SHORT-TERM BIOMASS PRODUCTION OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) AND ITS INHERITANCE

BY

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ABSTRACT

The objective of this study was to evaluate the short-term (one-year) biomass production in red clover and to obtain an estimate of the heritability of this characteristic. One thousand plants selected from ten adapted cultivars were space-planted (one-metre centers), and assessed for top growth, root and crown dry matter in fall of the establishment year. Fifty plants having the highest biomass and thirty-two having the lowest biomass were selected, brought into the greenhouse and paired crosses made within and between plants of the two groups. Single-cross progenies were evaluated in an RCB design with four replicates in the following year. Dry weights of top growth, root and crown were measured in the fall of that year. Progenies from parents with high biomass had higher biomass than those from parents with low biomass or having one parent in each category. The narrow-sense heritability estimated from mid-parent offspring regression was 0.23, from parent-offspring correlation was 0.22, and realized heritability was 0.15, indicating that progeny testing would be required for successful selection of populations with greater biomass production. Certain genotypes showed high general combining ability, indicating that these clones may be useful for synthetic cultivar development. Some crosses also showed high specific combining ability for this character. Plants selected for high biomass production tended to have higher shoot:root ratios and flower more profusely than the parental checks and the low biomass plants. This indicates that by selecting for high biomass, red clover will be selected for more annual growth habit at the same time.

RESUME

Les buts de cette recherche étaient d'évaluer la production à court terme (1 an) en biomasse de trèfle rouge et d'obtenir une estimation de l'héritabilité de ce caractère. Mille plants ont été sélectionnés parmis dix cultivars et ensuite repiqués en plantations espacées ayant des espacements d'un mètre (centre à centre). A l'automne de l'année d'établissement, ces plants furent evalués pour les paramètres suivant: matière sèche de l'herbage au-dessus du collet et matière sèche du collet et racines (ensemble). D'après ces évaluations, cinquante plants ayant les plus hautes biomasses et trente-deux ayant les plus basses furent déterrés et repiqués en serre. Des croisements couplés ont été faits à l'intérieur et entre chaque groupe de plants. Les progénitures des croisements simples furent évaluées l'année suivante à l'intérieur d'un dispositif expérimental de blocs aléatoires ayant quatre répétitions. La matière sèche de l'herbage au-dessus des collets et celle des collets et racines (ensemble) furent évaluées pour chaque plant à l'automne. Les progénitures issues des parents à haute biomasse avaient des biomasses plus élevées que celles issues des parents à faible biomasse; en outre, les progénitures issues des parents à haute biomasse étaient supérieures en biomasse aux progénitures ayant des parents de chaque groupe. L'héritabilité au sens étroit, estimée à partir d'une analyse de régression fut évaluée à 0.23; celle estimée à partir d'une corrélation entre parents et progénitures fut 0.22 et l'héritabilité réalisée fut 0.15. Ces valeurs nous indiquent qu'il faudrait évaluer les progénitures davantage. Il fut remarqué que certains génotypes possédaient une aptitude générale de combinaison; ceci indique que ces clones pourraient être utiles lors du développement d'un cultivar synthétique. En outre, certains croisements démontraient une aptitude spécifique de combinaison pour le caratère de haute biomasse. Il fut aussi noté que les progénitures de haute biomasse avaient des proportions de tiges:racines supérieures et fleurissaient plus abondamment que les parents témoins et les plants à faible biomasse. Ceci indique donc qu'une sélection de trèfle rouge pour le caractère de haute biomasse comporte aussi une sélection pour un type de croissance annuelle plus reproductif.

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1. INTRODUCTION

With the exception of water, nitrogen is probably the most limiting factor in crop production. Inexpensive nitrogen fertilizers associated with cultural practices and cultivars that maximize crop yields under high levels of nitrogen have substantially changed modern agriculture. However, the production of 1 kilogram of ammonia which is the basic raw material of nitrogen fertilizers, needs the use of 9,580 kilocalories of natural gas. The price of anhydrous ammonia has increased 312% during the period from 1970 to 1980 because of increasing cost and competition for natural gas as a source of energy. Increasing costs and environmental concerns over nitrogen fertilization have renewed interest in the use of forage legumes as an inexpensive source of nitrogen in crop production by plowing down as green manure. Legume plowdown is also useful for organic and sustainable agriculture (Auld *et al.*, 1982; Douglas, 1980; Hesterman *et al.*, 1986; USDA, 1980).

For centuries the value of legumes in crop rotation has been recognized. Nitrogen fixed by legumes in symbiosis with *Rhizobium* bacteria is contributed to succeeding non-fixing crops upon decomposition of legumes. In addition to this contribution of nitrogen, legume plowdown is also beneficial to cropping systems in the breaking of disease cycles, accumulation of soil organic matter, and improvement of soil structure. Green manure crops also help reduce soil erosion, improve moisture holding capacity, and increase the availability of other plant nutrients. Green manure crops will improve subsequent crop yields for up to three years after they have been plowed down. It was found that legume plowdown supported corn yields equivalent to those achieved with 90 to 125 kg ha^{-1} of fertilizer nitrogen (Baldock and Musgrave, 1980; Bruulsema and Christie, 1987).

Red clover, *Trifolium pratense* L., is recognized as one of the most important forage legumes in the world. Grown alone or with grasses, red clover is adapted to a wide range of soil types, pH levels, and environmental conditions. Through symbiotic N_2 fixation, red clover also provides nitrogen for growth of companion grasses and subsequent crops. Estimated amounts of nitrogen fixed range from 125 to 220 kg ha^{-1} $year^{-1}$. These qualities have made red clover useful for hay, silage, pasture, and soil improvement in many areas of the temperate region of the world (LaRue and Patterson, 1981; Rohweder *et al.*, 1977; Smith *et al.*, 1985).

With the increasing costs of nitrogen in grain production, especially corn, there has been interest in the use of legumes in crop rotations. This is illustrated by increases in the use of red clover in Ontario : in 1976, red clover seed sales were 528,000 kg, but in 1984 sales were up to 1,360,000 kg, an increase of almost three fold. Red clover has been the preferred legume for plowdown, because it is easy to establish, it can be grown on soils that are either too wet or too acid for alfalfa, and seed costs are low compared to other legumes. Results to date also indicate that the benefits from red clover plowdown are equal or better than those from other legumes species (Baldock and Musgrave, 1980; Bruulsema and Christie, 1987; Christie and Maitland, 1990; Groya and Shaeffer, 1985; LaRue and Patterson, 1981; Smith *et al.*, 1985).

The objectives of this study were to evaluate the inheritance of short-term (one year) biomass production of red clover, to evaluate the relationship between short-term biomass production and red clover growth type, and to select high biomass producing red clover genotypes for use in the development of synthetic cultivars with higher biomass production.

2. LITERATURE REVIEW

2.1 Economic importance of red clover

The overall economic importance of red clover to world agriculture is difficult to estimate. Red clover is consumed by animals in the forms of hay, silage, or pasture, and then it is consumed by human beings the forms of meat, fiber, and dairy products. Its introduction to central and northern Europe and the United States was said to have a profound impact on civilization and agriculture by steadily supplying high quality feed for livestock (Fergus and Hollowell, 1960; Taylor and Smith, 1979).

