could be constructed for use in selecting more acceptable lines of Yorkshire fog to sheep:-

$I = b_1X_1 + b_2X_2 + b_3X_3$, where

$X_1$, $X_2$, $X_3$ represent the phenotypic values of the characters important in determining sheep preference and $b_1$, $b_2$, $b_3$ are optimum weights assigned to the characters in selection. These optimum weights could be computed (in matrix form) as:-

$$\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}\boldsymbol{z}$$

where $\mathbf{b}$ is a vector of partial regression coefficients of the X's in the index $I = \mathbf{bX}$, $\mathbf{P}^{-1}$ is the inverse matrix of phenotypic variance-covariance values of the characters considered, $\mathbf{G}$ is the matrix of genotypic variance-covariance values of the characters considered, and $\boldsymbol{z}$ is the vector of relative importance values ( $\boldsymbol{z} = b_j'/b_c'$, refer to section 4.2) determining sheep preference for the characters considered (Hazel and Lush, 1942; Robinson *et al.*, 1951). The use of such procedures *may* lead to some genetic improvement in selection against crown rust infection and flower and seed head presence. However it is unlikely that selection for more acceptable genotypes to sheep using either the sheep preference score or clump green material score would be worthwhile due to the particularly high level of environmental variance relative to genetic variance level associated with these characters.

## 5.4 AGRONOMIC ASPECTS

The presence of inflorescences, green material and crown rust infection are largely influenced by environmental factors. Environmental control of these characters may be achieved in part through animal treading and grazing effects. Heavy treading about the time of floral initiation may considerably reduce the number of flower heads produced by Yorkshire fog (Edmond, 1964). Following floral initiation, the close defoliation of grasses causing removal of reproductive meristems may lead to a marked reduction in the number of flowering

to vegetative tillers produced (Davies *et al.*, 1971).

Observations in the present study suggested that hard, monthly grazing over the summer and autumn prevented excessive dead material accumulation. Ungrazed or laxly grazed Yorkshire fog plants may produce tillers from nodes above the soil level ("aerial-tillers") thereby resulting in a "mop-habit" of growth (Arber, 1965). The production of roots from such elevated nodes probably leaves the plant at a disadvantage under summer drying upper-soil conditions, and this may provide one reason for the production of large amounts of dead leaf and sheath tissue. Allowing excessive dead material to accumulate has the added disadvantage that it provides a substrate for *Pithomyces chartarum* which causes facial eczema in sheep (Sinclair, 1961). This fungus produces the toxin sporidesmin, which can affect the germination of Yorkshire fog seed (Wright, 1969).

It has been observed that hard, frequent grazing during the summer and autumn may lead to a reduction in the incidence of crown rust infection in ryegrass pastures (Lancashire and Latch, 1970). It is possible that hard, frequent grazing of Yorkshire fog during the summer and autumn may, similarly, result in a reduction in crown rust infection.

After following the control of flower and seed heads, dead leaf and sheath material and crown rust infection, through proper grazing management as suggested by Watkin and Robinson (1974), there may be little real evidence of an acceptability problem of this grass to sheep!

## CONCLUSIONS

1. The results revealed a lack of temporal consistency of sampling intensity for each sheep. Several sheep in the group exhibited abnormally fickle grazing behaviour.

2. Among the characters studied, the presence of inflorescences, clump green leaf and sheath material and crown rust infection appeared to be the most important plant characters determining the acceptability of Yorkshire fog over the summer to early-winter. Sheep rejected clumps containing a high proportion of inflorescences, dead leaf and sheath material and crown rust infection.

3. Leaf pubescence appeared to be unimportant in determining sheep preference in this study, negating previously held views to the contrary.

4. The importance of flavanols/oligoflavalans in determining the acceptability of Yorkshire fog needs re-assessment. Alternative methods of investigation have been suggested.

5. A large proportion of the variation in sheep preference was not explained by the characters assessed. Some of this unexplained variation may have been due to unassessed plant characters such as trans-aconitic acid or other characters, whose importance in determining sheep preference is unrecognised, or due simply to sheep fickleness of grazing.

6. Leaf tensile strength, leaf width, clump erectness, clump height and diameter, and soluble sugar level all appeared to be unimportant in determining the acceptability of Yorkshire fog to sheep in this study.

7. There was little genetic variation compared to environmental variation in the characters examined. Assuming that sampling of the gene-pool collection was representative of the genetic variation present, this suggests less genetic diversity in Yorkshire fog in New Zealand than was previously thought.

8. The clustering behaviour of four agglomerative clustering strategies was examined. The Centroid and Median methods produced reversals. The Centroid, Median, and Group Average methods had obvious chaining defects. Ward's method did not produce reversals or result in chaining.

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APPENDIX I Pilot study - simple linear regressions of sampling intensity for each sheep

| Sheep | Regression | $r_{yx}$ | s.e.($b_1$) | s.e.($b_0$) | $r^2$ | F | $\hat{\sigma}^2_{y.x}$ |
|---|---|---|---|---|---|---|---|
| 1 | $\hat{Y} = -0.33X + 47.8$ | -0.76 | 0.069 | 3.736 | 0.584 | ** | 57.707 |
| 2 | $\hat{Y} = -0.21X + 38.3$ | -0.62 | 0.066 | 3.613 | 0.384 | ** | 53.982 |
| 3 | $\hat{Y} = -0.06X + 20.5$ | -0.18 | 0.079 | 4.296 | 0.032 | NS | 76.327 |
| 4 | $\hat{Y} = -0.23X + 37.0$ | -0.70 | 0.058 | 3.112 | 0.492 | ** | 40.041 |
| 5 | $\hat{Y} = -0.22X + 27.5$ | -0.67 | 0.061 | 3.308 | 0.446 | ** | 45.242 |
| 6 | $\hat{Y} = -0.04X + 19.7$ | -0.23 | 0.044 | 2.360 | 0.053 | NS | 23.029 |
| 7 | $\hat{Y} = -0.06X + 28.5$ | -0.23 | 0.071 | 3.859 | 0.052 | NS | 61.569 |
| 8 | $\hat{Y} = -0.10X + 23.8$ | -0.30 | 0.080 | 4.347 | 0.091 | NS | 78.130 |
| 9 | $\hat{Y} = -0.23X + 32.2$ | -0.68 | 0.060 | 3.349 | 0.473 | ** | 46.368 |
| 10 | $\hat{Y} = -0.11X + 29.8$ | -0.39 | 0.070 | 3.701 | 0.150 | NS | 56.637 |
| 11 | $\hat{Y} = -0.19X + 26.7$ | -0.66 | 0.053 | 2.914 | 0.429 | ** | 35.118 |
| 12 | $\hat{Y} = -0.13X + 23.9$ | -0.58 | 0.045 | 2.480 | 0.336 | * | 25.438 |
| 13 | $\hat{Y} = -0.22X + 34.3$ | -0.68 | 0.006 | 3.214 | 0.462 | ** | 42.714 |
| 14 | $\hat{Y} = -0.23X + 32.5$ | -0.83 | 0.038 | 2.063 | 0.687 | ** | 17.597 |
| 15 | $\hat{Y} = -0.07X + 23.8$ | -0.42 | 0.037 | 2.005 | 0.180 | NS | 16.619 |
| 16 | $\hat{Y} = -0.23X + 29.9$ | -0.91 | 0.026 | 1.416 | 0.829 | ** | 8.296 |
| 17 | $\hat{Y} = -0.18X + 30.9$ | -0.84 | 0.029 | 1.578 | 0.698 | ** | 10.300 |
| 18 | $\hat{Y} = -0.67X + 77.2$ | -0.16 | 1.050 | 57.190 | 0.024 | NS | 13524.8 |
| 19 | $\hat{Y} = -0.01X + 24.1$ | -0.05 | 0.064 | 3.477 | 0.003 | NS | 49.988 |
| 20 | $\hat{Y} = -0.14X + 20.2$ | -0.80 | 0.025 | 1.400 | 0.639 | ** | 8.109 |
| 21 | $\hat{Y} = -0.15X + 28.5$ | -0.64 | 0.044 | 2.386 | 0.405 | ** | 23.551 |
| 22 | $\hat{Y} = 0.01X + 19.1$ | 0.06 | 0.050 | 2.718 | 0.004 | NS | 30.554 |
| 23 | $\hat{Y} = -1.30X + 107.3$ | -0.29 | 1.070 | 55.335 | 0.084 | NS | 12661.6 |
| 24 | $\hat{Y} = -0.12X + 26.4$ | -0.48 | 0.054 | 2.930 | 0.232 | * | 35.488 |
| 25 | $\hat{Y} = -0.07X + 46.6$ | -0.04 | 0.433 | 22.913 | 0.002 | NS | 2170.992 |
| 26 | $\hat{Y} = -0.14X + 33.7$ | -0.67 | 0.039 | 2.141 | 0.445 | ** | 18.948 |
| 27 | $\hat{Y} = 0.15X + 16.4$ | 0.15 | 0.234 | 11.848 | 0.024 | NS | 580.467 |
| 28 | $\hat{Y} = -0.08X + 38.9$ | -0.24 | 0.076 | 4.159 | 0.058 | NS | 71.537 |
| 29 | $\hat{Y} = -0.14X + 29.6$ | -0.68 | 8.038 | 2.072 | 0.464 | ** | 17.758 |
| 30 | $\hat{Y} = -0.14X + 35.0$ | -0.47 | 0.068 | 3.692 | 0.217 | * | 56.364 |

