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THE EFFECT OF TEMPERATURE ON THE
GROWTH AND DEVELOPMENT OF FIVE
WHITE CLOVER (TRIFOLIUM REPENS L.) POPULATIONS

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SUMMARY

Three controlled climate rooms were used to determine the effects of varying temperature conditions on the growth and development of five white clover populations. Of particular interest were the adaptive responses of these clovers to low temperature (frost) conditions. Ten stolon morphological characters were measured at time intervals from tagged stolons. This enabled regression analysis to obtain polynomial functions describing the development pattern of these characters. Pooled environments ANOVA of data taken at the end of the growth periods, together with adaptation analysis, enabled evaluation of genotype-environment interactions for these characters.

The five clover populations had characteristic development patterns for the stolon DW components (stem, leaf, petiole and total DW) and leaf area within each of the three environments. All populations irrespective of origin, maintained similar growth rates within each environment which had the effect of producing 'parallel' curves. The initial placement coefficient consequently had a direct bearing on the size of the final stolon DW attained. The cold temperatures alone did not adversely effect or cause different growth responses of these five clover populations. Other climatic factors usually associated with cold temperatures such as short photoperiods and low light intensities may have collectively brought about differential responses of the clovers between environments.

Each of the clover populations developed a distinct stolon morphology regardless of the temperature conditions.

The Mediterranean clovers, Ladino and Spain consistently produced small numbers of stolons with large stems, leaves, petioles and leaf area in all environments. The North European clovers produced large numbers of stolons with small component parts. The heritability estimates for the DW characters were high as were the correlation coefficients between these characters. The negative correlations between the stolon DW components and the numbers of stolons which also showed low heritability estimates, collectively brought about the unpredictability of total plant DW production. The stolon characters leaf numbers, stolon length and stolon thickness showed largest variation between temperatures. All clovers produced similar numbers of leaves within each environment, but leaf production was greatest in the hot room. The clovers had long spindly stolons in the hot conditions but short thick stolons in the frost room which is probably an adaptive response to these conditions.

The changing pattern of total plant production between clover populations and environments appears to be the result of different stolon branching characteristics. Both maximum production under favourable environmental conditions and survival under harsh conditions, relies on a continual supply of new stolons. There was some evidence of different branching responses of the clovers in the three temperature conditions. The patterns, however, were not always clear which was probably due to the 'artificial' nature of the climate rooms where only one climatic factor varied.

The branching responses did indicate that the improved

autumn growth of Spanish clover (shown in field experiments) could be a consequence of increased stolon numbers in the earlier hot summer conditions. Spanish clover indicated specific adaptability to favourable environments, which occur in the moist autumn conditions of the Mediterranean. Ladino clover failed to produce many new stolons at all temperatures which undoubtedly affects the persistence of this clover. This has been found in field conditions when Ladino rarely lasts longer than a year and fails to survive through harsh winters. The temperate clovers, Kent and Huia showed increased branching in the cool temperatures like those of autumn and early spring in temperate regions. Improved stolon numbers at these times would ensure survival through the winter followed by rapid growth later in spring and summer.

All the populations achieved a certain degree of frost tolerance, the clovers from North Europe the most hardy and the Mediterranean clovers the least. One could speculate that under harsher environmental conditions, the Kent and Russian clovers may have achieved a greater level of frost tolerance while the Mediterranean clovers may have continued growth and suffered structural damage.

CHAPTER ONE

INTRODUCTION

The Role of White Clover (*Trifolium repens* L.) in Pastoral Agriculture

White clover is widely distributed throughout the temperate regions of the world where it is of major agronomic importance (Erith 1924; Martin 1960; Williams 1970; Levy 1970). The most important function of legumes in a pasture is their ability to fix atmospheric nitrogen through symbiosis with root nodule bacteria called Rhizobium (Martin 1960) and thus increase the potential pool of nitrogen for the pasture. Sears et al. (1953, 1965) estimated that approximately 410 kg/ha/year of nitrogen can be fixed by white clover under initial pasture development conditions in New Zealand. This nitrogen becomes available for associated grass growth through transfer of breakdown products of the clover shoot and root parts and transfer of nitrogen compounds through the grazing animal (Butler and Bathurst, 1956).

White clover is also important because of its high quality feed for grazing animals (Williams 1970). Compared with grass herbage, white clover has higher levels of protein, and minerals such as calcium and magnesium (Johns 1966). A low supply of these elements is frequently associated with metabolic disorders of animals. White clover herbage is also of high digestibility and acceptability by animals (Ulyatt et al. 1976).

Although white clover has become the most important pasture legume in New Zealand, its effective use is dependent on an adequate supply of nutrients in the soil (Sears 1953), effective symbiosis with R. trifolii (Munro and Hughes, 1966) and optimum climatic conditions (Mitchell, 1956). The environmental influences, particularly the limitations of cold temperature on clover growth, are the main interest in this study.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. PHYSIOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF
WHITE CLOVER2.1.1. Vegetative Characteristics

White clover has a prostrate, spreading habit of growth (Erith 1924; Leffel and Gibson, 1973). Following germination, the seedling develops a pair of cotyledons, a taproot, simple leaf and short main stem (Sanderson 1966). Compound trifoliate leaves, borne on upright petioles, are produced from nodes along the main stem and as the internodes do not elongate, the leaves become crowded (Spedding and Diekmahns, 1972). After 6-8 weeks, buds in the axils of these leaves on the main stem, develop into primary stolons which spread horizontally over the soil surface. The number of primary stolons developing from the main stem can vary greatly depending upon the environment and the genotype (Hollowell 1966). The main stem ceases development after stolon growth has begun.

The stolon, in contrast to the main stem, has elongated internodes and an apical bud that remains vegetative, continually producing new leaves. The nodes along a stolon seemingly act as crowns but produce only one adventitious root and trifoliate leaf on a long petiole at each node (Sanderson 1966; Chow 1967). In the axil of leaves on the primary stolon are buds which may remain dormant or develop into either a flower or a secondary

(branch) stolon. The secondary stolon develops in the same manner, producing trifoliolate leaves and roots at each node (Leffel and Gibson, 1973). These secondary stolons may produce further branch or tertiary stolons and thus by repeated branching, stolons spread in all directions and "fill" the pasture sward (Hollowell 1966).

2.1.2. Persistence of Clover Stolons

The initial seedling taproot is shortlived (Westbrook and Tesor, 1955) becoming unimportant and withered once the axillary buds of the main stem cease developing into primary stolons. The stolons are capable of developing and leading an independent existence, relying on nodal roots for continued growth (Sanderson 1966). Absorbed minerals from the roots and photosynthetic metabolites from the leaves are translocated toward the growing meristematic tissues at the tip of the stolon (Hollowell 1966). This translocation in one direction leads to deterioration and eventual death at the proximal end of the stolon. The destruction of roots and stolons through infection by pathogenic organisms is usually a secondary cause of plant death. The perenniality of white clover depends upon continual vegetative renewal at the stolon tip and production of viable, vigorous secondary stolons from the nodes along the stolon (Beinhart 1963; Spedding and Diekmahns, 1972; Hollowell 1966). The secondary stolons achieve physical independence when the primary stolon has decayed (Leffel and Gibson, 1973).

Perenniality is also dependent upon the proportion and rate of axillary buds developing into secondary stolons rather than flowers (Chow 1967). Since each node has only one axillary bud it gives rise to either a secondary stolon or an inflorescence. The formation of inflorescences is directly at the expense of secondary stolons so prolific flowering can lead to loss of vegetative persistence (Gibson 1957). Flowering depends on many factors among which genotype, stage of development, temperature and photoperiod are most important (Thomas 1962). In general, when the plant is flowering, not all axillary buds develop into flowers but there is an irregular development of both secondary stolons and flowers along the stolon.

2.2. ADAPTATION OF PLANTS TO THE ENVIRONMENT WITH PARTICULAR REFERENCE TO TEMPERATURE

The natural distribution of pasture species is largely determined by their adaptation to the major climatic and edaphic factors of the environment (McWilliam 1977; Mitchell 1956; Eagles and Ostgard, 1971). Temperature is always prominent in that it limits both distribution of plants and also the diversity of plant forms that can colonize a particular region (Langridge and McWilliam, 1967). Plants must be able to develop adaptive systems in response to seasonal variation in temperature to ensure their continued growth and survival.

2.2.1. Genetic Basis of Adaptation

An organism may adapt to a particular climate either by evolving a genetic system which produces stable phenotypes regardless of the prevailing environment or by a

system which develops phenotypes adjusted to the environment encountered (Langridge 1963). The genetic ability to resist injurious effects of an environment by systems in which the phenotype remains constant is called "homeostasis". Where the genotype adapts to environmental change by an alteration of the phenotype, the genetic phenomenon is one of "adaptive flexibility" (Langridge and McWilliam, 1967).

Homeostasis or often termed "buffering" of genotypes, ensures that the growth pattern predetermined by the genes is sustained even if environmental effects are tending to cause modifications (Langridge 1963; Allard and Bradshaw, 1964). This process applied to individuals ensures that each is adapted to a range of environments. Where there are a number of coexisting genotypes each adapted to a different range of environments, it is known as population buffering (Allard and Bradshaw, 1964). Outbreeding species such as T. repens carry considerable genetic variation in the heterozygous condition, which is manifested in a range of genotypes by segregation and recombination.

Finlay and Wilkinson (1963) used regression techniques to show that cultivars with high phenotypic stability (homeostasis) generally have low mean yields when tested in a range of environments. These genotypes produce such stable phenotypes that they are unable to exploit environments which enable high yields. A cultivar having general adaptability to several environments is usually one with high yields and average phenotypic adaptability (Ebeinhart and Russell, 1966). Allard and Bradshaw (1964) believe that cultivars which consist of a diversity of genotypes (population buffering) show general adaptability to many environments.

When the temperature changes markedly, buffering of the phenotype cannot be maintained. For survival, the genotype needs to be able to develop adaptive phenotypes (Langridge and McWilliam, 1967). Phenotypic modifications enable plants to survive unfavourable portions of the environmental cycle (e.g. cold winters) and to utilize efficiently the more favourable times for growth and reproduction. These responses to changing climatic conditions show continuous variation and are often polygenically controlled (Cooper 1963).

An adapted outbreeding population consists of many different genotypes or combinations of genes but all are phenotypically similar (Cooper 1963). If there is a slow progressive change in the environment such as the climatic transect from the Mediterranean to the Subartic circle, selective forces cause phenotypes more effectively adapted to the environment to predominate. This results in a readjustment of genotypes in the population. This is demonstrated by the tolerances of plant genotypes that form a "temperature cline" to continuously changing temperature environments (Cooper 1963; Langridge and McWilliam, 1967).

2.2.2. Physiological Basis of Climatic Response

Environmental factors which fluctuate seasonally include temperature, light intensity and photoperiod (Eagles 1967; Cooper 1964). These factors can influence growth through changes in photosynthetic activity, respiration, leaf initiation, distribution of assimilates and other processes which enable the plant to survive in a particular environment.

In temperate regions, plants are able to exploit a wide range of low to moderate temperatures for their CO₂ assimilation (Pisek 1973), but many species have a minimum temperature of -3°C to -5°C when almost all metabolic activity in the plant ceases (Langridge and McWilliam, 1967; Pisek 1973). Plant growth has been explained by physiologists in terms of concepts such as Net Assimilation Rate, Relative Growth Rate and Leaf Area Ratio. Black (1955) and Eagles (1967) defined relative growth rate (RGR) as the product of net assimilation rate (NAR) and the Leaf Area Ratio (LAR). NAR is the dry matter gain per unit of photosynthetic surface and LAR is the ratio of photosynthetic to total plant tissue. Cooper (1964), Eagles (1967), and Robson and Jewiss (1968) found that Mediterranean populations of Lolium, Dactylis, and Festuca had greater dry matter production (higher RGR) in autumn-winter in Britain than the North European populations. MacColl and Cooper (1964) showed that this ability to grow at low temperatures (5°C) was closely related to the winter temperature in the place of origin, but inversely correlated with percentage survival after freezing at -5°C.

Cooper (1964) and MacColl and Cooper (1967) postulated that the higher RGR of Mediterranean grasses compared to North European grasses was due to a greater rate of leaf expansion at low temperatures and there was little or no difference between populations in net assimilation rate. However, Morley (1958), Eagles (1967) and Eagles and Ostard (1971) working with Trifolium subterraneum and D. glomerata did find differences in NAR. The light

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environments were different between the two sets of experiments. MacColl and Cooper (1967) had low light intensities and eight hour photoperiods characteristic of an English winter. Eagles (1967) used high light intensities and sixteen hour photoperiods in controlled environment rooms. Net assimilation rate was shown to be positively correlated with light intensity (Black 1955; Blackman 1959) while LAR was negatively correlated. Thus, where there were high light energy inputs at low temperatures (5°C) there was a high NAR in the North European material, which outweighed the effects of its low LAR, resulting in a high relative growth rate. In low light intensities, the higher LAR of the Mediterranean populations outweighed the effect of its low NAR to give a higher RGR of the Mediterranean rather than the North European population.

When comparing populations of Festuca arundinacea, Robson and Jewiss (1968a) found that when the day temperatures were increased while night temperatures remained low ($18^{\circ}\text{C}/-3^{\circ}\text{C}$), growth of the North European material increased more than that of the Mediterranean material and the difference between them was eliminated (Robson and Jewiss, 1968b). They suggested that assimilates normally accumulate within North European material at low temperatures rather than being used for leaf growth. This accumulation restricts photosynthesis until temperatures rise again in spring. Thorne and Evans (1964) previously showed that an increased sink for assimilates increased the rate at which they were produced by preventing assimilates accumulating in the leaf.

The basic difference between climatic races of grasses in growth at low temperatures appears to be due to differences in the use or proportionate distribution of assimilates to the root and/or shoot. Mediterranean material at 5°C uses assimilates for expansion of new leaf surface as the environment is most favourable for growth at this stage (Cooper 1964). Williams and Hoglund (1978) demonstrated a similar response which occurred with a Spanish population of T.repens. However, the North European material at 5°C uses less assimilates for leaf production and an increasing proportion of carbohydrates is diverted to roots and shoot bases (Eagles 1967). This accumulation of carbohydrates at low temperatures contributes to the greater cold hardiness of Northern material (Cooper 1964; Robson and Jewiss, 1968; Lorenzetti et al. 1971; Thomas and Lazenby, 1968). In harsh climates this reserve carbohydrate is re-mobilized in the spring and used to produce photosynthetic tissue.

These differences between Mediterranean and European populations in seasonal growth and winter hardiness are related to the climatic conditions of the areas in which they have evolved (MacColl and Cooper, 1967; Robson and Jewiss, 1968). Selection occurs for active winter growth in Mediterranean regions but is for winter hardiness in northern climates, while in cool temperate regions no strong adaptation mechanism for either winter growth or winter dormancy has occurred. The possibility of combining the active winter growth of Mediterranean material with increased cold hardiness

depends on how far these characters are physiologically related (Robson 1967; Cooper 1964). The association between leaf expansion at low temperature, respiration and carbohydrate accumulation in these climatic races suggests that winter growth and frost susceptibility may be physiologically interdependent and therefore difficult to separate (Morley et al. 1957).

2.2.3. Adaptive Variation

Environmental factors such as temperature, light intensity and day length initiate developmental responses in plants which fit the plant for survival in its particular environment. Levins (1969) expressed the view that plants must have the ability to survive the extreme environments ("resistance adaptation") as well as the ability to grow, metabolize and develop ("capacity adaptation"). The relative importance of these two types of adaptation will depend on the environmental extremes to which the plant is exposed (Eagles and Ostgard, 1971).

The distribution of forage species including T. repens, from the Mediterranean along the Atlantic coast to the continental regions of North Europe, show a pattern of climatic variation (Cooper 1963). In the Mediterranean environment, winter is the most favourable growing season, and MacColl and Cooper (1967) found that forage species such as Festuca, Lolium and Dactylis show better growth production at low temperatures (capacity adaptation). The adaptation to cool season growth is also shown by T. repens and a Spanish population has shown outstanding autumn-winter growth in New Zealand (Barclay 1960; Williams and Barclay, 1975). These Mediterranean

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populations show resistance adaptation during summer when drought conditions make it unfavourable for growth (Williams et al. 1977).

At the other extreme, populations of these same species in Northern Europe show quite a different seasonal growth pattern. Winter cold is the main limiting factor and species require resistance adaptation (eg. frost tolerance) to survive (Lorenzetti et al. 1971; Thomas and Lazenby, 1968; Robson and Jewiss, 1973). Growth is poor at low temperatures but reaches high levels during summer with higher temperatures and light intensity. In the intermediate temperate regions such as New Zealand and southern Britain where neither winter cold nor summer drought is limiting, the growing season is longer and strong adaptation mechanisms are not apparent.

2.3. DISTRIBUTION AND ADAPTIVE VARIATION IN WHITE CLOVER

T. repens is a temperate species, widely adapted to regions from the sub-arctic to the sub-tropics (Williams 1970). The centre of origin, and region of greatest diversity, is the Mediterranean (Vavilov 1952). The plant is indigenous to the whole of Europe and Central Asia and has spread with western civilisation into Eastern Asia and America (Daday 1958). The early settlers introduced the species to Australia and New Zealand. In New Zealand it has become the most important pasture legume (Davies and Levy, 1931). White clover is ideally suited to the temperate climate and even rainfall which occurs in New Zealand (Levy 1970) and well adapted ecotypes have developed (Davies and Levy, 1931).

2.3.1. Morphological Adaptations

Characteristic morphological patterns are associated with the climatic adaptations of white clover. The Mediterranean region offers a diversity of suitable habitats for white clover whereas Northern Europe is marginal for the species and adaptation to environmental factors, particularly low winter temperatures dominates the morphological structure of the populations (W. Williams, unpublished).

Many Mediterranean populations are winter active, but summer dormant due to high temperatures and drought which occur during that season. Some populations are large-leaved, tall (long petioles) and thick stemmed, while in dry areas with hard grazing, only small-leaved types persist (Davies and Young, 1967). Variability declines with movement from the centre of origin so that natural populations from Northern Europe are uniformly prostrate, small-leaved and thin stemmed with many fine branched stolons (Daday 1954; Davies and Young, 1967). In field trials, Davies (1958) found that populations adapted to harsh conditions tended to be small-leaved and less productive than the large-leaved clovers. However, the small leaves and thin stems are associated with increased production of branch stolons and the resultant high numbers of active meristems, ensures improved vegetative persistence (W. Williams, unpublished).

2.3.2. Ecotypes of White Clover

In conjunction with the variation of plant types associated with the temperature cline, is a discontinuous distribution of ecotypes which have evolved as adaptations to local conditions (W. Williams, unpublished; Hill 1975). These ecotypes are all genetically different. Some of these are discussed in the following.

2.3.2.1. Ladino Clover

'Ladino gigante lodigiano' is an ecotype from the Lodi district in Northern Italy (Leffel and Gibson, 1973). It is adapted to mild winters and hot summer conditions but is unable to withstand continual periods of moisture stress or hard grazing. Under these conditions it behaves as an annual (Leffel and Gibson, 1973; Gibson et al. 1963). Many selections from this ecotype have been made and are commonly known as 'ladino' white clover. Gibson et al. (1963) found that ladino generally lacked persistence due to poor stolon branching in summer conditions and a possible lack of winter hardiness in northern parts of America. Under conditions in Britain, Davies (1958) suggested that the poor performance of ladino was due to its failure to over-winter satisfactorily resulting in loss of persistency.

2.3.2.2. Mediterranean

These ecotypes are generally tall, large-leafed with thick stolons (Davies and Young, 1967) and have adapted to winter growth when temperatures are cool as this is the only period when moisture levels are adequate (Barclay 1963; Williams and Barclay, 1975.) The potential

of pasture plants from the Mediterranean for improvement of winter growth in maritime temperate regions has been realised in many species (Corkill 1945; Barclay 1960; Cooper 1964; Eagles 1967). Barclay (1960) found a population of white clover from Valencia, Spain which had superior winter growth to New Zealand white clover (Grasslands Huia). This led to a hybridization programme between selected Spanish plants and elite New Zealand clover plants (Barclay 1960, 1963), resulting in a winter active cultivar, Grasslands Pitau (N.Z. x Spain) x N.Z.

The New Zealand and Spanish populations have similar annual productivity but the Spanish seasonal growth cycle is displaced two months behind that of New Zealand (Williams and Barclay, 1975). This superior winter growth of the Spanish population is most evident in the northern parts of New Zealand, but in the southern climatic regions of New Zealand, the Spanish population was only slightly better in autumn (Barclay 1960; Williams and Barclay, 1975).

Pasture evaluation with the cultivar Grasslands Pitau, showed that the yield advantages over Grasslands Huia were greatest in Northland and declined with increasing latitude (Lambert et al. 1969; Weeda et al. 1969). Harris et al. (1973) found that Huia-based pastures had higher total yield and similar clover content to Pitau based pastures in spring-summer and the yield of Pitau not much greater than Huia in summer-autumn and therefore suggested there was no advantage of Pitau in the Southland region. The situation is complex and other environmental factors such as frost tenderness and/or stolon branching

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characteristics may influence the growth of white clover and accentuate the asynchrony of seasonal growth patterns of the Spanish, New Zealand and hybrid clover populations (William and Barclay, 1975; Williams and Hoglund, 1978).

2.3.2.3. New Zealand Ecotypes

A large amount of variation among populations of white clover has developed throughout New Zealand. Davies and Levy (1931) classified six major types, including imported strains and ecotypes which had developed as ecological adaptations to environmental conditions in New Zealand. The bulk of the imported seed was of lax Dutch type of white clover (Davies and Levy, 1931) which is short-lived and low producing. In contrast were the ecotypes, New Zealand 'Wild white No. 1'; a persistent, highly productive, large-leaved clover with strongly spreading stolons and New Zealand 'Wild white No. 2'; a vigorous, small-leaved, thin stoloned, dense clover adapted to closely grazed pastures (Davies and Levy, 1931; Corkill 1952). It is unlikely that these ecotypes originated from the Dutch clover but probably developed from clover lines imported to New Zealand from other countries, and became ecologically adapted. For instance, there are strains of clover in Western France which have similar morphological and cyanogenetic properties to the New Zealand 'Wild white No. 1' (Corkill 1952).

Following the classification of the white clover types, a scheme was begun to identify and certify areas producing the New Zealand 'Wild white No. 1' type

(Corkill 1949). The certified non-pedigree strain had a 50% improvement of production over the uncertified New Zealand strain. Pedigree strains bred from superior selections of this New Zealand type 1 gave a further 15% improvement. Additional breeding work based on selections from the best ecotype material (New Zealand 'Wild white No. 1' and New Zealand 'Wild white No. 2') resulted in the production of a synthetic variety of white clover. This variety (Grasslands Huia) has improved winter and summer production over the older certification types (Barclay 1960).

2.3.2.4. British Ecotypes

A wild form of white clover is abundant in Britain and exists under almost all the climatic and soil conditions that are to be found (Williams 1945). It is a prostrate, small-leafed form of clover with thin stolons and many branches which makes it extremely persistent. It has become ecologically adapted to continuously grazed pastures and seed from these old permanent pastures is known as English wild white (Williams 1945; Hawkins 1960). A particularly small-leafed, dense clover is found in southern England and is known as Kent wild white (Hawkins 1960).

The English wild white is different in morphology and persistency from Dutch white clover which was imported and used extensively in Britain in the early 20th century (Williams 1945). A certification scheme was commenced in 1930 to ensure the isolation and purity of the wild white clover. Because of the great variation found among individuals of English wild white clover, a more

uniform, pedigree strain, S 184 was produced (Williams 1945). This cultivar was based on the denser more vigorous plants derived from the various wild populations of clover native to Britain.

The variety, S 100 bred at Aberystwyth (Williams 1945) is a larger leaved variety based upon individual plants selected from New Zealand white clover, Dutch white and English wild white clover. This variety is higher producing than wild white on developed soils under good management but not as persistent on poorer more exposed soils (Williams 1945). Seventy-five percent of the annual production of wild white clover occurs in the three summer months (Ollerenshaw et al. 1976), whereas S 100 has a longer growing season, beginning growth earlier in spring and under favourable conditions extending into early winter.

2.4. GROWTH PROCESSES IN WHITE CLOVER

2.4.1. Effect of Temperature on White Clover Growth and Development

As described earlier, an individual white clover plant is made up of primary, secondary and tertiary stolons, each capable of developing and leading an independent existence (Sanderson 1966). Each stolon produces nodal roots and leaves to ensure continued growth. From controlled environment studies, Mitchell (1956) found that the stolon population of young plants growing in pots was unstable and therefore believed it more important to consider the individual rooted stolon of white clover as the basic productive unit, rather than the total production from all stolons.

Beinhart (1962, 1963) showed a close relationship between the environment and the meristematic activity, leaf area and growth of ladino clover plants. Maximum dry weight production of clover plants was associated with highest total leaf area and he concluded temperature modified growth rates of white clover by influencing leaf area production. Both Mitchell (1956) and Beinhart (1963) considered the two important morphological responses affecting the growth of clover to be the rate of leaf production by active meristems and the rate at which secondary meristems become active and produce branch stolons.

2.4.2 Stolon Growth and Development

Much of the work relating to the environmental effects on white clover growth has been carried out on New Zealand white clover (Mitchell 1955, 1956, 1960, 1962; Brougham 1958, 1962) and ladino white clover (Beinhart 1962, 1963, 1964).

The optimum temperature for individual stolon growth is approximately 24°C and growth is reduced at temperature extremes (Mitchell 1956; Beinhart 1962) with little stolon elongation below 5°C. This slow growth of clover at low temperatures could be due to its own physiological limitations or to the inactivity of the resident *Rhizobium* (Davies and Young, 1967; Martin 1960). Gibson (1963) found with subterranean clover that growth of clover at 5°C was much reduced regardless of whether the plant was depending on N fixation or fertiliser N. Scott (1970) measured the net photosynthesis (CO_2 gained in photosynthesis minus that lost in respiration) and found maximum rates occurred at 20°C. If the light intensity increases, the temperature range most suitable for CO_2 uptake is

displaced upwards (Beinhart 1962; Pisek 1970) but becomes stationary at 30° - 35° C when light levels approach saturation point.

2.4.3. Leaf and Petiole Growth

Leaves originate immediately behind the apices of the stolons where they are closely crowded encircling each other and the growing point. When the leaves have fully developed within the growing point, the petiole begins to elongate. Growth occurs first in the leaf blades, then petioles and finally the stem internodes (Mitchell 1955).

Brougham (1958, 1962) showed that leaf unfolding and petiole elongation were determined by the light environment. Leaf development of white clover ensures a state of dynamic equilibrium between the environment and leaf growth, such that all light is continually intercepted by active photosynthesizing leaf (Brougham 1962). Following defoliation, the first crop of new leaves form a canopy which intercepts a considerable amount of light. Leaves developing later are then subjected to a low light intensity and petiole elongation continues until the laminae of these leaves have penetrated the existing canopy to reach full light. Photosynthesis then becomes the main activity (Brougham 1962).

Gross photosynthesis in white clover increases with leaf area until all the incident radiation is absorbed and then remains constant irrespective of further increases in LAI (Brougham 1958b; Brown and Blaser, 1968; Donald 1962). In these studies, respiration rate was assumed to be proportional to leaf area and independent of gross photosynthesis with the result

that net photosynthesis passed through an optimum value then declined with increasing LAI. If the yield or growth rate was defined as dry weight of living material only, then a sigmoid growth curve was obtained (Brougham 1960). However, McCree and Troughton (1966) with controlled environment experiments showed that respiration rate was determined mainly by the gross photosynthetic rate and only partly by the heavily shaded tissue. Thus, both photosynthesis and respiration increased with leaf area until there were sufficient leaves to absorb all the incident radiation. The growth rate then remained constant if all the material, dead and living, was collected (Troughton 1971).

The processes of cell division and elongation in the leaves are temperature dependent, so that an increase of 6°C in air temperature from winter to spring is paralleled by an eight fold increase in the rate of cellular activities (Brougham 1962). At optimum temperatures ($20\text{-}25^{\circ}\text{C}$), the rate of leaf appearance is about 1.5 leaves/week (Mitchell 1960). The changing of the growth processes involved with the development of the leaf canopy are largely dependent on changes in the light environment (Brougham 1962), whereas temperature determines the rate of cell division and expansion of the developing leaves.

Temperature influences the morphology of the leaves as well as the rate of leaf production. In full light, temperature had little effect on leaf size of Grasslands Huia, but with decreased light intensity the leaves at 12°C were much larger than those at 22°C (Mitchell 1955). The importance of leaf size and weight is stressed when

comparing different genotypes of white clover. Williams and Hoglund (1978) compared Spanish and New Zealand white clover over a range of temperatures. At low temperatures ($12^{\circ}/6^{\circ}\text{C}$) both genotypes produced similar numbers of leaves, but the Spanish clover had 30% superior leaf DM production. The probable reason for this superiority of the Spanish material at low temperatures, is its ability to produce a higher proportion of total weight as leaf weight and thus have a photosynthetic advantage (Williams and Hoglund, 1978). Cooper (1964) and Eagles (1967) showed similar results with L. perenne and D. glomerata at low temperatures. Mediterranean material used assimilates in the expansion of leaf material whereas continental material had less leaf expansion and stored assimilates for later spring growth. At intermediate temperatures ($22^{\circ}/14^{\circ}\text{C}$), New Zealand clover had a greater leaf production rate than Spanish clover resulting in superior leaf DM production even though the Spanish clover had larger leaves (Williams and Hoglund, 1978).

2.4.4. Growth of Axillary Buds and Secondary Stolons

The number of stolons developing from secondary meristems in the axil of leaves is dependent on light intensity and temperature. Primary leaf production on a single stolon increases with increase in temperature (Mitchell 1956) but activation of secondary meristems to produce branch stolons in ladino white clover showed an inverse relationship with temperature (Beinhart 1963). At 10°C , branches emerged from 71% of primary nodes, whereas at 30°C only 14% of axillary buds developed branches. Mitchell (1961) obtained similar results with New Zealand white clover where tillering was stimulated by both short days and low temperatures.

Light intensities have no effect on the rate of primary leaf production from a single stolon (Mitchell 1956; Brougham 1962) but do affect the rate of branching (Beinhart 1963). High light intensities increased branching and together with low temperatures, resulted in maximum axillary bud development into new stolons. However, at 10°C, high branching percentages did not offset the low rate of primary leaf production. Maximum leaf production from a whole plant occurred between 17°C and 23°C where moderate meristem activity was coupled with moderate branching percentages (Mitchell 1956; Beinhart 1963).

In the comparison of Spanish and New Zealand white clovers under controlled environment conditions, Williams and Hoglund (1978) found that the dry weight (DW) production per stolon of Spanish clover was superior at low temperatures but the Spanish plants produced fewer stolons resulting in less total production than the New Zealand clover. It appears that superior growth per stolon corresponds to inferior branching and vice versa.

The varying development patterns of stolon DW and stolon branching shown by different clover genotypes, contributes to the seasonal production patterns found in the field. Spring and autumn are periods characterised by active branching of ladino and New Zealand white clover (Beinhart 1963; Williams and Hoglund, 1978) resulting in great increases in the total number of active meristems. This becomes manifested in high leaf areas and growth rates of stolons during the following summer period. However under controlled environment conditions, Williams

and Hoglund (1978) showed evidence of Spanish clover actively branching at high temperatures. They suggested that improved stolon branching in summer coupled with superior growth per stolon under cool conditions resulted in the improved autumn-early winter growth shown by Mediterranean clovers. More information is needed on the growth and development characteristics of a wide range of white clover ecotypes under different environmental conditions.

2.5. CYANOGENESIS IN WHITE CLOVER

The term cyanogenesis describes the release of hydrocyanic acid (HCN) when leaves of white clover are damaged (Hughes 1969). This release is due to the hydrolysis of a glucoside containing cyanide by an enzyme, β -glucosidase (Melville and Doak, 1940). White clover contains two cyanogenetic glucosides (80% lotaustralin, 20% linamarin) and the enzyme linamarase (Coop 1940). Genetical studies by Corkill (1942) have shown that two independent gene loci determine the production of glucoside and enzyme. Lotaustralin and linamarin are homologues and are governed by alleles of a single locus designated Ac/ac. The presence or absence of linamarase is governed by alleles of an independent locus designated Li/li. Only plants which possess dominant alleles of both genes, liberate HCN when damaged.

There are four possible phenotype-genotype combinations:

- 1) Glucoside and enzyme (AcLi) cyanogenetic
- 2) Glucoside only (Acli) acyanogenetic
- 3) Enzyme only (acLi) acyanogenetic
- 4) Neither glucoside or enzyme (accli) acyanogenetic

However, there is considerable variation in cyanogenic activity and many workers believe there are modifying genes making it a quantitative polygenic characteristic (Maher and Hughes, 1973). The significance of HCN production by clover is not yet understood but may be important in nitrogen metabolism or serve as a protective substance against herbivorous animals.

Daday (1954a, 1954b) studied the geographic distribution of the glucoside and enzyme genes in Europe. Mediterranean populations consist predominantly of Ac Li (cyanogenic) individuals whereas many populations from the colder regions of Northern Europe are ac li (acyanogenic). A decrease in winter average temperature is associated with a reduction in the dominant gene frequency of the glucoside and enzyme (Daday, 1954a, 1954b).

In the indigenous regions of white clover (Mediterranean and Europe), the gene distribution of glucoside and enzyme ranges from 100 to 0% in association with decreasing winter temperatures. Natural selection has produced a balanced equilibrium in these populations (Daday 1958) whereas in recently colonized regions such as New Zealand, the gene frequencies exhibit an irregular pattern.

The dominant alleles are frequent in warm climates but rare in cold environments. Daday (1965) found that the enzyme linamarase is activated by cold temperature and the HCN liberated caused an irreversible inhibition of the respiratory system of the Ac Li genotype resulting in tissue death (Davies and Young, 1967). Daday (1965) suggests that during the evolution of T. repens, polygenes enhancing fitness at high or low temperatures have become linked with the dominant and recessive alleles respectively.

The environmental factors influencing the distribution of the Ac and Li genes is complex but it appears to be advisable to include the genes in warm climate cultivars of T. repens and exclude them from cultivars intended for cooler climates (W. Williams, unpublished).

2.6. COLD TOLERANCE OF WHITE CLOVER

2.6.1. The Effect of Freezing on Plant Cell Processes

Initially, lowering the temperature of cells, slows metabolic processes (Levitt 1956; Pisek 1970; Heber and Santarius 1973). Hardy and non-hardy plants of temperate climates tolerate exposure to temperatures round the freezing point but when ice formation occurs, the non-hardy plants are killed (Levitt 1956). Ice formation occurs in the intercellular spaces, as the water outside the protoplast has a lower concentration of solutes (Levitt 1956; Heber and Santarius, 1973). If freezing is rapid and there is a drop to very low temperatures, intracellular ice formation can occur. This causes death by disruption of structural elements within the cell. Plant survival following intercellular ice formation depends on the hardening properties of the individual involved (Levitt 1956). Hardening is defined as the sequence of cellular processes or metabolic changes which transforms sensitive cells into hardy cells (Levitt 1956; Dexter 1956; Smith 1964; Heber and Santarius, 1973).

2.6.1.1. Mechanisms of Injury

Plants appear to be insensitive to changes of temperature per se. Freezing injury is not a temperature effect but is linked to protoplast dehydration caused by ice formation (Levitt 1956; Dexter 1956; Heber and

Santarius, 1973). Levitt (1956) suggested that the dehydration accompanying freezing led to cell contraction, and the deformation of the shrunken cell caused tension in the protoplasm which resulted in mechanical damage of organised structures such as membranes. An alternative proposal on freezing injury (Levitt 1972) involved the formation of disulphide bonds between adjacent proteins in membranes during cell dehydration. Following thawing, when water uptake causes cell expansion, the proteins are unable to break apart and the secondary structures of the proteins break down. The resulting protein denaturation causes irreversible damage to the membrane structures of cell organelles.

Heber and Santarius (1973) considered the most important factor involved in freezing injury to be the solute concentration inside the cell. This reached abnormally high levels during freezing and Heber (1967) showed membrane inactivation brought about by membrane toxic compounds. Heber and Santarius (1973) suggest that in the course of dehydration, the salt concentrations within the cell rise to toxic levels causing structural alterations between membrane components and consequent permeability changes. These are three possible processes involved with freezing injury but the mechanisms responsible are not yet clear.

2.6.2. Development of Frost Hardiness

2.6.2.1. Environmental Factors

The major climatic factors involved in the development of hardiness appear to be daylength and temperature

(Dexter 1956; Lorenzetti et al., 1971). In the natural environment, hardiness develops most rapidly under conditions of shortening days and decreasing temperature, which exist during autumn in temperate regions (Levitt 1956; Smith 1964; Rowley 1975, 1976). The hardening process involving metabolic changes must occur before even the most hardy plants become fully tolerant of cold temperatures. Many temperate pasture species require approximately 14 days hardening at 2°C to achieve near maximum cold tolerance (Hacker et al. 1974; Rowley 1976).