Red clover is usually grown in mixtures with grasses. It is normally used as a feed in beef cow-calf and stocker production; however, it is also used in many dairy, sheep, and horse production systems. In many areas, it is used as a plowdown green manure crop between cash crops and as a companion crop with cereal crops. In 1976, about 20 million hectares of red clover were grown worldwide and approximately 7 million hectares in North America were sown to red clover for hay, silage, pasture, and soil improvement. In the USA, the annual hay yield is about 4.0 t/ha. Based on the hay prices of $66 per ton in 1976, the value of red clover hay is about $1.5 billion and $5 billion in North America and the world, respectively, or $270 per hectare. The value of red clover seed production in the USA was between $23 million and $27 million. In Canada, the amount of red clover seed production in 1990 was 5.64

million kilograms which was about 50% higher than the five-year average (1985-1989) of 3.75 million kilograms. Ninety percent of these seeds were single-cut red clover (5.1 million kilograms) and the double-cut red clover made up the rest (0.54 million kilograms). Approximately 80% of single-cut red clover seed was produced in Alberta, the rest was from Saskatchewan, British Columbia, and Manitoba (Anonymous, 1991; Smith *et al.*, 1985; Taylor and Smith, 1979).

Red clover is grown in many areas in Eastern Canada where it is not suitable to grow alfalfa because of acid or wet soil problems. In Atlantic Canada, red clover is recommended; as a one or two year break crop on cash crop farms; as a plowdown or green manure crop; for long-term stands on peat bogs and poorly drained upland areas and; on the soils with pH lower than 6.0 (Atlantic advisory committees on cereal, protein, corn and forage crops, 1985).

2.2 Origin and distribution of red clover

Red clover is thought to have an origin in southeastern Europe and Asia Minor surrounding the Mediterranean and Red seas, where many *Trifolium* species and their relatives have been found. An early flowering type was known in Spain by 1500 and spread further north. Red clover was introduced to England from Germany about 1645 and was cultivated in northern Europe around 1650. It was probably carried to North America by the Dutch colonists in 1625, however, the first record of clover seed for sale appeared in 1729 (Fergus and Hollowell, 1960; Smith *et al.*, 1985; Taylor, 1985; Taylor and Smith, 1979).

Red clover will adapt best to the areas where summer is moderately cool or warm with best growth when the temperatures are between 21 to 24°C and moisture is adequate throughout the growing season (Duke, 1981; Kendall, 1958; Smith *et al.*, 1985; Taylor, 1985).

In Europe, red clover is an important forage legume in Scandinavia, England, Scotland, Wales, and Ireland and it is also cultivated in other temperate regions which include the USSR, Yugoslavia, Poland, Hungary, and most countries in western Europe. In North America, red clover is grown in the humid northeastern region which extends west into North and South Dakota, Nebraska, and Kansas, north into Ontario and Quebec, and south into Tennessee and North Carolina and it is also grown other southern states at high elevations. At lower elevations in the southeastern USA, it is used as a winter annual and in the Pacific Northwest, it is grown primary under irrigation as a seed crop. Red clover is also grown less extensively in other temperate parts of the world such as Japan, South Africa, Argentina, Columbia, Mexico, Chile, New Zealand, and Australia (Smith *et al.*, 1985; Taylor, 1985; Taylor and Smith, 1979).

2.3 Plant description of red clover

Red clover is a herbaceous plant formed from leafy stems growing from a crown. The plant consists of hollow stems and branches, leaves, and flower heads when it is fully grown. The germination of red clover seed is the same as a typical leguminous pattern which produces two cotyledonary leaves, followed by one true unifoliolate leaf. The rest of the leaves, usually a maximum of four or five leaves on a primary shoot, are palmately trifoliolate with a slender petiole and broadly triangular stipule. Stems and leaves of most American red clover strains are densely covered with short hairs while the European ecotypes are less hairy. The root system of red clover is a taproot system with many lateral roots. Tap roots will normally die and disintegrate in the second year and the plants will depend on secondary roots for their survival (Duke, 1981; Fergus and Hollowell, 1960; Montpetit and Coulman, 1991; Taylor, 1985).

The flower head of red clover with different colour consists of 50 to 275 sessile florets which have a similar shape to pea flowers but longer and more slender. The floret is normally a perfect flower with one ovary of two ovules. Only one ovule will usually develop into seed after fertilization and seed set per head is between 37 to 65 seeds. Seed is about 2-3 mm long with an oval shape, a notch on one side and different colour (Duke, 1981; Fergus and Hollowell, 1960; Starling *et al.*, 1950; Taylor, 1985).

2.4 Cultivation of red clover

2.4.1 Soil requirement and fertilization

Red clover can adapt to a wide range of soil types, however, it grows best on fertile and well-drained soils with high moisture holding capacity. This indicates that loams, silt loam, or moderately heavy textured soils are more suitable than sandy or gravelly soils. Red clover can grow satisfactorily on fairly acid soils (pH 5.7-6.5) with sufficient supply of potassium and phosphorus but it will give maximum yields on soils with an optimum pH of 6.6 to 7.6. If soils are too acid (pH below 5.5), red clover will perform poorly with inadequate nodulation for nitrogen fixation. In this case, lime should be applied to the soils in the year before sowing (Duke, 1981; Fergus and Hollowell, 1960; Smith *et al.*, 1985; Taylor, 1985).

2.4.2 Seeding

Red clover is normally undersown to small-grain crops such as oats, barley, or winter wheat, in the northern and central parts of the red clover production areas of North America. Red clover can tolerate lower light intensity than other legumes thus it is more successful than other forage legumes for underseeding and pasture renovation. (Fergus and Hollowell, 1960; Smith *et al.*, 1985).

Red clover is traditionally sown in early spring, or late winter in some areas. In the southern parts of the clover producing area, it can be sown in late summer if sufficient moisture is available. It is usually seeded from 15 October to 15 December depending on locations in the southern states of the USA where it is used as a winter annual (Smith *et al.*, 1985; Taylor, 1985).

2.4.3 Stand and harvest management

Red clover is a short-lived perennial that will give satisfactory yields for about three years. The highest herbage yields will be obtained in the second year. If red clover is sown with small-grain companion crops they should be removed early to reduce competition with the red clover. In the second year, the first crop should be harvested at prebloom or early-bloom stage to compromise between forage quality and yield. At this stage, red clover is leafy, high in protein content and digestible nutrients and has the highest protein yield per area (Smith *et al.*, 1985; Taylor, 1985).

2.5 Reproduction of red clover

2.5.1 Floral and seed development

Each floret of red clover is a complete leguminous flower, which consists of calyx, corolla, ten stamens, and a pistil. The calyx tube has five lobes or teeth. The corolla tube is formed from five united petals consisting of a standard petal, two wing petals, and two keel petals. Nine stamens and a stigma fuse together to form a column inside the corolla tube leaving the tenth stamen free. An anther consists of four microsporangia. The anther will dehisce and let the pollen fill the cavity between fused keel petals before the petals become fully grown (Duke, 1981; Hindmarch, 1963; Taylor and Smith, 1979).

Photoperiod has been found to stimulate flower formation in red clover. The critical photoperiod is 14 hours for most red clover cultivars. Longer daylength will normally induce red clover to flower earlier and more profusely, however, the effect depends upon the age of plants. It was also found that seeding dates had an effect on red clover flowering. Plants seeded in mid-May flowered profusely and the percentage declined with later seedings whereas plants seeded after mid-July did not flower (Gorman, 1955; Keller and Peterson, 1950; Ludwig *et al.*, 1953; Smith, 1957).

Red clover is a cross-pollinated species possessing a self-incompatibility system. Insects are required to pollinate red clover flowers. Generally, honeybees (*Apis mellifera* L.) will avoid red clover flowers if there are other sources of nectar since the flowers are too large for them. However, they may sometimes visit red clover flowers to collect pollen. Bumblebees (*Bombus spp.*) are more effective pollinators for red clover. Alkali bees (*Nomia melanderi* Ckll.) and leaf cutter bees (*Megachile rotundata* [F.]) can also be used to pollinate red clover (Fergus and Hollowell, 1960; Taylor, 1985; Taylor and Smith, 1979).