Sheep 1-10, Block III; 10-20, Block II; 20-30, Block I

SIGNIFICANCE SYMBOLS: NS, not significant = $P > 0.05$

* = $0.05 \geq P > 0.01$

** = $0.01 \geq P$

APPENDIX II :
ESTIMATED T STATISTICS FOR DIFFERENCES AMONGST PAIRS OF B0'S AND B1'S FROM THE REGRESSION EQUATIONS FOR EACH SHEEP

| B1 DIFFS. / B0 DIFFS. | SHEEP NUMBER 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 1.26NS | 2.57* | 1.11NS | 1.19NS | 3.54** | 2.73* | 2.18* | 1.09NS | 2.24* |
| 2 | 1.83NS | | 1.46NS | 0.23NS | 0.11NS | 2.14* | 1.55NS | 1.06NS | 0.22NS | 1.04NS |
| 3 | 4.80** | 3.17** | | 1.73NS | 1.60NS | 0.22NS | 0.00NS | 0.36NS | 1.71NS | 0.47NS |
| 4 | 2.22* | 0.27NS | 3.11** | | 0.12NS | 2.61* | 1.85NS | 1.32NS | 0.00NS | 1.32NS |
| 5 | 4.07** | 2.20* | 1.29NS | 2.09NS | | 2.39* | 1.71NS | 1.19NS | 0.12NS | 1.18NS |
| 6 | 6.36** | 4.31** | 0.16NS | 4.43** | 1.92NS | | 0.24NS | 0.66NS | 2.55* | 0.85NS |
| 7 | 3.59** | 1.85NS | 1.39NS | 1.71NS | 0.20NS | 1.95NS | | 0.37NS | 1.83NS | 0.50NS |
| 8 | 4.19** | 2.57* | 0.54NS | 2.47* | 0.68NS | 0.83NS | 0.81NS | | 1.30NS | 0.09NS |
| 9 | 3.11** | 1.24NS | 2.15* | 1.05NS | 1.00NS | 3.05** | 0.72NS | 1.53NS | | 1.30NS |
| 10 | 3.42** | 1.64NS | 1.64NS | 1.49NS | 0.46NS | 2.30* | 0.24NS | 1.05NS | 0.48NS | |
| 11 | 4.45** | 2.50* | 1.19NS | 2.42* | 0.18NS | 1.87NS | 0.37NS | 0.55NS | 1.24NS | 0.66NS |
| 12 | 5.33** | 3.23** | 0.69NS | 3.29** | 0.87NS | 1.23NS | 1.00NS | 0.02NS | 1.99NS | 1.32NS |
| 13 | 2.74** | 0.83NS | 2.57* | 0.60NS | 1.47NS | 3.66** | 1.15NS | 1.94NS | 0.45NS | 0.92NS |
| 14 | 3.59** | 1.39NS | 2.52* | 1.21NS | 1.28NS | 4.08** | 0.91NS | 1.81NS | 0.08NS | 0.64NS |
| 15 | 5.66** | 3.51** | 0.70NS | 3.57** | 0.96NS | 1.32NS | 1.16NS | 0.00NS | 2.15* | 1.43NS |
| 16 | 4.48** | 2.16* | 2.08NS | 2.08NS | 0.67NS | 3.71** | 0.34NS | 1.33NS | 0.63NS | 0.03NS |
| 17 | 4.17** | 1.88NS | 2.27* | 1.75NS | 0.93NS | 3.95** | 0.58NS | 1.54NS | 0.35NS | 0.27NS |
| 18 | 0.51NS | 0.68NS | 0.99NS | 0.70NS | 0.87NS | 1.00NS | 0.85NS | 0.93NS | 0.79NS | 0.83NS |
| 19 | 4.64** | 2.83* | 0.65NS | 2.76* | 0.71NS | 1.05NS | 0.85NS | 0.05NS | 1.68NS | 1.12NS |
| 20 | 6.92** | 4.67** | 0.07NS | 4.92** | 2.03NS | 0.18NS | 2.12* | 0.79NS | 3.31** | 2.43* |
| 21 | 4.35** | 2.26* | 1.63NS | 2.17* | 0.25NS | 2.62* | 0.00NS | 0.95NS | 0.90NS | 0.30NS |
| 22 | 6.21** | 4.25** | 0.28NS | 4.33** | 1.96NS | 0.17NS | 1.09NS | 0.92NS | 3.04** | 2.33* |
| 23 | 0.94NS | 1.11NS | 1.43NS | 1.13NS | 1.31NS | 1.45NS | 1.29NS | 1.37NS | 1.22NS | 1.26NS |
| 24 | 4.51** | 2.56* | 1.13NS | 2.48* | 0.25NS | 1.78NS | 0.13NS | 0.50NS | 1.30NS | 0.72NS |
| 25 | 0.06NS | 0.35NS | 1.11NS | 0.41NS | 0.82NS | 1.16NS | 0.77NS | 0.97NS | 0.61NS | 0.72NS |
| 26 | 3.27** | 1.10NS | 2.75* | 0.87NS | 1.57NS | 4.39** | 1.18NS | 2.04NS | 0.38NS | 0.91NS |
| 27 | 2.53* | 1.77NS | 0.33NS | 1.68NS | 0.90NS | 0.27NS | 0.97NS | 0.59NS | 1.28NS | 1.08NS |
| 28 | 1.59NS | 0.11NS | 3.08** | 0.37NS | 2.15* | 4.02** | 1.83NS | 2.51* | 1.25NS | 1.63NS |
| 29 | 4.26** | 2.09NS | 1.91NS | 1.98NS | 0.54NS | 3.15** | 0.25NS | 1.20NS | 0.66NS | 0.05NS |
| 30 | 2.44* | 0.64NS | 2.56* | 0.41NS | 1.51NS | 3.49** | 1.22NS | 1.96NS | 0.56NS | 0.99NS |