The hardiness responses are affected both by the developmental stage of the plant which varies throughout the year (Levitt 1956; Lorenzetti et al. 1971) and the interrelated environmental factors. During the pre-hardening period, light is important for photosynthetic accumulation of reserves which seems to be essential to plants for cold tolerance (Smith 1964; Rowley 1976). However, Sjoseth (1964) found with T. pratense that as the hardening period lengthened, increase of cold hardiness was associated with decreases in temperature, light intensity and photoperiod. As a general rule, those environmental factors most favouring rapid plant growth appear least to favour cold tolerance (Cooper 1964; Morley et al. 1958; Thomas and Lazenby, 1968a; Breeze and Foster, 1971).

2.6.2.2. Cellular Processes affecting Frost Hardiness

Metabolic changes which include alterations in intracellular concentrations of cell constituents such as sugars, amino acids, organic acids and proteins cause the physical modifications resulting in increased hardiness.

Increasing sugar concentrations is regularly associated with increasing frost hardiness (Levitt 1956; Dexter 1956; Smith 1964). Consequently light is necessary for synthesizing transportable compounds such as carbohydrates which act as protective agents within the cell. Levitt (1956) and Parker (1963) suggested that accumulated carbohydrates such as starch were converted to sugars during the winter months to maintain high concentrations of protective agents in the cell. However, Lawrence et al. (1973) suggested that a high soluble carbohydrate content in L. perenne may simply be due to accumulation of assimilates at low temperatures.

With D. glomerata, Eagles (1967) found that in a winter dormant and very cold-hardy Norwegian population, photosynthesis continued at moderately low temperatures but the use of assimilates for leaf expansion almost ceased, resulting in the accumulation of soluble carbohydrates in the shoot bases and roots. By contrast, in a winter active but cold susceptible population of Dactylis from Portugal, the assimilates produced were used for active extension growth with little or no accumulation of soluble carbohydrates. Lorenzetti et al. 1971; Breeze and Foster, 1971; Thomas and Lazenby, 1968a all support the view that high levels of carbohydrate reserve are associated with cold hardiness in plants. Organic acids, amino acids and soluble proteins increase in concentration during frost hardening and it is difficult to determine whether or not these compounds are involved in protection (Heber and Santarius, 1973). It appears that almost every group of cell constituents is in some way related to hardiness.

2.6.2.3. Mechanism of Frost Hardiness

Heber and Santarius (1973) postulated that increased frost hardiness is associated with the formation of protective compounds in the cell. Interaction between membranes and toxic cell compounds has been shown to decrease membrane stability and lead to membrane inactivation (Heber 1967). Lovelock (1954b) believes that the colligative action of protective substances such as sugars produced during hardening, decreases the concentrations of toxic cell components resulting from cell dehydration during freezing. There is also evidence that protective substances not only have a colligative action on toxic components but there is a direct interaction of protectants and membranes which leads to membrane stabilization (Heber and Santarius, 1973).

According to Levitt (1972), the main mechanism of protection is the prevention of the oxidation of proteins and formation of disulphide bridges during freezing. Protectants produced during the hardening period prevent neighbouring proteins from becoming linked.

2.6.3. Methods of Testing For Frost Hardiness

Dexter (1956) and Smith (1964) state that the most common method of testing for winter hardiness is in the field. Those plants which survive and persist until spring are considered winter hardy. However, continual fluctuations in weather patterns and complex interactions between climatic variables, plant growth and crop management make it necessary to test over several years before adequate results are obtained (Lorenzetti *et al.* 1971). This has led to the use of controlled environments and

specially designed freezing chambers (Dexter 1956; Wit 1952; Smith 1964; Lorenzetti et al. 1971) to assist in the evaluation of cold tolerance in plants.

Controlled environments allow plants to be grown under accurately prescribed environmental conditions. Lorenzetti et al. (1971); Thomas and Lazenby (1968) and Lawrence et al. (1973) indicated the importance of temperature, light intensity and photoperiod during the growth and hardening periods and these factors had to be taken into account when considering the cold tolerance and winter hardiness of L. perenne populations. Dexter (1956) stressed that any method of assessing cold tolerance in controlled environments must have close agreement with patterns of field and cabinet survival. Failure to create exact reproduction of the field environment is not important provided that the conditions used produce results comparable to field survival.

Assessment of frost tolerance can be estimated by plant survival, percentage tiller survival, degree of leaf damage, yield of herbage or the vigour of renewed growth following freezing (Dexter 1956; Thomas and Lazenby, 1968; Lawrence et al. 1973). Dexter et al. (1932) demonstrated a correlation between frost resistance and the amount of electrolytes and other substances released from injured tissue. This has led to the electrical conductance technique of evaluating injury after freezing (Dexter et al. 1932; Graham and Daday, 1960; Rowley 1975). When cells are injured, membranes lose their semi-permeability so that electrolytes leach from the injured tissues into a surrounding solution. The concentration of electrolytes

in the resulting solution is determined by electrical conductivity measurement. The higher the conductivity reading, the greater the number of cells have been damaged and the greater the frost injury. This technique assesses the freezing sensitivity of leaves but not the overwintering ability or growth at low temperatures. It is especially effective for comparative studies between genotypes of a species or between species for their frost tolerance. Bula and Smith (1954) have obtained close agreement between this method and field assessment of frost hardiness of various legumes.

2.6.4 Cold Tolerance and Winter Hardiness of Trifolium Repens

Smith (1949) carried out some early experiments on the cold tolerance of ladino clover and common white clover, an ecotype found in the humid eastern half of North America. Both clover types were placed in a freezing chamber at -3°C and also encased in ice, following field hardening. Neither clover suffered any injury in air temperatures of -3°C but the ladino clover was injured more severely when encased in ice. Apparently the higher level of metabolic activity during the winter of the larger, more fleshy stolons of ladino clover caused a toxic accumulation of CO_2 within the ice. Ronningen (1953) and Knight (1953) studied the overwintering ability of various clover types in the field. Common white clover with its close reticulate stolon growth habit, showed less winter injury than those with an open, radiating growth habit such as ladino clover. It seems that the smaller, multi-branched stolons result in high numbers of active meristems and increased protective leaf cover which enhances the common white clover's winter survival.

Ruelke and Smith (1956) showed that the cold tolerance of common white clover stolons developed more rapidly and reached a higher level than in ladino clover stolons, although the trends were similar. This cold tolerance developed as soon as temperatures began to decline. A high level of tolerance was maintained during the winter months but began to decrease as soil temperatures rose in spring. It has been shown that carbohydrate content in plants is associated with cold hardiness (Smith 1964). The principal organs for carbohydrate storage are the stolon stems and crowns of ladino and common white clover. For a plant to maintain hardiness throughout winter there needs to be a slow conversion of available carbohydrate into protective sugars (Levitt 1956; Wood and Sprague, 1952; Ruelke and Smith, 1956). Kilpatrick et al. (1966) and Sprague (1956) demonstrated the importance of rate of cooling and thawing on the survival of various legumes including white clover. Stolons, roots and crowns which were cooled slowly withstood lower frosting temperatures without injury than tissues cooled rapidly.

Lorenzetti et al. (1971) and Breese and Foster (1971) studied the role of cold tolerance in the adaptive differentiation of climatic populations of L. perenne. The populations fell into three main groups according to their regions of origin, with North European varieties being most cold resistant and South European varieties the least. In the extreme environments such as Northern Europe, growth is limited by temperature and plants become winter dormant to survive the harsh winter conditions (Robson and Jewiss, 1968; MacColl and Cooper 1964). Similar cold tolerance studies would be most

beneficial in T. repens.

2.7 Summary

The distribution and adaptive variation of white clover has been outlined in this review. A diversity of ecotypes with a wide range of physiological and morphological adaptations have evolved in response to contrasting environmental conditions. Of particular interest is the adaptive responses of clover ecotypes to low temperature (frost) conditions.

The production from individual rooted stolons and the branching characteristics were considered important morphological responses affecting the adaptation of white clover. Emphasis has been placed on the effects of temperature but other environmental factors such as light intensity and photoperiod have interacting influences. Further information on the growth and developmental processes involved with the adaptive differentiation of climatic populations of T. repens would be most beneficial.

Objectives

The objectives were:

- (1) To determine the limitations of low temperature conditions (particularly frost) on the growth characteristics of five contracting white clover populations;
- (2) To compare morphological characters and developmental patterns among the five populations;
- (3) To identify some characters associated with adaptation of clover to cold climates and
- (4) To observe adaptability in white clover with respect to large changes in temperature.

CHAPTER THREE

METHODS AND MATERIALS

3.1 EXPERIMENTAL

3.1.1 White clover populations

The white clover populations represented a geographical distribution from the Mediterranean to sub-arctic regions. Each of the populations was regarded as a random sample of the selected genotypes from each of the climatic regions. A description of the populations is given in Table 1.

3.1.2 Establishment Period

Seeds of each clover population were germinated in petri dishes in an incubator at 25°C. After two days, four seedlings were placed (on a 6cm grid) in 4.5 litre pots containing sterilised sand. 54 pots of each clover population were sown making a total of 270 pots. The seedlings established in a glasshouse (25°C max., 15°C min.), for 54 days before transfer to the controlled environment rooms.

During establishment, the plants received daily watering with a half strength Hoglund nutrient solution minus nitrogen. This was further diluted 200 fold (see Table 2). Approximately 200ml of nutrient solution per pot per day were applied.

A mixture of four Rhizobium trifolii strains was applied a week after planting. These included:-

- (i) TAI, a strain from Tasmania which is effective on red, white and subterranean clover.
- (ii) 561 an isolate from Australia found to be effective at high altitudes in the Snowy Mountains.
- (iii) 560 an isolate from Tara Hills, Central Otago.

TABLE 1

The five clover population

<u>Population</u>	<u>Origin</u>	<u>Description</u>
1. Spanish	Valencia, Spain Latitude 38°N	Spanish ecotype, C1067(Williams and Barclay, 1975) Mediterranean climate low altitude, low rainfall.
2. Ladino	Lodi valley, Northern Italy Latitude 45°N	Lodigiano Lodi, C1726 Mediterranean valley with cold winters Climate transitional between mediterranean and continental.
3. Grasslands Huia	New Zealand Latitude 40°S	Synthetic cultivar (Barclay, 1960) Temperate climate, Low altitude, maritime
4. Kent wild white	Britain Latitude 52°N	British ecotype Temperate climate Low altitude, maritime
5. Russian ML - 48 - 65	Canada Latitude 57°N	Composite of Russian lines selected at Melfort, Canada extreme continental climate.

TABLE 2Concentrations of nutrients supplied

Major Elements	mg/l	Minor Elements	mg/l
P	15.5	Fe (EDTA)	2.5
K	119	B	0.25
S	68	Mn	0.25
Mg	24	Cu	0.01
Ca	100	Zn	0.025
Na	11.5	Mo	0.005
Cl	195		

TABLE 3Temperature Conditions(Day/Night) $\pm 0.5^{\circ}\text{C}$

HOT	24/14
COOL	12/6
COLD	12/-2

- (iv) 562a, isolated from white clover on Mt Holdsworth in the Tararua Mountains.

Nodules had formed on the roots of each white clover population three weeks after planting.

Following the establishment period in the glasshouse all pots were transferred to the growth rooms and allowed to acclimatize for 21 days. However, due to variation in plant growth between pots within populations, a graded series of pots was allocated to each room. This ensured that some good, intermediate and poor pots of each population were represented within each block in each growth room.

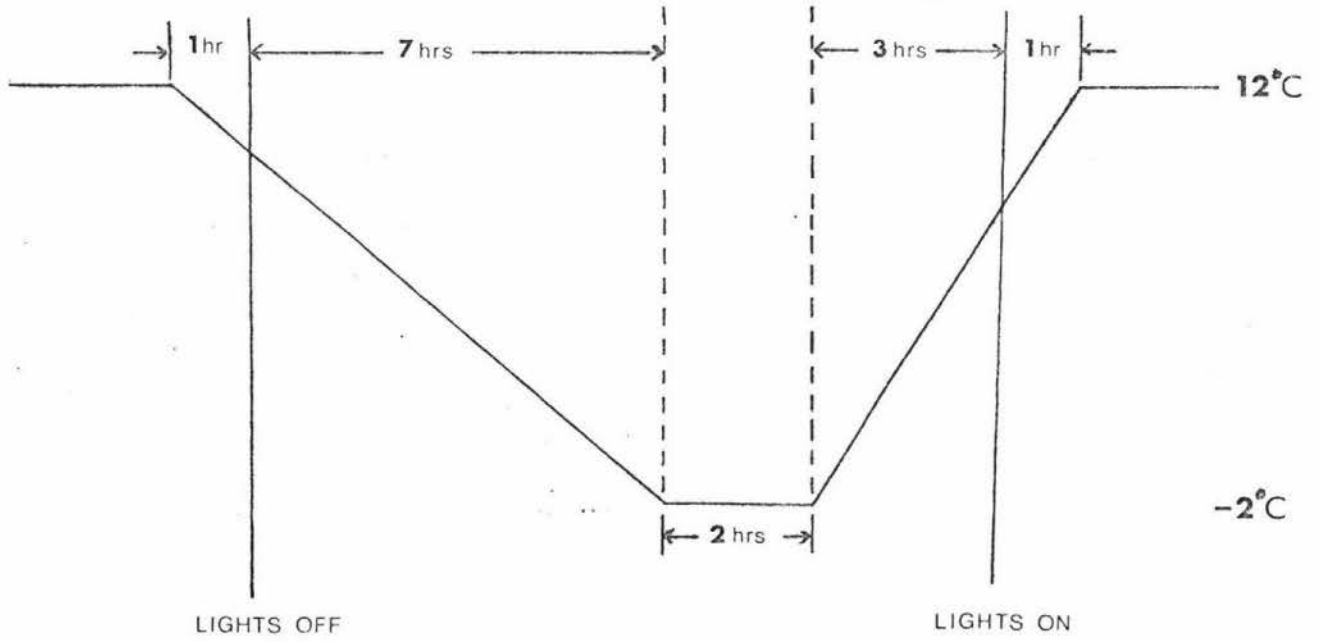
3.1.3 Controlled Environment Rooms

The experiment was carried out in three climate-controlled rooms. The temperature conditions are described in Table 3.

The hot environment provided temperature conditions near optimal for white clover growth (Mitchell, 1956) and the cool temperature was typical of autumn or early spring in temperate regions. The cold environment subjected plants to frost conditions so as to evaluate the cold tolerance of these five white clover populations.

Light intensities and daylength were similar in all growth rooms so that the only climatic variable was temperature. Light intensities of 160 watts m^{-2} (400 - 700 nanometers) were provided by four 1000W Sylvania Metalarc high pressure discharge lamps. The photoperiod was 12 hours with an abrupt light-dark change. The temperature and humidity changes between day and night occurred over 2 hours with the photoperiod

FIGURE 1

Frosting time sequence

beginning or ending halfway through each changeover. The relative humidities were 83% during the day and 69% at night in all three rooms. Carbon dioxide concentration was constant at 350ppm.

3.1.4 Frost Conditions

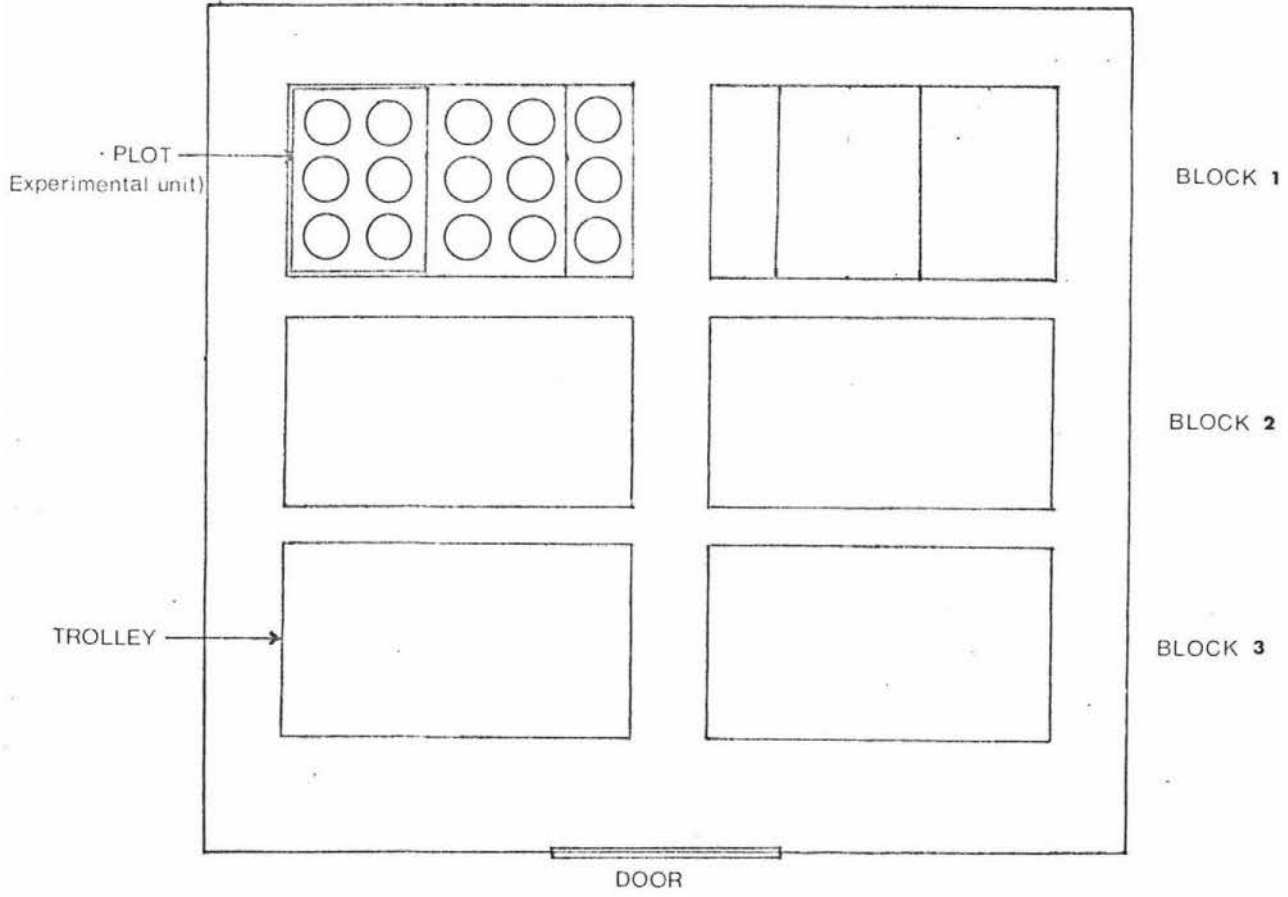
Following the initial establishment, plants were placed in the cold room at 12°/6°C for a 3 week hardening period before any frost treatments were imposed. The frost treatment was given only in the room designated "COLD." After this acclimatization period, a pattern of 3 days with frosts and 4 days without frosts was implemented.

On frost free nights, the minimum temperature was 6°C. On frost days, a white frost, which is visually similar to frosts observed in the field, was produced by directing steam into the controlled climate room, and maintaining a vapour pressure deficit (VPD) of zero during the frost period (Advective frost) (Hacker, 1974; Robotham et al, 1978). The temperature was maintained at 12°C during the light period then at the changeover was lowered over 8 hours in 15 x 32 minute steps to the frosting temperature. The minimum temperature was maintained for 2 hours after which the temperature was returned to 12°C in 15 steps over a 4 hour period. (See figure 1).

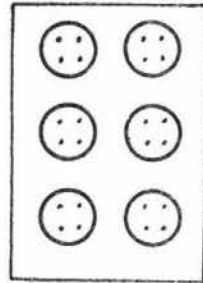
The experiment consisted of two growth cycles. During the first growth cycle, plants were developing hardiness and frosts were maintained at -2°C. In the second cycle, frosts were increased in intensity to -6°C. The frosting levels and occurrences are given in Appendix 1.

FIGURE 2

Growth Room Layout



EACH PLOT



1 PLOT/POPULATION

6 POTS/PLOT

4 PLANTS/POT

PLATE 1

View of a controlled growth room



3.1.5 Experimental Design

The experiment involved three environments each containing three complete randomised blocks with five clover populations. Each plot (experimental unit) consisted of six pots containing one population (see figure 2).

Each growth room contained 6 trolleys carrying 15 pots each. There were two trolleys per block.

Each experiment (environment) was conducted in a separate climate room. The climate room under operation is depicted in Plate 1.

3.1.6 Methods of Sampling

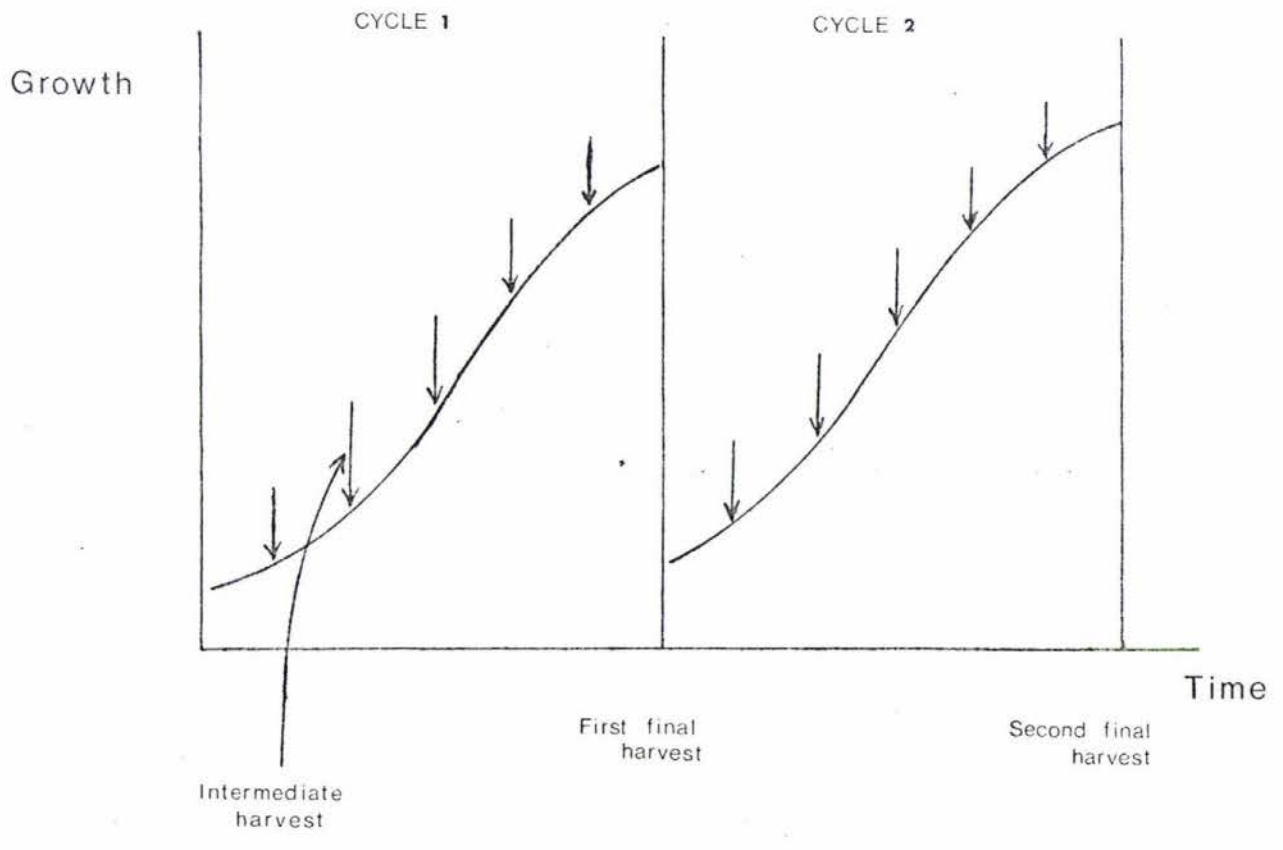
The two major growth parameters of white clover are the increase in dry weight of the component parts of individual stolons (i.e. roots, stems, leaves and petioles) and the increase in the number of stolons (Mitchell, 1956; Beinhart, 1963). One major objective was to determine the effects of temperature on these growth characteristics of the five contrasting clover populations.

Following the establishment period in the glasshouse and acclimatization period in the growth rooms, all leaves and petioles were removed, leaving a network of young stolons in each pot. In the subsequent growth period, five intermediate harvests were made in order to follow the stolon development of each clover population. At the end of the growth cycle, there was a final harvest of leaf and petiole material (Figure 3).

At the beginning of a growth cycle, 50 (10 stolons x 5 clovers) randomly selected primary stolons within each plot were marked with various coloured tags, designating

FIGURE 3

The pattern of sampling



the time of subsequent sampling (intermediate harvest). At each sampling time, 10 stolons with comparable times of development (same coloured tag) were cut from each plot and appropriate characteristics were measured. The sampling times in the three growth rooms were judged so that harvested stolons were at approximately equivalent stages of development. At the end of the first growth cycle, all leaf and petiole material was removed (final pot harvest) and fresh stolons tagged in readiness for the second growth cycle. A similar procedure was used in the second cycle. The sampling times are given in Table 4.

Sequential sampling at known time intervals allowed the use of regression analysis to estimate equations describing the development patterns of each character with respect to time.

3.1.7 Morphological characters measured

The sampled stolons were dissected into component parts as follows:

- (i) Herbage Yield
- (a) Stem dry weight (g)
 - (b) Leaf dry weight (g)
 - (c) Petiole dry weight (g)
 - (d) Total stolon dry weight (g)
 - (e) Lateral (secondary stolon) dry weight (g)

All stolon material was dried in a large vacuum oven at 45°C and 2.5mm Hg for 12 hours.

(ii) Leaf Number was determined from the number of nodes with newly-unfolded through to fully expanded leaves on the primary stolon.

TABLE 4Sampling Timetable

Growth Room		HOT	COOL	COLD
		24°/14°C	12°/6°C	12°/-2°C
CYCLE 1	Harvest a	5th day	8th day	8th day
	b	9	14	17
	c	13	21	25
	d	17	28	33
	Final Harvest	1	22	35
CYCLE 2	Harvest a	9th day	8th day	9th day
	b	13	15	18
	c	18	22	27
	d	22	29	37
	Final Harvest	2	27	39

- (iii) Leaf Area (cm^2) was determined by removing the trifoliate leaves from the petioles and passing through a Hayashi Denko automatic leaf area meter (Anom, 1975).
- (iv) Length of Stolon (cm) was measured after the leaves and petioles had been removed.
- (v) Number of Laterals (secondary branches) produced from the primary stolon were counted.
- (vi) Ratio of leaf dry weight to total dry weight was estimated from (i) and indicated the proportion of total stolon dry weight made up of photosynthetic leaf tissue.
- (vii) Stolon mass per unit length (g cm^{-1}) was estimated from (i) and (iv) and has been interpreted as an index of stem thickness by Mitchell (1955).
- (viii) Final harvest dry weight per pot (g)

The leaf and petiole material were removed from each pot at the end of a growth cycle. By dividing the dry weight (DW) yield per pot by the leaf and petiole yield per stolon, an estimate of the number of stolons per pot was obtained (Williams and Hoglund, 1978).

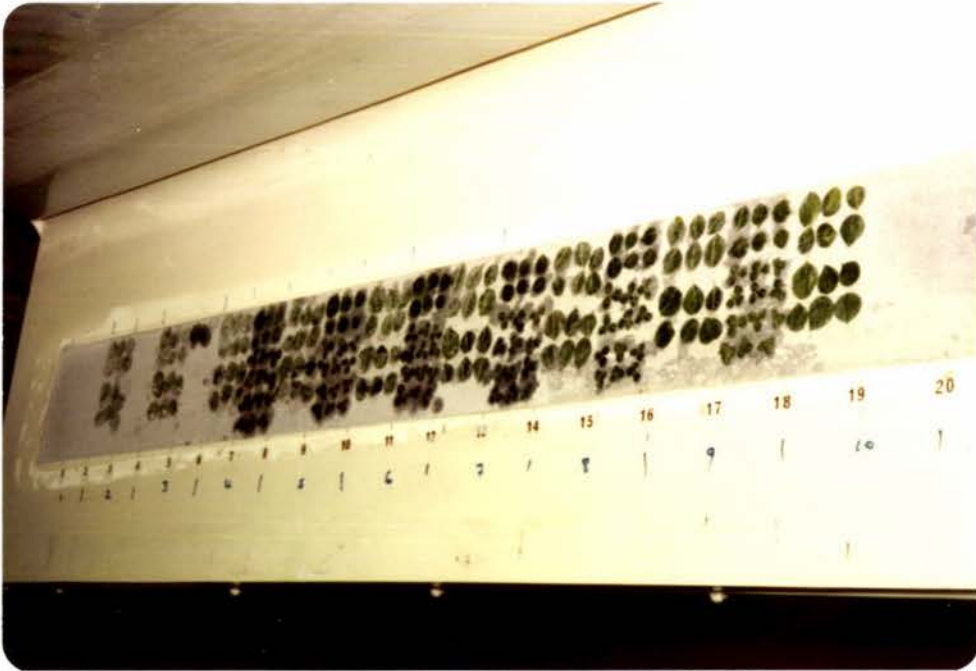
3.1.8 Electrical conductance technique for evaluating injury after freezing

When cells are damaged by freezing, plasma membranes lose their semi-permeability and permit amino acids and other electrolytes to leach from the injured tissue (Dexter *et al.*, 1932; Rowley, 1975). Measurement of the diffused electrolytes provides an estimate of the injury sustained during the freezing conditions.

A temperature gradient bar described by Rowley (1975) was used, which can expose a series of detached leaf

PLATE 2

View of the temperature gradient bar



samples to a range of freezing conditions, (Plate 2). The bar is designed to freeze the leaf samples along a temperature gradient to obtain their low temperature killing point. Twenty three thermocouples are set into the bar at regular intervals along its length to measure the temperature gradient. For instance, if a range from 0 to -12°C was set, each partition on the bar would decrease by approximately 0.5°C .

3.1.8.1 Procedure

Pieces of leaf tissue (single leaf blades from Spanish, Ladino and Huia clovers; trifoliate leaves from Kent and Russian clovers), were removed from plants in the cold room. Two pieces per replicate of each population were used for each temperature position on the gradient bar. The pieces were washed in distilled water after cutting, then rinsed in weak detergent (Multifilm X-77) and re-washed in de-ionized water, (Rowley et al., 1975) before being placed on the bar. After 18 hours of freezing, the temperature of the bar was raised to 0°C and the leaf samples removed. These were dropped into tubes containing 10ml of distilled water (1 tube per replicate (2 leaves), 3 replicates per population, and approximately 12 freezing temperatures) and put in a water bath at 25°C .

After 2 hours, the tubes were shaken and a conductivity reading (C1) of each solution taken, using a Philips conductivity meter (GM 4Z49) and matching conductivity electrode (PW 9510). The leaf tissue samples were then killed by placing the tubes in an ethylene glycol bath, inside a freezing chamber for 20 hours at -15°C . The tubes were then placed in a

water bath for 2 hours at 25°C and a final conductivity measurement made (C2). The ratio of the two conductivity measurements (C1/C2), was used as the index of injury (L). Values of L higher than 0.5 (50% leaching) indicated severe injury (Rowley, 1975). It should be emphasized that the technique assesses the freezing sensitivity of leaves, not whole plant overwintering ability or growth at low temperatures.

The index of injury (L) was plotted against the temperature of the gradient bar to obtain frost injury curves for each clover population. The killing point for each population estimated from the drawn curve, was at $L = 0.5$. Five conductance tests were carried out during the experiment at 3 weekly intervals, so that the pattern of frost injury or degree of hardiness could be followed for each population.

A side-line study was carried out with objective of comparing methods of assessing frost tolerance and is described in APPENDIX 2.

3.2 STATISTICAL PROCEDURES

The growth and development pattern for each clover population in each environment were described by regressing each growth character separately against time. The resultant growth functions were then compared to assess the genetic and environmental influences.

These functions were further used to estimate development levels at specific times. These estimates were subjected to a pooled analysis of variance over all three environments. Comparisons between populations and environments for each growth character were made at

the end point of the growth cycles. Estimates of broadsense heritability for each growth character were obtained together with estimates of simple phenotypic correlations amongst characters.

3.2.1 Curve Fitting

On plotting the data points, all the growth characters indicated non linear responses. The method of fitting many curves is to linearise them by applying appropriate transformations (Draper and Smith, 1966; Landsberg, 1977), there being a number of possible equations. Several criteria of best fit were then considered to make the final choice of equation type.

The types of curves considered most appropriate to this study were the exponential family of curves and the simplest form of equation was favoured. The regression coefficients for the relevant equations were estimated using the least-square principle, which involved the minimization of the sums of squares of the deviations (Draper and Smith, 1966) also, those curves with higher coefficients of determination, R^2 (that proportion of the total variation explained by the regression equation) were favoured. The final important criterion of best fit was to consider the type of curve which best described the development pattern of a character for all populations in all environments. For comparative purposes, the same type of equation must be fitted in all cases.

3.2.2 Exponential equations

One common exponential curve is described by:-

$Y = AB^X$ with linear form

$\log Y = \log A + (\log B) X$ and simple regression form

$Y = b_0 + b_1 X$.

The quadratic exponential equation introduces an additional term to account for further curvature in the exponential. The equation becomes:-

$Y = AB^X C^{X^2}$ with linear form

$\log Y = \log A + (\log B) X + (\log C) X^2$ and

multiple regression form, $y = b_0 + b_1 X + b_2 X^2$ where b_0 is a curve placement coefficient and b_1 and b_2 are rate of change coefficients. The quadratic term is a curvature modifier.

The simple and multiple regression equations were fitted using the REGRESSION sub-programme of Statistical Packages for the Social Sciences (SPSS/V6).

3.2.3 Comparison of Equations among Genotypes

Differences among the fitted equations were tested for significance by comparing the estimated regression coefficients (\hat{b}_0 , \hat{b}_1 , and \hat{b}_2). A significant difference in any of them indicated a difference in the curve and hence in the pattern of development. As the variances of the statistics were heterogenous, pair-wise t-tests were estimated using:-

$$t = \frac{\hat{X}_1 - \hat{X}_2}{\sqrt{\hat{\sigma}_1^2 + \hat{\sigma}_2^2}} \quad \text{where } \hat{X}_1 \text{ and } \hat{X}_2 \text{ are the regression statistics and } \hat{\sigma}_1^2 \text{ and } \hat{\sigma}_2^2 \text{ their respective variances.}$$

(Steel and Torrie, 1960)

The genotypes were ranked before comparisons so that the one-tailed t-test was applicable. Significance was tested at $P = 0.05$ and the sum of degrees of freedom for variance about regression was $(n_1 + n_2 - 4)$.

Comparisons were made between populations within each temperature regime. Also, for each population, comparisons were made across all three temperature regimes. This was done for each character separately.

The SPSS/REGRESSION output included estimates of all regression coefficients ($\hat{b}_0, \hat{b}_1, \hat{b}_2$), residual standard error ($\hat{\sigma}_{y \cdot x}$), standard errors of \hat{b}_1 and \hat{b}_2 ($\hat{\sigma}_{\hat{b}_1}, \hat{\sigma}_{\hat{b}_2}$) and the coefficient of determination (R^2).

The variance of b_0 is not given by SPSS/REGRESSION. For linear equations of the form, $Y = b_0 + b_1 X$, the variance of b_0 was estimated by:-

$$\sigma_{b_0}^2 = \sigma_{y \cdot x}^2 \left(\frac{\sum_i X_i^2}{n} - \bar{X}^2 \right)$$

where $\sigma_{y \cdot x}^2$ = variance about regression, n = number of observations in the estimator sample and $\sum_i (X_i - \bar{X})^2$ = sum of squares of X (Draper and Smith, 1966).

For the quadratic equations, the programme REGSPS (Gordon, unpublished) was used to obtain the variance - co-variance matrix of the b's $\left(V \begin{pmatrix} b_0 \\ b_1 \\ b_2 \end{pmatrix} \right)$, where the variances of the b's are on the diagonal. All comparisons were then possible using the t-test (Draper and Smith, 1966).

3.2.4 Estimation of points of Biological interest

Estimates of the dependent variable (Y), for particular values of time (X_k), were obtained from forward solutions of the regression equations. For equations with one X variable, $\hat{Y} = \hat{b}_0 + \hat{b}_1 X$, an estimate of the variance of a predicted \hat{Y}_k value was given by:

$$\sigma_{\hat{Y}_k}^2 = \sigma_{y \cdot x}^2 \left(\frac{1}{n} + \frac{(X_k - \bar{X})^2}{\sum_i (X_i - \bar{X})^2} \right), \quad i = 1 \text{ to } n$$

(Draper and Smith, 1966).

For quadratic equations, the estimated variance of \hat{Y}_k was obtained by:

$$\hat{\sigma}_{\hat{Y}_k}^2 = \sigma_{y \cdot x}^2 (1/n + C_{11} x_1^2 + C_{22} x_2^2 + 2 C_{12} x_1 x_2)$$

(Steel and Torrie, 1960),

where $\sigma_{y \cdot x}^2$ = variance about regression in each case, C_{11} , C_{12} and C_{22} are terms from the $(X'X)^{-1}$ inverse matrix (Draper and Smith, 1966) and were provided by REGSPS, and x_1 , x_2 are the linear and quadratic values of X_k expressed as deviates from their respective \bar{X} 's.

$$\text{i.e. } x_1 = X_k - \bar{X}$$

$$x_2 = X_k^2 - (\bar{X}^2)$$

All possible pair-wise t-tests were performed as before, to find significant differences among the \hat{Y}_k estimates for the various clover populations.