Half-open flowers of red clover appear to be the optimum stage for pollination. Stigma will be receptive for about 10 days which is the same period as the pollen viability. Viable pollen can be kept in liquid nitrogen up to 26 weeks. It takes about 18 to 50 hours between pollination and fertilization, depending upon the temperature, in diploid red clover and about 17 to 26 hours in tetraploid red clover. Seeds will reach physiological maturity within 14 days after pollination and can be harvested at about 21 days with sufficient dryness (Taylor and Smith, 1979; Zohary and Heller, 1984).

2.5.2 Self-incompatibility system in red clover

The self-incompatibility system in red clover was specified as being homomorphic, gametophytic one-locus (S) with many alleles. In this system, plants with the same S-allele will be self- and cross-sterile because the pollen tubes grow through the styles too slowly so that they will disintegrate before reaching the ovary. However, self-compatible, autogamous plants have been found. Two types of self-compatibility have been found, true-self-compatibility and pseudo-self-compatibility, both types are heritable. Pseudo-self-compatible plants has been defined as plants that had the same S-allele but pollen tubes can reach and fertilize the ovary and selfed seeds can be obtained. Normally, pseudo-self-compatibility can be affected by environmental, artificial and genetic causes. Pseudo-self-compatibility varies among clones and seasons. A higher percentage of self-compatible plants has been obtained at higher temperatures of 32-38°C (Brandon and Leffel, 1968; Crowe, 1964; Diachun and Henson, 1966; Fergus and Hollowell, 1960; Leffel, 1963; Leffel and Muntjan, 1970; Pandey, 1955; Thomas, 1955).

The self-incompatibility system in red clover is controlled by an extensive series of S-alleles. From the studies of I_0 and I_1 clones of red clover, both homozygous (S_1S_1 and S_2S_2) and heterozygous (S_1S_2) genotypes have been found as well as some plants that deviated from the normal system. These self-incompatibility genes have also been found to mutate both spontaneously and by X-irradiation. Different alleles in pollens and styles tended to mutate with different rates. Most mutation changed towards self-fertility. However, new S-alleles have been discovered among I_1-sib plants from I_0 clones. The self-incompatibility system in tetraploid red clover was found to be controlled by the same system as in diploid plants but weaker because of minor genes (de Nettancourt, 1969; Denward, 1963; Fergus and Hollowell, 1960; Leffel, 1963; Johnston *et al.*, 1968; Pandey, 1956a, 1956b).

2.5.3 Red clover seed production

In the humid northeastern region and the midwest region of the USA, seed is often harvested in the second crop after the first crop has been harvested for hay or silage. Seed production is the main purpose of red clover growers in the northwestern USA and the Prairie Provinces of Canada. Seed yields are higher in the northwest because of more favourable weather conditions. Normally, cloudy and rainy weather at flowering time will reduce the number of seed heads, affect ovary development, decrease bee activity for pollination and increase losses from harvesting (Fergus and Hollowell, 1960; Smith *et al.*, 1985; Taylor, 1985).

The problem of genetic shift is found when seed are produced outside the area of cultivar adaptation. It happens because all red clover cultivars are heterogenous populations consisting of heterozygous individuals. It was found that early clones tended to produce more seed heads and higher seed yields than the late clones. To avoid this problem, certification standards should be followed closely and seed should be produced in the northern latitudes (Bula *et al.*, 1969; Dovart and Waldman, 1969; Dovart *et al.*, 1968; Fergus and Hollowell, 1960; Taylor *et al.*, 1966; Taylor *et al.*, 1979).

2.6 Taxonomy of red clover

Red clover (*Trifolium pratense* L.) is in t. ᵓ genus *Trifolium* of section *Trifolium*, tribe *Trifolieae*, family Fabaceae. The tribe *Trifolieae* has eight sections with 237 species. There are 72 species in the section *Trifolium* which is divided into 17 subsections. Red clover is in subsection *Trifolium* which includes two annual species, *T. diffusum* Ehrh. and *T. pallidum* Waldst. & Kit. with the diploid chromosome number of 16, and two perennial species, *T. noricum* Wulf. and *T. mazanderanicum* Rech. fil., with diploid chromosome number of 16. There are six botanical varieties in *T. pratense* L., *pratense, americanum, sativum, maritimum, villosum, and rhodopeum*. Species that are more distantly related are *T. medium* L. (zigzag clover) with 2n = 64-80, *T. alpestre* L. (2n = 16), *T. rubens* L. (2n = 16), *T. heldreichianum* (2n = 16), *T. saroseinse* Hazsl. (2n = 48), *T. hirsutum* All., and *T. cheleri* L. (2n = 16) (Fergus and Hollowell, 1960; Taylor and Smith, 1979; Zohary and Heller, 1984).

Cultivated red clover may be divided into two groups; early flowering or medium or double-cut type, and late flowering or mammoth or single-cut type. The double-cut type is the main type grown in North America with the single-cut type is grown mainly in western Canada. Normally, the mammoth type is taller, more winterhardy, flowers 10-14 days later, and give higher first cut yield than the medium type. The mammoth type will produce no flowers in the seeding year but will have a rosette plant instead. The medium type can usually be harvested several times a year depending upon the length of growing season and moisture availability (Smith *et al.*, 1985; Taylor and Smith, 1979).

2.7 Red clover cultivars

2.7.1 Medium or double-cut red clover

Before 1950, most cultivars were naturally-adapted or farmer-selected ecotypes. After 1950, many cultivars with better persistence, disease resistance and winter-hardiness, and higher yields have been developed and released (table 1). Normally, red clover cultivars will not adapt to areas that are far from the areas of their development (Taylor, 1985).

2.7.2 Mammoth or single-cut red clover

Mammoth red clover is used less extensively in North America compared to the medium type. Altaswede cultivar was developed by the university of Alberta, Canada from Swedish red clover. It is highly adapted to conditions in Alberta and the western provinces of Canada, has very good winter-hardiness, and can survive up to 4-5 years. Alaskland which is winter-hardy and adapted to Alaska, was bred and released by the Alaska Agricultural Experiment Station from mass selection of three introduced USSR cultivars. Norlac is a cultivar that was developed and released in 1976 by Agriculture Canada, Research Station, Lacombe, Alberta. It is tolerant to northern anthracnose and powdery mildew and adapted to the northern regions particularly the western provinces of Canada (Fergus and Hollowell, 1960; Folkins *et al.*, 1976).

Table 1 Medium red clover cultivars which were released before 1990

cultivar	adaptation	reference
Arlington	north central & eastern U.S.	Smith *et al.*, 1973
Chesapeake	south central & southeastern U.S.	Hollowell, 1958
Flare	north central & east central U.S.	Moutray and Harding, 1985
Florex	north central & eastern U.S.	Smith *et al.*, 1985
Kenland	south central U.S.	Hollowell, 1951
Kenstar	south central U.S.	Taylor and Anderson, 1973
Lakeland	north central & northeastern U.S.	Hanson *et al.*, 1960
MorRed	north central & east central U.S.	Moutray *et al.*, 1985
Ottawa	eastern Canada	Fergus and Hollowell, 1960
Pennscott	northeastern U.S.	Hollowell, 1953
Prosper I	north central & northeastern U.S.	Taylor, 1985

cultivar	adaptation	reference
Redland	central & south central U.S.	Taylor, 1985
Redland II	north central & east central U.S.	Moutray and Mansfield, 1985
Reddy	north central & eastern U.S.	Stratton *et al.*, 1986
Redman	central & south central U.S.	Buker *et al.*, 1979
Tristan	north central & eastern U.S.	Smith *et al.*, 1985

2.7.3 Tetraploid red clover

Tetraploid red clover was first developed in Europe. It was used successfully in many countries. It is said to have higher persistence and disease resistance. However, it is not used much in North America probably because of difficulty in producing seed. Bytown red clover, released in 1979 by Agriculture Canada, was developed through doubling the chromosome number of the cultivar Ottawa. It is tolerant to powdery mildew but more persistent and have the same yield as Ottawa (Childers and Dickson, 1980; Taylor and Smith, 1979; Thomas, 1969).