B1 DIFFS. SHEEP NUMBER

B0
DIFFS.

| | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.61NS | 2.43* | 1.59NS | 1.27NS | 3.23** | 1.36NS | 2.00NS | 0.32NS | 3.40** | 2.59* |
| 2 | 0.24NS | 1.00NS | 0.15NS | 0.26NS | 1.85NS | 0.28NS | 0.42NS | 0.44NS | 2.18* | 0.99NS |
| 3 | 1.37NS | 0.77NS | 2.02NS | 1.94NS | 0.11NS | 2.04NS | 1.43NS | 0.58NS | 0.49NS | 0.97NS |
| 4 | 0.51NS | 1.36NS | 0.17NS | 0.00NS | 2.33* | 0.00NS | 0.77NS | 0.42NS | 2.55* | 1.42NS |
| 5 | 0.37NS | 1.19NS | 0.00NS | 0.14NS | 2.10NS | 0.15NS | 0.59NS | 0.43NS | 2.38* | 1.21NS |
| 6 | 2.18* | 1.43NS | 4.05** | 3.27** | 0.52NS | 3.72** | 2.66* | 0.60NS | 0.39NS | 1.98NS |
| 7 | 1.47NS | 0.83NS | 2.25* | 2.11NS | 0.12NS | 2.25* | 1.56NS | 0.58NS | 0.52NS | 1.06NS |
| 8 | 0.94NS | 0.33NS | 1.50NS | 1.47NS | 0.34NS | 1.55NS | 0.94NS | 0.54NS | 0.88NS | 0.48NS |
| 9 | 0.50NS | 1.33NS | 0.17NS | 0.00NS | 2.27* | 0.00NS | 0.75NS | 0.42NS | 2.51* | 1.38NS |
| 10 | 0.91NS | 0.24NS | 1.57NS | 1.51NS | 0.51NS | 1.61NS | 0.92NS | 0.53NS | 1.05NS | 0.40NS |
| 11 | | 0.86NS | 0.56NS | 0.61NS | 1.86NS | 0.68NS | 0.46NS | 0.17NS | 2.17* | 0.85NS |
| 12 | 0.73NS | | 1.98NS | 1.70NS | 1.03NS | 1.92NS | 0.93NS | 0.51NS | 1.53NS | 0.19NS |
| 13 | 1.75NS | 2.56* | | 0.26NS | 4.00** | 0.37NS | 1.35NS | 0.43NS | 3.27** | 3.11** |
| 14 | 1.62NS | 2.67* | 0.47NS | | 3.02** | 0.00NS | 1.05NS | 0.42NS | 2.96** | 1.98NS |
| 15 | 0.82NS | 0.03NS | 2.77* | 3.02** | | 3.54** | 2.34* | 0.57NS | 0.81NS | 1.57NS |
| 16 | 0.99NS | 2.10NS | 1.25NS | 1.04NS | 2.49* | | 1.28NS | 0.42NS | 3.18** | 2.50* |
| 17 | 1.27NS | 2.38* | 0.95NS | 0.62NS | 2.78* | 0.47NS | | 0.47NS | 2.42* | 1.04NS |
| 18 | 0.88NS | 0.93NS | 0.75NS | 0.78NS | 0.93NS | 0.83NS | 0.81NS | | 0.63NS | 0.50NS |
| 19 | 0.57NS | 0.05NS | 2.15* | 2.08NS | 0.07NS | 1.54NS | 1.78NS | 0.93NS | | 1.89NS |
| 20 | 2.01NS | 1.30NS | 4.02** | 4.93** | 1.47NS | 4.87** | 5.07** | 1.00NS | 1.04NS | |
| 21 | 0.48NS | 1.34NS | 1.45NS | 1.27NS | 1.51NS | 0.50NS | 0.84NS | 0.85NS | 1.04NS | 3.00** |
| 22 | 1.91NS | 1.30NS | 3.61** | 3.93** | 1.39NS | 3.52** | 3.75** | 1.01NS | 1.13NS | 0.36NS |
| 23 | 1.32NS | 1.37NS | 1.18NS | 1.22NS | 1.37NS | 1.26NS | 1.25NS | 0.29NS | 1.37NS | 1.44NS |
| 24 | 0.07NS | 0.65NS | 1.82NS | 1.70NS | 0.73NS | 1.08NS | 1.35NS | 0.89NS | 0.51NS | 1.91NS |
| 25 | 0.85NS | 0.98NS | 0.52NS | 0.60NS | 0.98NS | 0.72NS | 0.67NS | 0.50NS | 0.96NS | 1.14NS |
| 26 | 1.94NS | 2.99** | 0.16NS | 0.40NS | 3.38** | 1.48NS | 1.05NS | 0.76NS | 2.35* | 5.28** |
| 27 | 0.84NS | 0.62NS | 1.46NS | 1.34NS | 0.62NS | 1.13NS | 1.21NS | 1.04NS | 0.62NS | 0.32NS |
| 28 | 2.40* | 3.10** | 0.88NS | 1.38NS | 3.27** | 2.05NS | 1.80NS | 0.67NS | 2.73* | 4.26** |
| 29 | 0.81NS | 1.76NS | 1.23NS | 0.99NS | 2.01NS | 0.12NS | 0.50NS | 0.83NS | 1.36NS | 3.76** |
| 30 | 1.76NS | 2.50* | 0.14NS | 0.59NS | 2.67* | 1.29NS | 1.02NS | 0.74NS | 2.15* | 3.75** |

B1 DIFFS.
B0
DIFFS.