3.2.5 Linear Model

Data collection was conducted so that comparisons of morphological characters could be made between the five clover populations within each temperature regime, and across the three temperature regimes.

Each phenotypic observation was based on the following random effects model (Le Clerg et al, 1962; Comstock and Moll, 1963):

$$X_{ijk} = \mu + \gamma_i + \beta_{j(k)} + \eta_k + \gamma\eta_{ik} + \epsilon_{ijk}, \text{ where}$$

μ = mean of all populations over the three environments;

γ_i = i^{th} population effect over all environments ($i = 1 \dots g$, g = number of populations);

$\beta_{j(k)}$ = effect of the j^{th} block in the k^{th} environment ($j = 1 \dots b$, b = number of blocks)

η_k = k^{th} environmental effect ($k = 1 \dots e$, e = number of environments);

$\gamma\eta_{ik}$ = ik^{th} population x environment interaction;

ϵ_{ijk} = ijk^{th} residual deviation (experimental error).

X_{ijk} = ijk^{th} phenotypic variate, due to the i^{th} population, in the j^{th} block, in the k^{th} environment.

All effects are considered to be random, normal, independent deviates with expectations equal to zero

and generating variances of corresponding designation.

3.2.6 Pooled Analysis of Variance over the three Environments

The data used for this analysis were estimated from the regression equations describing the development pattern of each growth characteristic. For any particular growth character, a regression function was fitted on an experimental unit (plot mean) basis. This was done for each clover population, in each block, within the three environments. Then an estimated Y value, taken at the end point of the growth cycles, was obtained from these regression functions and this data used for the Pooled Analysis. This estimated value is more accurate to use compared with the final field data as the development pattern leading to the end point has been taken into account.

The linear model outlined previously was applied to each growth character separately. Estimates of variance components were obtained from the expectations of mean squares which arise from such random effect designs (Comstock and Robinson, 1952a), (Table 5).

The E(MS) in Table 5 define the F-tests as described in Le Clerg et al (1962):

$$\text{F test for GxE, } F_{GE} = \frac{MS_{GE}}{MS_{\text{error}}}$$

$$\text{F test for populations, } F_G = \frac{MS_G}{MS_{GE}}$$

$$\text{F test for blocks, } F_{B(E)} = \frac{MS_{B(E)}}{MS_{\text{error}}}$$

$$\text{F test for environments, } F_E = \frac{MSE + MS_{\text{error}}}{MS_{B(E)} + MS_{GE}}$$

TABLE 5
Pooled Anova

Source of Variation	df	SS	E(MS)
Environments	e-1	$\sum_k X_{..k}^2 / bg - CF$	$\sigma^2 + g\sigma_{B(E)}^2 + b\sigma_{GE}^2 + bg\sigma_E^2$
Blocks within environments	e(b-1)	$\sum_j \sum_k X_{jk}^2 / g - CF - SS_E$	$\sigma^2 + g\sigma_{B(E)}^2$
Populations	g-1	$\sum_i X_{i..}^2 / be - CF$	$\sigma^2 + b\sigma_{GE}^2 + be\sigma_G^2$
Population x Environments	(g-1)(e-1)	$\sum_t \sum_k X_{t.k}^2 / b - CF - SS_G - SS_E$	$\sigma^2 + b\sigma_{GE}^2$
Error	e(g-1)(b-1)	$\sum_{ijk} X_{ijk}^2 - CF - SS_G - SS_E - SS_{GE} - SS_{B(E)}$	σ^2

CF = correction factor = $X_{...}^2 / g b e$

Where $X_{...} = \sum_{ijk} X_{ijk}$

$X_{i..} = \sum_{jk} X_{ijk}$ for each i population

$X_{.j.} = \sum_{ik} X_{ijk}$ for each j block

$X_{..k} = \sum_{ij} X_{ijk}$ for each k environment

$X_{i.k} = \sum_j X_{ijk}$ for each i population in each k environment

and σ^2 is the variance arising from ϵ_{ijk} , the error component

σ_G^2 from γ_i , the population effects

$\sigma_{B(E)}^2$ from $\beta_{j(k)}$, the block effect

σ_E^2 from η_k , the environment effect and

σ_{GE}^2 from $\gamma\eta_{ik}$, the population x environment interaction.

The degrees of freedom for the F_E test are weighted degrees of freedom (Satterthwaite, 1946).

$$df' \text{ of numerator} = \frac{[MS_E + MS_{\text{error}}]^2}{\frac{(MS_E)^2}{df_E} + \frac{(MS_{\text{error}})^2}{df_{\text{error}}}}$$

$$df' \text{ of denominator} = \frac{[MS_{B(E)} + MS_{GE}]^2}{\frac{(MS_{B(E)})^2}{df_{B(E)}} + \frac{(MS_{GE})^2}{df_{GE}}}$$

3.2.7 Heritability estimates

Two forms of broadsense heritability can be derived from the variance components in Table 5 (Allard, 1960; Gordon, 1972).

- (a) Full heritability where the phenotypic variance contains all the variance components including the block and environmental effects.

$$h_F^2 = \frac{\sigma_G^2}{\sigma^2 + \sigma_G^2 + \sigma_{GE}^2 + \sigma_{B(E)}^2 + \sigma_E^2}$$

- (b) Restricted heritability where the phenotypic variance only contains the variance components associated with the genotypic differences.

$$h_R^2 = \frac{\sigma_G^2}{\sigma^2 + \sigma_G^2 + \sigma_{GE}^2}$$

The data used for this analysis were based upon plot means. As the variance of a mean is given by the equation, $\sigma_{\bar{x}}^2 = \frac{\sigma^2}{n}$ where n is the number of individual observations making up the mean, the phenotypic variance used in these heritability ratios has similarly been reduced. Thus the heritability values obtained are higher than one would expect to obtain for the heritability of individuals.

Variance estimates of these heritabilities have been defined by Gordon et al (1972). The basic estimator is given by the following expression:

$$\hat{\sigma}_{h_R}^2 = X^2 \sigma_Y^2 + Y^2 \sigma_X^2 - 2XY \text{Cov}(X,Y) / Y^4$$

(Osborne and Paterson, 1952).

where $X = \sigma_G^2$ (Genotypic variance)

$$Y = \sigma_P^2 \text{ (phenotypic variance)} = \sigma^2 + \sigma_G^2 + \sigma_{GE}^2$$

For the present model and restricted heritability case,

$$\sigma_X^2 = V_G$$

$$\sigma_Y^2 = V + V_{GE} + V_G + 2 \text{Cov}(G,GE) + \text{Cov}(G, \text{Error}) + \text{Cov}(GE, \text{error})$$

$$= V + V_{GE} + V_G + 2 \left\{ [(1 - be)/b^2e] V - V_{GE}/e \right\}$$

and $\text{Cov}(X,Y) = V_G + V/b^2e - V_{GE}/e$ (Gordon et al, 1972)

From Comstock and Moll (1963):

V_G = sampling variance of the genotypic variance

$$= \frac{1}{(b \cdot e)^2} \sum_{n=1}^g \frac{2 (MS_G)^2}{df_G + 2}$$

V = sampling variance of the error variance

$$= \frac{2 (MS \text{ error})^2}{df \text{ error} + 2}$$

V_{GE} = sampling variance of the population X environment variance

$$= \frac{1}{b^2} \sum_{n=1}^{g^e} \frac{2 (MS_{GE})^2}{df_{GE} + 2}$$

where b = number of blocks

e = number of environments

g = number of populations

A computer programme PHANIE (PHenotypic ANalyses In Environments) (Gordon, unpublished) was used to carry out the pooled analysis of the three environments together with the h^2 and $\sigma_{h^2}^2$ estimates.

3.2.8 Simple Correlation Co efficient

Correlation is a measure of the intensity of the association between two characters (Steel and Torris, 1960). The phenotypic correlation is given by:

$$r_p = \frac{\text{Cov}(X,Y)}{\sqrt{\sigma_x^2 \cdot \sigma_y^2}} = \frac{\sum(X - \bar{x})(Y - \bar{y})}{\sqrt{\sum(X - \bar{x})^2 \sum(Y - \bar{y})^2}} \quad (\text{Steel and Torrie, 1960})$$

where $\sum(X - \bar{x})(Y - \bar{y})$ = sum of cross products of the deviations

and $\sum(X - \bar{x})^2$ = sum of squares of the deviations.

The computer programme PHANIE was used to carry out this analysis.

3.2.9 Adaptation Analysis

The pooled analysis of variance enables the total variation ascribable to populations and environments to be partitioned into three independent comparisons. One measuring the differences between populations, one measuring environmental differences and one assessing their joint effects, the GE interaction. The GE interaction effect can be further split into the variation components for each population within the environments. These separate population - environment interaction effects are known as ecovalences. The terms population and genotype are synonyms in the following text. The ecovalences are given by:

$$\sum_K (\bar{X}_{i.k} + \bar{X}_{...} - \bar{X}_{i..} - \bar{X}_{..k})^2 \text{ for each } i \text{ genotype.}$$

(a) Genotype yield in each environment

(b) Mean of all genotypes in all environments (Grand mean)

(c) Mean genotype yield over all environments (Genotype mean)

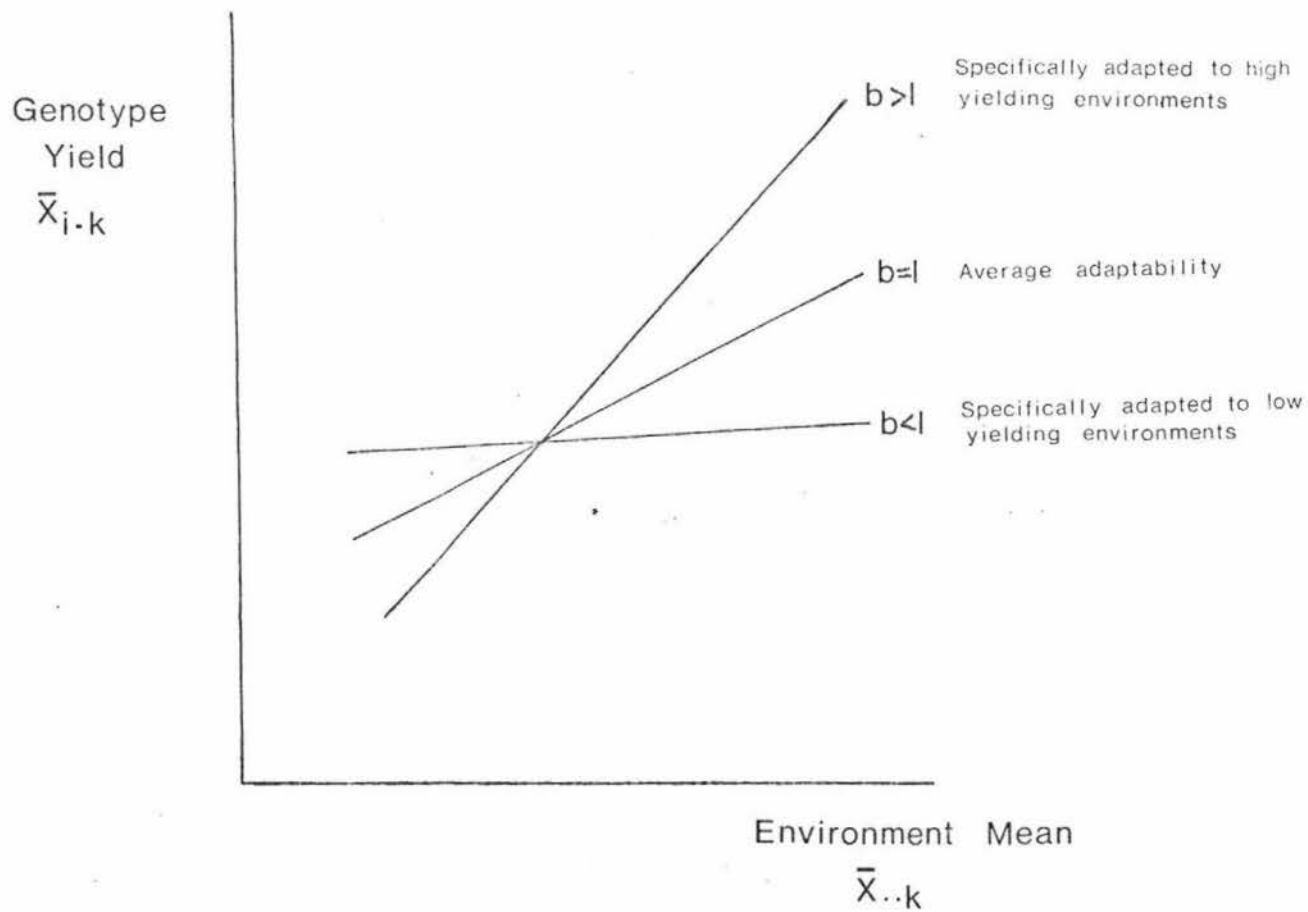
(d) Yield of all Genotypes in each environment (Environment mean)

Finlay and Wilkinson(1963) and Eberhart and Russell (1966) developed regression techniques in order to study the patterns of adaptability between genotypes and the environment. For each genotype, a linear regression of individual yield ($\bar{X}_{i.k}$) on the mean yield of all genotypes for each environment ($\bar{X}_{..k}$) was computed. The mean yield of all genotypes for each environment (Environment mean) is a method of evaluating an environment without the inaccuracies of defining it in physical terms. Probably the most important point on the validity of this analysis is whether the environmental means are a fair representation of the actual environments.

From the model, $X_{i.k} = \mu + G_i + E_k + GE_{ik} + \epsilon$, the regression is in effect regressing $G_i + E_k + GE_{ik}$ as the Y variate against E_k as the X variate (Hill, 1975). As the genotypic effect, G is constant for a particular genotype, the resultant regression coefficient b, of the i th genotype line, includes environmental variation E, and that portion of the GE interaction variation which is a linear function of the environment. Part of the GE interaction variation forms the unexplained deviations from the regression line. Eberhart and Russell (1966) defined a "stable" genotype as one with deviations from the regression line as small as possible. The series of regression lines presented in Figure 4 illustrate the possible responses which can occur.

The significance of b, the regression coefficient may be determined by testing its departure from zero or unity. Eberhart and Russell (1966) defined a well adapted genotype as one with a high mean yield ($\bar{X}_{i..}$),

FIGURE 4

Adaptation Analysis

unit regression coefficient ($b = 1.0$) and deviations from regression as small as possible (i.e. a high coefficient of determination, R^2). The adaptation analysis was estimated using the programme PHANIE, which printed the ecovalences, adaptation coefficient b , the standard error of b , significance levels and R^2 .

CHAPTER 4

RESULTS

4.1 Curve fitting

The final estimated regression functions describing the stolon growth characters were fitted on an experimental unit (plot mean) basis. This method was chosen because of the large variation in growth between individual stolons of each clover population. The effect was to reduce the stolon variation, as the variance of a plot mean is given by σ^2/n where n is the number of observations (Steel and Torrie, 1960). The quadratic exponential functions were found to describe the development pattern of the stolon characters with most accuracy and appropriateness of biological interpretation (as outlined in section 3.2).

4.2 Stolon growth Characters

4.2.1 Stem dry weight production

The fitted curves for each population in each environment are plotted in Figure 5 and the data points given in Appendix 3. The regression statistics describing these curves (b_0 , b_1 , and b_2) are given in Table 6. Comparisons across populations for each of the regression statistics were achieved using pair-wise t-Tests. Significance grouping at $P = 0.05$ are indicated in Tables 6 and 7.

The b_0 regression coefficient is a curve placement parameter which describes the initial growth potential of each population. Ladino was consistently superior to all other populations in each environment.

The b_1 regression coefficient is a rate of change parameter which describes the growth rate in g/day. The b_2 coefficient is an 'acceleration' parameter describing the change in growth rate (g/day/day).

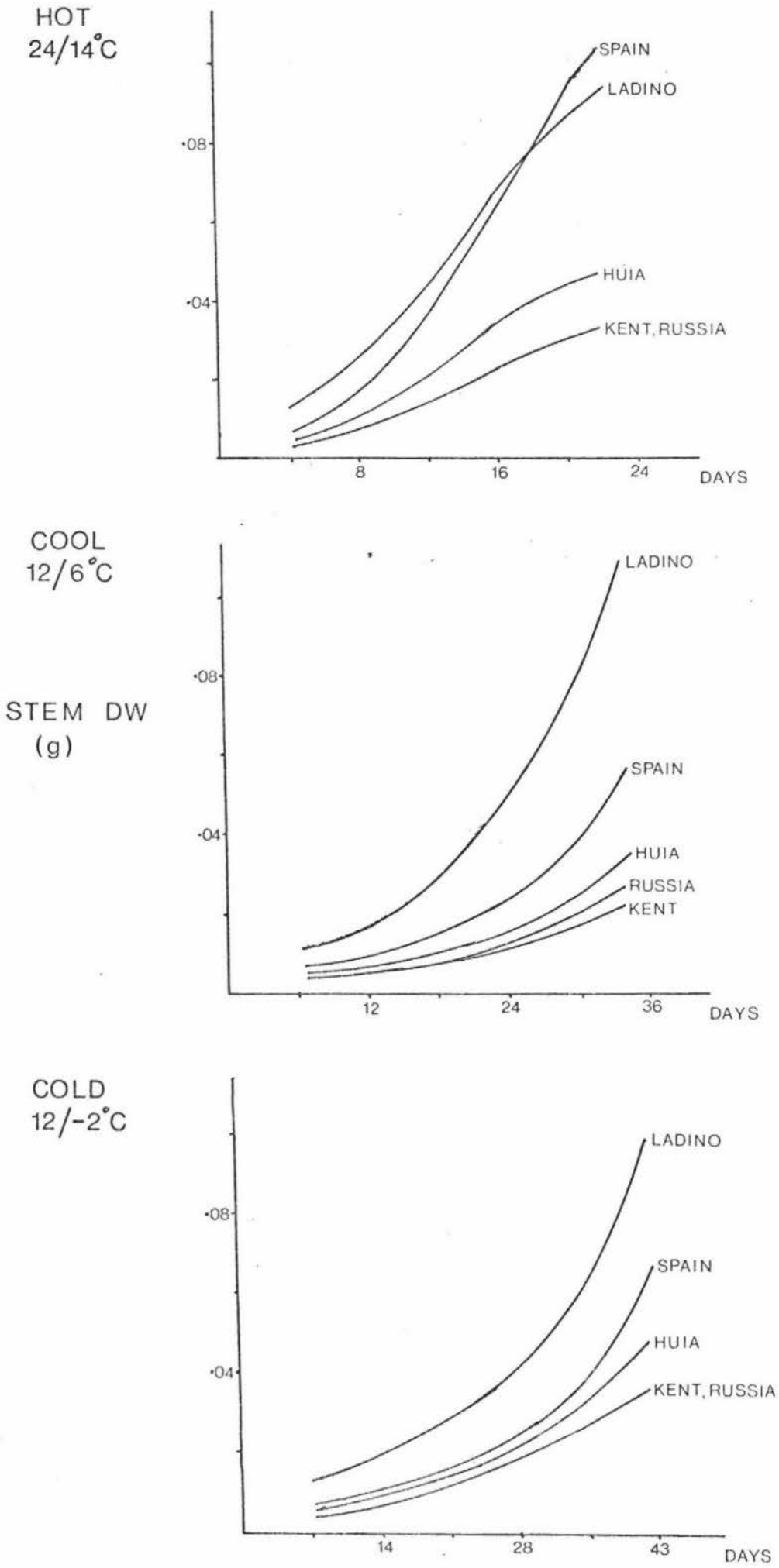
From Table 6, in both the hot ($24^{\circ}/14^{\circ}\text{C}$) and cool ($12^{\circ}/6^{\circ}\text{C}$) environments, there were no significant differences among populations for b_1 . In the cold ($12^{\circ}/-2^{\circ}\text{C}$) environment, Huia was significantly superior to the Spanish and Ladino clovers for b_1 , but the reverse was true for b_2 .

The regression coefficients were also compared across environments (Table 7). All the clover populations gave higher values for b_1 in the hot environment than the cool and cold environments. However, values for b_2 declined at a faster rate in the hot environment than the colder environments.

Comparisons of the final stem dry weights (\hat{Y}) estimated at the end of the growth period in each environment (similar developmental stage), are indicated in Table 8. The Spanish clover had the greatest stem Dry Weight (DW) per stolon in the hot environment, but ladino was superior in both the cool and cold environments. Huia was intermediate in all environments, the Kent and Russian clovers consistently had the least stem DW in each environment.

Similar growth patterns occurred in the second growth period (regression statistics given in Appendix 4A) except the Spanish no longer had the greatest DW in the hot environment and Ladino was consistently superior in all environments.

FIGURE 5



Estimated regression statistics describing the development pattern of stem dry weight of five white clover populations in three environments

$\log_e(g)$	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b_0	-6.589	-5.455	-7.087	-7.206	-6.649
	SE of b_0	0.280	0.217	0.212	0.229	0.282
		* b	a	b	b	b
	b_1	0.329	0.239	0.350	0.320	0.251
	SE of b_1	0.047	0.036	0.035	0.038	0.047
		ab	b	a	ab	ab
	b_2	-.0062	-.0046	-.0076	-.0071	-.0050
	SE of b_2	0.0017	0.0013	0.0013	0.0014	0.0017
		a	a	a	a	a
	R^2		.968	.961	.977	.967
COOL 12°/6°C	b_0	-6.259	-5.369	-6.078	-6.617	-6.352
	SE of b_0	0.356	0.165	0.275	0.211	0.318
		b	a	b	b	b
	b_1	0.114	0.107	0.080	0.118	0.114
	SE of b_1	0.038	0.018	0.029	0.022	0.034
		a	a	a	a	a
	b_2	-.0005	-.0005	-.0007	-.0008	-.0009
	SE of b_2	0.0009	0.0004	0.0007	0.0005	0.0008
		a	a	a	a	a
	R^2		.942	.984	.955	.926
COLD 12°/-2°C	b_0	-5.505	-4.657	-6.180	-6.316	-5.899
	SE of b_0	0.186	0.171	0.259	0.270	0.102
		b	a	c	c	c
	b_1	0.068	0.057	0.127	0.111	0.079
	SE of b_1	0.016	0.015	0.023	0.024	0.009
		bc	c	a	ab	bc
	b_2	-.00007	-.00007	-.00124	-.0010	-.0005
	SE of b_2	0.00032	0.00029	0.00044	0.0005	0.0002
		a	a	b	ab	ab
	R^2		.968	.960	.939	.928

* Statistics sharing the same letter are not significantly different (P=0.05)

Statistical differences amongst populations across environments for stem dry weight

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		NS	NS	*	*	NS
HOT/COLD	b ₀	*	*	*	*	*
COOL/COLD		*	*	NS	NS	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	b ₁	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	b ₂	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	NS	NS	NS	NS
HOT/COLD	Y	*	NS	NS	NS	NS
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at P=0.05

* = significance at P=0.05

The asterick is indicated in line with the environment containing the larger estimate

Estimated stem dry weights at final harvest time

	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	antilog (cg)	9.9	9.0	4.4	2.9	2.9
24°/14°C	$\hat{Y} \log_e (g)$	-2.313	-2.408	-3.124	-3.541	-3.541
at 22 days	SE of \hat{Y}	0.113	0.087	0.085	0.092	0.113
		* a	b	c	d	cd
COOL	antilog (cg)	5.6	10.5	3.5	2.8	3.2
12°/6°C	$\hat{Y} \log_e (g)$	-2.882	-2.254	-3.352	-3.576	-3.442
at 35 days	SE of \hat{Y}	0.137	0.064	0.105	0.081	0.122
		b	a	c	c	c
COLD	antilog (cg)	6.8	9.5	4.9	3.5	3.4
12°/-2°C	$\hat{Y} \log_e (g)$	-2.688	-2.354	-3.016	-3.352	-3.381
at 43 days	SE of \hat{Y}	0.093	0.086	0.130	0.135	0.051
		b	a	bc	cd	d

* Statistics sharing the same letter are not significantly different (P=0.05)

4.2.2 Leaf and Petiole Dry Weight Production

The fitted regression curves are plotted in Figures 6 and 7, respectively with data points given in Appendix 3. The regression statistics and significance groups for leaf dry weight (DW) are indicated in Tables 9 and 10. The populations showed similar response curves for leaf and petiole production in all environments.

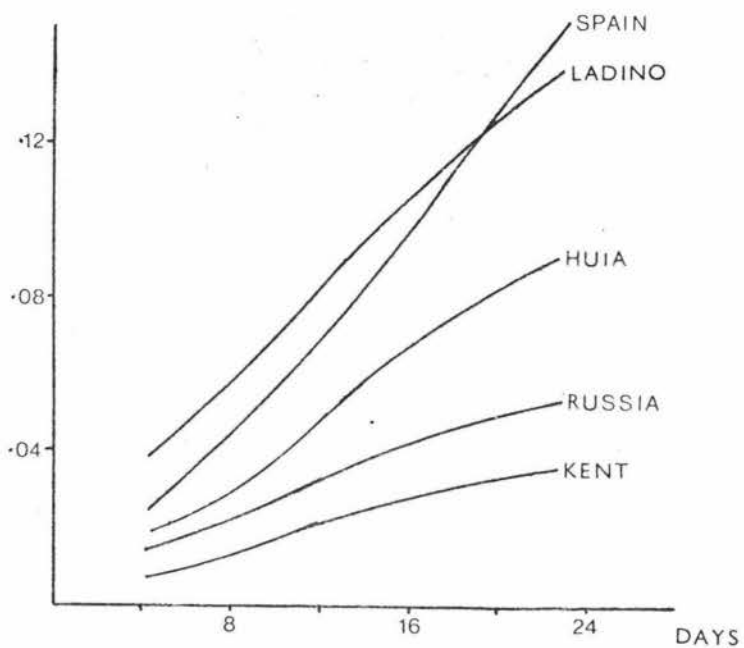
From Table 9, Ladino clover had a consistently superior b_0 coefficient for leaf DW production in each environment but the five populations had similar b_1 and b_2 coefficients. When comparing across environments (Table 10); Huia, Kent and Russian clovers had greater values for b_1 in the hot environment than in the colder environments, but Spain and Ladino were no different.

The final leaf dry weights (\hat{Y}) estimated at the end of the growth period in each environment are given in Table 11. This showed the superiority of the Spanish and Ladino clovers in all environments, followed by Huia and finally the Kent and Russian clovers producing the least DW production.

The regression statistics and significance groups for petiole dry weight are given in Tables 12 and 13. Ladino clover had the greatest values for b_1 in all environments followed by Spain and Huia then the Kent and Russian clovers. The reverse trend was applicable for the b_2 coefficient. When comparing petiole DW production between environments (Table 13), all populations had greater values of b_1 in the hot environment relative to the colder environments.

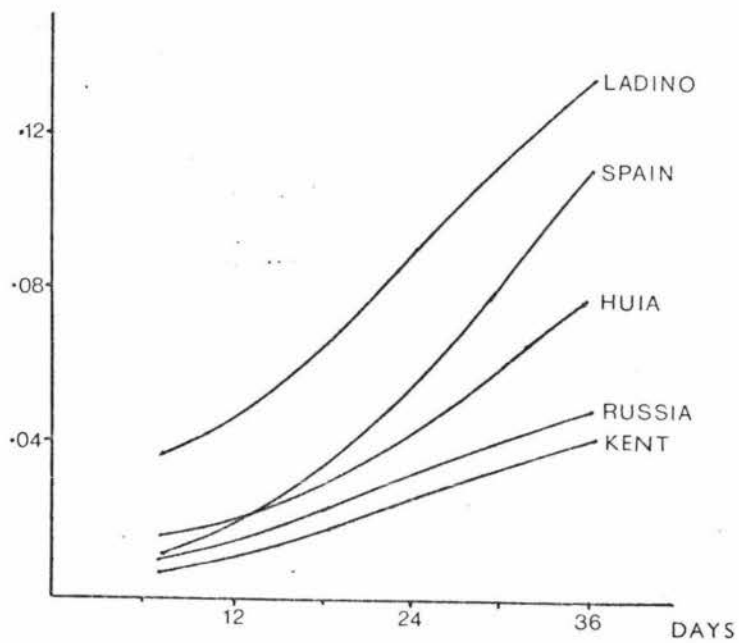
FIGURE 6

HOT
24/14°C

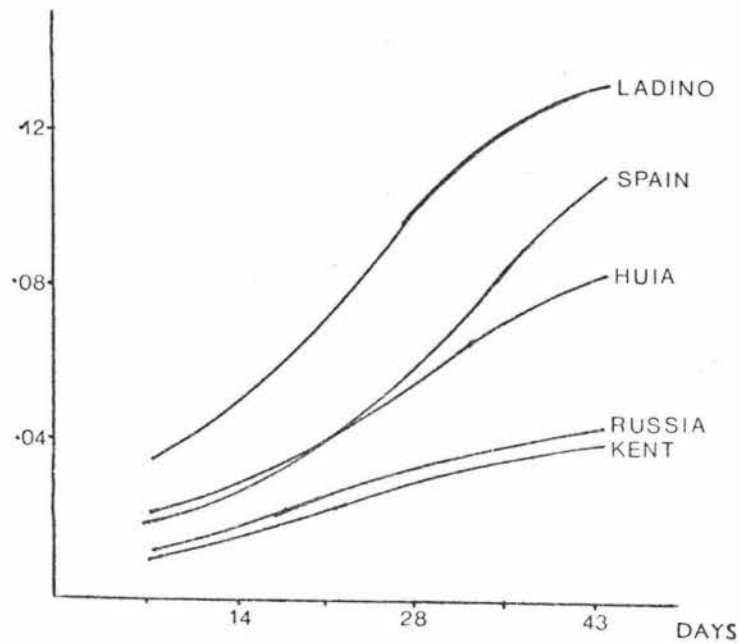


COOL
12/6°C

LEAF DW
(g)



COLD
12/-2°C



Regression statistics describing the development patterns
for leaf dry weight

Log _e (g)	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT						
24°/14°C	b ₀	-4.682	-3.890	-5.408	-5.756	-5.221
	SE b ₀	0.208	0.257	0.215	0.278	0.285
		* b	a	c	c	bc
	b ₁	0.199	0.140	0.267	0.194	0.194
	SE b ₁	0.035	0.043	0.036	0.046	0.049
		ab	b	a	ab	ab
	b ₂	-.0033	-.0024	-.0061	-.0044	-.0044
SE b ₂	0.0013	0.0016	0.0013	0.0017	0.0018	
	ab	a	b	ab	ab	
R ²		.881	.960	.956	.871	.859
COOL						
12°/6°C	b ₀	-5.238	-3.771	-4.686	-5.515	-4.942
	SE b	0.318	0.133	0.234	0.256	0.246
		bc	a	b	c	bc
	b ₁	0.121	0.058	0.070	0.098	0.083
	SE b ₁	0.034	0.014	0.025	0.027	0.026
		a	b	ab	ab	ab
	b ₂	-.0010	-.0002	-.0003	-.0011	-.0009
SE b ₂	0.0008	0.0003	0.0006	0.0006	0.0006	
	a	a	a	a	a	
R ²		.931	.970	.932	.908	.879
COLD						
12°/-2°C	b ₀	-4.670	-3.840	-4.644	-5.024	-4.819
	SE b ₀	0.154	0.170	0.198	0.193	0.129
		b	a	b	b	b
	b ₁	0.087	0.074	0.095	0.058	0.064
	SE b ₁	0.014	0.015	0.018	0.017	0.017
		a	a	a	a	a
	b ₂	-.00072	-.00072	-.0011	-.0004	-.0006
SE b ₂	0.0003	0.00029	0.0003	0.0003	0.0002	
	a	a	a	a	a	
R ²		.965	.926	.918	.902	.945

* Statistics showing the same letter are not significantly different (P=0.05)

Statistical differences amongst populations across environments for leaf dry weight

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		NS	NS	*	NS	NS
HOT/COLD	b ₀	NS	NS	*	*	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		NS	NS	*	*	*
HOT/COLD	b ₁	*	NS	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		NS	NS	*	*	*
HOT/COLD	b ₂	*	NS	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	NS	NS	NS	NS
HOT/COLD	Y	*	NS	NS	*	NS
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at P=0.05

* = significance at P=0.05

The asterick is indicated in line with the environment containing the larger estimate

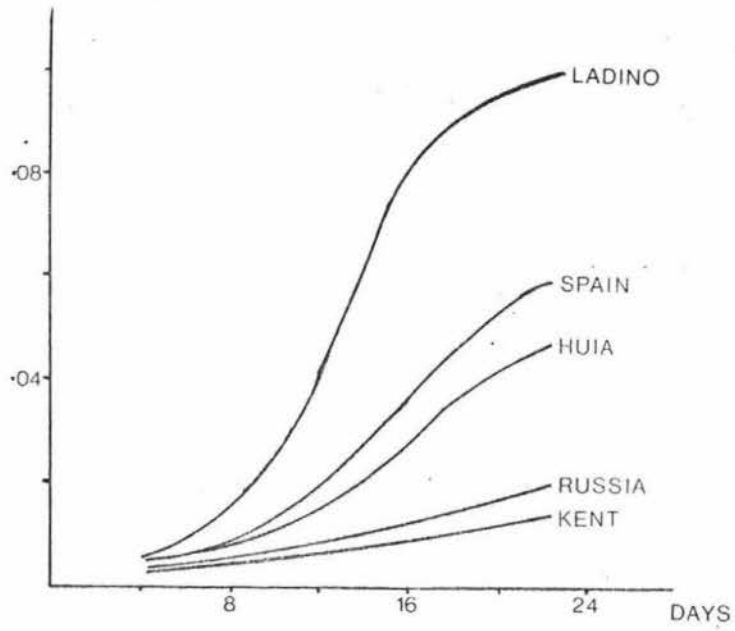
Estimated leaf dry weight at final harvest

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	Antilog (cg)	15.0	13.9	8.4	2.7	4.6
24°/14°C	$\hat{Y} \log_e (g)$	-1.897	-1.973	-2.477	-3.612	-3.079
at 22 days	SE of \hat{Y}	0.083	0.103	0.086	0.112	0.125
		* a	a	b	d	c
COOL	Antilog (cg)	10.8	13.8	7.4	3.4	4.3
12°/6°C	$\hat{Y} \log_e (g)$	-2.226	-1.981	-2.604	-3.381	-3.147
at 35 days	SE of \hat{Y}	0.106	0.051	0.090	0.098	0.095
		a	a	b	c	c
COLD	Antilog (cg)	10.6	13.7	8.1	3.7	4.1
12°/-2°C	$\hat{Y} \log_e (g)$	-2.244	-1.988	-2.513	-3.297	-3.194
at 43 days	SE of \hat{Y}	0.077	0.085	0.099	0.097	0.065
		b	a	b	c	c

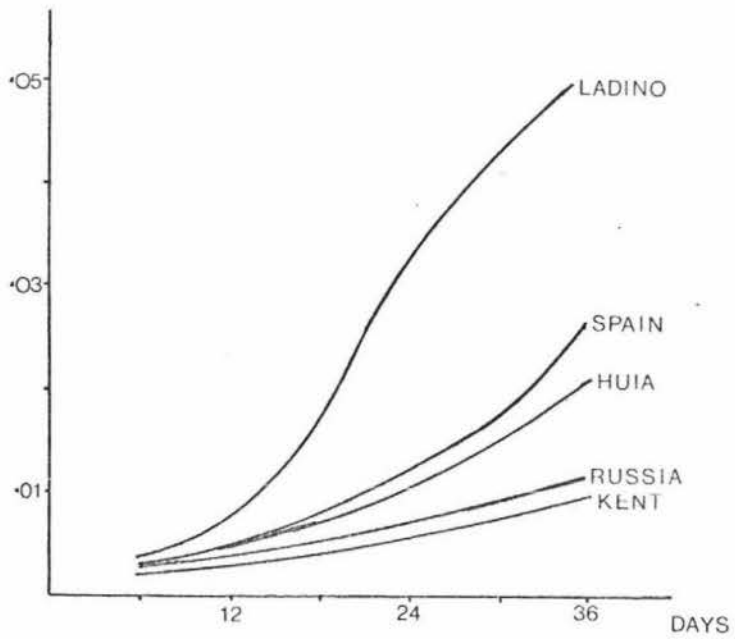
* Statistics sharing the same letter are not significantly different (P=0.05)

FIGURE 7

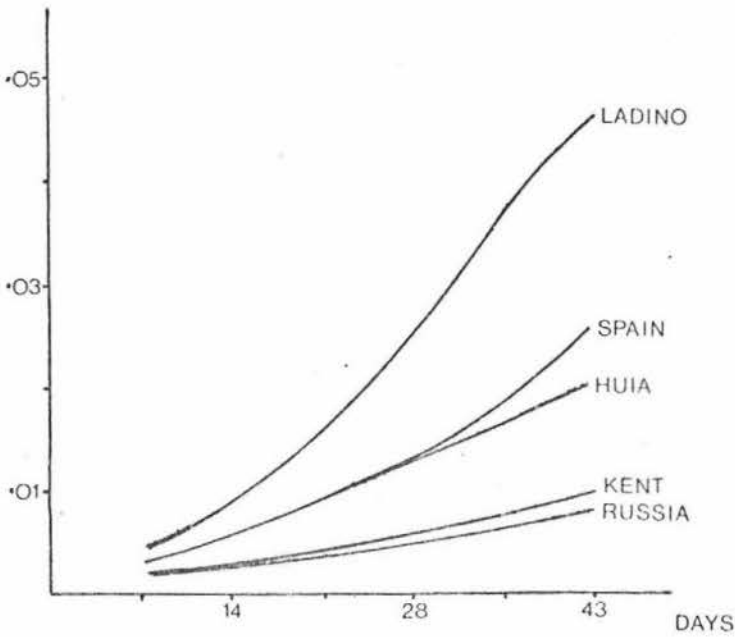
HOT
24/14°C



COOL
12/6°C
PETIOLE DW
(8)



COLD
12/-2°C



Regression statistics describing the development patterns for
Petiole dry weight

$\log_e(g)$	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT						
24°/14°C	b_0	-7.554	-8.087	-7.284	-6.306	-6.728
	SE b_0	0.186	0.466	0.295	0.323	0.421
		* c	c	bc	a	ab
	b_1	0.396	0.565	0.344	0.074	0.156
	SE b_1	0.031	0.078	0.049	0.054	0.070
		b	a	b	c	c
	b_2	-.0083	-.0138	-.0071	+.0006	-.0016
	SE b_2	0.0011	0.0028	0.0018	0.0020	0.0025
		b	c	b	a	a
	R^2	.942	.939	.942	.862	.873
COOL						
12°/6°C	b_0	-6.560	-7.350	-6.524	-5.678	-5.961
	SE b_0	0.290	0.291	0.246	0.288	0.360
		b	c	b	a	ab
	b_1	0.094	0.236	0.091	-0.028	0.010
	SE b_1	0.031	0.031	0.026	0.031	0.038
		b	a	b	c	c
	b_2	-.0003	-.0032	-.0005	+.0015	+.0006
	SE b_2	0.0007	0.0007	0.0006	0.0007	0.0009
		b	c	b	a	ab
	R^2	.965	.948	.952	.811	.713
COLD						
12°/-2°C	b_0	-6.468	-6.938	-6.566	-5.796	-6.078
	SE b_0	0.179	0.310	0.192	0.229	0.187
		bc	c	c	a	ab
	b_1	0.097	0.174	0.109	-0.009	0.029
	SE b_1	0.016	0.028	0.017	0.020	0.167
		b	a	b	c	c
	b_2	-.0008	-.0020	-.0011	+.0011	-.00007
	SE b_2	0.0003	0.0005	0.0003	0.0004	0.0003
		bc	d	cd	a	b
	R^2	.936	.964	.952	.845	.821

* Statistics sharing the same letter are not significantly different (P=0.05)

Statistical differences amongst populations across environments
for Petiole dry weight

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		*	NS	*	NS	NS
HOT/COLD	b_0	*	*	*	NS	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	NS	*
HOT/COLD	b_1	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	NS	NS
HOT/COLD	b_2	*	*	*	NS	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	NS	*
HOT/COLD	Y	*	*	*	NS	*
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at P=0.05

* = significance at P=0.05

The asterick is indicated in line with the environment containing the larger estimate.