2.8 Genetics of red clover

2.8.1 Morphological and physiological characters

Red clover is a diploid species with a chromosome number of 14. Tetraploid types with 28 chromosomes have been developed and mostly used in Europe. Red clover leaf growth is controlled by 13 genes while six genes determine flower development and only one gene governs stem growth (Fergus and Hollowell, 1960). Annual growth habit is characterized by one or more recessive genes (Taylor and Smith, 1979). Gene dw_1 controls dwarf characteristics and is recessive to normal growth (Smith, 1974). Two recessive genes, *f* and *n*, condition a polyphyllic character, having leaves with five leaflets (Simon, 1962). Leaf marking, which is monofactorial inheritance with developmental and suppressed gene action, is dominant over nonmarking (Fergus and Hollowell, 1960; Taylor and Smith, 1979). The length of petioles is determined by two pairs of complementary genes and one or more modifiers (Hanson and Hanson, 1957). The colour of the red clover leaf is governed by two groups of genes, green and red colour groups, whereas several recessive genes control chlorophyll-deficiency which is transmitted on a simple Mandelian principle (Fergus and Hollowell, 1960). Flower colour is conditioned by about 25 genes with epistatic effect and is linked to seed characteristics. Petal colour intensity was found to be controlled by about 25 genes with complex inheritance (Cornelius and Taylor, 1981). Long corolla tube is dominant or partially dominant over the short one and linked with general vigor (Starling *et al.*, 1950). Nodulation by *Trifolium trifolii* and nitrogen fixation are determined by two recessive groups of genes (Nutman, 1954, 1957, 1968). Internal breakdown of crown is controlled by few genes (Graham and Newton, 1971). Split- leaflet, round pollen and purple-red flower colour which may be useful for genetic markers were each found to be controlled by single genes, designated *sl, rp* and *p*, respectively (Parrot and Smith, 1986b). A gene designated *sy* which affects meiosis and gives univalents at diakinesis and the subsequent formation of 2n eggs, is also a single recessive gene.

Genetic male-sterility of red clover is determined by two recessive genes, ms_1 and ms_2, with an epistatic effect between the two loci (Smith, 1971; Taylor *et al.*, 1978). Frequency of *2n* pollen formation with the red clover plants was found to be controlled by 2-6 genes (Parrot and Smith, 1986a). By using 4x-2x crosses Taylor and Wiseman (1987) found that it was possible to broaden the genetic base of tetraploid red clover. Derived triploids between 3x-2x and 2x-3x plants and 2n gametes also gave viable seeds which made development of trisomic plants for gene mapping possible (Taylor and Chen, 1988). 4x-2x crosses were found to be a more efficient method of producing triploid and tetraploid red clover than colchicine and nitrous oxide induced methods (Taylor and Berger, 1989). Bilaterally derived tetraploid red clover plants were studied by Tofte and Smith (1989). They found that the aneuploid frequency was 23%, and hypoploid frequency was higher than that of hyperploids.

2.8.2 Pest resistance

Normally, only one or few genes will determine disease resistance in red clover. Resistance to southern anthracnose caused by *Colletotrichum trifolii* B&E is controlled by one recessive gene (Athow and Davis, 1958). However, resistance to northern anthracnose [*Kabatiella caulivora* (Kirch.)] is conditioned by few dominant genes (Smith and Maxwell, 1973). Few different genes seem to control different races of powdery mildew caused by *Erysiphe polygoni* DC in red clover (Stavely and Hanson, 1967). Resistance to rust (*Uromyces trifolii* var. *fallens* Arth. is governed by a single gene that is linked with seedling lethality, thus it cannot be used for cultivar improvement (Engelke *et al.*, 1977). Different types of resistance to bean yellow mosaic virus are conditioned by different genes (Diachun and Henson, 1974; Taylor *et al.*, 1986). A single dominant gene determines resistance to vein mosaic virus in red clover (Khan *et al.*, 1978). Tolerance to white clover mosaic virus was found to be determined by polygenes (Martin *et al.*, 1990). Resistance to stem nematode (*Ditylenchus dipsaci*) is controlled by two dominant genes, one of them is closely linked to S-locus (Nordenskiold, 1971).

2.9 Breeding of red clover

Various breeding methods for red clover before 1980 have been reviewed comprehensively by Fergus and Hollowell (1960) and Taylor and Smith (1979). Most red clover cultivars in use before 1950 were resulted from natural and farmer selection. After that time, many cultivars have been developed by using some forms of controlled mass selection. Yield, pest resistance, regrowth potential, persistence and winter-hardiness for northern atmosphere are the characters that are emphasized in most recent red clover breeding programs (Smith *et al.*, 1985). Very few programs have paid attention to improve red clover quality since it is one of the best quality forage legumes.

2.9.1 Mass selection

Mass selection is an effective breeding method for improving characters that are easily selected and simply inherited. Repetition of mass selection for several cycles is called phenotypic recurrent selection (Fergus and Hollowell, 1960; Smith *et al.*, 1985; Taylor and Smith, 1979).

Mass selection has been used effectively to improve red clover resistant to southern anthracnose, northern anthracnose, crown rot, stem nematode, and winter-killing (Fergus and Hollowell, 1960). Height of nectar in red clover florets, dry matter yield and protein content were also increased by using mass selection (Hawkins, 1971; Taylor and Smith, 1979). Norlac and Kenstar cultivars were developed through mass selection followed by progeny testing (Folkins *et al.*, 1976; Taylor and Anderson, 1973).

Phenotypic recurrent selection has been found to be effective in improving resistance to northern anthracnose, powdery mildew, crown rot, yellow clover aphid, pea aphid, and reducing the content of formononetin (Gorz *et al.*, 1976; Maxwell and Smith, 1971; Smith and Maxwell, 1973; Smith *et al.*, 1973; Taylor and Smith, 1979). Cultivar 'Arlington' was developed from three cycles of phenotypic recurrent selection on six heterogeneous populations (Smith *et al.*, 1973). By using six cycles of phenotypic recurrent selection, Taylor *et al.* (1990) successfully increased the resistance to northern anthracnose of ten red clover populations. They found that the mean disease severity index reduced by 36% over six generations and realized heritability averaged 20% per cycle. Martin *et al.* (1990) also suggested the use of recurrent selection with progeny testing for improving tolerance to white clover mosaic virus in red clover. Petal colour intensity of red clover flowers was changed from 3.1 to 8.2 on a scale of two to nine (light pink to purple) by imposing seven cycles of phenotypic recurrent selection (Cornelius and Taylor, 1981). This method also used to increase the number of head parts in red clover with multi-parted flower heads from 1 to 7.4 in six cycles with a regression coefficient of 0.92 head parts per generation (Taylor *et al.*, 1985). However, number of seeds per plant, persistence and vigor decreased probably from inbreeding depression. Bowley *et al.* (1984a, 1984b) used this breeding method to increase stem length of red clover plants 3.7 and 2.9 cm cycle^{-1} at first and second harvest. This increase resulted from the increase of cell number per internode and was associated with decreases in stem number per plant and persistence. Parrot and Smith (1986a) also obtained 47.38% of average *2n* pollen production per plant after three cycles of selection from the original population with 0.04% production.