| | SHEEP NUMBER | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 1 | 2.20* | 3.99** | 0.90NS | 2.40* | 0.59NS | 2.40* | 1.97NS | 2.44* | 2.41* | 1.96NS |
| 2 | 0.76NS | 2.66* | 1.02NS | 1.06NS | 0.32NS | 0.91NS | 1.48NS | 1.29NS | 0.92NS | 0.74NS |
| 3 | 1.00NS | 0.75NS | 1.16NS | 0.63NS | 0.02NS | 0.91NS | 0.85NS | 0.18NS | 0.91NS | 0.77NS |
| 4 | 1.10NS | 3.13** | 1.00NS | 1.39NS | 0.37NS | 1.29NS | 1.58NS | 1.57NS | 1.30NS | 1.01NS |
| 5 | 0.93NS | 2.92** | 1.01NS | 1.23NS | 0.34NS | 1.10NS | 1.53NS | 1.44NS | 1.11NS | 0.88NS |
| 6 | 1.77NS | 0.75NS | 1.18NS | 1.15NS | 0.07NS | 1.70NS | 0.80NS | 0.46NS | 1.72NS | 1.23NS |
| 7 | 1.08NS | 0.81NS | 1.16NS | 0.67NS | 0.02NS | 0.99NS | 0.86NS | 0.19NS | 0.99NS | 0.81NS |
| 8 | 0.55NS | 1.17NS | 1.12NS | 0.21NS | 0.07NS | 0.45NS | 1.01NS | 0.18NS | 0.45NS | 0.38NS |
| 9 | 1.08NS | 3.07** | 1.00NS | 1.36NS | 0.37NS | 1.26NS | 1.57NS | 1.55NS | 1.27NS | 0.99NS |
| 10 | 0.48NS | 1.39NS | 1.11NS | 0.11NS | 0.09NS | 0.37NS | 1.06NS | 0.29NS | 0.38NS | 0.31NS |
| 11 | 0.58NS | 2.74* | 1.04NS | 0.93NS | 0.28NS | 0.76NS | 1.42NS | 1.19NS | 0.77NS | 0.58NS |
| 12 | 0.32NS | 2.08NS | 1.09NS | 0.14NS | 0.14NS | 0.17NS | 1.18NS | 0.57NS | 0.17NS | 0.12NS |
| 13 | 1.58NS | 4.57** | 1.01NS | 1.84NS | 0.35NS | 2.03NS | 1.58NS | 1.84NS | 2.08NS | 1.17NS |
| 14 | 1.30NS | 3.82** | 1.00NS | 1.67NS | 0.37NS | 1.65NS | 1.60NS | 1.77NS | 1.67NS | 1.16NS |
| 15 | 1.39NS | 1.29NS | 1.15NS | 0.76NS | 0.00NS | 1.30NS | 0.93NS | 0.12NS | 1.32NS | 0.90NS |
| 16 | 1.57NS | 4.26** | 1.00NS | 1.84NS | 0.37NS | 1.92NS | 1.61NS | 1.87NS | 1.95NS | 1.24NS |
| 17 | 0.57NS | 3.29** | 1.05NS | 0.98NS | 0.25NS | 0.82NS | 1.40NS | 1.23NS | 0.84NS | 0.54NS |
| 18 | 0.49NS | 0.65NS | 0.42NS | 0.52NS | 0.53NS | 0.50NS | 0.76NS | 0.56NS | 0.50NS | 0.50NS |
| 19 | 1.80NS | 0.25NS | 1.20NS | 1.31NS | 0.14NS | 1.73NS | 0.66NS | 0.70NS | 1.75NS | 1.39NS |
| 20 | 0.20NS | 2.68* | 1.06NS | 0.34NS | 0.16NS | 0.00NS | 1.23NS | 0.75NS | 0.00NS | 0.00NS |
| 21 | | 2.40* | 1.07NS | 0.43NS | 0.18NS | 0.17NS | 1.26NS | 0.80NS | 0.17NS | 0.12NS |
| 22 | 2.60* | | 1.22NS | 1.77NS | 0.18NS | 2.37* | 0.59NS | 0.99NS | 2.39* | 1.78NS |
| 23 | 1.29NS | 1.46NS | | 1.10NS | 1.07NS | 1.08NS | 1.32NS | 1.14NS | 1.08NS | 1.08NS |
| 24 | 0.56NS | 1.83NS | 1.33NS | | 0.11NS | 0.30NS | 1.12NS | 0.43NS | 0.30NS | 0.23NS |
| 25 | 0.78NS | 1.18NS | 0.89NS | 0.87NS | | 0.16NS | 0.45NS | 0.02NS | 0.16NS | 0.16NS |
| 26 | 1.62NS | 4.22** | 1.20NS | 2.01* | 0.55NS | | 1.22NS | 0.70NS | 0.00NS | 0.00NS |
| 27 | 1.00NS | 0.22NS | 1.48NS | 0.82NS | 1.16NS | 1.44NS | | 0.93NS | 1.22NS | 1.19NS |
| 28 | 2.17* | 3.99** | 1.10NS | 2.46* | 0.32NS | 1.11NS | 1.79NS | | 0.71NS | 0.59NS |
| 29 | 0.35NS | 3.07** | 1.27NS | 0.89NS | 0.73NS | 1.38NS | 1.10NS | 2.00NS | | 0.00NS |
| 30 | 1.48NS | 3.47** | 1.17NS | 1.82NS | 0.49NS | 0.30NS | 1.50NS | 0.70NS | 1.26NS | |

# APPENDIX III

HARVEST 1. GENOTYPE POPULATION MEAN DATA.

CHARACTERS :
1 = SHEEP PREFERENCE ASSESSMENT (LAX)(1-6)(ORIG:0-5)
2 = FLOWER & SEED HEADS (1-6)
3 = CLUMP GREEN MATERIAL (1-5)
4 = CLUMP RUST (1-6)(ORIG:0-5)
5 = LEAF WIDTH (1-5)
6 = CLUMP HEIGHT
7 = CLUMP DIAMETER
8 = CLUMP ERECTNESS (1-5)
9 = PUBESCENCE (TOTAL SCORE 3-15)
10 = LEAF TENSILE STRENGTH