Estimated Petiole dry weight at final harvest

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	antilog (cg)	5.7	9.6	4.2	0.7	1.7
24°/14°C	$\hat{Y} \log_e (g)$	-2.865	-2.343	-3.170	-4.962	-4.075
at 22 days	SE of \hat{Y}	0.075	0.187	0.118	0.130	0.169
		* b	a	c	e	d
COOL	antilog (cg)	2.6	5.0	2.0	0.8	0.8
12°/6°C	$\hat{Y} \log_e (g)$	-3.650	-2.996	-3.912	-4.828	-4.880
at 35 days	SE of \hat{Y}	0.111	0.112	0.095	0.118	0.138
		b	a	b	c	c
COLD	antilog (cg)	2.5	4.4	2.0	0.9	0.7
12°/-2°C	$\hat{Y} \log_e (g)$	-3.689	-3.124	-3.912	-4.710	-4.962
at 43 days	SE of \hat{Y}	0.090	0.155	0.096	0.115	0.094
		b	a	b	c	c

* Statistics sharing the same letter are not significantly different (P=0.05)

The final petiole dry weights (\hat{Y}) estimated at harvest time in each environment are indicated in Table 14. The rankings were the same within each environment; Ladino clover being superior to Spain and Huia followed by Kent and Russian clovers.

During the second growth period, similar patterns of development occurred for both leaf and petiole production (Appendix 4B and 4C).

4.2.3 Total Stolon Dry Weight Production

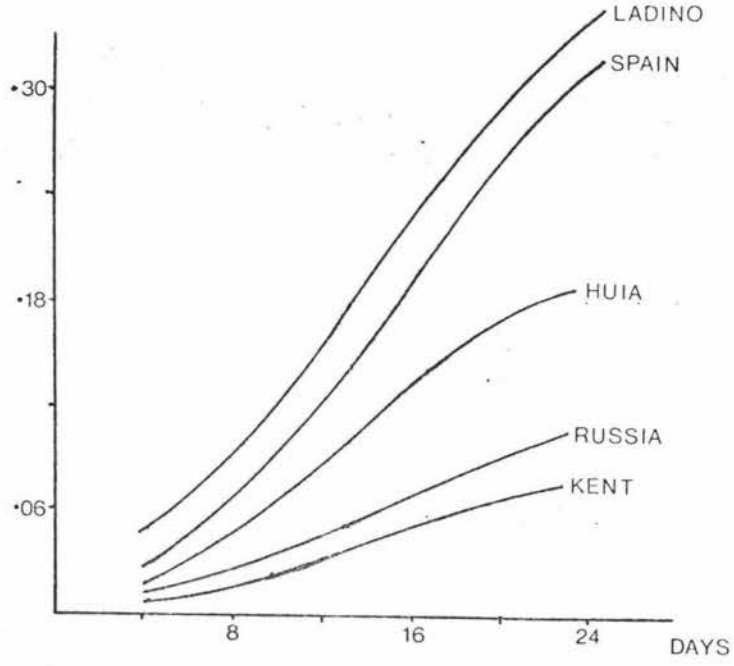
The fitted regression curves are plotted in Figure 8 for each clover population in each environment. The actual data points are given in Appendix 3. The total stolon dry weight (DW) consists of the sum of stem DW, leaf DW and petiole DW. The regression statistics and significance groups describing the development patterns for total stolon DW are indicated in Tables 15 and 16.

From Table 15, in each environment Ladino clover was significantly better than all other populations for the b_0 (placement) coefficient. The b_1 (rate of change) and b_2 (rate of change of rate of change) coefficients were similar for all populations in the cool and cold environments. However, in the hot environment, Huia clover had a greater b_1 value than Ladino clover but the reverse was true for the b_2 coefficient.

The comparison of regression coefficients between environments is indicated in Table 16. All populations had greater b_1 values in the hot environment compared with the colder environments. There were no differences between

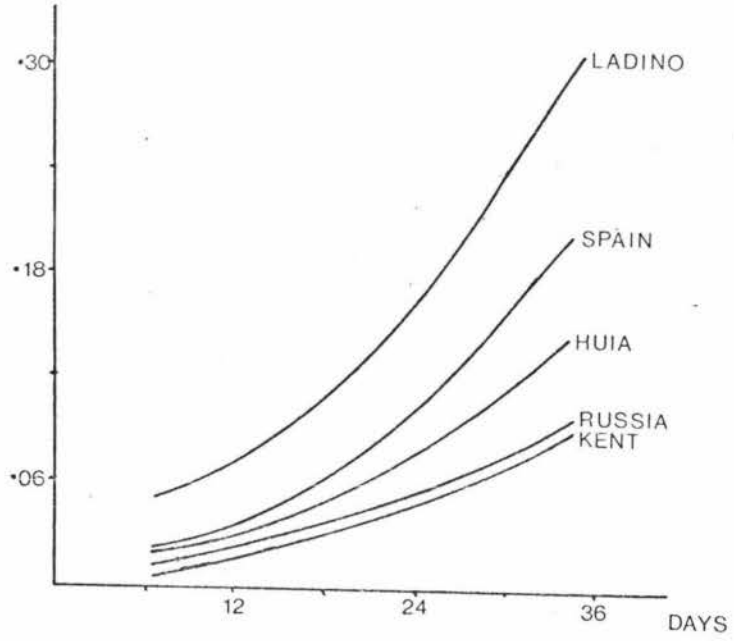
FIGURE 8

HOT
24/14°C

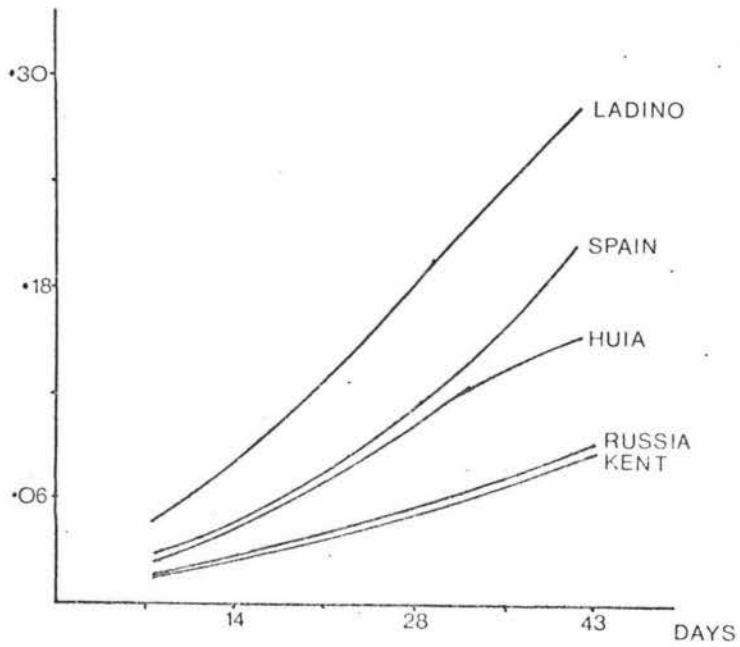


COOL
12/6°C

TOTAL DW
(g)



COLD
12/-2°C



Regression statistics describing the development patterns
for total dry weight

$\log_e (g)$	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT						
24°/14°C	b_0	-4.755	-3.926	-5.414	-5.780	-5.181
	SE b_0	0.190	0.217	0.216	0.239	0.283
		* b	a	c	c	bc
	b_1	0.275	0.222	0.331	0.269	0.239
	SE b_1	0.032	0.036	0.036	0.040	0.047
		ab	b	a	ab	ab
	b_2	-.0051	-.0042	-.0075	-.0059	-.0051
	SE b_2	0.0012	0.0013	0.0013	0.0015	0.0017
		ab	a	b	ab	ab
	R^2	.956	.978	.971	.951	.921
COOL						
12°/6°C	b_0	-5.042	-3.743	-4.582	-5.326	-4.795
	SE b_0	0.313	0.112	0.218	0.230	0.261
		bc	a	b	c	bc
	b_1	0.139	0.094	0.093	0.114	0.102
	SE b_1	0.033	0.012	0.023	0.024	0.028
		a	a	a	a	a
	b_2	-.0012	-.0006	-.0006	-.0010	-.0011
	SE b_2	0.0008	0.0003	0.0005	0.0006	0.0006
		a	a	a	a	a
	R^2	.988	.947	.959	.954	.916
COLD						
12°/-2°C	b_0	-4.389	-3.551	-4.537	-4.876	-4.600
	SE b_0	0.160	0.174	0.213	0.225	0.118
		b	a	bc	bc	bc
	b_1	0.096	0.083	0.116	0.087	0.080
	SE b_1	0.014	0.016	0.019	0.020	0.011
		a	a	a	a	a
	b_2	-.0007	-.0007	-.0013	-.0007	-.0007
	SE b_2	0.0003	0.0003	0.0004	0.0004	0.0002
		a	a	a	a	a
	R^2	.949	.971	.938	.923	.971

* Statistics sharing the same letter are not significantly different (P=0.05)

Statistical differences amongst populations across environments for Total dry weight

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		NS	NS	*	NS	NS
HOT/COLD	b_0	NS	NS	*	*	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	b_1	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	b_2	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	NS	*	NS	NS
HOT/COLD	Y	*	NS	NS	NS	NS
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at P=0.05

* = significance at P=0.05

The asterick is indicated in line with the environment containing the larger estimate.

TABLE 17

Estimated total dry weight at final harvest

	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	antilog (cg)	30.7	33.8	17.0	6.6	9.2
24°/14°C	$\hat{Y} \log_e (g)$	-1.181	-1.085	-1.772	-2.718	-2.386
at 22 days	SE of \hat{Y}	0.076	0.087	0.087	0.096	0.114
		* a	a	b	d	c
COOL	antilog (cg)	18.7	30.1	12.9	6.9	7.6
12°/6°C	$\hat{Y} \log_e (g)$	-1.677	-1.201	-2.048	-2.674	-2.577
at 35 days	SE of \hat{Y}	0.120	0.043	0.084	0.088	0.100
		b	a	c	d	d
COLD	antilog (cg)	19.8	27.4	14.7	8.1	8.1
12°/-2°C	$\hat{Y} \log_e (g)$	-1.619	-1.295	-1.917	-2.513	-2.513
at 43 days	SE of \hat{Y}	0.080	0.087	0.107	0.113	0.059
		b	a	c	d	d

* Statistics sharing the same letter are not significantly different (P=0.05)

the cool and cold environments. However, the growth rates declined at a faster rate (b_2 coefficient) in the hot environment compared with the colder environments.

The final total stolon DW estimated at the endpoints of the growth period are given in Table 17. The significance groupings of the populations remained the same for each environment. Ladino was consistently superior followed by Spain then Huia white clover. The Kent and Russian clovers were consistently the poorest. This pattern of development also occurred in the second growth period (Appendix 4D).

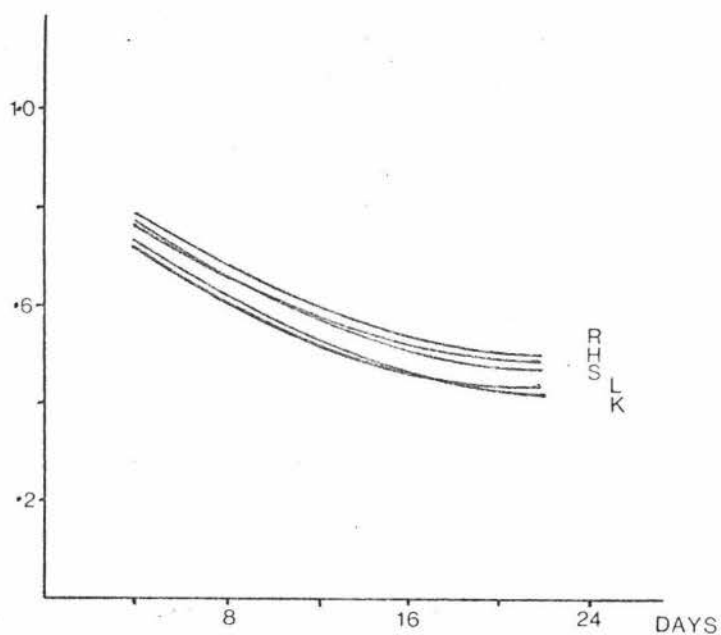
4.2.4 Ratio of Leaf dry weight to Total stolon dry Weight

The ratios were estimated for each plot and then regressed. The fitted regression curves are plotted in Figure 9 with appropriate data points given in Appendix 3. The development patterns of all populations within each environment, as described by the b_0 , b_1 , and b_2 regression coefficients (Table 18) were very similar. The populations indicated a decline (negative values for b_1) in the ratio of leaf to total dry weight with time. Comparison of statistics across environments (Table 19) indicated that all clover populations except Russia had a greater rate of decline in the hot compared with the cooler environments.

The final ratios estimated at the end points (Table 20), showed that the populations had similar rankings within each environment. The Spanish, Huia and Russian clovers had consistently greater values than Ladino and Kent clover in all environments. These latter clovers had a greater

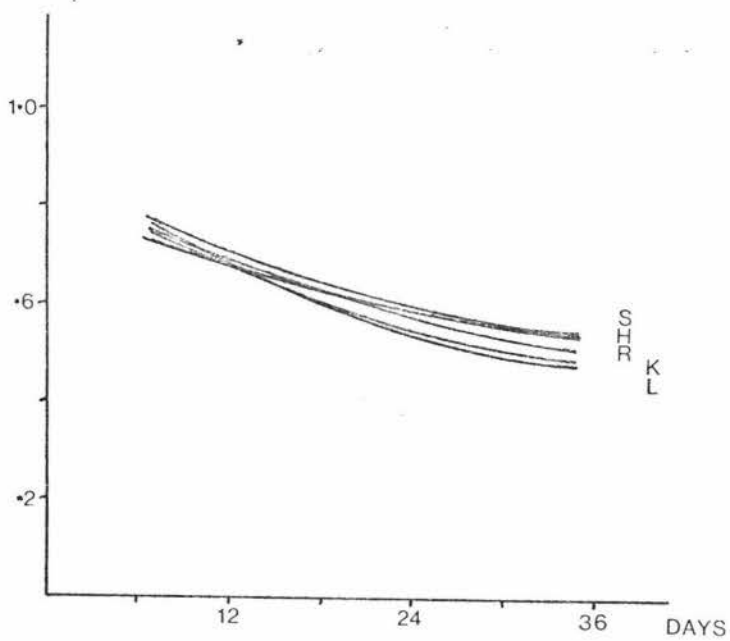
FIGURE 9

HOT
24/14°C



COOL
12/6°C

$\frac{\text{LEAF DW}}{\text{TOTAL DW}}$



COLD
12/-2°C

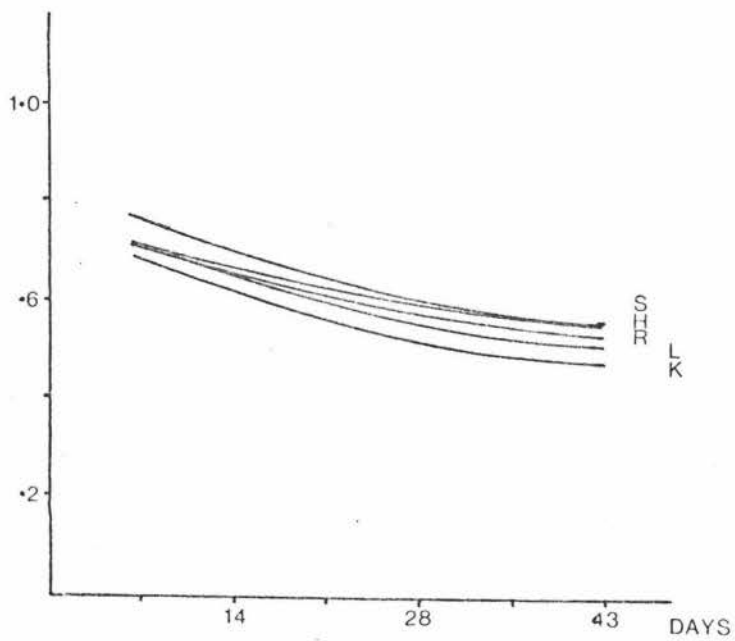


TABLE 18

Regression statistics describing the development patterns
for the ratio of leaf to total dry weight

\log_e	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b_0	0.074	0.035	0.006	0.024	-0.040
	SE b_0	0.054	0.146	0.084	0.103	0.097
		*a	a	a	a	a
	b_1	-0.075	-0.082	-0.063	-0.075	-0.045
	SE b_1	0.009	0.024	0.014	0.017	0.016
		a	a	a	a	a
	b_2	0.0018	0.0018	0.0014	0.0015	0.0007
SE b_2	0.0003	0.0009	0.0005	0.0006	0.0006	
	a	a	a	a	a	
	R^2	.961	.819	.893	.912	.864
COOL 12°/6°C	b_0	-0.196	-0.028	-0.104	-0.189	-0.148
	SE b_0	0.061	0.076	0.073	0.133	-0.097
		b	a	ab	ab	ab
	b_1	-0.018	-0.035	-0.023	-0.017	-0.019
	SE b_1	0.006	0.008	0.008	0.014	0.010
		a	a	a	a	a
	b_2	0.0002	0.0004	0.0003	0.00002	0.0001
SE b_2	0.00015	0.0002	0.0002	0.0003	0.0002	
	a	a	a	a	a	
	R^2	.857	.926	.864	.769	.805
COLD 12°/-2°C	b_0	-0.281	-0.289	-0.107	-0.147	-0.219
	SE b_0	0.045	0.056	0.053	0.072	0.037
		b	b	a	ab	b
	b_1	-0.009	-0.008	-0.021	-0.029	-0.016
	SE b_1	0.0004	0.0005	0.005	0.006	0.003
		a	a	b	c	ab
	b_2	.00002	-.00002	.0002	.0003	.0001
SE b_2	.00008	.00009	.00009	.0001	.00006	
	b	b	ab	a	ab	
	R^2	.879	.884	.903	.882	.944

* Statistics sharing the same letter are not significantly different (P=0.05)

TABLE 19

Statistical differences amongst populations across environments for the ratio of leaf to Total dry weight

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		*	NS	NS	NS	NS
HOT/COLD	b ₀	*	*	NS	NS	NS
COOL/COLD		NS	*	NS	NS	NS
HOT/COOL		*	*	*	*	NS
HOT/COLD	b ₁	*	*	*	*	NS
COOL/COLD		NS	*	NS	NS	NS
HOT/COOL		*	*	*	*	NS
HOT/COLD	b ₂	*	*	*	*	*
COOL/COLD		NS	*	NS	NS	NS
HOT/COOL		*	NS	*	*	NS
HOT/COLD	Y	*	*	*	*	NS
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at P=0.05

* = significance at P=0.05

The asterick is indicated in line with the environment containing the larger estimate.

TABLE 20

Estimated Ratio of Leaf to total dry weight at final harvest

	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	Ratio	.48	.42	.49	.40	.50
24°/14°C	$\hat{Y} \log_e \hat{Y}$	-.734	-.867	-.713	-.916	-.693
at 22 days	SE of \hat{Y}	.0216	.0585	.0338	.0413	.039
		* a	b	a	b	a
COOL	Ratio	.56	.47	.55	.47	.53
12°/6°C	$\hat{Y} \log_e \hat{Y}$	-.580	-.755	-.598	-.755	-.635
at 35 days	SE of \hat{Y}	.0234	.0292	.0282	.0511	.0373
		a	b	a	b	a
COLD	Ratio	.54	.50	.54	.46	.51
12°/-2°C	$\hat{Y} \log_e \hat{Y}$	-.616	-.693	-.616	-.777	-.673
at 43 days	SE of \hat{Y}	.0225	.0279	.0268	.0359	.0184
		a	b	a	b	a

* Statistics sharing the same letter are not significantly different (F=0.05)

proportion of their growth made up of stem tissue. From Table 19, the ratio of leaf to total dry weight was greater in the colder environments.

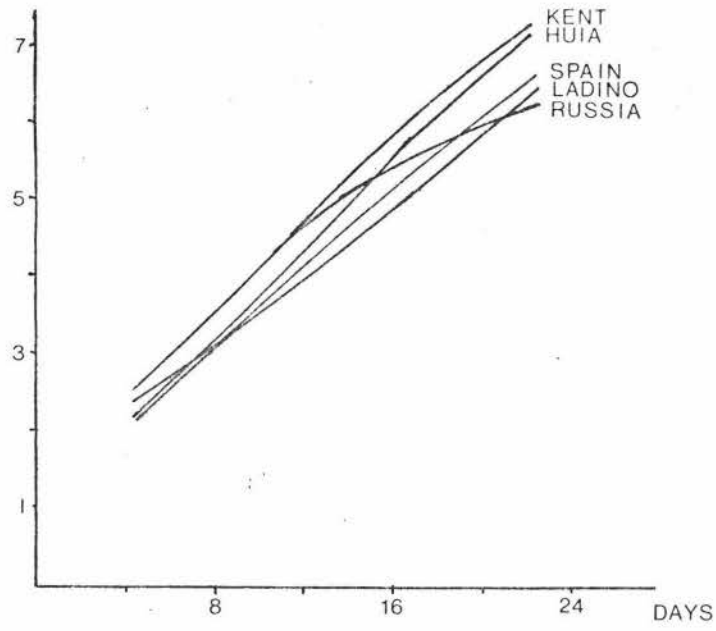
4.2.5 Leaf number per stolon

The fitted curves for each population and environment are plotted in Figure 10 (data points given in Appendix 3). The regression statistics and significance groups are given in Tables 21 and 22. Within each environment the development patterns of all the clover populations for leaf production were basically the same. On comparing the leaf production rates between environments (Table 22), Huia, Kent and Russian clovers had greater rates in the hot compared with the colder environments. Using the estimates of the b_1 and b_2 coefficients, these clover populations produced approximately two leaves per week in the hot environment compared with one leaf per week in the cool and cold environments.

The final estimated leaf numbers at the end of the growth period are given in Table 23. The populations produced similar numbers of leaves within each environment but the most leaves were produced in the hot environment. (Table 22).

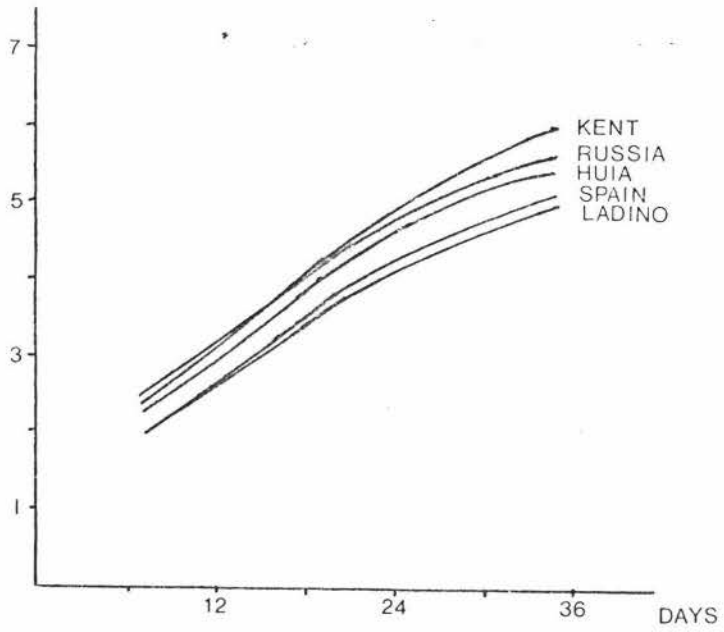
The regression statistics describing the development pattern for the second growth period are given in Appendix 4F.

HOT
24/14°C

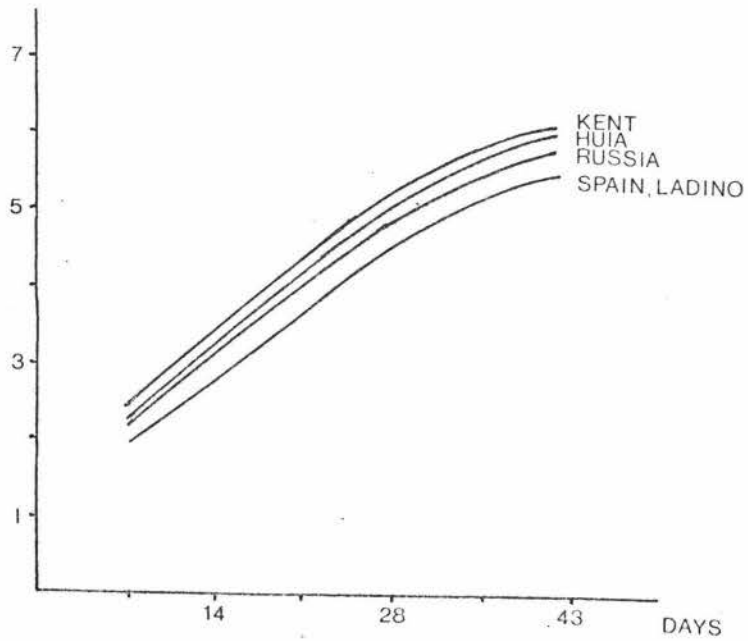


COOL
12/6°C

LEAF
NUMBERS



COLD
12/-2°C



Regression statistics describing the development patterns for
Leaf production

Log _e	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b ₀	0.234	0.470	0.164	0.356	0.267
	SE b ₀	0.139	0.117	0.167	0.120	0.111
		* a	a	a	a	a
	b ₁	0.111	0.075	0.124	0.109	0.130
	SE b ₁	0.023	0.019	0.028	0.020	0.019
		ab	b	ab	ab	a
	b ₂	-.0017	-.0006	-.0020	-.0016	-.0027
	SE b ₂	0.0008	0.0007	0.001	0.0007	0.0007
		ab	ab	ab	a	b
	R ²		.950	.957	.940	.961
COOL 12°/6°C	b ₀	0.170	0.222	0.283	0.264	0.333
	SE b ₀	0.130	0.125	0.043	0.056	0.122
		a	a	a	a	a
	b ₁	0.076	0.070	0.070	0.077	0.073
	SE b ₁	0.014	0.013	0.005	0.006	0.013
		a	a	a	a	a
	b ₂	-.0010	-.0009	-.0009	-.001	-.001
	SE b ₂	0.0003	0.0003	0.0001	0.0001	0.0003
		a	a	a	a	a
	R ²		.944	.940	.992	.989
COLD 12°/-2°C	b ₀	0.197	0.165	0.405	0.419	0.283
	SE b ₀	0.111	0.117	0.090	0.096	0.069
		a	a	a	a	a
	b ₁	0.068	0.070	0.061	0.065	0.064
	SE b ₁	0.010	0.010	0.008	0.009	0.006
		a	a	a	a	a
	b ₂	-.0008	-.0008	-.0007	-.0008	-.0007
	SE b ₂	0.0002	0.0002	0.0002	0.0002	0.0001
		a	a	a	a	a
	R ²		.935	.987	.956	.951

* Statistics sharing the same letter are not significantly different (P=0.05)

Statistical differences amongst populations across environments for Leaf production

Environmental Comparison	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		NS	NS	NS	NS	NS
HOT/COLD	b_0	NS	NS	NS	NS	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		NS	NS	*	NS	*
HOT/COLD	b_1	NS	NS	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		NS	NS	NS	NS	*
HOT/COLD	b_2	NS	NS	NS	NS	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	Y	*	*	*	*	NS
COOL/COLD		NS	NS	*	NS	NS

NS = non significance at P=0.05

* = significance at P=0.05

The asterick is indicated in line with the environment containing the larger estimate.

Estimated leaf production at final harvest

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	No. of leaves	6.4	6.2	7.1	7.2	6.1
24°/14°C	$\hat{Y} \log_e$	1.858	1.828	1.963	1.970	1.813
at 22 days	SE of \hat{Y}	0.056	0.047	0.067	0.048	0.045
		*ab	ab	ab	a	b
COOL	No. of leaves	4.9	4.7	5.1	5.7	5.3
12°/6°C	$\hat{Y} \log_e$	1.597	1.552	1.637	1.748	1.666
at 35 days	SE of \hat{Y}	0.047	0.048	0.016	0.022	0.047
		b	b	b	a	ab
COLD	No. of leaves	4.9	5.1	5.7	5.9	5.6
12°/-2°C	$\hat{Y} \log_e$	1.585	1.619	1.751	1.780	1.719
at 43 days	SE of \hat{Y}	0.056	0.059	0.045	0.048	0.035
		b	ab	ab	a	ab

* Statistics sharing the same letter are not significantly different (P=0.05)

4.2.6 Leaf Area per stolon

The fitted regression curves are plotted in Figure 11 and corresponding data points given in Appendix 3. The regression statistics and significance groups describing these curves are given in Tables 24 and 25. When these curves are compared with those of Total stolon dry weight (Figure 9), the development patterns are quite similar. There were no differences between clover populations for rate of change in Leaf Area (b_1 coefficient) within each environment. However, on comparing environments (Table 25), the Leaf Area increased more rapidly in the hot environment compared with the colder environments.

The final leaf areas estimated at the end points of the growth cycle (Table 26) indicated the larger values of the Mediterranean clovers compared with the North temperate clovers at all temperatures. Also, the populations tended to have larger Leaf areas in the hot compared with the colder environments (Table 25).

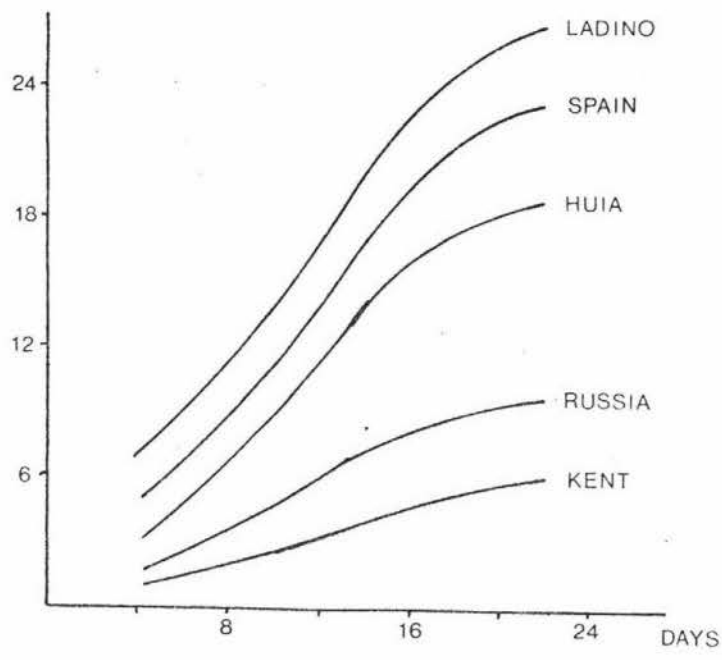
4.2.7 Length of stolon

The fitted curves are plotted in Figure 12 with the appropriate data points given in Appendix 3. The regression statistics and significance groups are indicated in Tables 27 and 28.

Ladino clover had the greatest value for the b_0 coefficient (the initial curve placement parameter) in each environment. All the populations indicated similar changes in stolon length with time (b_1 and b_2 coefficients) in the cool and cold environments. In the hot environment; Huia, Kent and Spanish clovers tended to have significantly greater b_1 coefficients to Ladino and Russia but the reverse

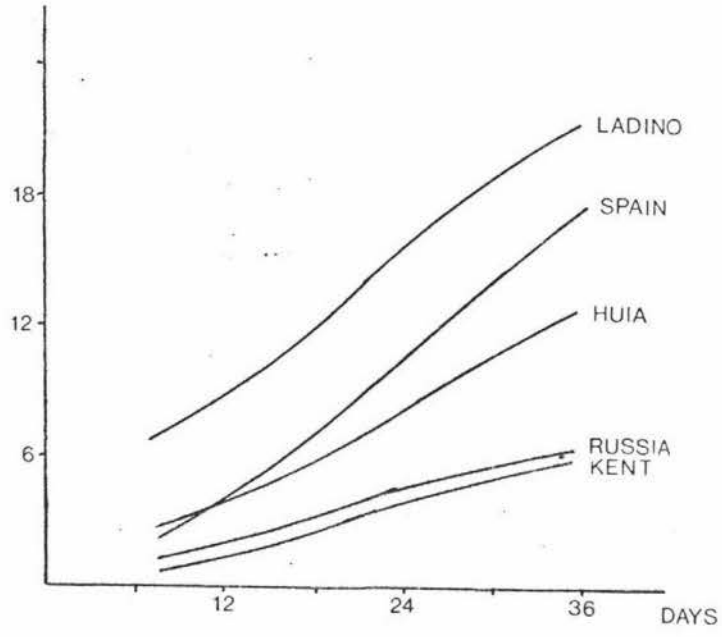
FIGURE II

HOT
24/14°C

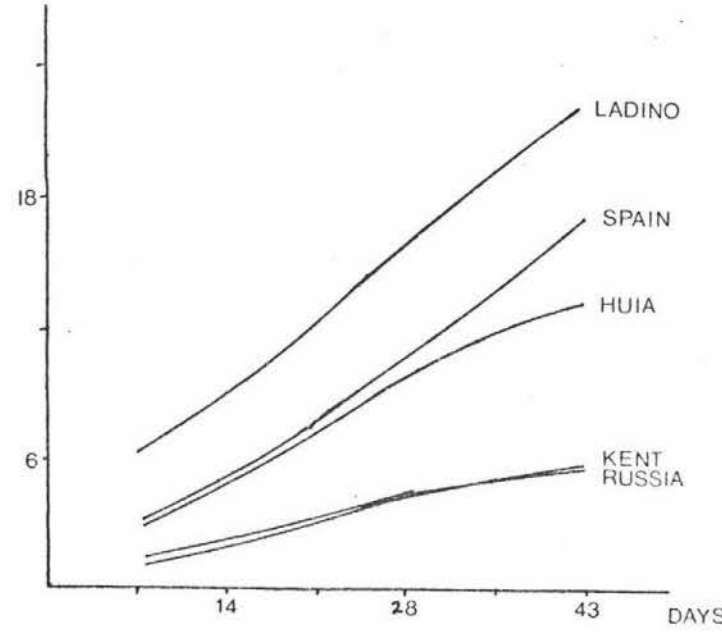


COOL
12/6°C

LEAF AREA
(sq cm)



COLD
12/-2°C



Regression statistics describing the development patterns
for Leaf Area

$\log_e(\text{cm}^2)$	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b_0	0.767	0.997	-0.136	-0.601	-0.083
	SE b_0	0.166	0.205	0.221	0.239	0.181
		* a	a	bc	b	c
	b_1	0.217	0.212	0.292	0.214	0.218
	SE b_1	0.028	0.034	0.037	0.040	0.030
		a	a	a	a	a
	b_2	-.0045	-.0050	-.0072	-.0052	-.0054
	SE b_2	0.0010	0.0012	0.0013	0.0015	0.0011
	a	a	a	a	a	
	R^2	.967	.932	.950	.901	.941
COOL 12°/6°C	b_0	-0.083	1.122	0.441	-0.571	0.003
	SE b_0	0.291	0.190	0.201	0.230	0.231
		bc	a	b	c	b
	b_1	0.141	0.090	0.080	0.105	0.078
	SE b_1	0.031	0.020	0.021	0.024	0.025
		a	a	a	a	a
	b_2	-.0016	-.0010	-.0006	-.0012	-.0009
	SE b_2	0.0007	0.0005	0.0005	0.0006	0.0006
	a	a	a	a	a	
	R^2	.933	.938	.948	.926	.885
COLD 12°/-2°C	b_0	0.636	1.302	0.602	0.172	0.264
	SE b_0	0.230	0.174	0.205	0.207	0.164
		b	a	b	b	b
	b_1	0.081	0.071	0.084	0.052	0.055
	SE b_1	0.020	0.016	0.018	0.018	0.015
		a	a	a	a	a
	b_2	-.0007	-.0007	-.0009	-.0004	-.0006
	SE b_2	.0004	0.0003	0.0004	0.0004	0.0003
	a	a	a	a	a	
	R^2	.906	.915	.902	.851	.868

* Statistics sharing the same letter are not significantly different (P=0.05)

TABLE 25

Statistical differences amongst populations across environments
for Leaf Area

Environmental Comparison	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		*	NS	*	NS	NS
HOT/COOL	b_0	NS	NS	*	*	*
COOL/COLD		*	NS	NS	*	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	b_1	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	*	*	*	*
HOT/COLD	b_2	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL		*	NS	*	NS	*
HOT/COLD	Y	*	NS	*	NS	*
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at $P=0.05$

* = significance at $P=0.05$

The asterick is indicated in line with the environment containing the larger estimate.