Maternal line selection and phenotypic selection were found to effectively improve persistence in red clover (Moktarzadeh *et al.*, 1967). However, this was obtained with the reduction in forage yield and flowering in the first year stand but improved yield and later flowering in the second year. Higher resistance to stem nematode in red clover was obtained when maternal line selection was employed (Dijkstra, 1956). This method was also employed to achieve a higher percentage of two-seeded pods in diploid red clover but it failed to do so in tetraploids (Dijkstra, 1969). Four cycles of half-sib family selection have been employed effectively to select red clover tolerant to 2,4-D herbicide (Taylor *et al.*, 1989). The level of tolerance increased 0.30 units per cycle to about 35%.

2.9.2 Progeny testing in red clover

The aim of a progeny test is to distinguish superior parents. The progeny test should be made when the heritability of a character being improved is low (Breese and Hayward, 1972). Using a progeny test will give a better annual result than individual phenotypic selection alone when a heritability estimate is as low as 10% (Morley and Heinrich, 1960).

Knowledge of combining ability of clones or lines is necessary when these clones or lines will be used for synthetic or hybrid cultivar development. Progeny testing in red clover has not been done much since it is difficult to maintain the parental plants or clones (Taylor *et al*, 1962; Taylor and Smith, 1979). Selfed progeny test is not employed widely in red clover because of the self-incompatibility problem (Taylor and Smith, 1979).

2.9.2.1 Topcrossed and open-pollinated progeny test

Topcross and open-pollinated progeny tests are not used extensively in red clover breeding because less information is obtained compared to polycross or diallel cross progeny tests. Dijkstra (1970) employed topcrossed progeny test to evaluate the possibility of using reciprocal recurrent selection in red clover. He found that the progeny performance did not support the use of this method. No significant epistatic effect was found among 6 I_1 clones of bulk introduced red clover by using topcrossed progeny test (Cornelius and Taylor, 1980).

2.9.2.2 Polycross progeny test

The same kind of information as obtained from topcross and open-pollinated progeny tests can be acquired by polycross progeny test but the male parents are more restricted and more seeds for testing can be obtained (Allard, 1960; Rumbaugh *et al.*, 1988). The polycross progeny test was used to develop cultivar 'Kenstar' from 20 red clover clones (Taylor and Anderson, 1973; Taylor *et al.*, 1968). They also found significant correlation for persistence, yield, seed yield, and powdery mildew resistance between the progenies and their parents. Competitive effects in red clover as related to the polycross progeny test were studied by Taylor and Kendall (1965). They suggested that the polycrosss progenies should be selected by the separate-progeny test first, then a mixed-progeny test should be used to assess the competitive ability of the selected clones. Torrie *et al.* (1952) used this method to examine sib lines that were used to maintain parental genotypes.

By using the polycross progeny test, seed yields of clones and their progenies were found not correlated, however early clones were correlated with high seed yields (Taylor *et al.*, 1966). A narrow-sense heritability estimate of 0.56 for growth type in red clover was found by using the polycross progeny test (Coulman and Oakes, 1987).

2.9.2.3 Diallel-cross progeny test

The diallel cross is a mating design in which individuals of a group are crossed with each other in all possible combinations. The diallel cross provides more information but it needs more work to obtain seeds. Crossing must be done by hand or by some form of controlled pollination, thus the number of parents have to be limited to conform with the available resources (Rumbaugh *et al.*, 1988). The advantage of the diallel cross over the polycross progeny test is that it can measure specific combining ability of the clones being tested (Busbice *et al.*, 1972).

The diallel cross progeny test has been used extensively in red clover breeding programs. By using diallel crosses of seven noninbred parents, Anderson (1960) found significant general combining ability and specific combining ability for yield, growth habit, persistence, and flowering. He also obtained relatively high heritability estimates for these characters. Anderson *et al.* (1974b) also used this method for ten noninbred clones and found that general combining ability was the most important source of genetic variation. They also found the heritabilities of 17% to 42% for persistence, yield, vigor and date of the first bloom. Additive genetic variance was found to be a significant part of genetic variance among progenies from wild and cultivated red clover plants (Taylor and Smith, 1979). Combining ability of six I_1sib_1 red clover lines was examined by diallel cross progeny test (Smith and Puskulcu, 1976). Significant general and specific combining ability for maturity, yield, and regrowth potential were found. Cornelius *et al.* (1977) found that nonadditive genetic variance was an important portion for ten I_1 red clover clones. High heritability estimates for crude protein content of red clover progenies from high and low protein parents were obtained (Taylor and Smith, 1979). By evaluating the diallel cross progenies of red clover, Pederson *et al.* (1981) found significant general combining ability for length of rotted roots after inoculating with *Fusarium roseum* (Lk.). The low heritability estimate of 0.075 for root rot severity was obtained, which indicated that evaluation of progenies would be necessary for selection.

2.9.3 Inbreeding and heterosis in red clover

Red clover shows 50-60% loss in vigor from inbreeding depression like many other cross-pollinated crops (Cornelius and Taylor, 1981; Fergus and Hollowell, 1960; Taylor *et al.*, 1985) However, clones in some inbred families have been found to equal or even exceed their non-inbred parents in vigor (Taylor *et al.*, 1970). Inbred lines are normally difficult to maintain since they are weak and susceptible to virus diseases (Taylor and Smith, 1979). The reason for inbreeding red clover is to obtain inbred line, which will result in maximum heterosis after crossing, and to get homozygous S-alleles clones which are essential for control of pollination in hybrid seed production (Taylor and Smith, 1979).

Maximum heterosis in red clover can be obtained when selected inbred lines or clones are combined into hybrid cultivars. However, difficulties associated with inbred line production and maintenance, and control of pollination in seed production fields have prevented the use of hybrid cultivars in a commercial scale (Smith *et al.*, 1985; Taylor, 1982; Taylor, 1985). Hybrid cultivars in red clover have long been proposed (Leffel, 1963; Leffel and Muntjan, 1970) and evaluated in the field on an experimental basis (Anderson *et al.*, 1972).

2.9.4 Polyploidy induction

Chromosome doubling in diploid red clover has produced promising tetraploids since the species has low chromosome number with an allogamous character and it is grown for forage thus seed production is not of prime importance (Fergus and Hollowell, 1960). Generally, tetraploid red clover has more disease resistance, more persistence, more cold tolerance, better root system, and higher yield, regrowth potential, and protein content, than the diploids (Anderson, 1973; Fergus and Hollowell, 1960; Thomas,1969).

However, they are less leafy and drought tolerant, have coarser stems and lower seed yield. The most important problem that make tetraploid red clover less useful is the difficulty to produce enough seeds to meet demand (Ellerstrom and Sjodin, 1974; Fergus and Hollowell, 1960).

Methods that have been used to induce tetraploidy in red clover are: colchicine treatment of the seedling shoot (Fergus and Hollowell, 1960); seed treatment with o.oo8% of ethyleneimine (Taylor and Smith, 1979); treatment of fertilized flowers with nitrous oxide (Taylor *et al.*, 1976); and through pollinations involving unreduced gametes (Parrot *et al.*, 1985).

2.9.5 Interspecific hybridization

Interspecific hybridization of red clover with other species in the genus *Trifolium* has been tried to study the relationship of red clover with those species and to broaden the genetic base for some characteristics such as persistence and resistance to some diseases (Taylor, 1985). Successful hybridization with two annual species, *T. pallidum* L. and *T. diffusum* Ehrh. has been achieved (Armstrong and Cleveland, 1970; Taylor *et al.*, 1963). The hybrid between *T. pratense* L. and *T. diffusum* Ehrh. was fertile, however, it did not show any promising characters that could be transferred to red clover. Crosses with the perennial species *T. saroseinse* Hazsl. were also made and the interspecific hybrid was obtained by using *in vitro* embryo rescue, but it was sterile (Phillips *et al.*, 1982). However, this hybrid has shown some desirable characters that would be useful for improving cultivated red clover.