| | CHARACTERS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| GENOT. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 9 | 2.58 | 3.79 | 3.08 | 2.63 | 2.00 | 5.29 | 13.25 | 2.50 | 10.88 | 42.40 |
| 13 | 2.45 | 3.13 | 3.05 | 2.49 | 2.52 | 6.73 | 15.38 | 3.09 | 10.08 | 53.05 |
| 15 | 2.50 | 3.21 | 2.92 | 2.33 | 2.29 | 6.38 | 14.71 | 3.13 | 10.17 | 44.53 |
| 16 | 1.91 | 4.42 | 3.25 | 2.38 | 2.13 | 6.63 | 14.50 | 2.96 | 10.83 | 47.48 |
| 19 | 2.00 | 3.92 | 3.00 | 2.17 | 2.04 | 6.54 | 14.54 | 3.13 | 11.17 | 45.73 |
| 29 | 2.09 | 3.68 | 3.04 | 2.63 | 2.00 | 5.75 | 13.73 | 2.65 | 11.42 | 46.14 |
| 35 | 2.83 | 3.50 | 3.17 | 1.75 | 1.92 | 6.33 | 13.96 | 2.96 | 11.17 | 43.12 |
| 42 | 2.38 | 3.83 | 2.96 | 2.58 | 2.04 | 6.02 | 13.75 | 2.71 | 10.96 | 45.53 |
| 50 | 1.90 | 3.63 | 3.08 | 2.36 | 1.96 | 5.47 | 14.86 | 2.38 | 10.38 | 42.50 |
| 55 | 1.88 | 3.63 | 2.88 | 2.21 | 2.08 | 5.71 | 13.33 | 2.75 | 10.00 | 43.63 |
| 56 | 2.08 | 3.46 | 2.83 | 2.63 | 1.96 | 5.83 | 13.71 | 3.00 | 10.92 | 39.99 |
| 57 | 2.67 | 3.88 | 2.88 | 2.08 | 2.04 | 6.67 | 14.13 | 3.00 | 10.67 | 45.55 |
| 62 | 2.21 | 3.83 | 2.63 | 2.08 | 2.13 | 5.75 | 14.50 | 2.63 | 10.46 | 43.98 |
| 65 | 2.59 | 3.68 | 3.13 | 2.12 | 2.10 | 6.24 | 13.66 | 2.36 | 10.77 | 44.41 |
| 67 | 2.35 | 4.08 | 2.96 | 2.17 | 2.35 | 6.79 | 14.49 | 2.88 | 10.50 | 48.18 |
| 69 | 2.75 | 3.33 | 3.33 | 2.13 | 2.29 | 7.17 | 15.46 | 2.75 | 10.00 | 44.32 |
| 70 | 2.58 | 2.96 | 2.88 | 2.25 | 2.29 | 6.00 | 14.33 | 2.71 | 10.33 | 44.75 |
| 71 | 2.50 | 3.13 | 3.21 | 1.96 | 2.08 | 5.79 | 14.50 | 2.42 | 9.58 | 39.54 |
| 72 | 2.67 | 3.33 | 3.33 | 2.08 | 2.29 | 5.83 | 14.71 | 2.21 | 10.71 | 43.38 |
| 74 | 1.79 | 3.33 | 2.83 | 2.54 | 2.00 | 6.46 | 13.66 | 2.92 | 10.00 | 46.49 |
| 76 | 2.21 | 3.21 | 2.83 | 2.46 | 1.88 | 5.79 | 14.13 | 2.92 | 10.04 | 40.46 |
| 79 | 2.71 | 3.25 | 3.00 | 2.17 | 2.42 | 6.58 | 14.46 | 2.83 | 10.08 | 49.60 |
| 82 | 2.50 | 2.67 | 2.88 | 2.42 | 1.92 | 6.04 | 15.00 | 3.00 | 9.92 | 43.86 |
| 84 | 2.96 | 2.13 | 2.96 | 2.25 | 2.71 | 7.21 | 16.54 | 3.38 | 9.67 | 47.26 |
| 86 | 1.92 | 4.42 | 2.83 | 2.83 | 2.04 | 6.13 | 13.67 | 2.92 | 10.88 | 50.75 |
| 88 | 2.88 | 3.25 | 3.08 | 3.12 | 2.25 | 5.89 | 14.26 | 2.20 | 9.85 | 45.55 |
| 91 | 1.79 | 3.17 | 2.79 | 2.33 | 1.92 | 6.50 | 14.67 | 2.96 | 10.75 | 42.36 |
| 92 | 2.14 | 2.12 | 2.21 | 1.71 | 2.88 | 5.50 | 12.31 | 2.72 | 9.58 | 45.46 |
| 99 | 2.54 | 3.58 | 2.92 | 1.67 | 2.08 | 6.54 | 14.83 | 2.92 | 10.79 | 45.29 |
| 101 | 2.46 | 3.50 | 3.13 | 2.29 | 2.33 | 7.00 | 15.21 | 3.08 | 10.46 | 45.82 |
| 103 | 2.38 | 3.13 | 3.21 | 2.17 | 1.92 | 5.92 | 14.54 | 2.92 | 10.79 | 42.52 |
| 106 | 2.13 | 3.92 | 2.67 | 2.00 | 2.13 | 6.17 | 13.75 | 2.58 | 11.21 | 46.94 |
| 110 | 2.16 | 3.13 | 3.25 | 2.46 | 2.17 | 6.17 | 15.00 | 2.71 | 9.92 | 44.90 |
| 114 | 2.42 | 3.63 | 3.29 | 2.88 | 2.25 | 5.33 | 13.08 | 2.50 | 10.63 | 46.08 |
| 117 | 2.42 | 3.63 | 2.67 | 2.25 | 2.08 | 5.58 | 13.63 | 2.63 | 9.71 | 47.57 |
| 119 | 2.00 | 2.96 | 2.79 | 2.71 | 1.83 | 5.75 | 13.46 | 2.33 | 10.29 | 43.82 |
| 120 | 1.96 | 3.92 | 3.33 | 2.00 | 1.83 | 6.54 | 14.29 | 3.00 | 11.58 | 44.08 |
| 122 | 2.67 | 3.79 | 2.96 | 1.75 | 1.83 | 5.63 | 14.29 | 2.79 | 11.25 | 45.15 |
| 123 | 2.33 | 3.08 | 3.04 | 2.04 | 2.29 | 6.50 | 15.42 | 2.71 | 10.75 | 46.76 |
| 126 | 2.08 | 4.08 | 2.88 | 1.96 | 2.00 | 6.17 | 14.67 | 2.63 | 10.88 | 45.35 |
| 138 | 2.17 | 3.50 | 2.75 | 1.88 | 1.96 | 5.67 | 14.33 | 2.75 | 10.67 | 44.00 |
| 142 | 2.13 | 4.08 | 2.96 | 2.42 | 2.42 | 6.08 | 14.29 | 2.92 | 11.21 | 45.35 |
| 147 | 2.08 | 3.63 | 3.17 | 2.46 | 2.25 | 6.47 | 14.79 | 2.54 | 11.13 | 40.36 |
| 155 | 2.05 | 3.54 | 3.50 | 2.04 | 2.13 | 6.07 | 15.42 | 3.04 | 10.46 | 42.14 |
| 165 | 2.08 | 3.71 | 3.00 | 2.58 | 2.00 | 5.58 | 13.96 | 2.72 | 10.92 | 42.47 |
| 166 | 2.42 | 3.00 | 2.88 | 2.63 | 2.71 | 5.83 | 13.58 | 2.63 | 10.38 | 45.20 |
| 176 | 2.00 | 4.25 | 3.21 | 2.96 | 2.42 | 6.58 | 14.17 | 2.88 | 11.42 | 45.34 |
| 180 | 2.08 | 2.79 | 2.91 | 2.19 | 1.90 | 5.79 | 14.09 | 2.64 | 10.67 | 43.99 |
| 185 | 2.17 | 4.25 | 2.83 | 1.92 | 2.08 | 5.75 | 13.75 | 2.83 | 10.96 | 44.95 |
| 187 | 2.38 | 3.54 | 3.46 | 2.17 | 1.75 | 6.04 | 13.50 | 2.83 | 10.92 | 44.33 |
| 188 | 2.00 | 4.00 | 3.00 | 2.13 | 2.00 | 6.33 | 14.13 | 3.04 | 10.79 | 57.02 |
| 189 | 1.71 | 4.29 | 2.92 | 2.38 | 1.54 | 5.50 | 12.33 | 2.71 | 10.50 | 45.41 |
| 193 | 2.21 | 3.71 | 2.96 | 2.00 | 2.21 | 6.17 | 14.50 | 3.25 | 10.33 | 43.52 |
| S.E. | 0.29 | 0.31 | 0.16 | 0.28 | 0.17 | 0.43 | 0.68 | 0.23 | 0.38 | 3.22 |
| SEDD | 0.41 | 0.44 | 0.23 | 0.40 | 0.24 | 0.61 | 0.97 | 0.32 | 0.53 | 4.55 |

HARVEST 2. GENOTYPE POPULATION MEAN DATA.