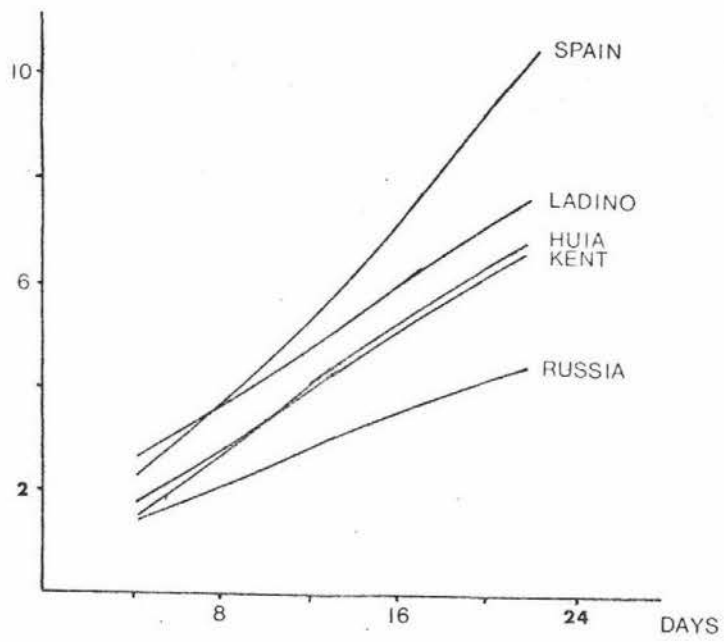
TABLE 26

Estimated Leaf Area at final harvest

	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	Leaf Area(cm ²)	22.69	25.61	18.60	5.40	8.20
24 ^o /14 ^o C	$\hat{Y} \log_e$ (cm ²)	3.122	3.243	2.923	1.686	2.104
at 22 days	SE of \hat{Y}	0.067	0.082	0.089	0.096	0.073
		* ab	a	b	d	c
COOL	Leaf Area(cm ²)	17.03	21.67	12.94	5.03	5.53
12 ^o /6 ^o C	$\hat{Y} \log_e$ (cm ²)	2.835	3.076	2.560	1.615	1.710
at 35 days	SE of \hat{Y}	0.112	0.073	0.077	0.088	0.089
		ab	a	b	c	c
COLD	Leaf Area(cm ²)	16.63	21.56	13.14	5.19	4.82
12 ^o /-2 ^o C	$\hat{Y} \log_e$ (cm ²)	2.811	3.071	2.576	1.647	1.573
at 43 days	SE of \hat{Y}	0.115	0.087	0.103	0.104	0.082
		ab	a	b	c	c

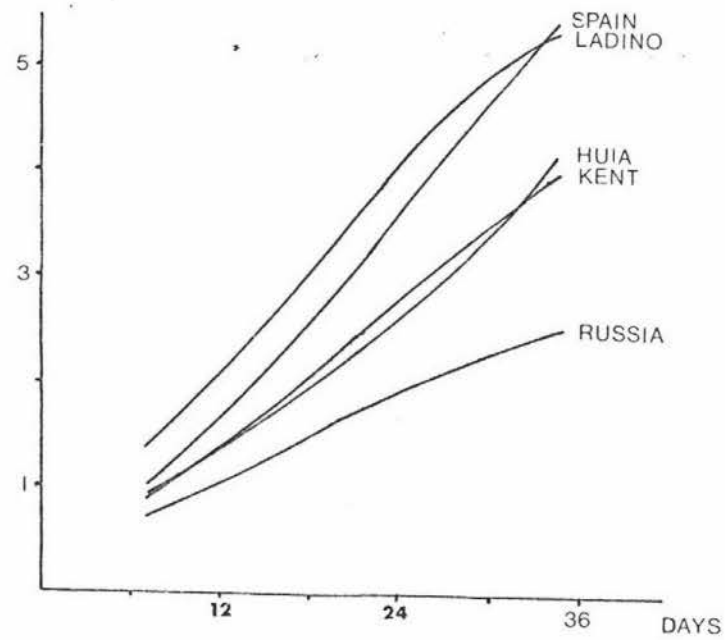
* Statistics sharing the same letter are not significantly different (P=0.05)

HOT
24/14°C



COOL
12/6°C

STOLON LENGTH
(cm)



COLD
12/-2°C

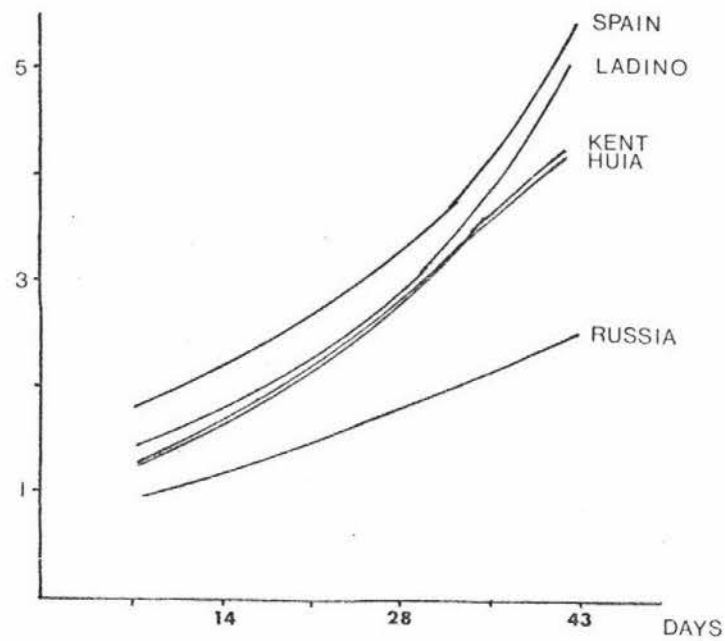


TABLE 27

Regression statistics describing the development patterns
for length of stolon

\log_e (cm)	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b_0	0.066	0.479	-0.532	-0.222	-0.115
	SE b_0	0.181	0.112	0.141	0.193	0.161
		*b	a	d	bcd	bc
	b_1	0.161	0.105	0.211	0.171	0.120
	SE b_1	0.030	0.019	0.023	0.032	0.027
		abc	c	a	ab	bc
	b_2	-.0026	-.0016	-.0046	-.0034	-.0022
	SE b_2	0.0011	0.0007	0.0009	0.0012	0.0010
		abc	a	c	abc	ab
	R^2	.955	.963	.973	.936	.926
COOL 12°/6°C	b_0	-0.730	-0.371	-0.545	-0.845	-0.905
	SE b_0	0.287	0.121	0.176	0.124	0.171
		ab	a	ab	b	b
	b_1	0.102	0.096	0.067	0.102	0.086
	SE b_1	0.030	0.013	0.019	0.023	0.018
		a	a	a	a	a
	b_2	-.0009	-.0011	-.0003	-.0011	-.0010
	SE b_2	0.0007	0.0003	0.0004	0.0005	0.0004
		a	a	a	a	a
	R^2	.918	.977	.955	.939	.943
COLD 12°/-2°C	b_0	0.146	0.427	-0.193	-0.219	-0.364
	SE b_0	0.151	0.109	0.198	0.225	0.089
		ab	a	bc	bc	c
	b_1	0.029	0.022	0.057	0.055	0.040
	SE b_1	0.014	0.010	0.018	0.020	0.008
		a	a	a	a	a
	b_2	0.0002	0.0001	-.0004	-.0004	-.0002
	SE b_2	0.0003	0.0002	0.0003	0.0004	0.0002
		a	a	a	a	a
	R^2	.957	.945	.887	.866	.961

* Statistics sharing the same letter are not significantly different (P=0.05)

TABLE 28

Statistical differences amongst populations across environments for stolon length

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		*	*	NS	*	*
HOT/COLD	b_0	NS	NS	NS	NS	NS
COOL/COLD		*	*	NS	*	*
HOT/COOL		NS	NS	*	*	NS
HOT/COLD	b_1	*	*	*	*	*
COOL/COLD		*	*	NS	NS	*
HOT/COOL		NS	NS	*	*	NS
HOT/COLD	b_2	*	*	*	*	*
COOL/COLD		NS	*	NS	NS	*
HOT/COOL		*	*	*	*	*
HOT/COLD	Y	*	*	*	*	*
COOL/COLD		NS	NS	NS	NS	NS

NS = non significance at P = 0.05

* = significance at P = 0.05

The asterick is indicated in line with the environment containing the larger estimate.

Estimated stolon lengths at final harvest

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	Length (cm)	10.39	7.50	6.58	6.65	4.30
24°/14°C	$\hat{Y} \log_e$ (cm)	2.341	2.015	1.884	1.895	1.459
at 22 days	SE of \hat{Y}	0.073	0.045	0.057	0.078	0.065
		* a	b	b	b	c
COOL	Length (cm)	5.63	5.50	4.15	3.91	2.43
12°/6°C	$\hat{Y} \log_e$ (cm)	1.728	1.705	1.423	1.364	0.888
at 35 days	SE of \hat{Y}	0.110	0.047	0.068	0.082	0.066
		a	a	b	b	c
COLD	Length (cm)	5.41	5.01	4.31	4.37	2.54
12°/-2°C	$\hat{Y} \log_e$ (cm)	1.688	1.611	1.461	1.475	0.932
at 43 days	SE of \hat{Y}	0.076	0.055	0.099	0.113	0.045
		a	a	a	a	b

* Statistics sharing the same letter are not significantly different (P = 0.05)

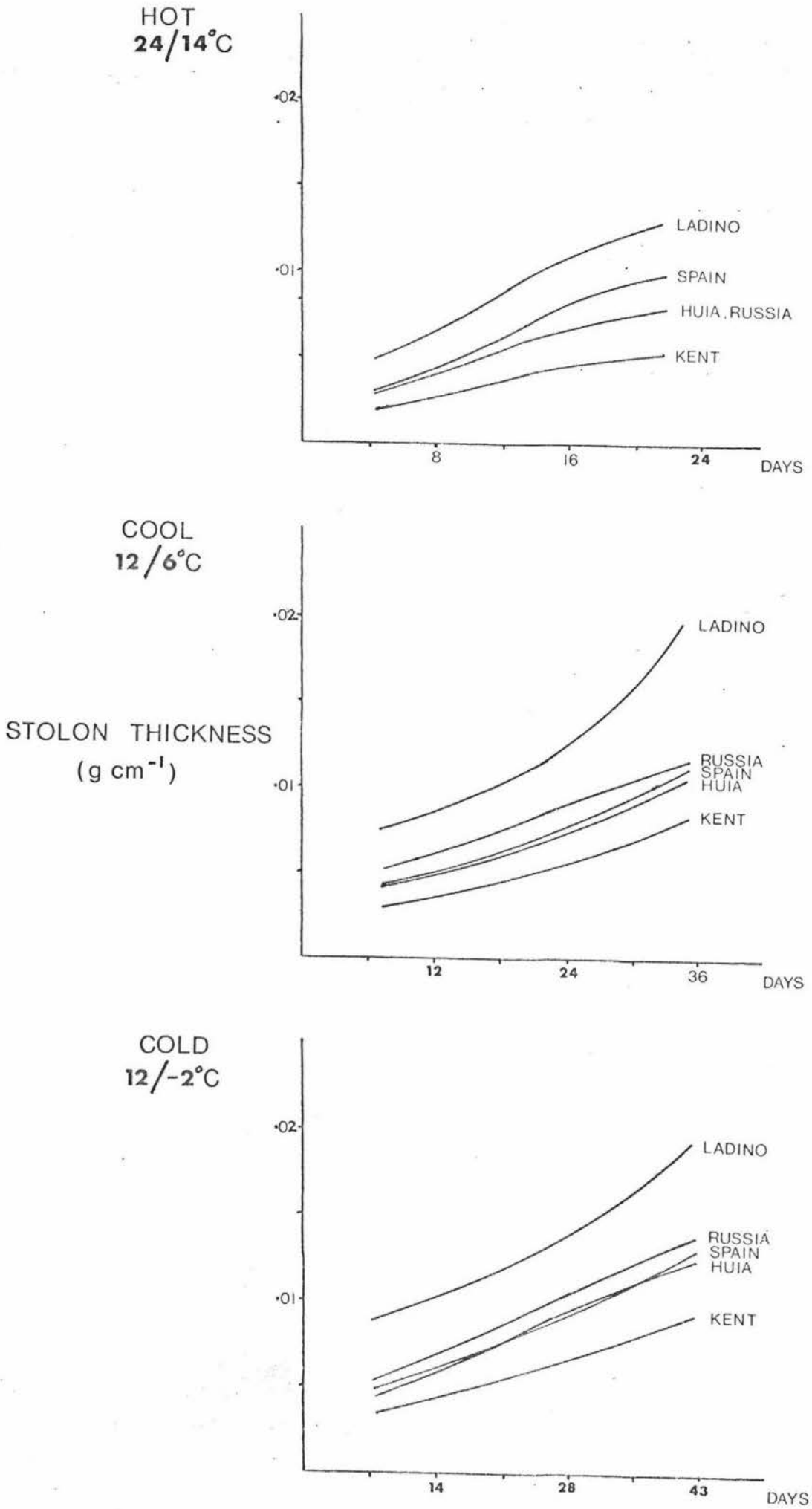
was true for the b_2 coefficient (the rate of change of the rate of change in stolon length). When comparing environments (Table 28), the clover populations had greater b_1 coefficients in the hot than the cold environment.

The final stolon lengths (Table 29) in the hot environment showed the greater stolon length of Spanish clover while Russian clover had the shortest stolons. This pattern also occurred in the cool environment but in the cold environment, the clover populations had similar stolon lengths except Russia which was significantly shorter. All populations had longer stolons in the hot environment compared to the colder environments. (Table 28).

4.2.8 Stolon thickness

The fitted regression curves are plotted in Figure 13 with data points given in Appendix 3. The regression statistics and significance groups describing these curves are given in Tables 30 and 31. Ladino clover had greater values for the b_0 coefficient (describing the initial stolon thickness) compared with the other populations in each environment. However, the estimates for the b_1 and b_2 coefficients (the rate of change of stolon thickness) were constant for all populations in each environment. On comparing regression statistics between environments (Table 31), all populations had greater values of b_0 in the cool and cold compared with the hot environment. However, all populations had greater b_1 values in the hot compared with the colder environments but the reverse was true for the b_2 coefficient.

FIGURE 13



Regression statistics describing the development patterns
for stolon thickness

\log_e (g cm ⁻¹)	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b_0	-6.655	-5.934	-6.556	-6.984	-6.535
	SE b_0	0.229	0.171	0.193	0.134	0.146
		* bcd	a	bc	d	b
	b_1	0.169	0.134	0.139	0.149	0.132
	SE b_1	0.038	0.029	0.032	0.022	0.024
		a	a	a	a	a
	b_2	-.0035	-.0029	-.0031	-.0036	-.0028
	SE b_2	0.0014	0.0010	0.0012	0.0008	0.0009
	a	a	a	a	a	
	R^2	.903	.904	.889	.932	.931
COOL 12°/6°C	b_0	-5.530	-4.999	-5.533	-5.772	-5.447
	SE b_0	0.121	0.127	0.212	0.151	0.226
		b	a	b	b	b
	b_1	0.012	0.010	0.013	0.017	0.028
	SE b_1	0.013	0.013	0.022	0.016	0.024
		a	a	a	a	a
	b_2	0.0005	0.0006	0.0004	0.0003	0.0002
	SE b_2	0.0003	0.0003	0.0005	0.0004	0.0006
	a	a	a	a	a	
	R^2	.940	.946	.823	.884	.791
COLD 12°/-2°C	b_0	-5.650	-5.084	-5.987	-6.097	-5.534
	SE b_0	0.069	0.111	0.109	0.152	0.079
		b	a	c	c	b
	b_1	0.039	0.034	0.070	0.056	0.040
	SE b_1	0.006	0.010	0.010	0.014	0.007
		b	b	a	ab	b
	b_2	-.0002	-.0002	-.0008	-.0006	-.0003
	SE b_2	0.0001	0.0002	0.0002	0.0003	0.0001
	a	a	b	ab	a	
	R^2	.976	.923	.948	.874	.964

* Statistics sharing the same letter are not significantly different (P = 0.05)

Statistical differences amongst populations across environments for stolon thickness

<u>Environmental Comparisons</u>	<u>Statistic</u>	<u>Spain</u>	<u>Ladino</u>	<u>Huia</u>	<u>Kent</u>	<u>Russia</u>
HOT/COOL		*	*	*	*	*
HOT/COLD	b_0	*	*	*	*	*
COOL/COLD		NS	NS	*	NS	NS
<hr/>						
HOT/COOL		*	*	*	*	*
HOT/COLD	b_1	*	*	*	*	*
COOL/COLD		*	NS	*	NS	NS
<hr/>						
HOT/COOL		*	*	*	*	*
HOT/COLD	b_2	*	*	*	*	*
COOL/COLD		*	*	*	NS	NS
<hr/>						
HOT/COOL		NS	*	*	*	*
HOT/COLD	Y	*	*	*	*	*
COOL/COLD		*	NS	NS	NS	NS

NS = non significance at P = 0.05

* = significance at P = 0.05

The asterick is indicated in line with the environment containing the larger estimate.

Estimated stolon thickness at final harvest

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	Thickness (mg cm ⁻¹)	9.7	12.4	6.8	4.3	6.8
24°/14°C	$\hat{Y} \log_e(\text{gcm}^{-1})$	-4.636	-4.390	-4.991	-5.449	-4.991
at 22 days	SE of \hat{Y}	0.092	0.069	0.077	0.054	0.059
		* a	a	b	c	b
COOL	Thickness (mg cm ⁻¹)	10.2	18.5	9.8	7.4	11.8
12°/6°C	$\hat{Y} \log_e(\text{gcm}^{-1})$	-4.585	-3.990	-4.625	-4.906	-4.440
at 35 days	SE of \hat{Y}	0.047	0.049	0.081	0.058	0.087
		b	a	b	c	b
COLD	Thickness (mg cm ⁻¹)	12.5	19.0	11.4	8.1	13.3
12°/-2°C	$\hat{Y} \log_e(\text{gcm}^{-1})$	-4.382	-3.963	-4.474	-4.816	-4.320
at 43 days	SE of \hat{Y}	0.034	0.056	0.055	0.076	0.040
		b	a	b	c	b

* Statistics sharing the same letter are not significantly different (P =0.05)

The final stolon thickness estimated at harvest time in each environment are given in Table 32. The clover populations showed similar rankings within each environment. Ladino consistently had the thickest stolons while Kent clover had the thinnest. When comparing environments, the stolons were thickest for all populations in the cold environment (Table 31).

In the second growth period, the developmental growth patterns of the ten stolon characters were generally in close agreement with the first growth period (Appendix 4). However, over a similar time period, less herbage dry weight production per stolon occurred in the second growth period. This could have been due to a decline in the vigour of growth following establishment, a restrictive influence on growth caused by the confinement of the pots and/or an effect of defoliation.

4.2.9 Stolon numbers

Two methods of assessing the numbers of stolons were attempted. Firstly, the number of secondary stolons produced from the tagged primary stolons of each population were counted. Secondly, an estimate of the stolon population was obtained by dividing the total pot dry weight by the stolon leaf and petiole dry weight.

4.2.9.1 Production of secondary stolons

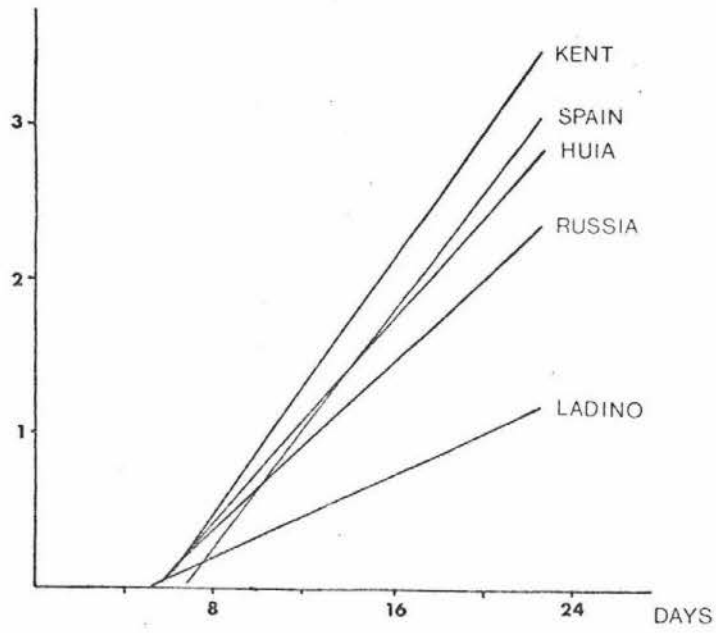
The fitted curves for the first and second growth periods are given in Figures 14 and 15 respectively (data points in Appendix 3). The straight line was most applicable for this stolon character. The regression statistics and significance groups are shown in Tables 33, 34, 35 and 36. The populations had similar placement(b_0)

coefficients within each environment (Tables 33 and 35) and between environments (Tables 34 and 36). The rate of change in stolon numbers (b_1 coefficient) and the final estimated number of secondary stolons (\hat{Y}) indicated similar significance groupings, amongst populations within each environment.

In the first growth period, the plants were still establishing and the clover populations produced quite high numbers of new stolons (Table 33). Kent and Spanish clover produced the most laterals in the hot environment while Ladino was significantly the poorest. Kent clover was significantly better than the other populations in the cool environment while in the cold environment, Kent, Huia and Russian clover had greater numbers of new stolons than Spanish and Ladino clover. When comparing environments (Table 34), all the populations except Ladino showed a greater rate of stolon production (b_1 coefficient) in the hot environment.

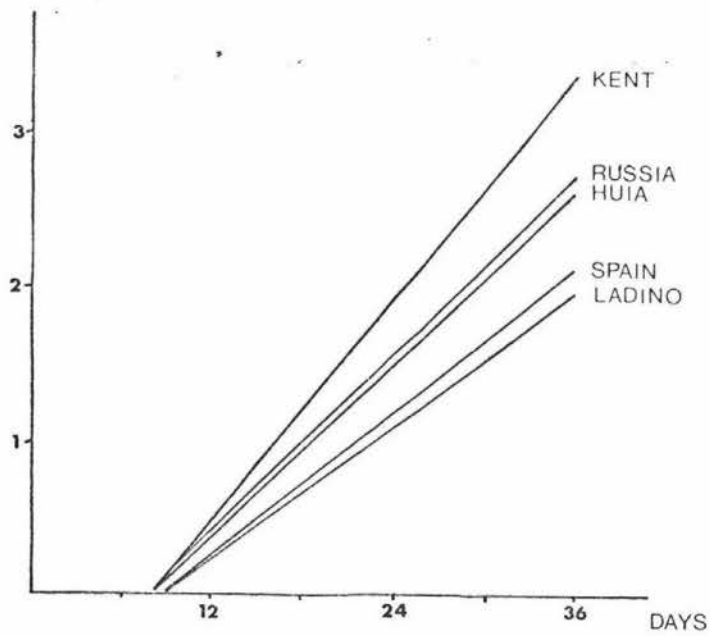
During the second growth period, a contrasting pattern of secondary stolon production occurred (Figure 16 and Table 35). Fewer secondary stolons were formed in each of the environments, particularly the hot environment. There were no significant differences between populations for lateral production in the hot environment. Kent, Huia and Russian clovers produced more secondary stolons than Spanish and Ladino clover in the cool and cold environments which was consistent with first growth period. However, when comparing environments, the populations tended to show similar rates of stolon production (b_1 coefficient) across all environments (Table 36).

HOT
24/14°C

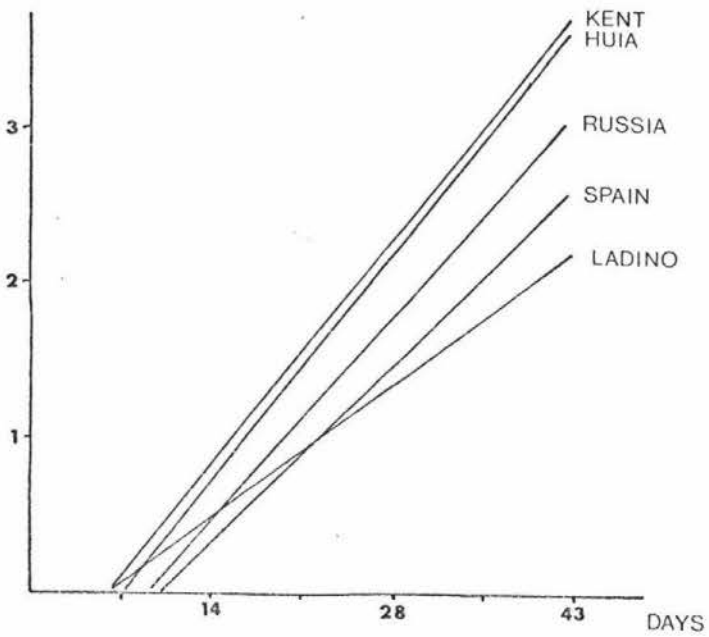


COOL
12/6°C

STOLON NUMBERS



COLD
12/-2°C



Regression statistics describing the development patterns
for stolon numbers (First growth cycle)

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b ₀	-1.472	-0.419	-1.004	-1.290	-0.875
	SE b ₀	0.387	0.206	0.190	0.184	0.209
		* b	a	b	b	ab
	b ₁	0.205	0.075	0.174	0.219	0.149
	SE b ₁	0.027	0.014	0.013	0.013	0.014
		ab	d	bc	a	c
	Y	3.04	1.24	2.81	3.54	2.39
	SE Y	0.28	0.15	0.14	0.14	0.15
		ab	c	b	a	b
	R ²	.819	.683	.931	.958	.891
COOL 12°/6°C	b ₀	-0.869	-0.768	-0.895	-1.022	-0.844
	SE b ₀	0.233	0.129	0.164	0.155	0.172
		a	a	a	a	a
	b ₁	0.084	0.076	0.095	0.120	0.097
	SE b ₁	0.010	0.006	0.007	0.007	0.007
		bc	c	b	a	b
	Y	2.15	1.96	2.54	3.31	2.64
	SE Y	0.30	0.23	0.25	0.25	0.26
		b	b	b	a	ab
	R ²	.843	.935	.934	.961	.930
COLD 12°/-2°C	b ₀	-0.806	-0.383	-0.713	-0.491	-0.794
	SE b ₀	0.219	0.148	0.210	0.273	0.270
		a	a	a	a	a
	b ₁	0.076	0.060	0.099	0.096	0.088
	SE b ₁	0.007	0.005	0.007	0.009	0.009
		b	c	a	a	ab
	Y	2.47	2.21	3.57	3.62	2.97
	SE Y	0.28	0.23	0.28	0.32	0.31
		b	bc	a	a	ab
	R ²	.871	.923	.942	.874	.884

* Statistics sharing the same letter are not significantly different (P = 0.05)

Statistical differences amongst populations across environments for stolon numbers

Environmental Comparisons	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL		NS	NS	NS	NS	NS
HOT/COLD	b_0	NS	NS	NS	*	NS
COOL/COLD		NS	*	NS	NS	NS
<hr/>						
HOT/COOL		*	NS	*	*	*
HOT/COLD	b_1	*	NS	*	*	*
COOL/COLD		NS	NS	NS	*	NS
<hr/>						
HOT/COOL		*	*	NS	NS	NS
HOT/COLD	Y	NS	*	*	NS	NS
COOL/COLD		NS	NS	*	NS	NS

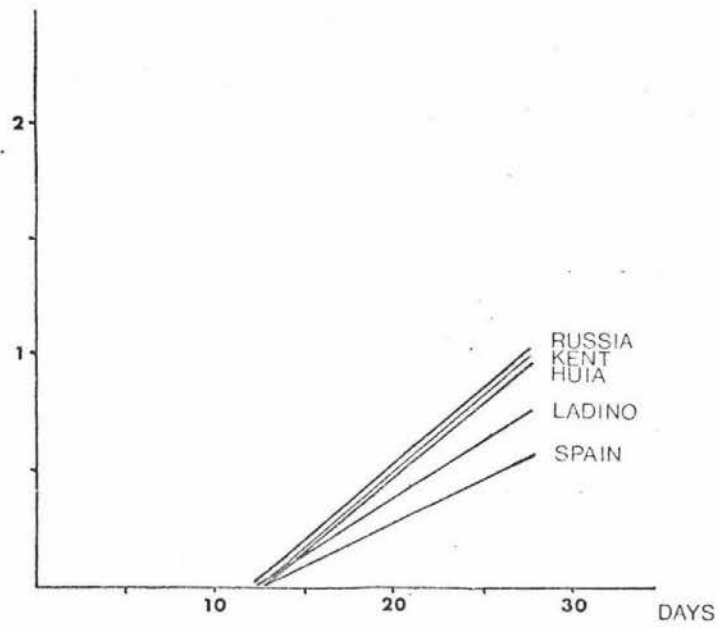
NS = non significance at P = 0.05

* = significance at P = 0.05

The asterick is indicated in line with the environment containing the larger estimate.

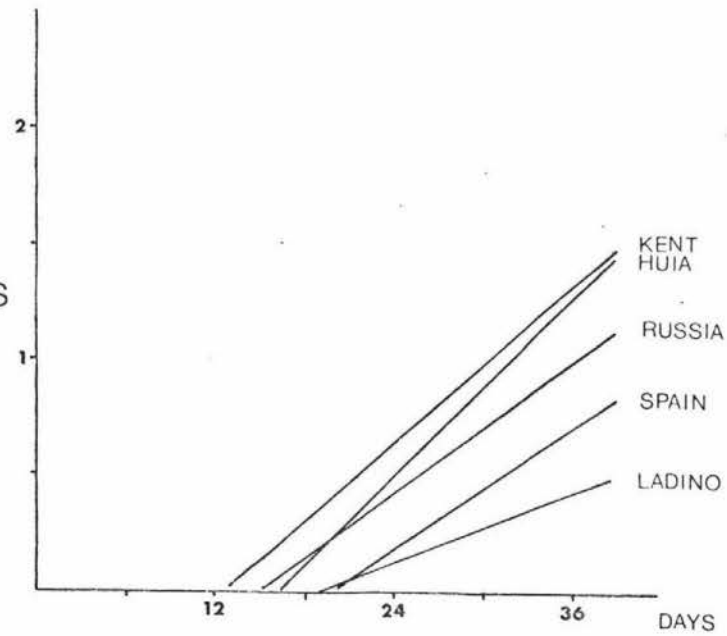
FIGURE 15

HOT
24/14°C

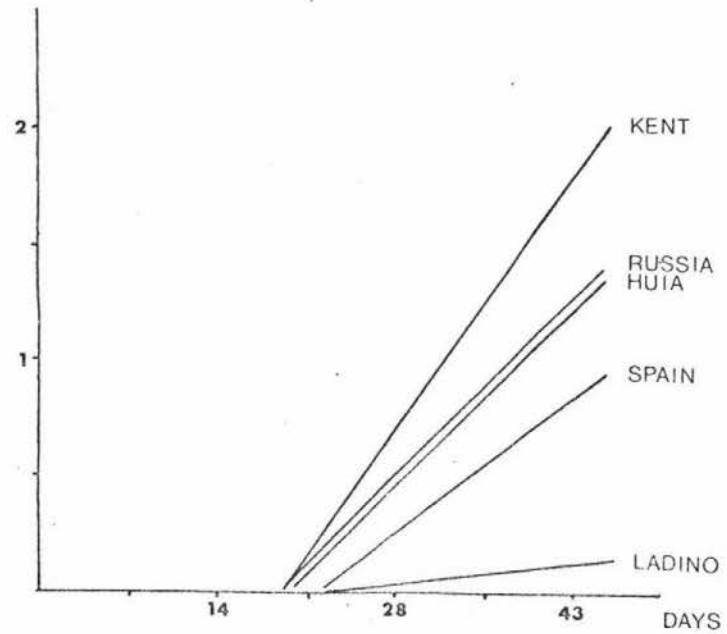


COOL
12/6°C

STOLON NUMBERS



COLD
12/-2°C



Regression statistics describing the development patterns
for stolon numbers (second growth cycle)

Statistic		Spain	Ladino	Huia	Kent	Russia
HOT 24°/14°C	b_0	-0.413	-0.444	-0.689	-0.650	-0.504
	SE b_0	0.251	0.338	0.299	0.377	0.224
		* a	a	a	a	a
	b_1	0.037	0.044	0.060	0.059	0.055
	SE b_1	0.013	0.018	0.016	0.020	0.012
		a	a	a	a	a
	Y	0.59	0.75	0.93	0.95	0.99
	SE Y	0.14	0.19	0.16	0.21	0.12
		a	a	a	a	a
	R^2	.516	.459	.664	.549	.749
COOL 12°/6°C	b_0	-0.720	-0.300	-1.044	-0.767	-0.763
	SE b_0	0.246	0.111	0.226	0.263	0.291
		ab	a	b	ab	ab
	b_1	0.039	0.020	0.063	0.055	0.049
	SE b_1	0.007	0.003	0.008	0.008	0.008
		b	c	a	ab	ab
	Y	0.79	0.49	1.40	1.39	1.16
	SE Y	0.13	0.06	0.12	0.14	0.16
		bc	c	a	a	ab
	R^2	.742	.791	.899	.836	.769
COLD 12°/-2°C	b_0	-0.807	-0.095	-0.947	-1.405	-0.764
	SE b_0	0.239	0.139	0.262	0.405	0.385
		b	a	b	b	b
	b_1	0.039	0.007	0.050	0.077	0.047
	SE b_1	0.006	0.004	0.007	0.010	0.010
		b	c	b	a	b
	Y	0.90	0.19	1.27	1.97	1.29
	SE Y	0.11	0.06	0.12	0.18	0.17
		c	d	b	a	b
	R^2	.810	.258	.857	.852	.704

* Statistics sharing the same letter are not significantly different

Statistical differences amongst populations across environments for stolon numbers

Environmental Comparison	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT/COOL	b ₀	NS	NS	NS	NS	NS
HOT/COLD		NS	NS	NS	NS	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL	b ₁	NS	NS	NS	NS	NS
HOT/COLD		NS	*	NS	NS	NS
COOL/COLD		NS	NS	NS	NS	NS
HOT/COOL	Y	NS	NS	*	NS	NS
HOT/COLD		NS	*	*	*	NS
COOL/COLD		NS	*	NS	*	NS

NS = non significance at P = 0.05 .

* = significance at P = 0.05

The asterick is indicated in line with the environment containing the larger estimate.

4.2.9.2 Estimated stolon numbers per pot

Further information on the stolon numbers of each clover population in the three environments was derived from the dry weight (DW) per pot and dry weight (DW) per stolon data. This was an approximation of the total number of stolons in each pot and the estimates for the first and second growth period are indicated in Table 37.

The principal result shown in Table 37 is the apparent inverse relationship between growth per stolon and stolon numbers of these clover populations.

The Mediterranean clovers (Spain and Ladino) had greater growth per stolon but fewer numbers of stolons compared with the clovers from temperate climates (Huia, Kent, and Russia) which produced less growth per stolon but greater stolon numbers.