Recently, two interspecific hybrid germplasms have been released (Taylor and Collins, 1989). They were the hybrids between *T. pratense* L. X *T. diffusum* Ehrh. and between *T. saroseinse* Hazsl. X *T. pratense* L. Both had the intermediate characteristics of the parents. The first hybrid was fertile and annual with the chromosome number of 30. Although this hybrid did not have any desirable characters it could be used as a bridge for further interspecific hybridization. The second hybrid was sterile and strongly perennial with a chromosome number of 31. It should be useful for transferring the perennial character to red clover if the fertility can be restored.

2.10 Estimation of inheritance

2.10.1 Combining ability

Combining ability is the ability of an inbred or clone to transmit desirable performance to the hybrid progeny. The general combining ability of a particular inbred or clone is determined by its average performance in a series of hybrid combinations. General combining ability is said to measure mainly additive genetic effects (Poehlman, 1987).

Specific combining ability refers to the performance of the particular inbreds or clones in a specific cross. The particular combination of inbreds or clones giving the best single-cross yield performance would be designated as having the best specific combining ability. Specific combining ability measures non-additive types of gene interactions. Combining ability tests are used to identify desirable combinations of inbred lines or clones in the breeding of hybrids or synthetic varieties (Poehlman, 1987).

A method of estimating general combining abilities that is convenient for use with perennial forage plants is known as the polycross method. A number of plants from all the lines or clones to be tested are grown together and allowed to pollinate naturally, self-pollination being prevented or immunized by natural mechanisms favoring cross-pollinated. Replication and polycross plot design favor random interpollination. The seed from the plants of one line or clone are therefore a mixture of random crosses with other lines or clones, and their progeny performance tests the general combining ability of that line or clone. The general combining abilities measured are those of lines or clones used as female parents (Falconer, 1989).

2.10.2 Heritability

Heritability is the degree to which the variability of a quantitative character is transmitted to the progeny. It may be defined as the proportion of the total variation in a progeny that has a genetic basis (Poehlman, 1987).

2.10.2.1 Narrow-sense heritability

Most of the quantitative characters are due to additive genetic effects. It is more useful to calculate the narrow-sense heritability which is the ratio of additive genetic variance to phenotypic variance. Various methods have been developed for estimating heritability based on the total observable variation of a quantitative character and its partitioning into genetically and environmentally controlled components. The common procedures are to use parent-offspring regression and parent-offspring correlation which will estimate the narrow-sense heritability.

2.10.2.1.1 Parent-offspring regression

The linear regression of the performance of offspring on that of the parents was proposed as a method of estimating heritability. The linear regression model is

$$Y_i = a + bX_i + e_i$$

where Y_i = performance of offspring of the *i*th parent

a = mean performance of all parents evaluated

b = linear regression coefficient

X_i = performance of *i*th parent

e_i = experimental error associated with measurement of X_i

In plant species "parent" refers to a random plant or a line from a population, and "offspring" are half-sib or selfed progeny. A midparent-offspring regression also can be used, which is the relationship between the average performance of two parents and their full-sib offspring (Fehr, 1990).

The relationship of the regression coefficient to heritability depends on the type of offspring that is evaluated. The type of offspring also determines if a narrow- or broad-sense heritability is obtained. When the type of offspring is the full-sib progeny obtained by mating two random plants, the two plants of the mating are measured and their average or mid-parent value is determined. The performance of the full-sib progeny is regressed on the mid-parent value. The alleles in the full-sib offspring are obtained from the two parents of a mating, the regression coefficient is equal to the heritability, $b = h^2$ (Fehr, 1990).

Use of parent-offspring regression is based on several assumptions: (a) The character of interest has diploid Mendelian inheritance, (b) the population is random-mated, (c) the population is in linkage equilibrium or there is no linkage among loci controlling a character, (d) parents are noninbred, and (e) there is no environment correlation between the performance of parents and offspring (Vogel *et al.*, 1980). Failure to meet the assumptions can bias the heritability estimates obtained.

2.10.2.1.2 Parent-offspring correlation

Correlation of the performance of a parent with that of its offspring was proposed by Frey and Horner (1957) as an alternative to the parent-offspring regression method for computing heritability. When parents are measured in one season and their offspring in another, environmental differences between the two seasons can cause the range in phenotypes among the parents to be greater or less than that for the offspring. As a result, heritability percentages obtained by parent-offspring regression could have maximum values greater than 100 percent. To eliminate this effect of environment, the use of standard unit heritabilities obtained by calculating parent-offspring regressions on data coded in terms of standard deviation units was suggested. Such a procedure leads to results equivalent to the coefficient obtained from a simple parent-offspring correlation (Fehr, 1990).

2.10.2.2 Realized heritability

Realized heritability is the heritability of a character estimated by the amount of genetic improvement that is realized by selection within a population (Falconer, 1989). It can be shown as

$$h^2 = \frac{X_{SR} - X_{UR}}{X_{SP} - S_{UP}}$$

where h^2 is realized heritability,

X_{SR} is the mean of selected progenies,

X_{UR} is the mean of unselected progenies,

X_{SP} is the mean of selected parents, and

X_{UP} is the mean of unselected parents.

Realized heritability is estimated in the absence of a reference population and it is useful in the selection experiments. It is a descriptive measure of a particular selection situation that has limited usefulness for extrapolation to other populations.

2.11 Growth types of red clover

In the seeding year, red clover plants can be divided into five growth types on the basis of flowering and rosette development (Bird, 1948). Among the five growth types, three are flowering and two are non-flowering types. Plants that do not flower in the year of establishment were shown to be more persistent and less winter-killed (Bird, 1948; Smith, 1957, 1963; Therrien and Smith, 1960). Dates of seeding was found to influence the number of plants that flowered in the first year (Smith, 1957). Only plants that were well established could respond to long days of the summer and flowered. Therrien and Smith (1960) also found that plants, prevented from flowering in the seeding year by removing flower buds, flowering stems, or elongating tillers, could survived winter better and had higher vegetative growth in the following year. Plants which were allowed to flower naturally had fewer non-flowering tillers, smaller crowns, lower crown and root dry weight, lower total available carbohydrate in the roots, fewer root branches, and more winter-killing. Highly significant correlation between winter survival and growth type was also found (Smith, 1963). It was also suggested that flowering in the seeding year could be used reliably to select winter-hardy plants from red clover populations. A negative relationship between earliness of flowering and persistence has also been found (Mokhtarzadeh *et al.*, 1967; Taylor *et al.*, 1966, 1968). Taylor *et al.* (1968) also found that plants which bloomed in the first year died during the following winter. Progenies which produced the most ground cover in late summer or fall of the first year were found to have least persistence. Normally, plants that produce rosettes in short days of fall months should also be latest to bloom in the spring and thus most persistent.

Choo (1984) also found that in the fall of the third year of red clover stand, 14% of the rosette-forming, non-flowering (type 1) plants were alive, 8-11% of the intermediate types survived, and only 2% of the nonrosette-forming, profusely flowering (type 5) plants remained growing. The non-flowering classes (type 1 and 2) persisted better than the flowering classes (type 3, 4 and 5). The two flowering classes, in the production years, had the same proportion of the most vigorous plants. He concluded that the flowering response can be used as a selection criterion for persistent plants, and plants not flowering in the seedling year can attain a forage and seed yield as high as flowering plants in the production years. Narrow-sense heritability estimates for growth type in red clover ranging from 0.56 to 0.62 were found by using polycross and single-cross progeny tests (Coulman and Oakes, 1987). They also obtained high yielding synthetics from clones chosen on the basis of growth type. However, they found that a high proportion of non-flowering plants in synthetics did not always produce highly persistent red clover lines.