CHARACTERS :
1 = SHEEP PREFERENCE ASSESSMENT (LAX)(1-6)(ORIG:0-5)
2 = CLUMP GREEN MATERIAL (1-5)
3 = CLUMP RUST (1-6)(ORIG:0-5)
4 = CLUMP ERECTNESS (1-5)
5 = LEAF FLAVANOL LEVEL (1-5)
6 = LEAF WIDTH (1-5)
7 = CLUMP DIAMETER
8 = CLUMP HEIGHT

| | CHARACTERS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| GENOT. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 9 | 3.00 | 3.96 | 2.25 | 2.08 | 2.46 | 1.63 | 12.13 | 3.33 |
| 13 | 3.25 | 4.24 | 1.77 | 3.39 | 3.14 | 1.85 | 13.60 | 4.32 |
| 15 | 3.13 | 4.33 | 2.21 | 3.33 | 2.29 | 2.08 | 12.54 | 4.46 |
| 16 | 3.08 | 4.21 | 1.58 | 2.75 | 2.83 | 1.88 | 12.46 | 3.75 |
| 19 | 2.21 | 4.13 | 2.08 | 2.75 | 2.54 | 1.75 | 13.42 | 4.17 |
| 29 | 3.10 | 3.97 | 2.63 | 2.88 | 2.21 | 1.71 | 12.24 | 3.88 |
| 35 | 2.42 | 3.83 | 1.38 | 2.63 | 2.33 | 1.63 | 12.88 | 3.58 |
| 42 | 3.29 | 4.33 | 1.67 | 2.17 | 2.33 | 1.67 | 12.58 | 4.08 |
| 50 | 3.29 | 4.18 | 2.12 | 2.75 | 1.96 | 1.62 | 13.54 | 4.39 |
| 55 | 2.42 | 3.83 | 2.08 | 2.75 | 2.42 | 1.88 | 12.46 | 3.92 |
| 56 | 2.38 | 3.91 | 1.66 | 3.00 | 2.51 | 1.74 | 12.85 | 3.83 |
| 57 | 3.21 | 4.04 | 1.42 | 2.88 | 2.58 | 1.88 | 13.13 | 4.29 |
| 62 | 3.25 | 4.17 | 2.04 | 2.38 | 2.50 | 1.92 | 14.13 | 4.58 |
| 65 | 4.07 | 3.98 | 1.93 | 2.65 | 2.27 | 1.90 | 12.06 | 4.24 |
| 67 | 2.71 | 4.04 | 2.08 | 2.67 | 2.64 | 1.96 | 13.33 | 4.17 |
| 69 | 3.71 | 4.08 | 1.58 | 2.96 | 2.54 | 1.67 | 13.08 | 4.13 |
| 70 | 3.08 | 3.92 | 1.67 | 2.67 | 2.58 | 2.33 | 12.63 | 3.63 |
| 71 | 2.67 | 4.00 | 1.71 | 2.58 | 1.96 | 1.67 | 12.58 | 4.00 |
| 72 | 2.50 | 4.08 | 1.42 | 2.29 | 2.96 | 1.96 | 13.00 | 4.04 |
| 74 | 2.71 | 4.13 | 2.04 | 2.88 | 2.04 | 2.00 | 13.42 | 4.50 |
| 76 | 3.10 | 3.58 | 2.14 | 2.83 | 2.29 | 2.08 | 12.01 | 3.64 |
| 79 | 2.71 | 4.29 | 2.46 | 2.71 | 2.58 | 1.92 | 12.88 | 4.21 |
| 82 | 2.79 | 4.04 | 1.71 | 2.50 | 1.92 | 1.75 | 13.17 | 4.17 |
| 84 | 2.57 | 4.15 | 1.75 | 3.05 | 2.52 | 2.24 | 13.87 | 4.88 |
| 86 | 3.08 | 4.04 | 2.04 | 2.67 | 2.58 | 1.79 | 12.21 | 4.42 |
| 88 | 3.58 | 4.50 | 2.27 | 2.08 | 2.21 | 2.08 | 13.08 | 3.89 |
| 91 | 2.58 | 3.92 | 1.63 | 2.75 | 2.33 | 1.43 | 12.40 | 3.88 |
| 92 | 3.60 | 3.47 | 1.15 | 2.19 | 2.44 | 2.29 | 10.76 | 3.78 |
| 99 | 2.38 | 4.00 | 2.29 | 2.88 | 2.38 | 1.83 | 12.79 | 4.00 |
| 101 | 2.23 | 3.93 | 2.08 | 3.09 | 2.17 | 2.03 | 13.58 | 4.48 |
| 103 | 2.29 | 4.54 | 2.13 | 3.00 | 2.08 | 1.50 | 13.00 | 4.13 |
| 106 | 2.29 | 3.75 | 1.96 | 2.96 | 2.54 | 1.92 | 12.21 | 3.71 |
| 110 | 2.54 | 3.92 | 2.50 | 2.25 | 2.33 | 1.79 | 13.42 | 4.04 |
| 114 | 2.88 | 4.33 | 2.33 | 2.21 | 1.96 | 1.63 | 13.13 | 4.29 |
| 117 | 2.96 | 3.96 | 2.71 | 2.04 | 2.42 | 1.79 | 13.29 | 4.42 |
| 119 | 2.17 | 3.79 | 1.83 | 2.75 | 1.79 | 1.54 | 11.88 | 4.04 |
| 120 | 3.21 | 4.35 | 1.79 | 2.88 | 2.46 | 2.17 | 13.45 | 4.49 |
| 122 | 3.25 | 4.21 | 2.37 | 2.38 | 2.71 | 1.70 | 12.71 | 3.84 |
| 123 | 3.25 | 4.17 | 2.08 | 2.79 | 2.54 | 1.75 | 13.08 | 4.54 |
| 126 | 3.02 | 4.45 | 2.35 | 2.52 | 2.55 | 1.70 | 14.20 | 4.33 |
| 138 | 1.96 | 4.32 | 2.68 | 2.92 | 2.49 | 2.04 | 13.17 | 3.92 |
| 142 | 2.83 | 3.79 | 1.58 | 2.75 | 2.29 | 2.08 | 12.92 | 4.21 |
| 147 | 2.69 | 3.67 | 2.51 | 2.40 | 1.49 | 1.69 | 12.35 | 4.40 |
| 155 | 2.33 | 4.04 | 2.04 | 2.96 | 2.63 | 2.13 | 12.96 | 4.00 |
| 165 | 2.40 | 4.31 | 2.01 | 2.48 | 2.10 | 1.92 | 12.52 | 4.38 |
| 166 | 2.04 | 3.25 | 1.58 | 2.33 | 2.08 | 2.04 | 11.96 | 3.79 |
| 176 | 2.67 | 3.79 | 2.00 | 3.00 | 3.17 | 2.13 | 13.54 | 4.21 |
| 180 | 2.67 | 3.67 | 2.00 | 2.75 | 2.05 | 1.50 | 12.15 | 3.41 |
| 185 | 2.79 | 4.38 | 1.88 | 2.63 | 1.79 | 1.83 | 12.79 | 4.17 |
| 187 | 3.38 | 4.21 | 2.38 | 2.50 | 2.17 | 1.71 | 12.88 | 3.96 |
| 188 | 3.09 | 3.69 | 1.35 | 2.73 | 2.63 | 1.84 | 12.82 | 4.05 |
| 189 | 3.09 | 3.90 | 1.71 | 2.10 | 2.74 | 1.69 | 11.63 | 3.76 |
| 193 | 2.82 | 3.75 | 1.65 | 2.90 | 2.57 | 1.73 | 13.79 | 4.02 |
| S.E. | 0.46 | 0.26 | 0.24 | 0.24 | 0.32 | 0.19 | 0.61 | 0.34 |
| SEOD | 0.65 | 0.37 | 0.34 | 0.34 | 0.45 | 0.26 | 0.87 | 0.48 |

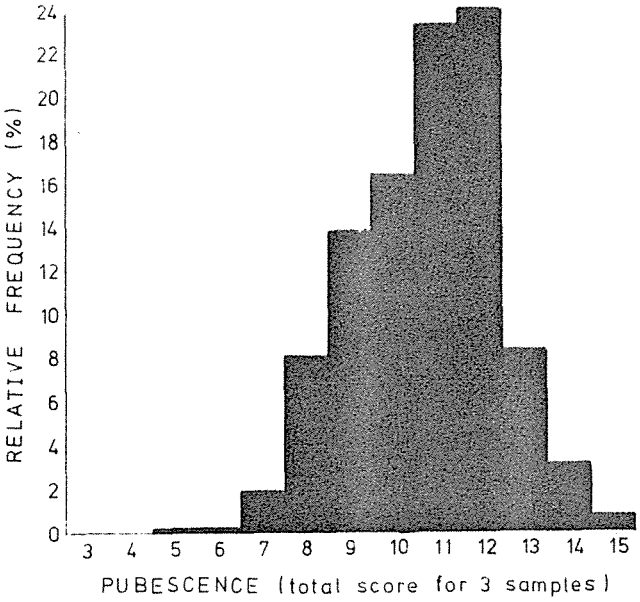
HARVEST 3. GENOTYPE POPULATION MEAN DATA.