Estimate of total stolon numbers per pot

<u>First growth period</u>		Spain	Ladino	Huia	Kent	Russia
HOT	DW/pot (g)	3.54	4.70	3.68	4.16	4.65
24°/14°C	DW/stolon(g)	0.207	0.235	0.126	0.034	0.067
	Stolon nos/pot	17	20	29	122	74
COOL						
12°/6°C	DW/pot (g)	2.28	4.81	2.61	2.60	2.29
	DW/stolon(g)	0.134	0.188	0.094	0.042	0.050
	Stolon nos/pot	17	25	28	62	46
COLD						
12°/-2°C	DW/pot (g)	2.62	5.18	3.35	3.22	2.16
	DW/stolon(g)	0.131	0.181	0.101	0.046	0.048
	Stolon nos/pot	20	28	34	70	45
<u>Second growth period</u>						
HOT	DW/pot (g)	5.25	5.42	4.68	4.06	4.52
24°/14°C	DW/stolon(g)	0.127	0.162	0.091	0.022	0.037
	Stolon nos/pot	42	34	51	185	122
COOL						
12°/6°C	DW/pot (g)	6.80	9.34	6.62	5.60	3.70
	DW/stolon(g)	0.118	0.175	0.083	0.025	0.029
	Stolon nos/pot	58	53	80	224	128
COLD						
12°/-2°C	DW/pot (g)	6.52	9.42	7.18	5.95	3.18
	DW/stolon(g)	0.101	0.154	0.074	0.026	0.026
	Stolon nos/pot	65	61	97	229	122

4.2.10 Pooled Environments ANOVA

4.2.10.1 Dry weight per pot (g)

At the end of the first growth period, the leaf and petiole material was removed from each pot and the data analysed using the Pooled Environments ANOVA. The ANOVA, population means and adaptation analysis are shown in Tables 38, 39 and 40 respectively.

The growth period for the hot, cool and cold environments was 22, 35, and 43 days respectively, and there was no significant difference ($P \leq 0.05$) between environments for dry weight of pots at these stages of growth (Table 38). From Table 39 there were no differences between populations in the hot environment but Ladino clover was superior to the other populations in the cool and cold environments. Comparing all three environments (Pooled ANOVA) Ladino was consistently superior to the other clover populations.

The genotype-environment interaction effect can be split into variation components for each genotype, which are known as ecovalences. These are given in Table 40 together with the adaptation coefficients as described in Section 3.2.9.

The Ladino and Russian clovers had the greatest GE interaction effects (largest ecovalences). The adaptation coefficients and mean yields tended to indicate that Ladino clover had average adaptability in all environments while Russian clover was specifically adapted to the hot environment. However, the significance tests of the adaptation coefficients (b), were equivocal because of the low df of regression (only three environments involved). Also, for Ladino clover, a large proportion of the GE effect

TABLE 38

Pooled Anova for dry weight (DW) per pot (g)

Source of Variation	DF	Mean Square	Significance Test
Environments	2	5.978	(NS)
Blocks (Env)	6	0.905	*
Genotypes	4	6.219	*
Gen x Env	8	1.034	*
Error	24	0.326	

Individual error variances formed a homogenous set

$$\chi^2 = 0.741 \quad (\text{Probability} = 0.696)$$

(NS) = significance at P = 0.10

* = significance at P = 0.05

** = significance at P = 0.01

TABLE 39

The Population means and significance levels
for DW per pot (g)

	Spain	Ladino	Huia	Kent	Russia
HOT	3.54 * a	4.72 a	3.69 a	4.16 a	4.65 a
COOL	2.28 b	4.81 a	2.61 b	2.60 b	2.29 b
COLD	2.62 b	5.18 a	3.35 b	3.22 b	2.16 b
POOLED	2.82 b	4.90 a	3.22 b	3.33 b	3.03 b

* Statistics sharing the same letter are not significantly different (P = 0.05)

TABLE 40

Adaptation analysis for DW per pot

Population	Ecovalence	Adaptation Coefficient b	SE of b	Significance Test		Stability Coefficient
				From 0	From 1	
Spain	.0016	1.03	.05	*	NS	.99
Ladino	.7173	-0.15	.36	NS	NS	.14
Huia	.0849	0.81	.36	NS	NS	.83
Kent	.0335	1.23	.11	NS	NS	.99
Russia	.8851	2.08	.79	NS	NS	.88

(NS) = significance at P = 0.10

* = significance at P = 0.05

** = significance at P = 0.01

was unexplained by the regression line (low stability coefficient). These factors have made it difficult to interpret the results from the adaptation analysis.

4.2.10.2 Total Stolon dry weight (g)

The pooled environments analysis of variance and adaptation analysis were also used to evaluate the genotype - environment interaction effects of the ten stolon characters previously described in the regression analysis. The procedure of the Pooled ANOVA has been outlined in section 3.2.6. The total stolon DW ANOVA, population means and adaptation analysis are given in Tables 41, 42 and 43 respectively.

The environments were not significantly different (Table 41) but the clover population differences were highly significant. The uniformity of the environments was attributed to the harvesting at similar developmental stages in each environment; i.e. at 22 days in the hot, 35 days in cool and 43 days in the cold environment. The clover populations in each environment (Table 42) had similar significance rankings as those determined by the regression analysis (i.e. for the \hat{Y} estimates in Table 17). The genotype-environment interaction effect was significant (but not as significant as the genotype differences) and the ecovalences and adaptation analysis are given in Table 43.

The Spanish and Kent white clovers had the greatest GE effects (largest ecovalences). The adaptation coefficient and mean yields for the Spanish clover indicated this clover to be specifically adapted to the hot environment. The Kent clover had low stolon dry weights in all environments,

Pooled Anova for total stolon dry weight (g)

<u>Source of Variation</u>	<u>df</u>	<u>Mean Square</u>	<u>Significance Test</u>
Environments	2	0.0068	NS
Blocks (Env)	6	0.0007	NS
Genotypes	4	0.0875	***
Gen x Env	8	0.0020	*
Error	24	0.0009	

Individual error variances formed a homogenous set

$$\chi^2 = 2.266 \text{ (Probability} = 0.323)$$

(NS) = significance at P = 0.10

* = significance at P = 0.05

** = significance at P = 0.01

*** = significance at P = -.001

The population means and significance levels for total Stolon DW (g)

	Spain	Ladino	Huia	Kent	Russia
HOT	0.303 * a	0.333 a	0.174 b	0.068 c	0.091 c
COOL	0.193 b	0.296 a	0.137 b	0.072 c	0.079 c
COLD	0.199 b	0.284 a	0.146 b	0.082 c	0.079 c
POOLED	0.232 b	0.304 a	0.152 c	0.074 d	0.083 d

* Statistics sharing the same letter are not significantly different (P = 0.05)

TABLE 43

Adaptation analysis for total stolon dry weight

Population	Ecovalence	Adaptation Coefficient b	SE of b	Significance Test		Stability Coefficient
				From 0	From 1	
Spain	.00202	2.89	.06	*	*	.98
Ladino	.00008	1.15	.35	NS	NS	.91
Huia	.00002	0.89	.17	NS	NS	.96
Kent	.00091	-0.24	.26	NS	NS	.46
Russia	.00026	0.32	.02	*	*	.99

* = significance at P = 0.05

** = significance at P = 0.01

but the adaptation coefficient (b) failed to show a significant value. The low stability coefficient also indicated that much of GE interaction variation was unexplained by the regression line. The Russian clover had an adaptation coefficient of $b < 1$ and low mean yields indicating it to be adapted to low yielding environments.

4.2.10.3 Leaf Area per stolon (cm²)

The pooled ANOVA, populations means and adaptation analysis for leaf area per stolon are shown in Tables 44, 45 and 46 respectively.

The environments were significantly different (Table 44); the clover populations indicating greater leaf areas in the hot environment (Table 45). The clover population differences were highly significant in each environment and the significance groupings support those found in the regression analysis (i.e. the \hat{Y} estimates in Table 26). The genotype-environment interaction effect was significant (but not as significant as the genotype differences) and the adaptation analysis is shown in Table 46.

The Spanish and Kent white clovers had the largest genotype-environment interaction effects. The Spanish clover indicated a high mean yield, an adaptation coefficient significantly greater than one and a high stability coefficient (much of the GE interaction explained by the regression line). In brief, Spanish clovers was specifically adapted to the hot environment. Kent clover, on the other hand, had small leaf areas in all environments and an adaptation coefficient of $b < 1$. Kent clover was adapted to the cool and cold environments. Ladino clover was generally adapted to all environments. Huia clover also showed average adaptability but a lower mean yield than Ladino.

TABLE 44Pooled Anova for Leaf Area per stolon

<u>Source of Variation</u>	<u>df</u>	<u>Mean Square</u>	<u>Signifance Test</u>
Environments	2	100.06	*
Blocks (Env)	6	7.52	NS
Genotypes	4	613.21	***
Gen x Env	8	19.06	*
Error	24	5.80	

Individual error variances formed a homogenous set

$$\chi^2 = 0.408 \quad (\text{Probability} = 0.818)$$

- (NS) = significance at P = 0.10
 * = significance at P = 0.05
 ** = significance at P = 0.01
 *** = significance at P = 0.001

The population means and significance levels for Leaf Area (cm^2)

	Spain	Ladino	Huia	Kent	Russia
HOT	28.69	26.07	16.56	4.99	8.36
	* a	a	b	c	c
	Environment Mean = 16.94				
COOL	17.50	21.76	13.05	5.01	5.51
	a	a	b	c	c
	Environment Mean = 12.57				
COLD	16.62	21.79	13.30	5.25	4.89
	b	a	b	c	c
	Environment Mean = 12.37				
POOLED	20.94	23.21	14.30	5.08	6.25
	a	a	b	c	c

* Statistics sharing the same letter are not significantly different ($P = 0.05$)

TABLE 46

Adaptation analysis for Leaf Area per stolon

Population	Ecovalence	Adaptation Coefficient b	SE of b	Significance Test		Stability Coefficient
				From 0	From 1	
Spain	21.533	2.61	0.07	*	*	.99
Ladino	0.028	0.96	0.04	*	NS	.98
Huia	0.553	0.76	0.08	(NS)	NS	.98
Kent	8.883	-0.03	0.05	NS	*	.33
Russia	0.771	0.71	0.09	(NS)	NS	.98

(NS) = significance at $P = 0.10$

* = significance at $P = 0.05$

** = significance at $P = 0.01$

4.2.10.4. Secondary Stolon Production

The pooled ANOVA, population means and adaptation analysis for secondary stolon production are shown in Tables 47, 48 and 49 respectively.

There were no significant differences between environments for secondary stolon production (Table 47). Within each environment, the clover population differences were significant and the significance groupings (Table 48) support those found in the regression analysis (ie. the \hat{Y} estimates in Table 33). The genotype-environment interaction effect was significant and the adaptation analysis is shown in Table 49.

The Mediterranean clovers, Spain and Ladino had the largest GE effects. The errors associated with their adaptation coefficients were large which was due to the low df of regression (only three environments compared). The stability coefficients were also low indicating that a large proportion of the GE effect was unexplained by the regression line. It is difficult to make valid interpretations about Spain and Ladino from these results. Huia clover had an adaptation coefficient significantly greater than one and a high stability coefficient indicating specific adaptability to the cold environment for this character (highest environmental mean). Kent clover produced the most stolons in all environments (Table 48) but the adaptation coefficient failed to show significance.

Valid interpretations from the adaptation analyses so far described have been very difficult to make. The regression analyses have been mainly insignificant due to the small number of environments involved. The type of data used (end points) has also tended to reduce the differences

Pooled Anova for secondary stolon production

<u>Source of variation</u>	<u>df</u>	<u>Mean Square</u>	<u>Significance Test</u>
Environments	2	1.144	NS
Blocks (Env)	6	0.335	NS
Genotypes	4	3.386	**
Gen x Env	8	0.432	*
Error	24	0.156	

Individual error variances are heterogenous

$$\chi^2 = 9.299 \quad (\text{Probability} = 0.010)$$

The pooled analysis is invalid but is presented in comparison with the other analyses.

* = significance at P = 0.05

** = significance at P = 0.01

The population means and significance levels for
lateral production

	Spain	Ladino	Huia	Kent	Russia
HOT	3.04 * ab	1.23 c	2.81 ab	3.54 a	2.39 b
COOL	2.06 b	1.89 c	2.44 b	3.19 a	2.54 b
COLD	2.45 c	2.20 c	3.57 a	3.62 a	2.97 b
POOLED	2.52 b	1.77 c	2.94 ab	3.45 a	2.64 b

* Statistics sharing the same letter are not significantly different (P = 0.05)

TABLE 49

Adaptation analysis for secondary stolon production

Population	Ecovalence	Adaptation Coefficient b	SE of b	Significance Test		Stability Coefficient
				From 0	From 1	
Spain	0.320	0.40	1.73	NS	NS	.10
Ladino	0.232	0.89	1.55	NS	NS	.25
Huia	0.110	2.07	0.01	**	**	.99
Kent	0.024	0.72	0.42	NS	NS	.75
Russia	0.035	0.91	0.60	NS	NS	.70

* = significance at P = 0.05

** = significance at P = 0.01

between the environments. In view of these reasons, the remaining adaptation analyses for the stolon characters are given in Appendix 5.

4.3. Heritability Estimates

The method of deriving these estimates from variance components has been described in section 3.2.7. The magnitude of the heritabilities may be high compared to those based on single plants, as the estimates are based on plot means with their reduced variance. The heritabilities for each stolon character in the first and second growth periods are given in Table 50. The estimated variance components and their standard errors for the stolon characters are given in Table 51.

The herbage components; stem DW, leaf DW, petiole DW and total stolon DW consistently showed high (0.8) heritability estimates in both the full and restricted sense (Table 50). Leaf Area which is closely associated with herbage production also had high heritability estimates. The standard errors associated with these estimates were small so some degree of reliability can be placed on them.

The other stolon DW character involved with whole plant DW production is the number of laterals produced from existing stolons. During the second growth period this showed both full and restricted heritability estimates of 0.45 but with large standard errors (0.2). The whole plant DW showed similar heritability estimates. Data from this experiment tended to show that heritabilities of whole plant DW characters were low, while the heritabilities of individual stolon DW characters were high.

Heritability Estimates for the stolon characters

Stolon Character	<u>First Growth Period</u>				<u>Second Period</u>			
	Full	S.E.	Restricted	S.E.	Full	S.E.	Restricted	S.E.
Stem dry weight	.82	.10	.80	.11	.77	.12	.82	.10
Leaf dry weight	.87	.07	.88	.07	.95	.03	.95	.03
Petiole dry weight	.61	.18	.77	.13	.87	.07	.93	.04
Total dry weight	.86	.08	.88	.07	.90	.05	.92	.04
Leaf Production	.15	.11	.53	.17	.16	.11	.46	.18
Length of Stolon	.39	.20	.69	.15	.39	.20	.71	.14
Leaf Area	.81	.11	.87	.08	.89	.06	.94	.04
Number of Laterals	.51	.18	.57	.18	.45	.20	.45	.21
Ratio Leaf to Total DW	.52	.19	.74	.13	.36	.18	.58	.17
Stolon thickness	.61	.20	.87	.08	.69	.17	.90	.06
Dry weight per Pot	.37	.18	.51	.20	.45	.19	.56	.20

TABLE 51

Estimated variance components and their standard errors
for each stolon character

<u>Stolon character</u>	<u>Variance component</u>	<u>Estimate</u>	<u>S.E. of $\hat{\sigma}^2$</u>
Stem DW (mg)	σ_E^2	32.21	33.03
	$\sigma_{B(E)}^2$	4.59	11.03
	σ_G^2	498.49	299.53
	σ_{GE}^2	34.10	27.90
	σ^2	78.41	21.75
Leaf DW (mg)	σ_E^2	0.73	3.76
	$\sigma_{B(E)}^2$	-4.36	8.04
	σ_G^2	1303.62	756.17
	σ_{GE}^2	-10.49	11.43
	σ^2	86.33	23.95
Petiole DW (mg)	σ_E^2	23.84	17.56
	$\sigma_{B(E)}^2$	-3.44	1.81
	σ_G^2	381.99	222.56
	σ_{GE}^2	1.37	5.31
	σ^2	27.24	7.55
Total DW (mg)	σ_E^2	142.91	125.74
	$\sigma_{B(E)}^2$	-3.98	55.36
	σ_G^2	6089.56	3547.72
	σ_{GE}^2	-1.03	87.18
	σ^2	499.25	138.47
Leaf Production	σ_E^2	.569	.425
	$\sigma_{B(E)}^2$.072	.047
	σ_G^2	.130	.083
	σ_{GE}^2	.005	.022
	σ^2	.112	.031

TABLE 51 Continued

Stolon Character	Variance component	Estimate	S.E. of $\hat{\sigma}^2$
Stolon length (cm)	σ_E^2	2.335	1.723
	$\sigma_{B(E)}^2$	-0.003	0.068
	σ_G^2	2.055	1.287
	σ_{GE}^2	0.306	0.234
	σ^2	0.609	0.169
Leaf Area (cm ²)	σ_E^2	5.286	4.759
	$\sigma_{B(E)}^2$	0.343	0.818
	σ_G^2	66.017	39.349
	σ_{GE}^2	4.419	2.892
	σ^2	5.802	1.609
Secondary Stolon production	σ_E^2	0.036	0.057
	$\sigma_{B(E)}^2$	0.036	0.035
	σ_G^2	0.328	0.218
	σ_{GE}^2	0.092	0.066
	σ^2	0.156	0.043
Ratio $\frac{\text{Leaf DW}}{\text{Total DW}}$	σ_E^2	.0008	.0006
	$\sigma_{B(E)}^2$	-.0001	.0001
	σ_G^2	.0015	.0009
	σ_{GE}^2	.0001	.0001
	σ^2	.0005	.0002
Dry Weight per pot (g)	σ_E^2	.291	.285
	$\sigma_{B(E)}^2$.116	.092
	σ_G^2	.576	.402
	σ_{GE}^2	.236	.157
	σ^2	.326	.090

The number of leaves produced by a stolon showed low (0.15) full sense heritabilities. Stolon length also showed comparatively low full sense heritabilities but quite high restricted heritabilities, which apply to this particular set of environments. The standard errors associated with the heritability estimates of these stolon characters were high.

For all the stolon characters showing large heritability estimates, the error component was the next greatest source of variation to the genotypic component (Table 51). Leaf production and stolon length indicated large environmental effects.

4.4. Simple Correlation Coefficients

The method used to derive the coefficients is given in section 3.2.8. The phenotypic and genotypic correlations and significances were derived from the pooled covariance analysis and the r 's are shown in Table 52.

The stolon components stem DW, leaf DW and petiole DW were highly correlated with one another both phenotypically and genotypically. The correlations with total DW are excluded as this character is not independent of the other three. Leaf Area showed high correlations with each of the stolon DW components. The DW per pot indicated significant phenotypic correlations with each of the stolon DW components, although the values were only moderate. The genotypic correlations were non-significant even though the values were high. This was due to the low number of clover populations involved (ie. low df).

The negative correlations between secondary stolon production and the stolon DW components were all significant.

TABLE 52

Correlation coefficients

(a) First growth period on upper side of diagonal

(b) Second growth period on the lower side of diagonal

	STDW	BLDW	PTDW	TOTDW	LNST	BLAR	NLT	DW/POT
Stem Dry Weight STDW		G.98*** P.94***	G.91* P.77***		G.73NS P.61***	G.96** P.91***	G-.86* P-.42***	G.67NS P.49**
Leaf Dry Weight BLDW	G.98*** P.93***		G.95* P.82***		G.77NS P.65***	G.99*** P.96***	G-.83* P-.43***	G.53NS P.41**
Petiole Dry Weight PTDW	G.90* P.75***	G=.93* P=.80***			G.76NS P.72***	G.94* P.88***		
Total Dry Weight TOTDW					G.74NS P.68***	G.98*** P.97***	G-.85* P-.50***	G.64NS P.48**
Length of Stolon LNST	G.84NS P.78***	G.85NS P.63***	G.86NS P.77***	G.83NS P.71***		G.79NS P.75***	P-.04NS	G.16NS P.39**
Leaf Area BLAR	G.97*** P.93***	G.99*** P.98***	G.93* P.87***	G.99*** P.98***	G.81NS P.78***		G-.78NS P-.44**	G.48NS P.44**
Number of Laterals NLT	G-.93* P-.52**	G-.92* P-.63***		G-.93* P-.61***	G-.66NS P-.36*	G-.91* P-.63***		G-.67NS P-.24NS
Dry Weight per Pot DW/POT	G.93* P.52***	G.90* P.63***		G.92* P.59***	G.83NS P.16NS	G.89* P.55***	G-.73NS P-.32*	

* = significant at P = .05

** = significant at P = .01

*** = significant at P = .001

This supports the inverse relationship between growth per stolon and stolon numbers described earlier. The Mediterranean clovers produce large stolons but few of them while the North European clovers produce big numbers of small stolons. However, there were no significant correlations between secondary stolon production and DW per pots. The negative correlations between the stolon DW components and the numbers of new stolons being produced which also showed low heritability estimates, collectively brought about the unpredictability of the DW production from a pot.

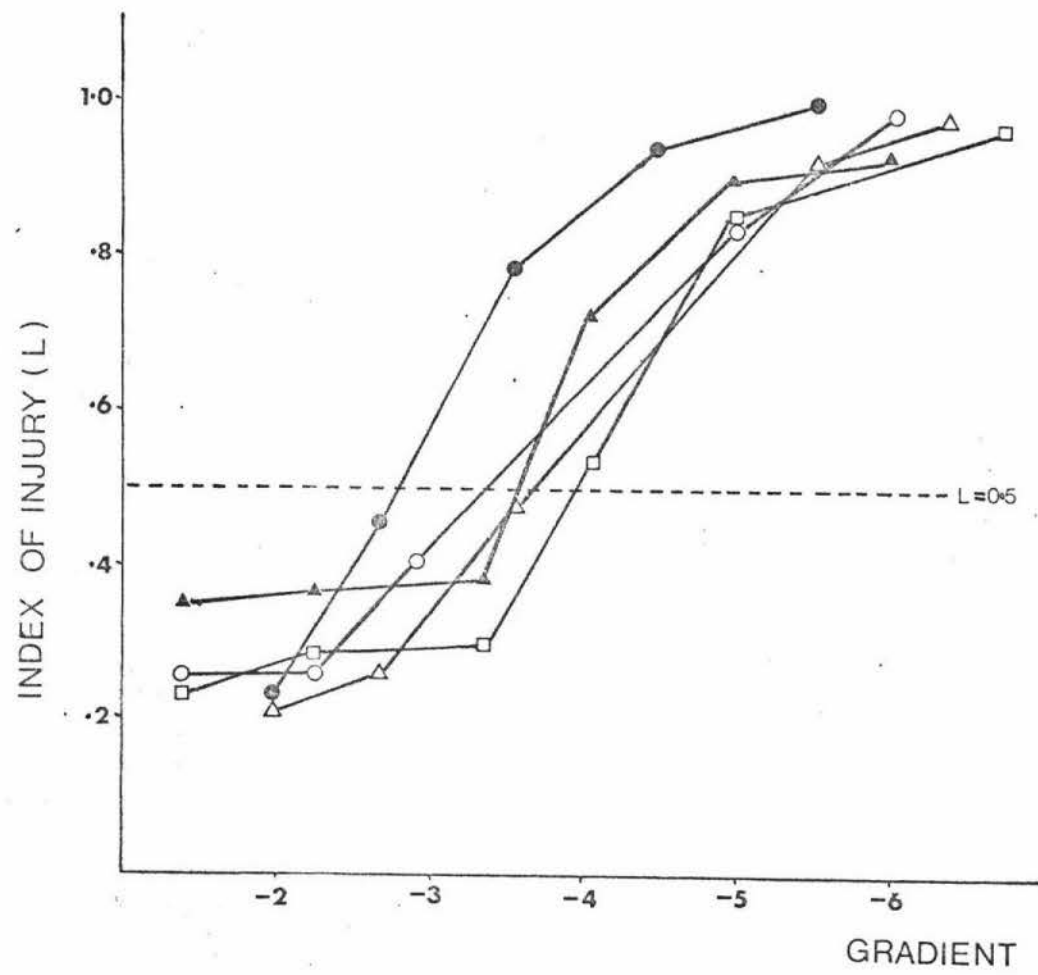
4.5. Electrical Conductance Technique of Assessing Frost Injury

The method has been outlined in section 3.1.8. The first conductance test was carried out when the plants had been in the controlled environments for two weeks but before any frost treatments had occurred. Thereafter, the clover populations were tested at three weekly intervals. The results are illustrated in graphic form in Figures 16, 17 and 18. Each data point represents the mean of three conductance measurements. Values of L , the index of injury, greater than 0.5 (50% leaching) indicated severe damage to the leaves. The approximate killing points of the existed leaf tissue were estimated from the eye-fit curves at $L = 0.5$ and are shown below the Figures.

As the frosting temperature was decreased over the course of the experiment, all the clover populations indicated a gradual increase in freezing tolerance of their leaves (Figure 18). As expected, the clovers from higher latitudes generally showed a greater degree of frost hardiness. Russian clover was the most hardy and Spanish the least, both at the beginning and end of the experiment. However,

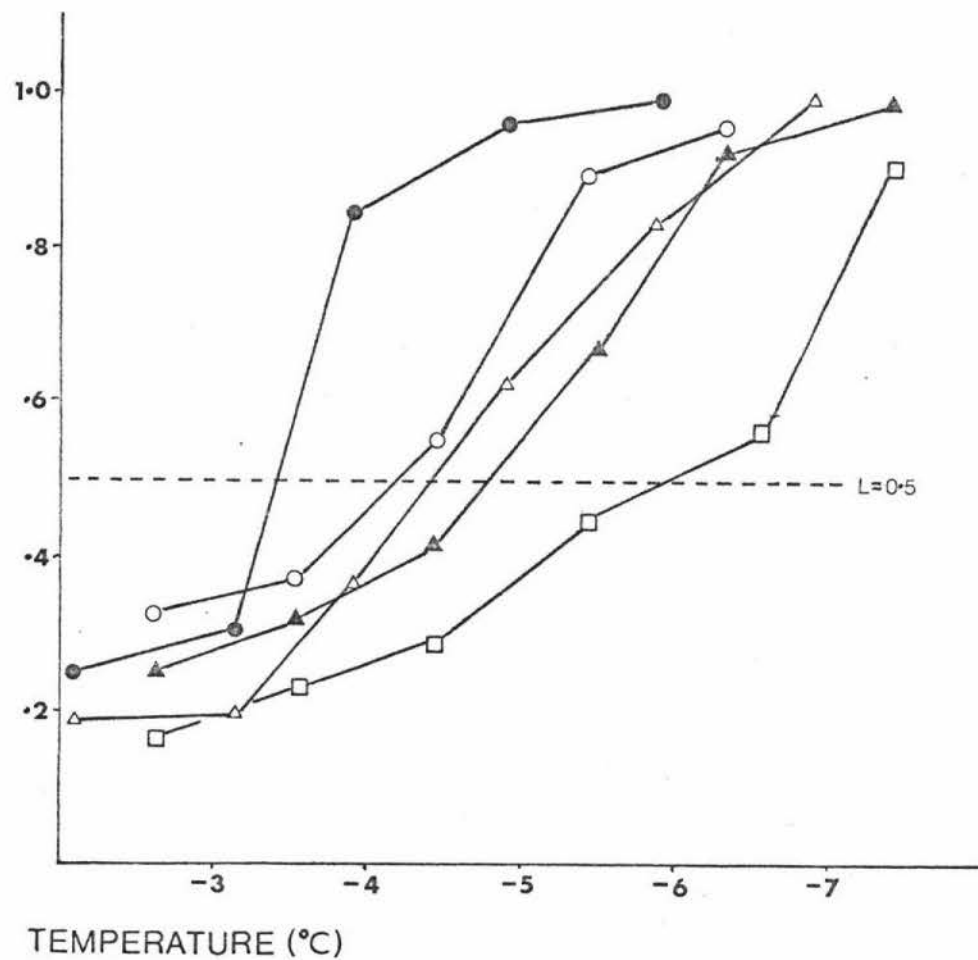
FIGURE 16

10 FEBRUARY



- SPAIN -2.8°C (Killing temperature)
- △ LADINO -3.6
- HUIA -3.4
- ▲ KENT -3.5
- RUSSIA -3.9

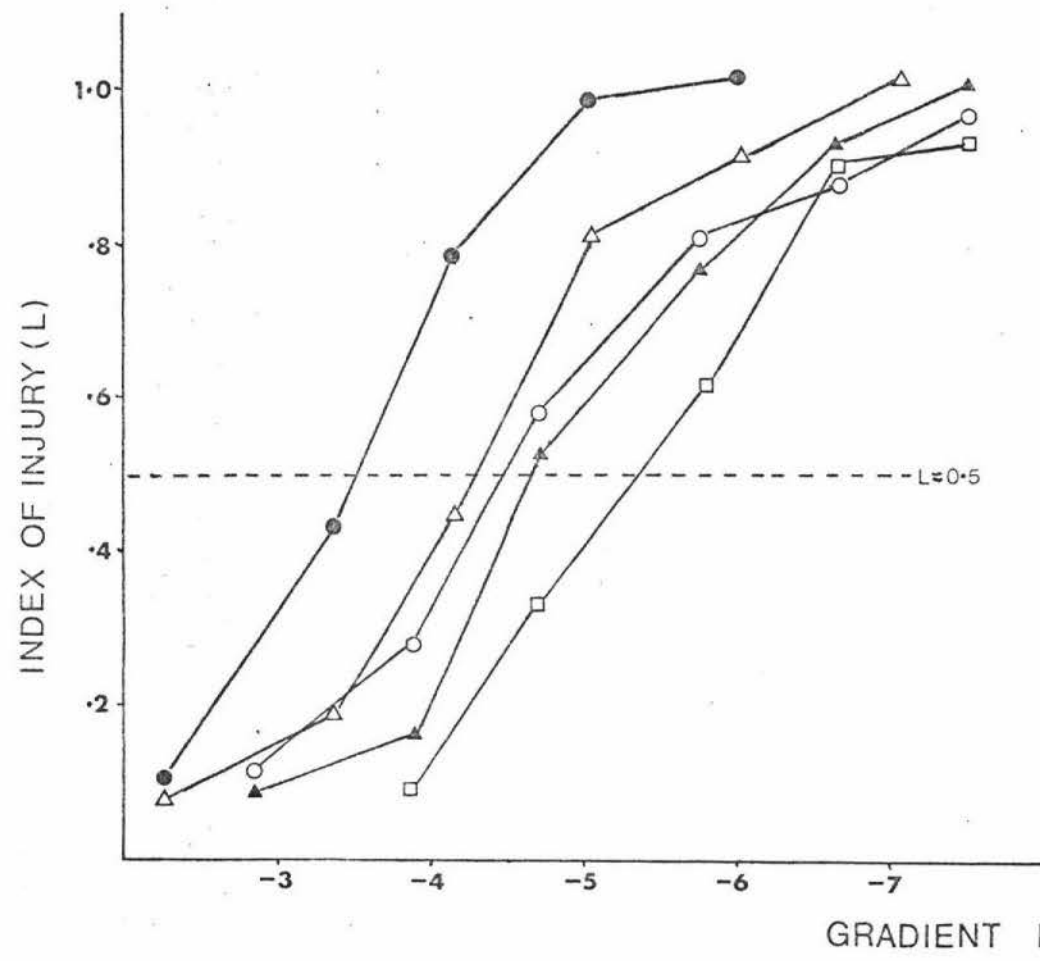
10 MARCH



- SPAIN -3.4°C
- △ LADINO -4.4
- HUIA -4.2
- ▲ KENT -4.7
- RUSSIA -5.7

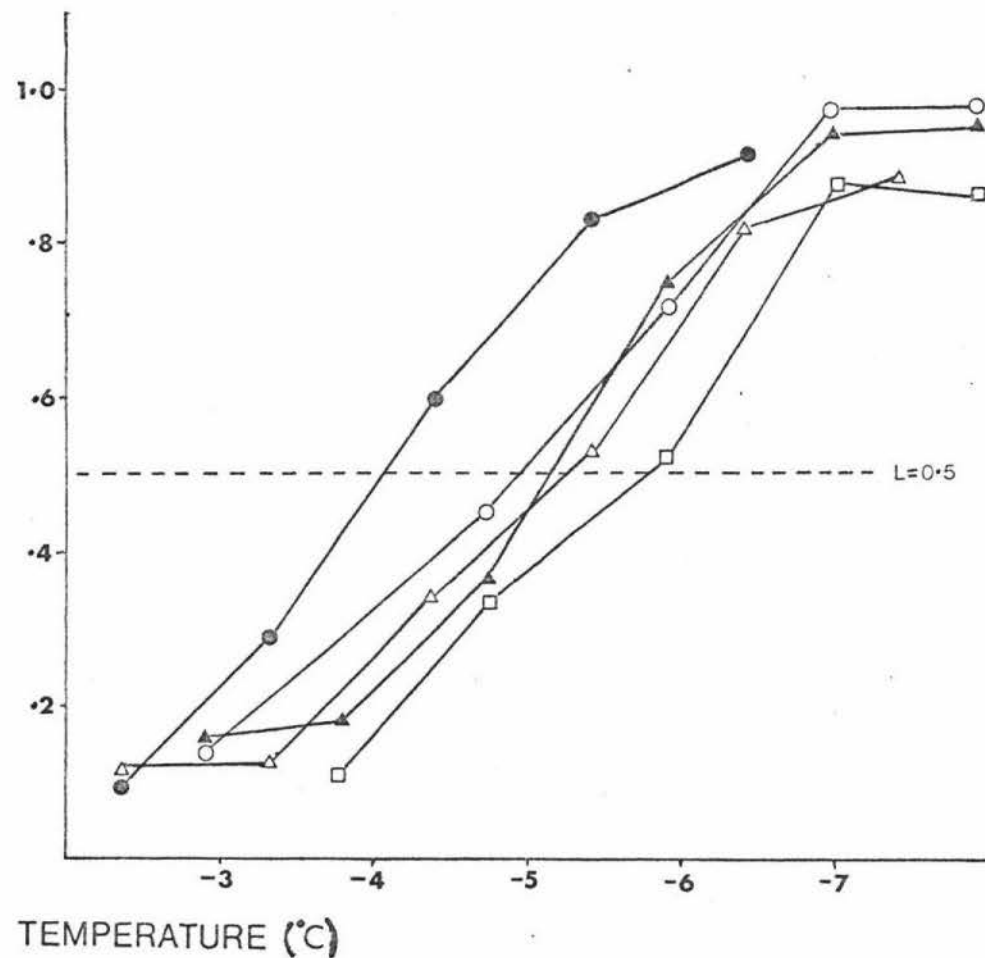
FIGURE 17

4 APRIL



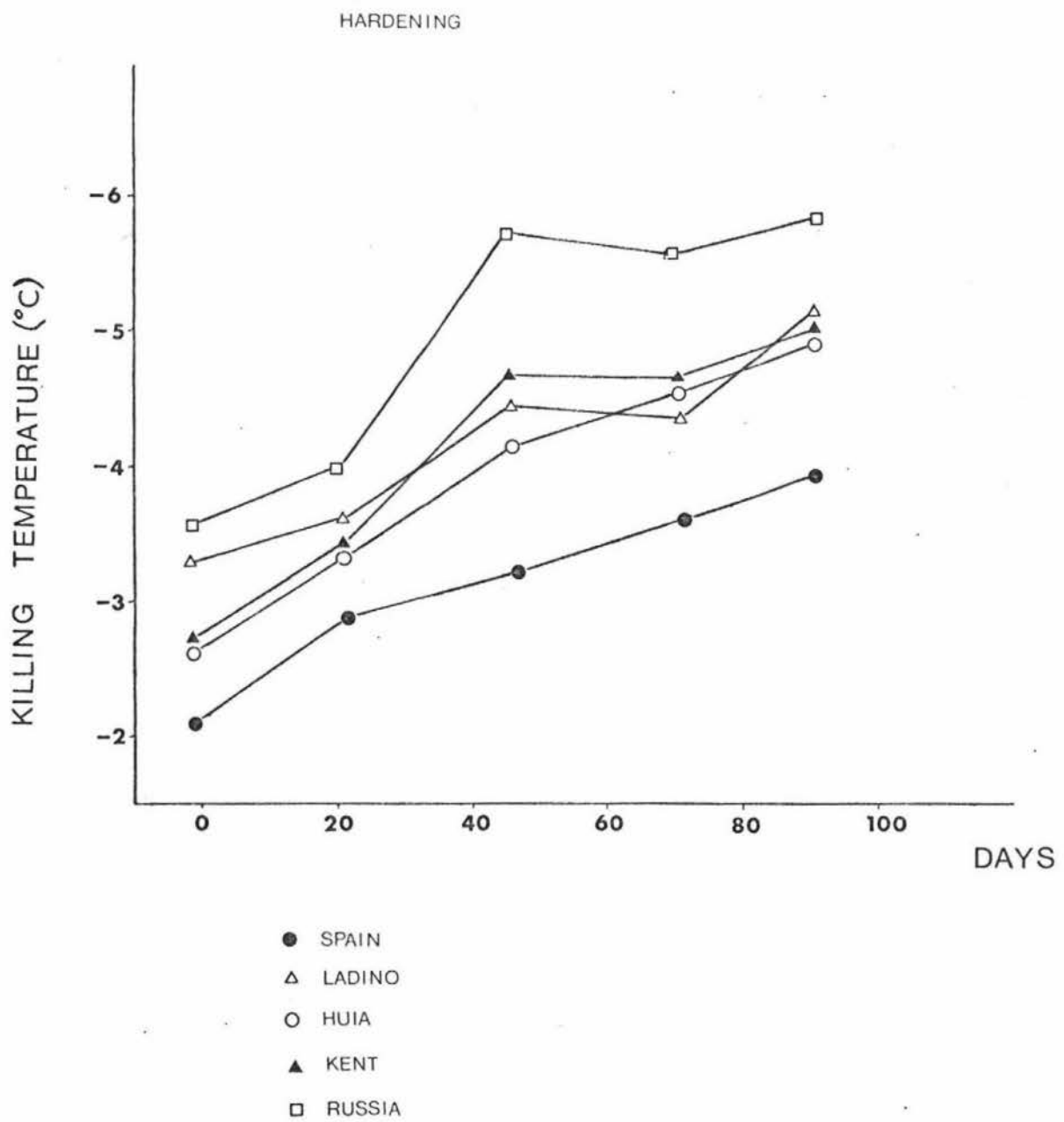
- SPAIN -3.6° (Killing temperature)
- △ LADINO -4.3
- HUIA -4.5
- ▲ KENT -4.6
- RUSSIA -5.4

21 APRIL



- SPAIN -3.9°
- △ LADINO -5.2
- HUIA -4.9
- ▲ KENT -5.1
- RUSSIA -5.7

FIGURE 18



Ladino clover which originates in the Mediterranean, had similar hardening properties to Kent and Huia clover. Further decreases in the frosting temperature with time may have eventually separated the clovers into a more distinctive order.