2.12 Effect of red clover plowdown

Red clover has been used to conserve or improve soils as a green-manure and rotational crop for centuries (Fergus and Hollowell, 1960). It has been found that the beneficial effects of red clover to following crops was not from nitrogen alone but also from some other unwellknown effects. Total dry matter yields of red clover in years with moist and cool spring were higher than that of alfalfa, but in years with severe mid-summer and early fall drought, red clover yielded less than alfalfa (Fribourg and Johnson, 1954). They also found that red clover top:root ratios generally were higher than those of alfalfa, and ranged between less than 2 to almost 4. The nitrogen percentages of tops including crowns, varied with the season, species, and location. The higher nitrogen content was associated with higher total dry matter yields. Alfalfa and red clover had an average nitrogen content about 3%. Root nitrogen contents were lower than those of tops, leaving about 2.6% for both red clover and alfalfa. Red clover yielded between 40 and 90 kg (with the average of 72 kg) of nitrogen per hectare in tops and roots, when good stands were obtained. A nitrogen yield of 60 kg ha^{-1} from red clover was less than that from alfalfa and sweetclover (Stickler and Johnson, 1959a).

Nitrogen availability to the following crops from red clover residuals during the second year after plowdown was about 3.5% of total nitrogen (Fribourg and Bartholomew, 1956). Smith (1956) compared two cutting practices with different legumes. He found that without cutting in the seeding year red clover produced less dry matter and nitrogen per acre than alfalfa but higher than alsike clover. All legumes being tested produced less dry matter and nitrogen yields when they were cut in September and October. However, red clover was found to give the highest nitrogen yield of 110 kg ha^{-1} compared to alfalfa, sweetclover and ladino clover when it was cut twice (Stickler and Johnson, 1959b). Stickler *et al.* (1959) found from two-year data that Kenland red clover added 92 kg of nitrogen per hectare to the soil and the grain yield of the following corn equalled to that obtained from the addition of 25 kg of inorganic nitrogen per hectare. If red clover was plowed down as green manure in late June or early July of the second year, it would provide 50 to 75 kg N ha^{-1} which was slightly lower than that of alfalfa and sweetclover to the soil (Bowren *et al.*, 1969). However, if it was removed for hay or silage and only the regrowth with crowns and roots were plowed down, the average nitrogen returned to the soil would be only 15 kg N ha^{-1}. Yield of a barley crop following red clover was the same as those following alfalfa, birdsfoot trefoil, alsike clover, sweetclover and grown on fallow when nitrogen fertilizer was applied (Hoyt and Leitch, 1983). Soil moisture in spring and soil moisture used by barley were also about the same following these legumes and fallow. Without nitrogen fertilizer, the yield of a barley crop following red clover was higher than that grown on fallow.

By using the ^{15}N isotope dilution method over a four-year stand, Heichel *et al.* (1985) found that red clover showed significant variation of N_2 fixation within a year and among years of the stand. Red clover fixed annual quantities of nitrogen ranging from about 60 to 120 kg N ha^{-1} compared to birdsfoot trefoil with the average of 92 kg N ha^{-1}. Throughout their experiments, red clover distributed 10% or less of its dry matter to root plus crown portions which usually had a nitrogen concentration only 0.5 to 0.6 that of tops. Therefore, the greatest return of fixed nitrogen to a subsequent crop would result from adaptation of management practices that achieve a plowdown of a regrowth of tops rather than only roots plus crowns soon after hay harvest.

Groya and Shaeffer (1985) also found that managements with multiple harvests resulted in greater nitrogen yield for red clover in the seeding year than with no harvest. Within the multiple harvest managements, nitrogen yields of red clover, alfalfa, and sweetclover were not significantly different. Managements in which herbage was accumulated by cutting and not removing and or not cutting resulted in greater nitrogen in the seeding year for plowdown than managements in which one or more harvests were removed. With the management of harvesting in the seeding year, red clover gave higher nitrogen yield (359 kg N ha^{-1}) than alfalfa and sweetclover. Most nitrogen yield was in the herbage (73%) which had nitrogen concentration of 35 g kg^{-1}. Sudangrass dry matter yields following alfalfa, sweetclover, and red clover were equal when harvested stubbles and fall regrowth were plowed down as green manure.

Bruulsema and Christie (1987) also found that corn grain yield following plowdown of red clover was 7% greater than that following alfalfa. However, whole-plant N-concentration of corn was 7% higher following alfalfa. The lower decomposability of red clover due to a higher lignin concentration and a lower C:N ratio may account for the lower nitrogen concentration observed in succeeding corn. Significant variation in plowdown nitrogen yield among cultivars within red clover was reported, but in the succeeding year there were no corresponding differences in corn yield or nitrogen concentration. Scott *et al.* (1987) used red clover as an intercrop and cover crop for corn grown on sloping land and harvested for silage. They found that red clover alone and combined with annual ryegrass (*Lolium multiforum* Lam.) were the most effective crop to give ground cover and dry matter production compared to the other 11 to 13 species tested. Badaruddin and Meyer (1990) also found that red clover herbage yield was not different significantly from those of alfalfa, sweetclover, and hairy vetch, however, its yield was highest when it was cut twice and there was adequate moisture. Wheat grain yields following alfalfa, sweetclover, hairy vetch, and red clover were generally equal, although there were some differences between soil NO_3-N following these legumes, indicating that the change in soil NO_3-N did not correlate with the grain yields of the subsequent wheat.

3. MATERIALS AND METHODS

3.1 Field and greenhouse experiments

A space-planted nursery (1 m centers) of 800 red clover plants chosen from ten adapted cultivars (80 plants of Arlington, Atlas, Florex, Kuhn, Lakeland, Marino, Marathon, Persist, Prosper, and Tristan) was established in 16 replicates at Lods Research Center, Macdonald Campus of McGill University on 27 June, 1989. The field which had the Chicot fine sandy loam soil was fertilized with 25 kg N, 100 kg P and 100 kg K ha^{-1} before planting. Herbicide Eptam (S-Ethyl dipropylthiocarbamate) was also incorporated into the soil before planting. These plants were scored for their growth types in the fall of that year on a scale of 0 to 4 as described by Bird (1948) and Choo (1984) as follows: type 0, plants that produced only a rosette of leaves and unelongated tillers; type 1, plants that produced mostly a leafy rosette but had one or two elongated non-flowering tillers or stems; type 2, plants that produced a prominent rosette but with several flowering stems; type 3, plants that produced a weak rosette with many flowering stems generally upright; type 4, plants that produced virtually no rosette with many upright flowering stems. In September, top growth dry weight of the 711 individual plants that survived was obtained by cutting with a sickle, drying in a forced air oven at 70°C for 48 hours and then weighing. Roots (plus crowns) were sampled about three weeks after cutting in October to determine root and crown fresh weight by using a non-destructive technique suggested by Young and Werner (1984). Sampled roots (including crowns) were dug out and washed. Root plus crown fresh weight was determined using a top-loading electronic balance. The plant root system then was submerged in a water tank set on the balance. The weight taken in this manner was considered an estimate of root fresh weight. Crown fresh weight was obtained by subtracting root fresh weight from total root plus crown fresh weight. Some of the sampled plants were dried to obtain an estimate of dry matter percentage that was used to calculate root and crown dry weights. Crowns of the 50 highest and 32 lowest total biomass producing plants were brought to the greenhouse and potted up in 15-cm pots for crossing in the winter.