CHARACTERS :
1 = SHEEP PREFERENCE ASSESSMENT (LAX)(1-6)(ORIG:0-5)
2 = CLUMP GREEN MATERIAL (1-5)
3 = CLUMP DIAMETER
4 = CLUMP ERECTNESS (1-5)
5 = LEAF WIDTH (1-5)
6 = CLUMP HEIGHT
7 = SOLUBLE SUGAR LEVEL

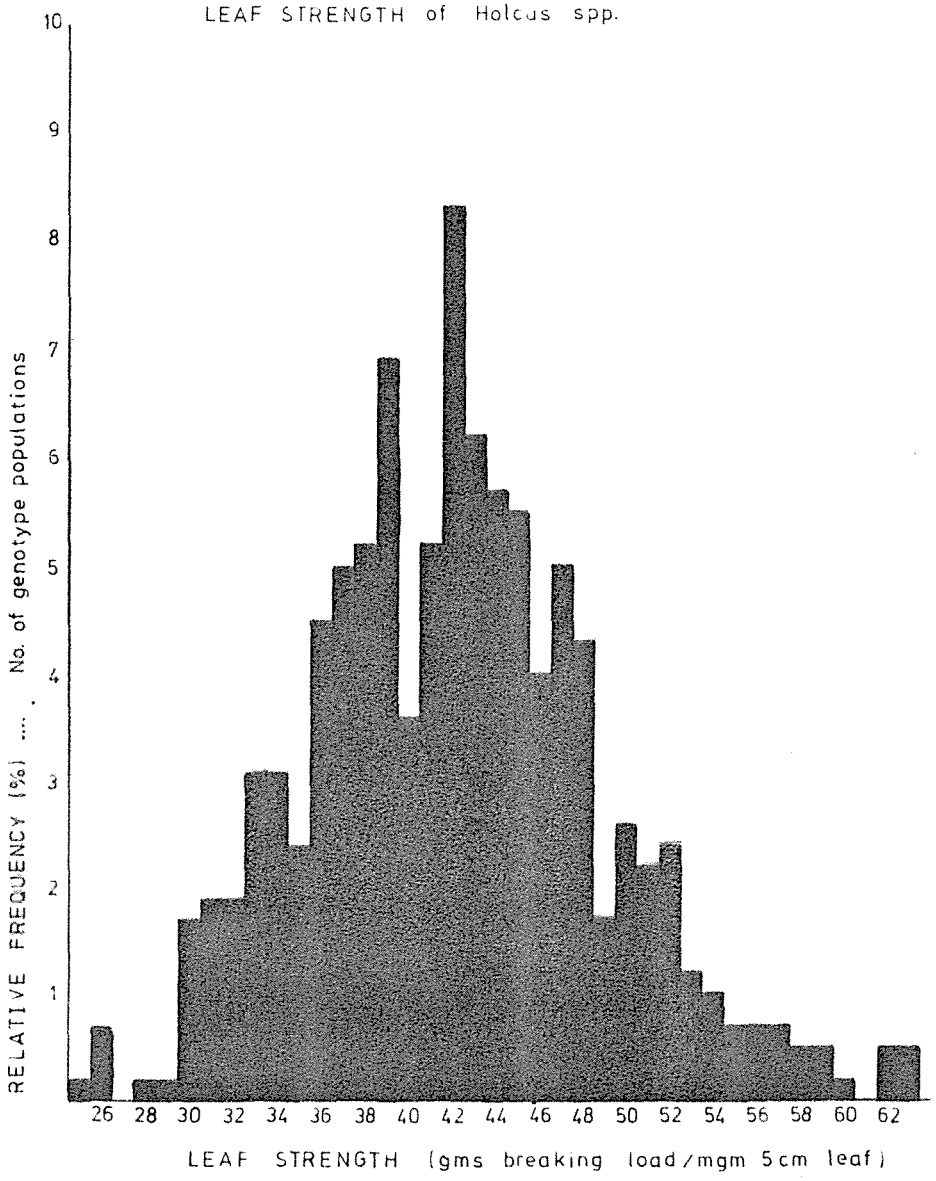
| | CHARACTERS | | | | | | |
|---|---|---|---|---|---|---|---|
| GENOT. | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 9 | 4.08 | 4.00 | 11.79 | 1.96 | 1.67 | 2.25 | 15.64 |
| 13 | 4.09 | 4.37 | 12.39 | 2.70 | 1.99 | 3.12 | 14.65 |
| 15 | 4.65 | 3.98 | 11.36 | 2.81 | 1.86 | 3.05 | 11.76 |
| 16 | 4.08 | 3.68 | 12.54 | 2.17 | 1.58 | 2.50 | 14.47 |
| 19 | 3.58 | 4.21 | 13.46 | 2.67 | 1.75 | 3.13 | 11.88 |
| 29 | 3.88 | 3.94 | 12.35 | 2.20 | 1.49 | 2.51 | 13.09 |
| 35 | 3.79 | 4.17 | 12.71 | 2.42 | 1.54 | 2.67 | 11.96 |
| 42 | 4.42 | 4.46 | 12.83 | 2.50 | 1.63 | 3.17 | 13.54 |
| 50 | 4.58 | 4.50 | 12.25 | 2.63 | 1.63 | 3.54 | 14.32 |
| 55 | 4.14 | 3.96 | 13.47 | 2.23 | 1.86 | 2.85 | 14.01 |
| 56 | 4.07 | 4.25 | 13.21 | 2.86 | 1.50 | 3.14 | 12.77 |
| 57 | 4.63 | 4.17 | 12.83 | 2.46 | 1.79 | 2.88 | 14.23 |
| 62 | 4.43 | 4.17 | 12.71 | 2.46 | 1.63 | 3.11 | 12.90 |
| 65 | 4.38 | 3.94 | 11.33 | 2.58 | 1.76 | 2.97 | 10.86 |
| 67 | 4.29 | 4.04 | 12.50 | 2.54 | 1.67 | 2.83 | 11.63 |
| 69 | 4.75 | 4.21 | 12.75 | 2.63 | 1.67 | 2.92 | 14.00 |
| 70 | 3.96 | 3.75 | 11.46 | 2.29 | 1.67 | 2.42 | 14.48 |
| 71 | 4.58 | 4.18 | 11.27 | 2.40 | 1.44 | 2.65 | 13.49 |
| 72 | 4.42 | 4.04 | 12.58 | 2.17 | 1.79 | 2.71 | 13.82 |
| 74 | 3.92 | 3.63 | 11.54 | 2.54 | 1.50 | 2.79 | 12.99 |
| 76 | 3.76 | 3.45 | 11.61 | 2.21 | 1.54 | 2.52 | 12.43 |
| 79 | 4.00 | 3.83 | 12.04 | 2.46 | 1.71 | 2.75 | 14.90 |
| 82 | 4.15 | 3.88 | 11.81 | 2.21 | 1.61 | 2.49 | 12.54 |
| 84 | 4.21 | 3.90 | 12.65 | 2.73 | 1.81 | 3.34 | 14.23 |
| 86 | 3.92 | 4.13 | 12.79 | 2.54 | 1.63 | 3.21 | 11.45 |
| 88 | 3.82 | 3.69 | 12.03 | 2.15 | 1.61 | 2.36 | 15.44 |
| 91 | 3.77 | 3.80 | 12.61 | 2.67 | 1.63 | 2.97 | 11.47 |
| 92 | 4.23 | 4.11 | 10.62 | 2.21 | 1.98 | 2.93 | 14.95 |
| 99 | 4.00 | 3.92 | 12.58 | 2.63 | 1.54 | 2.88 | 13.77 |
| 101 | 4.33 | 4.03 | 12.31 | 2.56 | 1.57 | 2.98 | 13.90 |
| 103 | 4.12 | 4.05 | 12.86 | 2.96 | 1.49 | 3.02 | 11.10 |
| 106 | 3.92 | 3.75 | 11.96 | 2.25 | 1.67 | 2.58 | 11.47 |
| 110 | 4.42 | 4.42 | 13.63 | 2.54 | 1.71 | 3.13 | 14.15 |
| 114 | 4.17 | 4.47 | 11.83 | 2.33 | 1.79 | 2.92 | 12.25 |
| 117 | 4.21 | 4.25 | 12.21 | 2.46 | 1.92 | 3.04 | 12.76 |
| 119 | 3.67 | 3.92 | 11.21 | 1.92 | 1.54 | 2.46 | 12.30 |
| 120 | 4.33 | 4.00 | 11.54 | 2.54 | 1.63 | 2.63 | 12.02 |
| 122 | 4.58 | 4.26 | 12.17 | 2.46 | 1.50 | 2.92 | 13.92 |
| 123 | 4.50 | 4.13 | 11.96 | 2.54 | 1.54 | 2.83 | 15.02 |
| 126 | 3.95 | 3.90 | 12.24 | 2.14 | 1.57 | 2.76 | 14.57 |
| 138 | 4.54 | 4.36 | 13.00 | 2.53 | 1.69 | 2.73 | 12.53 |
| 142 | 4.38 | 4.08 | 12.79 | 2.58 | 1.63 | 3.00 | 12.22 |
| 147 | 4.55 | 3.75 | 11.02 | 2.28 | 1.51 | 2.43 | 13.56 |
| 155 | 4.71 | 4.38 | 12.83 | 2.75 | 1.54 | 3.04 | 13.86 |
| 165 | 4.10 | 4.46 | 12.33 | 2.40 | 1.69 | 2.57 | 12.17 |
| 166 | 3.74 | 3.89 | 11.96 | 2.25 | 1.49 | 2.64 | 10.75 |
| 176 | 4.21 | 4.00 | 12.50 | 2.58 | 1.86 | 2.96 | 11.12 |
| 180 | 4.30 | 3.89 | 11.20 | 2.43 | 1.50 | 2.71 | 11.87 |
| 185 | 4.20 | 4.21 | 11.79 | 2.39 | 1.65 | 2.63 | 11.39 |
| 187 | 3.88 | 4.04 | 11.01 | 2.17 | 1.46 | 2.33 | 12.08 |
| 188 | 3.58 | 3.99 | 13.05 | 2.51 | 1.60 | 3.11 | 13.50 |
| 189 | 3.74 | 3.61 | 10.10 | 1.76 | 1.33 | 2.13 | 11.65 |
| 193 | 4.07 | 4.16 | 12.63 | 2.62 | 1.45 | 2.98 | 13.07 |
| S.E. | 0.34 | 0.25 | 0.74 | 0.21 | 0.14 | 0.26 | 1.13 |
| SEOD | 0.48 | 0.35 | 1.02 | 0.29 | 0.20 | 0.37 | 1.59 |