Previous studies (Rowley 1976; de Ruiter 1979) have indicated a disparity between frost temperatures required to kill exised leaf tissue compared with whole plants. A small side-line study to compare methods of assessing frost tolerance is described in Appendix 2.

CHAPTER 5

DISCUSSION

5:1 Development patterns of the stolon characters

Characteristic developmental patterns for stem dry weight (DW), leaf DW, petiole DW and total stolon DW occurred in the three environments for each clover population. Ladino clover consistently had greater values for the b_0 regression coefficient (a curve placement parameter describing the initial growth potential) for these stolon DW components. Generally, the clover populations demonstrated similar growth rates (as described by the b_1 and b_2 regression coefficients) for DW production of their component stolon characters within each of the controlled environments. Consequently, those populations which produced stolons with large stems, leaves and petioles such as the Ladino and Spanish clovers, were consistently superior to the small leaved Kent and Russian clovers in all environments.

All the populations showed greater growth rates (b_1 coefficient) for DW production in the hot compared to the colder environments but the growth rates declined at a faster rate (b_2 coefficient) in the hot environment. Brougham (1962) previously showed that the maximum possible yield of dry weight was a function of the light environment. In this experiment, light intensity and duration were equivocal so that the ultimate DW yields were similar for each environment. Thus, on examining the stolon DW curves, it appeared that near optimal DW production of the clover

populations was achieved in 20-25 days in the hot environment, whereas an appreciable growth rate still continued after 40 days in the cold environment.

The traditional technique of 'Growth Analysis' involved such concepts as relative growth rate, net assimilation rate and leaf area ratio which was based on the estimation of the major parameters of growth (dry weight and leaf area) over a stated harvest interval. This required the bold assumption that dry weight accumulation was constant between any two harvest times. Regression analysis on the other hand, utilizes information from a series of small frequent harvests to derive polynomial functions which describe the relationship of dry weight and leaf area with time (Radford, 1967; Nicholls and Calder, 1973). Standard errors can be estimated for the regression coefficients describing these functions, so that statistical tests can be used to compare curves. The use of regression analysis to derive polynomial curves in growth studies has enabled plant breeders to effectively assess and compare genetic and environmental effects.

Cooper (1964) found that Mediterranean populations of L. perenne and D. glomerata had superior growth rates to Norwegian populations under low temperature conditions. It has been suggested that Mediterranean populations use photosynthetic assimilates to produce greater leaf area and thus maintain growth in winter, while North European material use assimilates for storage in roots and shoot bases so as to survive the cold winter conditions of

their native habitats (MacColl and Cooper, 1967; Eagles, 1967; Robson and Jewiss, 1968). Williams and Hoglund (1978) found similar results with Spanish and New Zealand clovers, when the Spanish material produced a higher proportion of total weight as leaf weight under controlled low temperature conditions. However, in this experiment, the North European clovers had similar leaf growth rates to the Mediterranean clover in the frosting room, and the proportion of leaf to total tissue (ratio) was the same for both populations.

Morley (1958), Eagles (1967) and Lawrence et al (1973) stressed the problem associated with controlled environment conditions where environmental factors are held constant except those factors considered important treatments. Low temperatures are usually associated with low light intensities, short photoperiods, and small diurnal fluctuations in the field. Under low light intensity, a reduction in the NAR (net photosynthetic activity of the leaf surface) causes different responses; such that the Mediterranean material uses stored assimilates to expand more leaf tissue while North European material uses assimilates to maintain cold hardiness (Thomas, 1968; Lorenzetti et al. 1971). In this experiment, the light intensity remained moderately high so that photosynthesis continued at a moderate rate and all populations irrespective of origin, maintained similar growth rates in each environment. The frosting conditions did not adversely affect any of the clover populations nor substantially decrease their DW production. The day temperatures of 12°C and medium light intensity (160 W/m²)

enabled the Mediterranean and Northern clovers to continue leaf production and also attain a certain degree of frost tolerance. It appears that cold per se. is not the causal factor bringing about different growth responses of Mediterranean and European grasses and clovers, but all the climatic factors associated with frosts (i.e. short days, low light intensities, harsh winds..) collectively affect the growth responses.

The development patterns of all the clover populations for leaf production were constant within each environment. However the rates of leaf production differed between environments. In the hot environment, all populations produced approximately 2.0 leaves per week, while in the cool and cold environments, 1.0 and 0.8 leaves per week respectively were produced. This rate of leaf production is consistent with earlier work (Mitchell, 1956) but Williams and Hoglund (1978) found that New Zealand clover produced a greater number of leaves than Spanish clover in the hot environment. They found this increase of leaf numbers in the warmer environment ($22.5^{\circ}\text{C}/14^{\circ}\text{C}$) partially accounted for the superior stolon production of New Zealand clover to that of Spanish clover at this temperature. In this experiment no differences were found between populations for leaf production and the relative stolon herbage DW production between populations remained the same in each environment. A possible reason for the discrepancy of results involves the origin of the seed in the two experiments. Williams and Hoglund used progeny lines of the seven Grassland Huia parents while in this experiment, breeders

seed (three generations removed) was used. Some genetic drift may have occurred during seed multiplication, causing disparity in performance between the two sources of Grasslands Huia.

Beinhart (1963) showed that the herbage production from Ladino stolons was closely associated with the amount of photosynthetic leaf tissue formed. The results from this experiment also clearly show the close correlation ($r = .98$) between the total stolon herbage production and the leaf area attained (see section 4:4). The clover populations demonstrated similar development patterns within each environment. The regression coefficients describing the rate of change in leaf area were constant for each clover which is reflected by the 'parallel' curves in Figure 11. The initial placement coefficient had a direct bearing on the size of the final leaf area attained. Ladino had the largest stolons and leaf area in all environments, whereas Kent and Russia had the smallest.

When comparing environments the rate of leaf area production shown by these clovers, was greater in the hot environment compared to the colder environments. However, the rate declined at a faster rate within the hot environment. In time, the leaf area produced by any one clover would have been constant across all environments. Both Mitchell(1955) and Brougham (1958) found the leaf area attained by New Zealand white clover was a function of the light environment; the rate of leaf area production being determined by temperature.

Long spindly stolons with lengthy stolon internodes were formed in the hot environment. However, in the frosting environment, all populations produced shorter stolons with leaves more tightly packed together. As stem growth continued, the stolons became thicker and denser in the cold environment, including the Mediterranean clovers. This build up of stem tissue is probably a response to cold temperatures so that the clovers can survive frosting conditions. The Mediterranean clovers also showed this response, but under reduced light intensity and shortened photoperiod a contrasting developmental response may have occurred.

Each clover population was associated with a distinct morphological habit of growth. The populations showed high, full heritability estimates for the stolon DW characters and leaf area. The correlation coefficients between these characters were also high. Thus, Ladino clover showed large stems, leaves, petioles and leaf area in all environments, while in contrast Kent white clover had small stolon component parts. However, the negative correlations between the stolon DW characters and the numbers of new stolons being produced, which also showed low heritability estimates, collectively brought about the unpredictability of the total DW production from a pot. The total DW from a pot did not appear to be strongly correlated with any of the stolon characters.

The characters stolon length, stolon thickness and leaf production showed quite low full sense heritabilities but high restricted heritabilities. The full sense

heritability is estimated such that the phenotypic variance includes the variation due to different environments and blocks within environments. This heritability is probably a more realistic estimate of that portion of the total variation shown by a stolon character, which is due to genetic influences. The restricted heritability is based on the phenotypic variance containing no block or environmental variation. This heritability estimate only applies to one particular environment where the measurements were made. A large proportion of the variation in leaf production, stolon length and stolon thickness was due to the environmental effects.

5:2 Production of new stolon units

The rate at which secondary meristems became active and produced branch stolons was considered an important factor in the growth and persistence of white clover (Mitchell, 1956; Beinhart, 1963; Williams and Hoglund, 1978). The life span of any one stolon is limited (Chow, 1967; Beinhart, 1963) so that the survival of a clover plant relies on continual production of new stolons. This characteristic demonstrated variation between clover populations and environments in this experiment.

From seedling establishment, the clover populations differed in the number of primary stolons produced. Spanish clover in particular had fewer primary stolons while the Kent and Russian clovers produced high numbers. During the course of the experiment it became clear that clovers with greater growth per stolon had smaller numbers

of stolons while those clovers which produced less growth per stolon had large stolon numbers. This inverse relationship between growth per stolon and stolon numbers is consistent with results of Williams and Hoglund, 1978.

Stolon branching is also influenced by environmental conditions. During the first growth period of this experiment, the five clover populations produced quite high numbers of stolons in all environments. Spanish and Kent clovers produced the most laterals in the hot environment while Kent, Huia and Russian clovers had the greater numbers within the colder environments. Ladino clover produced the least new stolons compared to the other clovers in all environments. In the second growth period, a lot fewer secondary stolons were produced, particularly in the hot environment where there were no differences between populations. Kent, Huia and Russian clovers continued to produce more secondary stolons than Spanish and Ladino clovers in the cool and cold environments. The difference between the two cycles suggests that during establishment, the clovers were actively branching to form a base population of stolons. Subsequent environmental conditions, may then have had a strong influence on stolon branching.

Stolon branching appears to depend on the seasonal fluctuation of environmental factors, of which temperature is only one. Brock (1974) showed evidence that stolon populations of clover do shift from season to season. Williams and Hoglund (1978) found that plants growing under constant environment regimes were unsuitable for seasonal growth studies. They suggested this problem, could be

overcome by using appropriate pretreatment conditions. In this experiment, plants were established during summer in glasshouse conditions of high temperature, high light intensity and long (15 hour) photoperiods. On transferal to the controlled environment rooms, both the light intensity and photoperiod were reduced, a situation similar to autumn conditions. Thus, interpretation of the stolon branching responses in terms of temperature alone could be misleading as other environmental factors appear to be involved. This stresses the problem of extrapolating information from controlled environment studies as an explanation of possible responses in the field. Varying the light intensity and photoperiod would have factorialised the number of growth rooms required in the experiment making it difficult to manage the practical work.

However, the results from this experiment do support in part the view of Williams and Hoglund (1978), that the superiority in DW production of Spanish over New Zealand clover in autumn-early winter could be a consequence of improved stolon branching of the Spanish clover in hot summer conditions. During the second cycle, the New Zealand (Grasslands Huia) and Kent white clovers had greater numbers of secondary stolons in the cooler temperatures which were comparable to late autumn-winter conditions in temperate regions. Improved branching at this time would result in superior total plant growth during the following spring and summer. This adaptive response would ensure survival in an environment where winters are cold and summers conducive to rapid growth.

Ladino clover developed into a high producing clover

from establishment and showed the greatest total DW production in all environments during both growth periods. However, its lack of stolon branching at all temperatures particularly in the second growth period, could possibly lead to a decline in the DW production per pot from Ladino clover relative to the other clovers with time. The reported frosting damage or lack of winter hardiness of Ladino (Gibson et al., 1963) may be due to this fall in the number of active meristems rather than damage to the existing stem and leaf tissue. Most field reports of Ladino clover stress its lack of persistence (Beinhart, 1963; Knight, 1953). In U.S.A. it is treated as an annual pasture legume (Gibson, 1962).

The Russian clover had similar branching responses to Grasslands Huia and Kent white clover. However, even though the Russian clover tended to have increased branching at the low temperatures (significant at $F=0.10$), the total number of stolons were too few to compensate for the low DW per stolon production. Consequently, during the second growth period, the total DW per pot production from Russian clover in the cold environment was significantly poorer than the other clover populations. This reduction in growth per stolon and low production of new stolons (an apparent quiescent state) shown by Russian clover in the cold environment, may be an adaptive response to survive harsh winter conditions characteristic of its place of origin.

5:3 Adaptation Analysis

Some important factors relating to the adaptive responses of these five white clover populations were shown

by the regression analysis. Briefly, those populations which produce large stems, leaves and petioles such as the Ladino and Spanish clovers, have consistently greater stolon dry weight than the smaller leaved clovers (Kent and Russia) irrespective of temperature changes. Secondly, the changing pattern of total plant production between clover populations and environmental conditions is the result of different stolon branching characteristics. This factor governs the maximum dry weight production of a clover under favourable environmental conditions and may have a bearing on survival under harsh conditions. This character, unfortunately, is also the most difficult to assess. In this experiment, the numbers of secondary stolons produced from the primary stolons in the given time periods, were quite small. Also, the standard errors associated with the estimates for this character, calculated from the regression analysis, were fairly large. This made it difficult to clearly understand the pattern of branching shown by these clovers under diverse temperature conditions. An alternative method, such as the use of a quadrat to determine stolon numbers per unit area, may have given more definitive results.

The adaptation analysis developed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966) was used to aid in the understanding of the adaptive responses shown by these clovers. The genotype - environment interaction effects for each stolon character were evaluated using the regression analysis described in section 3:2:9. The Mediterranean clovers had high mean yields for the stolon herbage production characters (stem DW, leaf DW,

petiole DW and total DW). The Spanish clover also tended to show adaptation coefficients greater than one for these characters (including leaf area). This indicated that this clover functions well in favourable environments but much less in poorer environments. Spanish clover appeared to respond to good growing conditions but became less productive in stress environments. The Ladino clover had adaptation coefficients close to one in many cases indicating general adaptability to all environments. However, the coefficients often failed to achieve significance.

The North European clovers had low mean yields for the stolon DW components. The Kent clover in particular indicated adaptation coefficients less than one for these characters. This shows a specificity of adaptability to low yielding (cool) environments. The Huia clover had intermediate yields for the stolon DW characters in all environments and for some of these characters, adaptation coefficients close to one indicating general adaptability. However, this clover also had an adaptation coefficient significantly greater than one for the production of new stolons. This indicates specificity of adaptability to the high yielding environment (the cold environment) for this character. These adaptive features of Huia appear to be important for the growth and persistence of this clover in temperate regions, where there are seasonal changes of climate, from hot dry summers to cold frosty winters.

For the adaptation analysis there were only three temperature environments on the X-axis. A greater range of environments would have been more desirable so that the regression would have been based on a larger sample of

environments. The environmental means for some characters were also quite close together which resulted in the estimated regression function having a low coefficient of determination. The standard errors of the adaptation (regression) coefficients were large in many cases which made it difficult to achieve significance and difficult to reach valid conclusions.

5:4 Cold Tolerance

All the clover populations indicated a gradual increase of freezing tolerance of their leaves over the course of the experiment (Figure 18). The degree of hardiness attained by each clover was closely associated with their place of origin. Those clovers from Northern Europe, where the winters are harsh, were the most hardy, while the Mediterranean clovers were the least. As previously mentioned, the day temperatures of 12°C and medium light intensity (160 W/m^2) enabled the Mediterranean and Northern clovers to continue photosynthesizing and also attain a certain degree of frost tolerance. Rowley (1976) concluded that some growth was necessary for initial development of frost hardiness. However, a final high degree of hardiness may be prevented as the active physiological state of the plant inhibits the accumulation of photosynthetic products necessary for complete hardening (de Ruiter, 1979). We could speculate that under harsher environmental conditions, the Kent and Russian clovers may have ceased growth and attained a high level of cold hardiness, whereas the Spanish clover may have continued growth but suffered structural damage.

In general, the cold tolerance was also associated with a certain morphological type of clover. The most hardy clovers (Kent and Russia) produced the smallest DW per stolon (small component parts) but produced the largest number of stolons. The Spanish clover was in complete contrast, being the least hardy but producing large plant parts. However, Ladino clover showed relatively high tolerance to frosting of its leaf tissue even though it had large leaves. It would appear that there may be physiological processes unrelated to the accumulation of photosynthetic metabolites and/or morphological pattern, which give clovers some cold tolerance. For instance, the Spanish clover is cyanogenic whereas Ladino clover is acyanogenic, although it is of Mediterranean origin. This could be an important factor in its greater frost tolerance to leaf damage (Daday, 1965). However, the winter hardiness of Ladino clover has been reported to be poor (Beinhart, 1963; Gibson et al, 1963). As previously mentioned, this was probably due to the failure of Ladino to form new active meristems (stolon branching) during winter for the following spring growth. Even though it survived frost damage to its leaves, it failed to persist through the winter. This type of response may also occur with Spanish clover.

The New Zealand clover (G. Huia) appears to be ideally suited to a temperate climate with warm summers and cool winters. The important attribute seems to be a compromise between DW production per stolon and the numbers of stolons it is capable of producing. It is inferior to the Mediterranean clovers in stolon DW production but superior to the more cold hardy Kent and Russian clovers.

Conversely, it produces larger numbers of stolons than the Mediterranean clovers, particularly during winter to ensure continued growth in the following spring and summer. This adaptive response of *G. Huia* is an important factor in the success of this cultivar in temperate regions.

CONCLUSIONS

The following points emerged on the adaptive responses shown by these five white clover populations to varying temperature conditions.

1. The five clover populations showed similar development patterns for the stolon DW components (stem, leaf, petiole and total DW) and leaf area within each of the three environments. All populations irrespective of origin, maintained similar growth rates in each environment which had the affect of producing 'parallel' curves. The initial placement coefficient consequently had a direct bearing on the size of the final stolon DW attained. The Mediterranean clovers, Ladino and Spain consistently produced stolons with large stems, leaves, petioles and leaf area in all environments while the Russian and Kent clovers produced stolons with small component parts.
2. It appears that temperature alone does not cause different growth responses of Mediterranean, temperate and continental clovers. Medium light intensities and 12 hour photoperiods within the frost room enabled the Mediterranean and Northern clovers to continue stolon DW production and also attain a certain degree of cold tolerance. Other climatic factors associated with cold temperatures such as short photoperiods and low light intensities may have collectively brought about different growth responses of these clovers. This is a limitation with controlled environment studies where only one treatment is applied at a time.

3. Each of the clover populations was associated with a distinct stolon morphology. Regardless of temperature conditions, the Mediterranean clovers produced large stolon parts while North European clovers had small stolon parts. This was borne out by the high heritability estimates for the stolon DW components and leaf area, indicating that a large portion of the variation for these stolon characters was due to genetic influences.
4. A considerable proportion of the variation in leaf numbers, stolon length and stolon thickness was due to environmental effects. The five clovers produced similar numbers of leaves within each environment, but leaf production was far greater in the hot room. The clovers produced long spindly stolons in the hot room but short thick stolons in the frost room. This build up of stem tissue is probably a response to cold temperatures so that the clovers can survive frost conditions.
5. The changing pattern of total plant production between clover populations and environments appears to be the result of different stolon branching characteristics. This factor governs the maximum DW attained by a clover under favourable environmental conditions and influences survival under harsh conditions.
6. The negative correlation coefficients between the stolon DW characters and the numbers of new stolons being produced clearly showed that the clovers with large stolon parts had small numbers of stolons while the clovers with small stolon components had large stolon numbers.

The clovers indicated different branching responses under the three temperature conditions. Ladino clover failed to produce many new stolons at all temperatures which undoubtedly affects the persistence of this clover. Spanish clover appeared to have slightly better branching under hot conditions, while the Kent, Huia and Russian clovers had slightly improved branching at cooler temperatures. The results were not always clear and other climatic factors probably influence stolon branching.

7. In the field, plants adapt to the seasonal fluctuation of climatic factors by a sequence of development changes throughout the year, to ensure successful growth and survival. Pronounced changes in the growth responses of these clovers between environments did not occur, probably because of the 'artificial' nature of the climate rooms in which they were growing. However, the branching responses indicated in this experiment, suggest that the improved plant production of Spanish clover during autumn-early winter (found in field trials) could be due to the increased number of stolons formed earlier in the hot summer conditions. Conditions are favourable for growth during autumn in the Mediterranean and the adaptation analysis for Spanish clover indicated a specificity of adaptability to favourable environments.

Ladino clover did not show any particular branching pattern and in this experiment produced the greatest amount of herbage in all environments. This cultivar shows general adaptability to all environments. In time, there would have probably been a decline in the

production from Ladino which has been found in field conditions after one year. The Huia and Kent clovers appeared to have increased stolon branching in the cool temperatures like those of autumn and early spring in temperate regions. Improved stolon numbers at these times would ensure survival through the winter followed by rapid growth later in spring and summer.

8. All the clover populations indicated a gradual increase of freezing tolerance of their leaves during the experiment. The degree of hardiness attained by each clover was closely associated with their place of origin. Those clovers from Northern Europe, where the winters are harsh, were the most hardy while the Mediterranean clovers were the least. The most hardy clovers were also characterised by small stolon components and large numbers of stolons. The frost conditions did not cause structural damage to any of the five clovers. Wider differences between the five clovers for cold tolerance may have occurred under harsher environmental conditions.

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APPENDIX 1Frosting Timetable

<u>Cycle 1</u>	<u>Time</u> <u>(Days)</u>	<u>Frosting</u> <u>Intensity °C</u>	<u>Cycle 2</u>	<u>Time</u> <u>(Days)</u>	<u>Frosting</u> <u>Intensity °C</u>
Week 1	2	-2	Week 1	2	-3
	3	-2		3	-3
	4	-2		4	-3
Week 2	9	-2	Week 2	8	-4
	10	-2		9	-4
	11	-2		10	-4
				11	-4
Week 3	16	-2	Week 3	15	-4
	17	-2		16	-4
	18	-2		17	-4
				18	-4
Week 4	23	-2	Week 4	22	-5
	24	-2		23	-5
	25	-2		24	-5
				25	-5
Week 5	30	-2	Week 5	29	-5
	31	-2		30	-5
	32	-2		31	-5
				32	-5
Week 6	37	-3	Week 6	36	-6
	38	-3		37	-6
	39	-3		38	-6
				39	-6

APPENDIX 2Comparison of techniques assessing frost tolerance

The electrical conductance technique is relatively quick and simple but previous studies (J.M. de Ruiter per. comm.) have indicated that the method underestimates actual temperatures required for plant kill. A small side-line study was carried out with the objective of comparing the temperature gradient bar method of assessing frost tolerance with that of direct frosting intact plants within the cold room.

Additional plants of each population were planted in pots on the 10 February and left to establish and harden outside until the 1 June. During May, the mean maximum temperature had dropped to 14°C and the mean grass minimum to 4°C. Four frosts were recorded during this period, the lowest being -3°C. The plants were considered to be adequately frost hardened by this stage. Their freezing sensitivity was then determined by both temperature gradient bar and successive frostings under controlled cold-room conditions with subsequent visual assessment of damage (0-5 scale).

The cold-room conditions were similar to those outlined in section 3:1:4, but with five frost treatments: -4°, -6°, -8°, -10° and -12°C. Three pots of each clover population were subjected to the -4°C frost

then removed to a glasshouse for visual assessment of damage. Following this a fresh set of pots was subjected to the -6°C frost on the following day and the series continued. The temperatures required to cause frost injury of intact plants in the frosting room were then compared with the killing temperatures of exised leaf tissue (from a remaining set of plants left outside) estimated from the temperature gradient bar.

The injury curves for the two methods of assessing frost tolerance are shown in Appendix Figure 1. The following scale was used to assess the damage of plants removed from the frosting room:

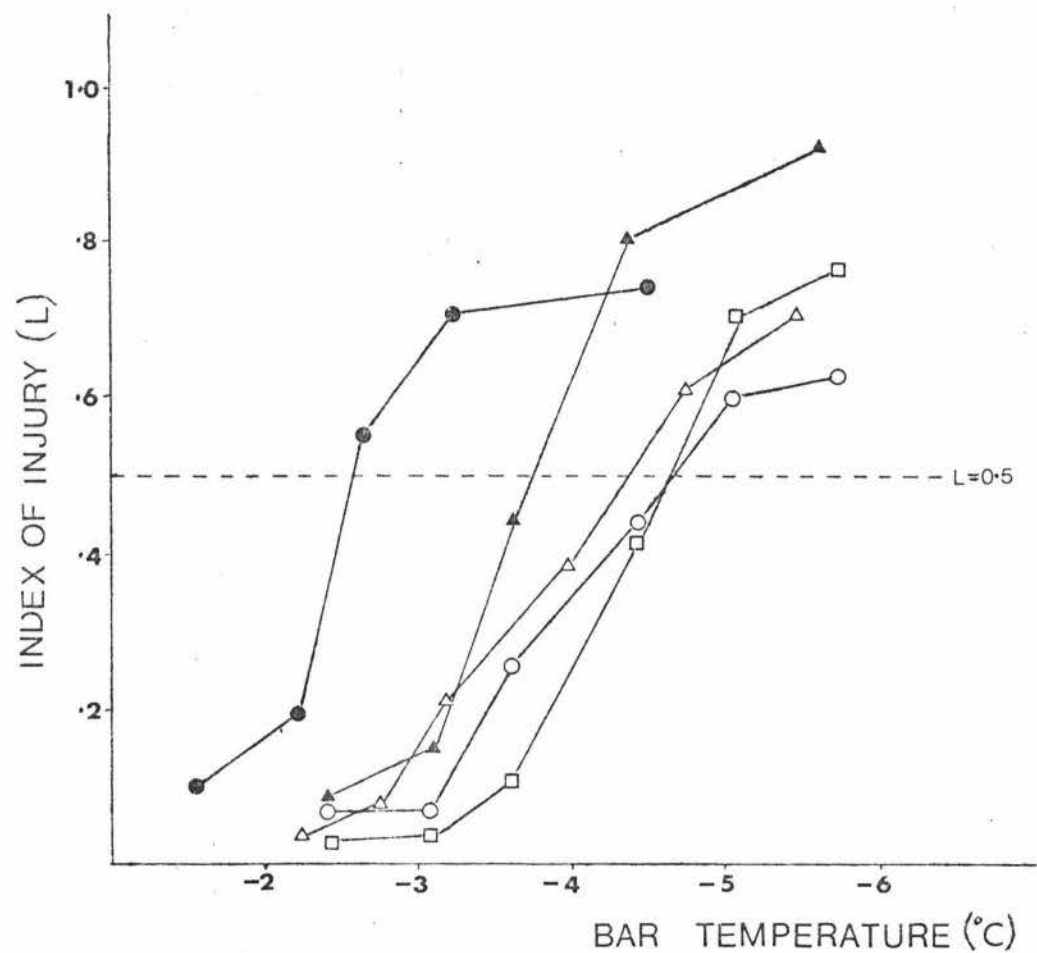
- 0 No damage
- 1 Necrosis on leaf edges
- 2 Younger leaves killed
- 3 Half of the foliage killed
- 4 Some survival of older leaves
- 5 Leaves completely killed

A value of $2\frac{1}{2}$ was used as an estimate of 50% necrosis. This was compared with the 50% killing index (L) assessed by the electrical conductance technique.

The estimated killing temperature of leaves on the gradient bar were substantially higher than that of whole plants in the frosting room. This discrepancy probably arose from the use of exised leaves on the

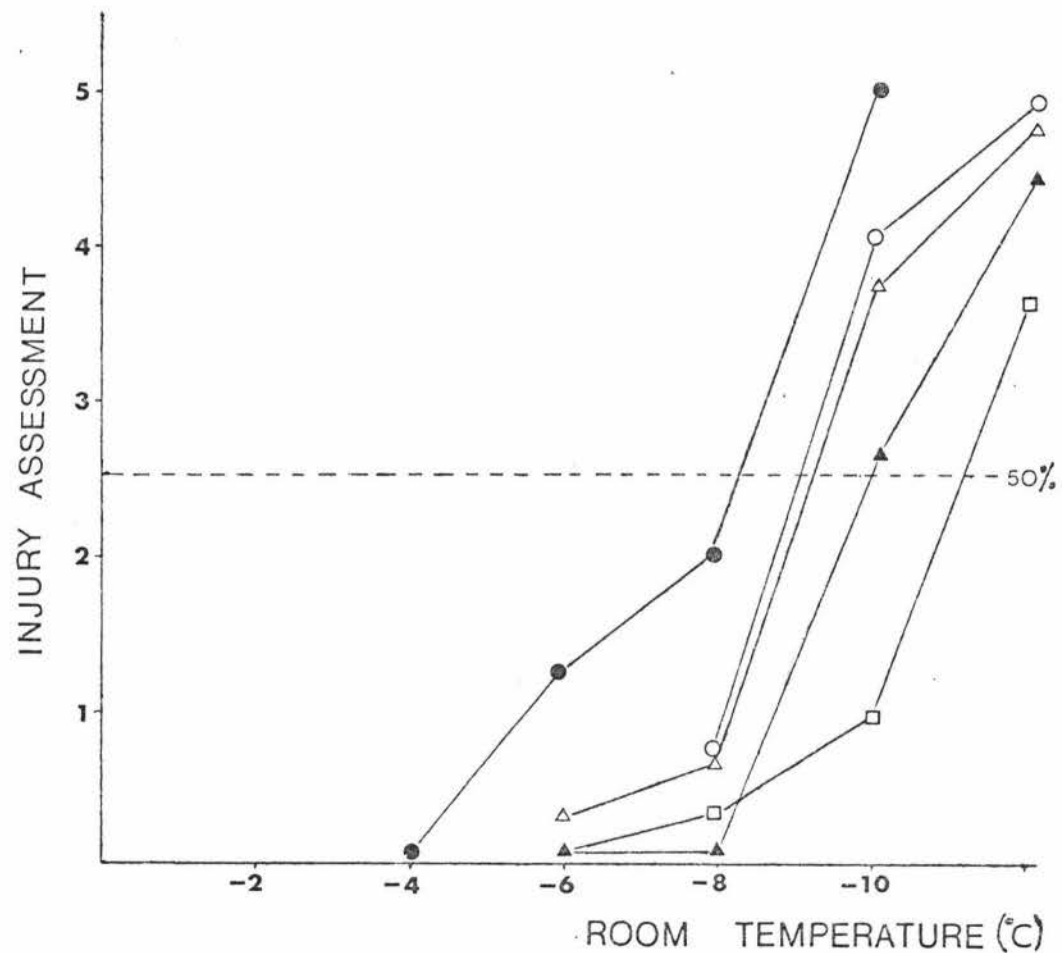
APPENDIX FIGURE I

GRADIENT BAR



- SPAIN -2.6° (Killing temperature)
- △ LADINO -4.4
- HUIA -4.6
- ▲ KENT -3.8
- RUSSIA -4.6

FROST ROOM



- SPAIN -8.2°
- △ LADINO -9.2
- HUIA -9.1
- ▲ KENT -9.8
- RUSSIA -11.0

gradient bar compared with intact plants in the frost room. Also the leaves were frozen immediately on contact with the bar whereas the rate of freezing and thawing is slow in the controlled frosting environment.

However, the absolute temperature required to cause injury is not the important factor, but that the two methods used in assessing cold tolerance produce comparable results. A significant correlation wasn't achieved in this experiment ($r = .65$ with 4 df) due to the low degrees of freedom. A similar experiment by de Ruiter (1979) using 15 forage legumes established a significant correlation ($r = .70$). A correction factor was also evaluated so that approximate field killing temperatures could be obtained from the electroconductivity measurements.