APPENDIX IV

FREQUENCY HISTOGRAM of
Holcus spp. PUBESCENCE




FREQUENCY HISTOGRAM OF THE
LEAF STRENGTH of Holcus spp.



APPENDIX V.

(1) Correlation matrix for all genotype populations combined in Harvest 1 across ten characters.

Characters:- 1: Sheep preference assessment under LAX grazing (1-6)
2: Leaf tensile strength
3: Leaf pubescence (3-15)
4: Leaf width
5: Clump greeness
6: Clump rust (1-6)
7: Clump erectness
8: Presence of inflorescences (1-6)
9: Clump height
10: Clump diameter

| X | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.002 | -0.081 | 0.138 | 0.242 | -0.226 | 0.001 | -0.326 | 0.087 | 0.046 |
| 2 | | -0.021 | 0.084 | 0.046 | 0.012 | 0.132 | 0.088 | 0.157 | 0.096 |
| 3 | | | -0.026 | 0.022 | -0.004 | 0.054 | 0.194 | -0.031 | -0.108 |
| 4 | | | | 0.078 | 0.018 | 0.089 | 0.011 | 0.264 | 0.185 |
| 5 | | | | | -0.135 | 0.102 | -0.005 | 0.302 | 0.302 |
| 6 | | | | | | -0.035 | 0.074 | 0.010 | 0.025 |
| 7 | | | | | | | 0.117 | 0.443 | 0.130 |
| 8 | | | | | | | | 0.135 | 0.031 |
| 9 | | | | | | | | | 0.628 |

(2) Correlation matrix for all genotype populations combined in Harvest 2 across eight characters.

Characters:- 1: Sheep preference assessment under LAX grazing (1-6)
2: Leaf width
3: Clump greenness
4: Clump rust (1-6)
5: Clump erectness
6: Clump height
7: Clump diameter
8: Leaf flavanols.

| X | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 1 | 0.076 | 0.422 | -0.209 | -0.002 | 0.173 | 0.149 | -0.085 |
| 2 | | 0.059 | -0.002 | -0.011 | 0.224 | 0.146 | 0.117 |
| 3 | | | 0.056 | 0.157 | 0.377 | 0.431 | -0.045 |
| 4 | | | | -0.073 | -0.037 | 0.052 | -0.047 |
| 5 | | | | | 0.322 | 0.254 | 0.007 |
| 6 | | | | | | 0.560 | -0.048 |
| 7 | | | | | | | -0.009 |

(3) Correlation matrix for all genotype populations combined in Harvest 3 across seven characters.

Characters:- 1: Sheep preference assessment under LAX grazing (1-6)
2: Leaf width
3: Clump greenness
4: Clump erectness
5: Clump height
6: Clump diameter
7: Soluble sugar level.

| X | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 1 | -0.027 | 0.420 | 0.256 | 0.239 | 0.099 | -0.048 |
| 2 | | 0.175 | 0.030 | 0.288 | 0.279 | 0.220 |
| 3 | | | 0.424 | 0.555 | 0.450 | 0.033 |
| 4 | | | | 0.542 | 0.353 | -0.012 |
| 5 | | | | | 0.635 | 0.067 |
| 6 | | | | | | 0.192 |