APPENDIX 3Data for the Regression analysis

e = Environments 1 = HOT (24°/14°C)
 2 = COOL (12°/6°C)
 3 = COLD (12°/-2°C)

b = Blocks 1 = Block I
 2 = Block II
 3 = Block III

g = Clover Populations 1 Spain
 2 Ladino
 3 Huia
 4 Kent
 5 Russia

TM = Time when stolons were measured (days)
 STDM = Stem DW
 BLDM = Leaf DW
 PTDM = Petiole DW
 TOTDM = Total DW
 NPT = Number of Leaves
 LNST = Length of stolon
 BLAR = Leaf Area
 NLT = Number of Laterals
 STTK = STDM/LNST = Stolon thickness
 RATIO = BLDM/TOTDM = Ration of leaf to total DW

Each piece of data is the mean of 10 stolons

e	g	b	TM	STDM	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
1	1	1	5	.007	.022	.000	.029	1.80	2.27	5.42	0
		2	5	.007	.024	.000	.031	2.33	2.43	5.38	0
		3	5	.006	.028	.000	.034	2.20	2.30	6.68	0
1	1	1	9	.012	.040	.011	.062	3.00	3.03	10.14	0.1
		2	9	.015	.034	.008	.056	3.10	3.37	8.64	0.0
		3	9	.012	.035	.011	.057	3.10	3.80	11.17	0.1
1	1	1	13	.053	.090	.026	.170	4.10	6.14	17.72	1.0
		2	13	.039	.075	.021	.135	4.33	5.49	17.60	0.9
		3	13	.031	.069	.023	.129	3.60	4.93	17.56	0.6
1	1	1	17	.053	.093	.032	.178	4.60	6.66	21.23	2.0
		2	17	.067	.116	.037	.219	5.67	9.29	24.02	2.1
		3	17	.065	.109	.043	.217	4.89	9.41	24.24	1.7
1	1	1	22	.110	.157	.069	.336	7.30	10.88	33.58	3.1
		2	22	.103	.156	.060	.320	6.60	10.47	30.38	4.7
		3	22	.081	.122	.052	.255	5.50	8.76	22.83	2.2
1	2	1	5	.011	.039	.000	.050	2.33	2.54	6.54	0
		2	5	.014	.038	.000	.052	2.11	2.51	6.11	0
		3	5	.012	.046	.000	.058	2.63	2.75	7.64	0
1	2	1	9	.028	.070	.030	.128	3.20	3.78	15.56	0.1
		2	9	.026	.049	.025	.100	2.78	3.53	11.90	0.2
		3	9	.026	.040	.019	.085	2.70	3.75	10.90	0.4
1	2	1	13	.052	.104	.046	.202	3.90	4.74	18.23	0.4
		2	13	.053	.097	.054	.204	4.13	5.76	22.39	0.3
		3	13	.033	.077	.035	.145	3.88	4.19	17.64	0.6
1	2	1	17	.062	.116	.065	.243	4.67	6.37	22.74	0.6
		2	17	.051	.091	.049	.191	4.44	5.41	17.80	0.8
		3	17	.078	.129	.073	.280	5.38	5.84	25.75	1.6
1	2	1	22	.097	.153	.129	.379	6.89	7.67	30.42	1.7
		2	22	.092	.123	.121	.336	5.78	7.49	24.20	0.6
		3	22	.089	.134	.084	.307	6.20	7.21	26.48	1.3

e	g	b	TM	STDM	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
1	3	1	5	.004	.016	.000	.020	1.90	1.32	3.21	0
		2	5	.004	.013	.000	.017	1.70	1.64	2.74	0
		3	5	.004	.016	.000	.020	2.70	1.60	3.08	0
1	3	1	9	.009	.025	.008	.042	2.90	2.18	5.80	0.2
		2	9	.012	.037	.011	.060	3.40	2.81	8.77	0.6
		3	9	.009	.025	.009	.043	3.00	2.96	7.83	0.3
1	3	1	13	.029	.066	.025	.120	4.44	4.86	12.60	0.9
		2	13	.027	.060	.020	.107	4.30	4.41	12.61	1.6
		3	13	.022	.047	.017	.086	4.38	4.34	10.90	1.5
1	3	1	17	.035	.065	.021	.121	5.80	5.31	12.43	2.2
		2	17	.028	.064	.021	.113	4.70	5.63	12.41	1.4
		3	17	.031	.081	.032	.144	5.20	5.26	17.76	1.9
1	3	1	22	.051	.089	.052	.192	7.60	6.52	18.55	3.1
		2	22	.049	.092	.047	.188	6.90	6.80	18.35	2.5
		3	22	.047	.079	.039	.165	6.70	7.18	15.25	3.1
1	4	1	5	.003	.007	.000	.010	2.11	1.77	1.27	0
		2	5	.003	.007	.000	.010	2.40	1.71	1.44	0
		3	5	.003	.007	.000	.010	2.50	1.58	1.27	0
1	4	1	9	.009	.018	.001	.028	3.40	2.94	3.28	0.7
		2	9	.009	.017	.001	.027	3.80	3.32	3.18	0.6
		3	9	.006	.011	.001	.018	3.10	2.78	2.23	0.5
1	4	1	13	.017	.020	.007	.043	4.40	4.30	3.70	1.6
		2	13	.017	.019	.006	.042	5.10	5.04	3.45	1.3
		3	13	.010	.012	.004	.026	4.22	3.00	3.08	1.3
1	4	1	17	.021	.025	.009	.055	5.56	5.71	5.19	2.6
		2	17	.023	.026	.012	.061	6.00	5.86	4.55	2.2
		3	17	.020	.021	.007	.048	5.00	4.56	3.89	2.1
1	4	1	22	.028	.028	.014	.070	7.50	6.95	6.08	3.8
		2	22	.029	.029	.012	.070	7.67	6.94	5.35	3.2
		3	22	.027	.025	.010	.062	6.89	6.02	3.93	4.2

e	g	b	TM	STDm	BLDM	FTDM	TOTDM	NPT	LNST	BLAR	NLT
1	5	1	5	.003	.609	.000	.012	2.30	1.32	2.01	0
		2	5	.005	.014	.000	.019	2.40	1.79	2.73	0
		3	5	.005	.016	.000	.021	2.44	1.72	2.48	0
1	5	1	9	.008	.022	.001	.031	3.20	1.98	4.20	0.2
		2	9	.009	.029	.001	.039	3.70	2.26	4.89	0.3
		3	9	.006	.020	.001	.027	3.10	1.91	3.98	0.2
1	5	1	13	.015	.030	.008	.053	4.40	2.76	6.17	1.0
		2	13	.019	.034	.009	.062	5.00	3.43	6.80	1.4
		3	13	.011	.021	.005	.037	4.00	2.60	5.12	0.6
1	5	1	17	.022	.040	.011	.073	6.10	3.80	7.27	1.5
		2	17	.024	.046	.015	.085	5.70	3.85	7.74	2.2
		3	17	.026	.052	.018	.096	5.30	4.02	10.14	2.0
1	5	1	22	.026	.042	.016	.084	6.70	3.95	8.90	1.8
		2	22	.029	.046	.014	.089	6.43	4.10	7.79	2.4
		3	22	.030	.046	.016	.092	5.60	4.54	7.92	2.7
2	1	1	8	.005	.013	.001	.019	2.00	1.16	2.30	0
		2	8	.004	.015	.001	.020	2.38	0.88	2.85	0
		3	8	.005	.012	.001	.018	1.70	1.01	2.30	0
2	1	1	14	.010	.026	.004	.040	3.00	2.02	5.76	0.1
		2	14	.008	.023	.006	.037	3.00	1.73	4.85	0.3
		3	14	.007	.018	.004	.029	2.67	1.40	4.36	0.1
2	1	1	21	.026	.057	.011	.094	3.78	3.47	10.48	1.0
		2	21	.015	.040	.009	.064	3.88	2.25	7.06	0.8
		3	21	.019	.051	.011	.081	3.50	3.13	10.73	0.5
2	1	1	28	.039	.083	.017	.139	4.56	4.61	15.24	1.9
		2	28	.027	.061	.015	.103	4.86	3.67	10.59	1.6
		3	28	.023	.049	.011	.103	4.00	3.18	9.29	0.9
2	1	1	35	.092	.153	.038	.283	5.33	8.07	23.42	2.9
		2	35	.057	.094	.024	.175	4.89	5.44	16.13	1.7
		3	35	.045	.084	.023	.152	4.88	4.45	15.13	1.9

e	g	b	TM	STDM	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
2	2	1	8	.012	.039	.000	.051	2.10	1.35	5.78	0
		2	8	.010	.040	.000	.050	2.20	1.45	6.82	0
		3	8	.011	.033	.000	.044	1.80	1.41	5.27	0
2	2	1	14	.018	.054	.013	.085	2.89	2.07	9.31	0.3
		2	14	.018	.042	.012	.072	3.11	2.23	7.83	0.2
		3	14	.016	.047	.012	.075	2.80	2.12	10.21	0.2
2	2	1	21	.033	.063	.019	.115	3.11	2.87	10.22	0.8
		2	21	.040	.075	.021	.136	4.11	3.66	13.90	0.6
		3	21	.040	.079	.023	.142	3.50	3.41	15.04	0.5
2	2	1	28	.055	.097	.029	.181	4.22	3.99	15.52	1.6
		2	28	.062	.109	.032	.203	4.90	4.73	18.87	1.1
		3	28	.073	.107	.034	.214	4.56	4.04	20.58	1.2
2	2	1	35	.116	.133	.048	.297	5.22	5.44	19.96	2.2
		2	35	.115	.136	.062	.313	5.00	6.26	23.92	2.1
		3	35	.091	.137	.049	.277	4.90	4.92	20.83	1.8
2	3	1	8	.006	.018	.000	.024	2.30	0.99	3.03	0
		2	8	.004	.017	.000	.021	2.11	1.07	3.29	0
		3	8	.004	.016	.000	.020	2.22	0.88	2.46	0
2	3	1	14	.008	.026	.006	.040	3.00	1.41	5.08	0.3
		2	14	.005	.015	.004	.024	2.89	1.19	3.02	0.1
		3	14	.006	.020	.004	.030	3.00	1.44	4.39	0.1
2	3	1	21	.014	.037	.009	.060	3.78	2.11	6.84	1.0
		2	21	.014	.039	.010	.063	3.80	2.18	7.19	1.1
		3	21	.016	.040	.008	.064	4.00	2.21	6.27	1.4
2	3	1	28	.022	.050	.012	.084	4.60	2.65	8.98	1.9
		2	28	.022	.059	.016	.097	4.75	3.03	10.14	1.9
		3	28	.020	.050	.010	.080	4.70	2.74	9.20	2.1
2	3	1	35	.036	.067	.020	.123	4.90	3.72	12.03	2.7
		2	35	.052	.084	.026	.162	5.29	5.31	14.56	1.9
		3	35	.038	.067	.018	.123	5.10	3.45	12.07	2.4

e	g	b	TM	STDM	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
2	4	1	8	.004	.010	.000	.014	2.30	0.95	1.42	0
		2	8	.003	.008	.000	.011	2.10	0.79	1.06	0
		3	8	.003	.006	.000	.009	2.30	0.97	1.13	0
2	4	1	14	.006	.015	.001	.022	3.30	1.42	1.89	0.9
		2	14	.005	.013	.001	.019	3.20	1.25	1.83	0.3
		3	14	.006	.013	.001	.020	3.30	1.68	2.01	0.7
2	4	1	21	.011	.021	.004	.036	4.20	1.85	2.73	1.7
		2	21	.011	.019	.004	.034	4.20	2.17	3.13	1.3
		3	21	.015	.024	.005	.044	4.30	2.89	4.29	1.0
2	4	1	28	.017	.020	.005	.042	4.88	3.93	3.08	2.4
		2	28	.015	.027	.001	.043	5.10	2.65	3.52	2.7
		3	28	.021	.023	.006	.050	5.33	3.70	4.38	2.7
2	4	1	35	.032	.043	.010	.082	5.60	4.14	5.32	3.2
		2	35	.028	.031	.007	.066	5.78	3.33	4.90	3.0
		3	35	.032	.035	.010	.077	6.10	4.49	5.54	3.1
2	5	1	8	.006	.017	.000	.023	2.60	0.76	2.28	0
		2	8	.003	.012	.000	.015	2.30	0.69	1.56	0
		3	8	.004	.012	.000	.016	2.00	0.86	1.54	0
2	5	1	14	.009	.024	.003	.036	3.78	1.24	3.03	0.9
		2	14	.007	.018	.001	.026	3.10	1.05	2.25	0.2
		3	14	.005	.014	.004	.023	3.20	0.97	2.50	0.1
2	5	1	21	.014	.024	.004	.042	3.78	1.84	2.98	1.3
		2	21	.014	.031	.005	.040	4.40	1.65	3.79	1.2
		3	21	.013	.029	.006	.048	4.11	1.63	4.01	1.2
2	5	1	28	.026	.045	.007	.078	4.90	2.65	5.95	2.0
		2	28	.018	.031	.006	.055	4.60	1.86	3.83	1.8
		3	28	.016	.034	.001	.051	4.60	1.80	4.12	1.7
2	5	1	35	.035	.044	.011	.090	5.60	2.71	5.99	2.9
		2	35	.028	.040	.007	.075	5.22	2.58	5.03	2.8
		3	35	.029	.040	.007	.076	5.10	2.46	5.54	2.0

e	g	b	TM	STDm	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
3	1	1	8	.007	.020	.001	.023	2.20	1.55	3.84	0
		2	8	.008	.017	.001	.026	1.67	1.62	3.07	0
		3	8	.006	.016	.001	.023	1.88	1.30	3.01	0
3	1	1	17	.014	.035	.007	.056	3.33	2.04	6.36	0.1
		2	17	.013	.035	.008	.056	3.30	1.91	7.57	0.2
		3	17	.013	.039	.009	.061	3.50	1.99	8.01	0.5
3	1	1	25	.027	.058	.011	.096	4.30	3.30	9.85	1.3
		2	25	.015	.042	.009	.066	3.50	2.00	6.59	0.3
		3	25	.019	.048	.009	.076	3.56	2.56	6.47	1.3
3	1	1	33	.038	.078	.015	.131	3.97	3.97	13.18	1.9
		2	33	.038	.085	.018	.141	4.67	3.60	15.22	2.0
		3	33	.039	.073	.017	.129	4.25	3.63	13.03	1.6
3	1	1	43	.069	.121	.027	.217	4.89	5.28	18.50	2.4
		2	43	.076	.115	.029	.220	5.20	6.18	16.81	2.9
		3	43	.056	.085	.023	.164	4.89	4.70	14.23	2.2
3	2	1	8	.013	.036	.001	.050	2.00	1.88	6.11	0.1
		2	8	.018	.046	.001	.065	2.00	1.93	7.37	0
		3	8	.014	.031	.001	.046	1.60	1.86	4.63	0
3	2	1	17	.027	.074	.017	.118	3.50	2.24	11.71	0.7
		2	17	.026	.060	.016	.102	3.44	2.33	11.31	0.8
		3	17	.021	.050	.012	.085	3.30	2.03	10.86	0.4
3	2	1	25	.041	.088	.020	.149	3.90	3.14	13.61	1.2
		2	25	.038	.094	.020	.152	3.60	3.16	13.90	1.4
		3	25	.033	.081	.016	.130	3.89	2.66	11.97	0.9
3	2	1	33	.052	.106	.030	.188	4.80	3.67	16.52	2.0
		2	33	.056	.112	.027	.195	4.33	3.56	17.17	1.7
		3	33	.065	.122	.037	.224	4.33	4.04	18.69	1.6
3	2	1	43	.102	.162	.053	.217	5.50	5.52	25.73	2.3
		2	43	.073	.107	.034	.214	4.78	4.13	17.71	2.2
		3	43	.117	.149	.058	.324	5.20	5.44	23.49	1.7

e	g	b	TM	STDM	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
3	3	1	8	.006	.021	.000	.027	2.50	1.50	3.96	0
		2	8	.006	.024	.000	.030	2.50	1.30	4.12	0.2
		3	8	.004	.014	.000	.018	2.00	1.07	2.32	0
3	3	1	17	.016	.041	.008	.065	3.67	2.18	6.86	0.9
		2	17	.013	.038	.008	.059	3.70	1.79	6.06	1.0
		3	17	.009	.029	.006	.044	3.44	1.52	5.06	0.9
3	3	1	25	.024	.052	.010	.086	4.44	2.79	8.43	1.7
		2	25	.031	.060	.013	.104	4.38	3.05	9.00	2.0
		3	25	.020	.051	.010	.081	4.22	2.57	8.80	1.3
3	3	1	33	.037	.070	.014	.121	5.56	3.67	10.60	3.0
		2	33	.033	.070	.013	.116	5.44	3.42	10.48	2.9
		3	33	.028	.065	.016	.109	5.22	2.71	11.20	2.9
3	3	1	43	.063	.105	.028	.196	6.50	5.21	17.22	3.9
		2	43	.054	.075	.022	.151	5.60	4.87	12.48	3.3
		3	43	.037	.067	.016	.120	5.33	3.21	10.70	2.9
3	4	1	8	.005	.010	.000	.015	2.30	1.53	1.81	0.2
		2	8	.004	.012	.000	.016	2.60	1.41	2.05	0
		3	8	.003	.008	.000	.011	2.20	0.88	1.23	0
3	4	1	17	.014	.021	.004	.039	4.20	2.18	3.31	1.6
		2	17	.010	.018	.003	.031	4.00	1.82	2.73	1.1
		3	17	.008	.013	.003	.024	3.70	1.39	3.06	0.8
3	4	1	25	.017	.020	.005	.042	5.00	2.91	3.29	2.6
		2	25	.015	.022	.004	.041	4.70	2.53	2.81	2.0
		3	25	.012	.019	.003	.034	4.30	2.25	2.61	1.9
3	4	1	33	.024	.029	.006	.059	5.70	3.28	3.95	2.8
		2	33	.032	.034	.008	.074	5.56	4.37	4.76	3.3
		3	33	.017	.023	.004	.044	4.89	2.71	4.37	2.4
3	4	1	43	.035	.039	.010	.054	6.29	4.26	5.24	3.4
		2	43	.039	.039	.010	.088	6.33	4.74	5.76	3.9
		3	43	.034	.035	.008	.077	5.70	3.90	4.87	2.8

e	g	b	TM	STDM	BLDM	PTDM	TOTDM	NPT	LNST	BLAR	NLT
3	5	1	8	.005	.013	.000	.018	2.1	0.98	2.12	0.1
		2	8	.005	.014	.000	.019	2.0	0.94	1.84	0
		3	8	.005	.012	.000	.017	2.1	0.85	1.84	0
3	5	1	17	.009	.020	.003	.032	3.4	1.30	2.44	0.8
		2	17	.010	.023	.004	.037	3.4	1.34	3.92	0.4
		3	17	.008	.017	.003	.028	3.2	1.29	2.43	0.6
3	5	1	25	.015	.026	.005	.046	4.4	1.70	3.65	1.4
		2	25	.019	.029	.004	.048	4.1	1.57	3.63	1.2
		3	25	.016	.033	.006	.057	4.2	1.86	4.17	1.7
3	5	1	33	.020	.029	.005	.054	4.8	1.79	3.54	1.9
		2	33	.021	.035	.005	.061	4.8	1.89	3.97	1.6
		3	33	.020	.035	.007	.062	4.9	1.91	4.66	2.0
3	5	1	43	.035	.043	.007	.085	6.4	2.86	4.80	3.9
		2	43	.035	.045	.008	.088	5.7	2.52	5.16	3.4
		3	43	.033	.038	.006	.077	5.2	2.47	4.94	2.2

APPENDIX 4A

Stem Dry Weight

regression statistics describing the development patterns
for the stolon characters in the second growth period.

$\log_e(g)$	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-6.441	-5.743	-6.511	-6.189	-6.120
	SE b_0	0.425	0.500	0.364	0.449	0.424
		* a	a	a	a	a
	b_1	0.150	0.125	0.132	0.030	0.051
	SE b_1	0.052	0.061	0.132	0.030	0.051
		a	a	a	a	a
	b_2	-0.0004	-0.0002	-0.0005	+0.0016	+0.0010
SE b_2	0.0014	0.0017	0.0012	0.0015	0.0014	
	a	a	a	a	a	
	R^2	.964	.936	.963	.908	.916
COOL	b_0	-6.461	-5.203	-6.933	-6.803	-6.217
	SE b_0	0.727	0.635	0.706	0.581	0.500
		ab	a	b	b	ab
	b_1	0.094	0.039	0.113	0.096	0.037
	SE b_1	0.058	0.051	0.056	0.046	0.040
		a	a	a	a	a
	b_2	-0.0002	0.0007	-0.0007	-0.0007	0.0004
SE b_2	0.0011	0.0009	0.0010	0.0009	0.0007	
	a	a	a	a	a	
	R^2	.925	.932	.919	.901	.923
COLD	b_0	-6.924	-6.924	-7.864	-6.324	-8.157
	SE b_0	0.660	0.734	0.596	0.460	0.804
		ab	ab	b	a	b
	b_1	0.145	0.090	0.182	0.056	0.172
	SE b_1	0.046	0.051	0.042	0.032	0.056
		ab	ab	a	b	ab
	b_2	-0.0014	-0.0005	-0.0018	0.0001	-0.0019
SE b_2	0.0007	0.0008	0.0007	0.0005	0.0009	
	ab	ab	b	a	b	
	R^2	.931	.918	.957	.961	.881

* Statistics sharing the same letter are not significantly different (P=0.05)

APPENDIX 4B

Leaf Dry Weight

$\log_e(g)$ Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-5.224	-3.914	-4.866	-6.189	-5.830
	SE b_0	0.394	0.507	0.277	0.473	0.590
		* bc	a	ab	c	bc
	b_1	0.163	0.054	0.086	0.107	0.162
	SE b_1	0.047	0.062	0.034	0.057	0.072
		a	a	a	a	a
	b_2	-0.0023	+0.0023	-0.0003	-0.0015	-0.0030
	SE b_2	0.0013	0.0017	0.0009	0.0016	0.0020
		a	a	a	a	a
R^2		.921	.797	.950	.782	.707
COOL	b_0	-4.656	-3.657	-4.823	-6.359	-5.910
	SE b_0	0.586	0.558	0.352	0.539	0.316
		a	a	a	b	b
	b_1	0.054	0.009	0.042	0.115	0.079
	SE b_1	0.047	0.045	0.028	0.043	0.025
		a	a	a	a	a
	b_2	0.0001	0.0008	0.0002	-0.0014	-0.0006
	SE b_2	0.0008	0.0008	0.0005	0.0008	0.0005
		ab	a	ab	b	ab
R^2		.898	.882	.957	.851	.954
COLD	b_0	-5.915	-4.694	-5.359	-5.710	-5.837
	SE b_0	0.444	0.537	0.412	0.373	0.544
		b	a	ab	ab	ab
	b_1	0.167	0.100	0.103	0.067	0.089
	SE b_1	0.031	0.037	0.029	0.026	0.038
		a	ab	ab	b	ab
	b_2	-0.0021	-0.0010	-0.0011	-0.0006	-0.0010
	SE b_2	0.0005	0.0006	0.0005	0.0004	0.0006
		b	ab	ab	a	ab
R^2		.937	.889	.933	.917	.820

* Statistics sharing the same letter are not significantly different ($F=0.05$)

APPENDIX 4C

Petiole Dry Weight

$\log_e(g)$ Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-8.009	-9.317	-7.388	-5.435	-5.238
	SE b_0	0.496	0.661	0.424	0.396	0.514
		* bc	c	b	a	a
	b_1	0.294	0.495	0.205	-0.073	-0.113
	SE b_1	0.060	0.080	0.051	0.048	0.062
		b	a	b	c	c
	b_2	-0.0043	-0.0094	-0.0023	-0.0034	-0.0052
	SE b_2	0.0017	0.0022	0.0014	0.0013	0.0017
		a	b	a	a	ab
	R^2	.956	.943	.956	.810	.860
COOL	b_0	-7.607	-5.818	-7.164	-5.340	-4.958
	SE b_0	0.598	0.573	0.434	0.576	0.217
		b	a	b	a	a
	b_1	0.133	0.057	0.091	-0.056	-0.086
	SE b_1	0.048	0.046	0.035	0.046	0.017
		a	a	a	b	b
	b_2	-0.0007	0.0005	-0.0001	0.0016	0.0020
	SE b_2	0.0009	0.0008	0.0006	0.0008	0.0003
		b	ab	b	ab	a
	R^2	.961	.952	.974	.759	.940
COLD	b_0	-7.085	-6.399	-8.334	-4.955	-5.394
	SE b_0	0.747	0.722	0.306	0.200	0.492
		cd	bc	d	a	ab
	b_1	0.126	0.120	0.169	-0.078	-0.036
	SE b_1	0.052	0.050	0.021	0.014	0.034
		a	a	a	b	b
	b_2	-0.0011	-0.0010	-0.0016	0.0017	0.0008
	SE b_2	0.0008	0.0008	0.0003	0.0002	0.0006
	R^2	.902	.921	.989	.975	.974
		b	b	b	a	ab

* Statistics sharing the same letter are not significantly different ($P=0.05$)

APPENDIX 4D

Total Stolon Dry Weight

$\log_e(g)$ Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-5.254	-4.324	-5.032	-5.390	-5.242
	SE b_0	0.414	0.505	0.283	0.443	0.537
		* a	a	a	a	a
	b_1	0.202	0.146	0.146	0.070	0.114
	SE b_1	0.050	0.061	0.034	0.054	0.065
		a	ab	ab	b	ab
	b_2	-0.0025	-0.0014	-0.0013	0.0003	-0.0010
SE b_2	0.0014	0.0017	0.0010	0.0015	0.0018	
	a	a	a	a	a	
R^2		.950	.904	.969	.897	.847
<hr/>						
COOL	b_0	-4.868	-3.376	-5.210	-5.802	-5.179
	SE b_0	0.609	0.565	0.420	0.564	0.371
		b	a	b	b	b
	b_1	0.092	0.019	0.096	0.098	0.046
	SE b_1	0.049	0.045	0.033	0.045	0.030
		a	a	a	a	a
	b_2	-0.0003	0.0008	-0.0005	-0.0008	0.0002
SE b_2	0.0009	0.0008	0.0006	0.0008	0.0005	
	a	a	a	a	a	
R^2		.931	.922	.963	.900	.954
<hr/>						
COLD	b_0	-5.379	-4.233	-5.784	-5.250	-5.957
	SE b_0	0.516	0.615	0.385	0.330	0.530
		ab	a	b	ab	b
	b_1	0.154	0.096	0.148	0.058	0.114
	SE b_1	0.036	0.043	0.027	0.023	0.037
		a	ab	a	b	ab
	b_2	-0.0017	-0.0008	-0.0015	-0.0002	-0.0012
SE b_2	0.0006	0.0007	0.0004	0.0004	0.0006	
	b	ab	b	a	ab	
R^2		.935	.908	.970	.971	.908

* Statistics sharing the same letter are not significantly different ($F=0.05$)

APPENDIX 4ERatio of leaf to total DW

\log_e	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	b_0	0.030	0.410	0.166	-0.606	-0.589
	SE b_0	0.099	0.137	0.157	0.276	0.186
		* b	a	ab	c	c
	b_1	-0.039	-0.092	-0.060	0.037	0.048
	SE b_1	0.012	0.017	0.019	0.033	0.022
		b	c	bc	a	a
	b_2	0.0003	0.0016	0.0010	-0.0018	-0.0020
SE b_2	0.0003	0.0005	0.0005	0.0009	0.0006	
	b	a	ab	c	c	
	R^2	.956	.943	.860	.728	.845
COOL	b_0	0.212	-0.282	0.387	-0.557	-0.730
	SE b_0	0.184	0.067	0.172	0.284	0.140
		a	b	a	bc	c
	b_1	-0.038	-0.010	-0.054	0.016	0.034
	SE b_1	0.015	0.005	0.014	0.023	0.011
		bc	b	c	ab	a
	b_2	0.0004	0.0001	0.0007	-0.0006	-0.0008
SE b_2	0.0003	0.0001	0.0003	0.0004	0.0002	
	ab	ab	a	bc	c	
	R^2	.883	.974	.895	.708	.836
COLD	b_0	-0.536	-0.461	0.425	-0.450	0.121
	SE b_0	0.150	0.132	0.167	0.221	0.206
		c	c	a	bc	ab
	b_1	0.013	0.004	-0.046	0.009	-0.025
	SE b_1	0.010	0.009	0.012	0.015	0.014
		a	ab	c	ab	bc
	b_2	-0.0003	-0.0002	0.0005	-0.0004	0.0002
SE b_2	0.0002	0.0001	0.0002	0.0003	0.0002	
	b	b	a	b	ab	
	R^2	.802	.914	.940	.910	.860

* Statistics sharing the same letter are not significantly different ($P=0.05$)

APPENDIX 4F

Leaf Production

\log_e	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-0.121	-0.108	0.207	0.197	0.397
	SE b_0	0.215	0.223	0.086	0.140	0.176
		* b	b	ab	ab	a
	b_1	0.115	0.128	0.093	0.097	0.072
	SE b_1	0.026	0.027	0.010	0.017	0.021
		a	a	a	a	a
	b_2	-0.0018	-0.0023	-0.0013	-0.0015	-0.0009
	SE b_2	0.0007	0.0008	0.0003	0.0005	0.0006
		a	a	a	a	a
	R^2		.935	.916	.987	.964
COOL	b_0	0.018	0.279	0.106	0.017	0.090
	SE b_0	0.279	0.083	0.138	0.175	0.215
		ab	a	ab	ab	ab
	b_1	0.054	0.035	0.071	0.065	0.050
	SE b_1	0.022	0.007	0.011	0.014	0.017
		ab	b	a	a	ab
	b_2	-0.0004	-0.0001	-0.0007	-0.0006	-0.0003
	SE b_2	0.0004	0.0001	0.0002	0.0003	0.0003
		ab	a	b	ab	ab
	R^2		.920	.991	.979	.968
COLD	b_0	0.061	0.389	0.012	0.266	-0.133
	SE b_0	0.219	0.300	0.159	0.266	0.241
		a	a	a	a	a
	b_1	0.050	0.035	0.058	0.062	0.067
	SE b_1	0.015	0.021	0.011	0.018	0.017
		a	a	a	a	a
	b_2	-0.0004	-0.0002	-0.0005	-0.0006	-0.0006
	SE b_2	0.0002	0.0003	0.0002	0.0003	0.0003
		a	a	a	a	a
	R^2		.954	.894	.976	.944

* Statistics sharing the same letter are not significantly different ($P=0.05$)

APPENDIX 4G

Leaf Area

Log _e (sqcm)	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	b ₀	0.189	0.756	0.592	-0.291	-0.134
	SE b ₀	0.331	0.591	0.232	0.580	0.593
		* a	a	a	a	a
	b ₁	0.177	0.124	0.076	0.035	0.094
	SE b ₁	0.040	0.071	0.028	0.070	0.071
		a	ab	b	b	ab
	b ₂	-0.0028	-0.0015	-0.0001	0.0009	-0.0008
	SE b ₂	0.0011	0.0020	0.0008	0.0019	0.0020
		a	a	a	a	a
	R ²	.935	.793	.963	.777	.755
COOL	b ₀	0.806	1.825	0.474	-0.828	-0.538
	SE b ₀	0.585	0.480	0.256	0.527	0.488
		ab	a	b	c	c
	b ₁	0.051	-0.005	0.049	0.082	0.035
	SE b ₁	0.047	0.038	0.020	0.042	0.039
		a	a	a	a	a
	b ₂	0.0001	0.0010	0.0001	-0.0008	0.0002
	SE b ₂	0.0008	0.0007	0.0004	0.0008	0.0007
		a	a	a	a	a
	R ²	.890	.896	.973	.844	.895
COLD	b ₀	0.308	1.029	-0.154	0.270	-0.398
	SE b ₀	0.553	0.682	0.487	0.456	0.458
		ab	a	ab	ab	b
	b ₁	0.107	0.065	0.101	-0.003	0.046
	SE b ₁	0.039	0.048	0.034	0.032	0.032
		a	ab	a	b	ab
	b ₂	-0.0012	-0.0005	-0.0011	-0.0005	-0.0004
	SE b ₂	0.0006	0.0008	0.0005	0.0005	0.0005
		b	ab	b	a	ab
	R ²	.850	.795	.896	.878	.818

* Statistics sharing the same letter are not significantly different (F=0.05)

APPENDIX 4H

Length of Stolon

\log_e (cm)	Statistic	Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-0.700	0.257	-0.769	-0.508	-0.227
	SE b_0	0.291	0.436	0.331	0.270	0.487
		* b	a	b	ab	ab
	b_1	0.140	0.041	0.131	0.081	0.030
	SE b_1	0.035	0.053	0.040	0.045	0.059
		a	a	a	a	a
	b_2	-0.0013	0.0008	-0.0013	-0.0003	0.0008
	SE b_2	0.0010	0.0015	0.0011	0.0012	0.0016
		a	a	a	a	a
	R^2		.964	.870	.944	.908
COOL	b_0	-0.318	-0.039	-0.804	-0.976	-0.670
	SE b_0	0.590	0.392	0.604	0.392	0.233
		a	a	a	a	a
	b_1	0.045	0.035	0.064	0.082	0.018
	SE b_1	0.047	0.031	0.048	0.031	0.018
		ab	ab	ab	a	b
	b_2	0.0001	0.0002	-0.0002	-0.0007	0.0003
	SE b_2	0.0008	0.0006	0.0009	0.0006	0.0003
		a	a	a	a	a
	R^2		.879	.921	.882	.916
COLD	b_0	-0.900	0.026	-1.069	-0.216	-1.475
	SE b_0	0.591	0.607	0.465	0.265	0.418
		ab	a	ab	ab	b
	b_1	0.084	0.033	0.082	0.011	0.065
	SE b_1	0.041	0.042	0.032	0.018	0.029
		a	a	a	a	a
	b_2	-0.0007	-0.0001	-0.0007	0.0004	-0.0006
	SE b_2	0.0007	0.0007	0.0005	0.0003	0.0005
		a	a	a	a	a
	R^2		.880	.811	.921	.972

* Statistics sharing the same letter are not significantly different ($P=0.05$)

APPENDIX 4IStolon thickness

$\log_e(g/cm)$ Statistic		Spain	Ladino	Huia	Kent	Russia
HOT	b_0	-5.741	-6.005	-5.742	-5.682	-5.893
	SE b_0	0.217	0.167	0.189	0.229	0.146
		* a	a	a	a	a
	b_1	0.010	0.083	0.001	-0.051	0.021
	SE b_1	0.026	0.020	0.023	0.028	0.018
		bc	a	bc	c	b
	b_2	0.0009	-0.0010	0.0008	0.0008	0.0002
	SE b_2	0.0007	0.0006	0.0006	0.0008	0.0005
		ab	c	ab	a	bc
R^2		.905	.957	.878	.622	.897
COOL	b_0	-6.143	-5.164	-6.129	-5.827	-5.547
	SE b_0	0.335	0.339	0.218	0.259	0.456
		b	a	b	ab	ab
	b_1	0.049	0.004	0.049	0.015	0.019
	SE b_1	0.027	0.027	0.017	0.021	0.036
		a	a	a	a	a
	b_2	-0.0003	0.0005	-0.0005	0.0001	0.0001
	SE b_2	0.0005	0.0005	0.0003	0.0004	0.0006
		a	a	a	a	a
R^2		.892	.900	.917	.766	.661
COLD	b_0	-6.024	-5.813	-6.795	-6.109	-6.682
	SE b_0	0.349	0.244	0.203	0.380	0.485
		ab	a	b	ab	ab
	b_1	0.061	0.057	0.099	0.045	0.106
	SE b_1	0.024	0.017	0.014	0.026	0.034
		a	a	a	a	a
	b_2	-0.0007	-0.0005	-0.0011	-0.0004	-0.0013
	SE b_2	0.0004	0.0003	0.0002	0.0004	0.0005
		a	a	a	a	a
R^2		.850	.960	.974	.792	.824

* Statistics sharing the same letter are not significantly different (P=0.05)

APPENDIX 5A

The population means and significance levels for stem DW(g)

	Spain	Ladino	Huia	Kent	Russia
HOT	0.098 * a	0.090 a	0.047 b	0.027 c	0.029 c
COOL	0.061 b	0.109 a	0.041 bc	0.030 c	0.031 c
COLD	0.067 b	0.097 a	0.050 bc	0.036 c	0.035 c
POOLED	0.075 b	0.099 a	0.046 c	0.031 c	0.032 c

* Statistics sharing the same letter are not significantly different ($P=0.05$)

Adaptation Analysis

Population	Ecovalence	Adaptation Coeff. b	S.E. b	Significance Tests		Stability Coefficient
				FROM 0	FROM 1	
Spain	.00042	8.78	4.77	NS	NS	0.77
Ladino	.00017	-4.85	0.11	*	*	0.99
Huia	.00001	1.58	1.41	NS	NS	0.56
Kent	.00003	-0.34	2.11	NS	NS	0.03
Russia	.00002	-0.18	1.37	NS	NS	0.02

* = significant differences at $P=0.10$

** = significant differences at $P=0.05$

APPENDIX 5BThe population means and significance levels for leaf DW(g)

	Spain	Ladino	Huia	Kent	Russia
HOT	0.149 * a	0.139 a	0.086 b	0.027 c	0.046 c
COOL	0.105 b	0.139 a	0.058 c	0.034 c	0.042 c
COLD	0.107 b	0.137 a	0.081 b	0.037 c	0.040 c
POOLED	0.120 a	0.138 a	0.075 b	0.033 c	0.043 c

* Statistics sharing the same letter are not significantly different (P=0.05)

Adaptation Analysis

Population	Ecovalence	Adaptation Coeff. b	S.E. b	Significance Tests		Stability Coefficient
				FROM 0	FROM 1	
Spain	.00043	3.39	1.13	NS	NS	.89
Ladino	.00006	-0.01	0.13	NS	NS	.06
Huia	.00011	1.84	1.08	NS	NS	.75
Kent	.00017	-0.59	0.44	NS	NS	.64
Russia	.00003	0.36	0.26	NS	NS	.66

* = significant differences at P=0.10

** = significant differences at P=0.05

APPENDIX 5C

The population means and significance levels for petiole DW(g)

	Spain	Ladino	Huia	Kent	Russia
HOT	0.058 * b	0.095 a	0.042 c	0.012 d	0.017 d
COOL	0.028 b	0.049 a	0.021 b	0.009 c	0.008 c
COLD	0.025 b	0.045 a	0.020 b	0.010 c	0.007 c
POOLED	0.037 b	0.063 a	0.028 b	0.010 c	0.011 c

* Statistics sharing the same letter are not significantly different (P=0.05)

Adaptation Analysis

Population	Ecovaleance	Adaptation Coeff. b	S.E. b	Significance Tests		Stability Coefficient
				FROM 0	FROM 1	
Spain	.00003	1.38	0.03	*	NS	.99
Ladino	.00028	2.14	0.03	*	*	.99
Huia	.00001	0.92	0.03	*	NS	.99
Kent	.00016	0.14	0.05	NS	*	.89
Russia	.00007	0.42	0.01	*	*	.99

* = significant differences at P=0.10

** = significant differences at P=0.05

APPENDIX 5DThe population means and significance levels for leaf production

	Spain	Ladino	Huia	Kent	Russia
HOT	6.36 * a	6.31 a	6.95 a	7.29 a	6.37 a
COOL	4.99 b	5.01 b	5.06 b	5.76 a	5.19 b
COLD	4.91 b	5.03 b	5.77 a	5.96 a	5.63 a
FOOLED	5.42 c	5.45 c	5.93 b	6.34 a	5.73 bc

* Statistics sharing the same letter are not significantly different ($P=0.05$)

Adaptation analysis

Population	Ecovaleance	Adaptation Coeff. b	S.E. b	Significance Tests		Stability Coefficient
				FROM 0	FROM 1	
Spain	.0384	1.03	0.23	NS	NS	.95
Ladino	.0184	0.95	0.15	NS	NS	.98
Huia	.0843	1.21	0.26	NS	NS	.95
Kent	.0055	1.07	0.05	*	NS	.99
Russia	.0664	0.75	0.16	NS	NS	.96

* = significant differences at $P=0.10$

** = significant differences at $P=0.05$

APPENDIX 5EThe population means and significance levels for stolon length (cm)

	Spain	Ladino	Huia	Kent	Russia
HOT	10.40 * a	7.40 b	6.77 b	6.60 b	4.34 c
COOL	5.78 a	5.56 a	4.25 a	3.95 ab	2.56 b
COLD	5.45 a	5.10 a	4.41 a	4.38 a	2.55 b
POOLED	7.21 a	6.02 ab	5.15 b	4.98 b	3.15 c

* Statistics sharing the same letter are not significantly different ($P=0.05$)

Adaptation Analysis

Population	Ecovalence	Adaptation Coeff. b	S.E. b	Significance Tests		Stability Coefficient
				FROM 0	FROM 1	
Spain	1.83	1.77	.08	*	NS	.99
Ladino	0.22	0.77	.14	NS	NS	.97
Huia	0.04	0.90	.06	*	NS	.99
Kent	0.10	0.90	.15	NS	NS	.97
Russia	0.35	0.66	.01	*	*	.99

* = significant difference at $P=0.10$

** = significant differences at $P=0.05$

APPENDIX 5F

The population means and significance levels for stolon thickness (gcm^{-1})

	Spain	Ladino	Huia	Kent	Russia
HOT	.0096 b	.0122 a	.0070 c	.0042 d	.0068 c
COOL	.0106 b	.0196 a	.0101 b	.0076 c	.0118 b
COLD	.0125 b	.0189 a	.0113 b	.0081 c	.0133 b
ICOLED	.0109 b	.0169 a	.0095 b	.0067 c	.0106 b

* Statistics sharing the same letter are not significantly different ($P=0.05$)

Adaptation Analysis

Population	Ecovalence	Adaptation Coeff. b	S.E. b	Significance Tests		Stability Coefficient
				FROM 0	FROM 1	
Spain	.000080	0.49	0.30	NS	NS	.74
Ladino	.000100	1.54	0.40	NS	NS	.94
Huia	.000003	0.85	0.09	NS	NS	.99
Kent	.000005	0.81	0.05	*	NS	.99
Russia	.000002	1.31	0.07	*	NS	.99

* = significant difference at $P=0.10$

** = significant difference at $p=0.05$