Several plants in the two groups died or did not flower, leaving 46 high and 23 low group plants available for crossing. In the winter of 1989, 564 crosses within and between the plants of the high and low biomass groups were made by using a toothpick technique as described by Taylor (1980). There were 394 crosses within the high group (S^{+}), 142 crosses within the low group (S^{-}), and 18 crosses between these two groups (SI). Seeds from each cross were harvested separately. In May 1990, these seeds and seeds of parental check cultivars were scarified by using sand paper, germinated on wet paper and then transferred to cells in a flat. Each flat was 28x55 cm in size and contained 54 growing cells. Seedlings were grown in the greenhouse for about six weeks then they were transplanted to the field at Lods Research Center on 21 June, 1990. The soil in this field was Ste-Rosalie clay. The fertilizer rate and herbicide used were the same as 1989. A randomized complete block design with 4 replicates was used. In each replicate, three plants from each cross were planted. Plants were spaced three plants per meter. The field was hand-weeded twice. Growth types of these plants were scored in September on the same scale as for the parents. Top growths from individual plots were cut and weighed for fresh weight. Samples from this top growth were taken and dried in the oven at 70°C for 48 hours to calculate the above ground dry matter yield. The middle plant of each plot was dug out after cutting and dried in the oven to get root (plus crown) dry weight per plant for each plot (cross). The 72 highest biomass producing progenies and the 36 progenies with the heaviest root (plus crown) were selected and one plant from selected plots that were left in the field was dug out and transplanted to the greenhouse for further use in the breeding program.

3.2 Calculation and estimation of inheritance

A SAS GLM procedure was used for all statistical analysis (SAS Institute Inc., 1985). All values were calculated on a per plant basis. Single-cross means of each clone were calculated from all crosses that had that clone as one parent.

The reciprocal difference was tested by partitioning variance from the analysis of variance among crosses which had both directions.

General combining ability of each clone was calculated from the deviation of its single-cross progeny mean from the overall progeny mean as suggested by Falconer (1989). A narrow-sense heritability estimate from parent-offspring regression was obtained by using the regression model from the SAS GLM procedure. In this case, mid-parent values were used, thus the heritability estimate was a regression coefficient as suggested by Fehr (1990). The correlation coefficient between parents and offsprings which was a narrow-sense heritability estimate was also obtained from the SAS GLM procedure.

A realized heritability estimate was calculated as suggested by Falconer (1989).

$$h^2 = \frac{X_{SR} - X_{UR}}{X_{SP} - X_{UP}}$$

where h^2 is a realized heritability estimate

X_{SR} is the mean of the progeny population from plants selected for high biomass

X_{UR} is the mean of the parental population tested with the progenies

X_{SP} is the mean of plants selected for high biomass production from the parental population

X_{UP} is the mean of the parental population

4. RESULTS AND DISCUSSION

4.1 Parental population

4.1.1 Biomass production

Significant difference ($p<0.05$) in one-year top growth dry weight between cultivars in the parental population was found (table 2). In this table, only top growth dry weight is presented because root dry weight was not available for all plants. From 711 surviving plants in the population, all cultivars, except Persist, were approximately equally represented. The cultivar Prosper gave the highest top growth dry weight which was about three times that of the lowest cultivar Kuhn. There were no plants from the cultivars Kuhn and Persist among plants that were selected for high biomass production. Approximately three quarters of the selected plants come from the three cultivars Prosper, Lakeland and Atlas. These cultivars produced the highest mean top growth per plant.

Significant variation ($p<0.05$) among plants of the entire parental population was found for top growth dry weight (table 3). Data for the 50 and 32 plants selected for high and low biomass production are shown in appendix I. Top growth dry weight of the high biomass producing parents was about 7 times that of the low group, and was 218% that of the overall population.

Considerable variation of root (plus crown) dry weight was also found (appendix I). Root (plus crown) dry weight of high biomass producing plants ranged from 101 to 305 g $plant^{-1}$. This indicates that selection for this character should be possible and may be useful.

Table 2 Top growth dry weight of each cultivar in the parental population and selected plants for high biomass production

cultivar	parental population		selected plants	
	number	dry weight	number	dry weight
		g $plant^{-1}$		g $plant^{-1}$
Arlington	77	106	4	196
Atlas	77	123	9	262
Florex	72	108	4	234
Kuhn	75	49	0	-
Lakeland	76	135	11	222
Marathon	75	116	5	236
Marino	68	69	1	219
Persist	36	77	0	-
Prosper	76	140	14	231
Tristan	79	109	2	204
$LSD_{0.05}$		21		48

Table 3 Top growth dry weight of the parental population, and populations selected for high and low biomass production

	population	high	low
number	711	50	32
range (g $plant^{-1}$)	5-383	169-383	5-92
mean (g $plant^{-1}$)	106 b	232 a	33 c
s.d.	58.24	51.98	24.45

Means within a row with the same letter were not significantly different at the 5% level

s.d. = standard deviation

4.1.2 Growth type

Most selected plants in the high biomass producing group had growth types 2 and 3 (appendix I), only one plant had growth type 1, and this plant had died before flowering, and two plants had growth type 4. There were no plants with growth type 0. In the low biomass producing group, there were plants with all growth types (0-4), however, there were more plants with growth types 0 and 1 than other growth types (appendix I). Most of the plants with growth types 3 and 4 had died before flowering in the greenhouse, and thus most of the plants that survived and flowered for crossing had growth types 0 and 1. The plants that died may have had physiological weakness because of low total available carbohydrate (Fergus and Hollowell, 1960).

4.1.3 Cultivar components

There were marked differences among cultivar components of high and low biomass producing parental groups (table 4). The majority of the plants in the high group came from the cultivars Prosper, Lakeland, and Atlas (78%), whereas Marino, Arlington, and Atlas made up more than 60% of the plants in the low group. In the high group there were no genotypes from Kuhn and Persist while Prosper was not found in the low group. These components were related to the percentage of non-flowering plants of individual cultivars in the parental population (table 4). The cultivars Kuhn and Persist which had the highest percentage of non-flowering plants did not appear among the plants selected for high biomass production while Prosper, Lakeland and Atlas with low non-flowering plant percentage were found in high percentages in the selected plants. The percentages of non-flowering plants in each cultivars were also highly correlated to top growth dry weights of the cultivars ($r = -0.84$, $p<0.01$). This character, the percentage of non-flowering plants, may be used as an index for evaluating new cultivars or strains compared to check cultivars or for selection for one-year biomass production.

Table 4 Cultivar components of plants selected for high and low biomass production and percentage of non-flowering plants and top growth dry weight in each cultivar in the parental population

cultivar	high group number	high group percent	low group number	low group percent	% non-flowering plants	dry weight
						g pl^{-1}
Arlington	4	8	5	16	22	106
Atlas	9	18	5	16	12	123
Florex	4	8	3	9	10	108
Kuhn	0	0	2	6	44	49
Lakeland	11	22	2	6	8	135
Marathon	5	10	2	6	15	116
Marino	1	2	6	19	22	69
Persist	0	0	4	13	42	77
Prosper	14	28	0	0	9	140
Tristan	2	4	3	9	5	109
Total	50	100	32	100		

4.2 Progeny testing

4.2.1 Biomass production

Significant variation among the single-cross progenies was found for root (including crown), top growth, and total dry weight of one-year red clover (table 5 and figure 1). Progenies of the S^+ (within the high biomass producing group) and SI (between high and low biomass producing groups) crosses had significantly higher top growth and total dry weight than those of the S^- (within the low biomass producing group) and the check (parental) cultivars. The S^- single-cross progenies had significantly higher top growth and total biomass than the parental check cultivars. Yield of the S^- progenies were also much higher than yield of the parents in the previous year. This may indicate that there was considerable heterosis expressed for certain crosses. However, the actual parental genotypes were not grown with the progeny.

The work of Taylor *et al.* (1970) indicated a significant inbreeding depression and implied that heterosis is important in red clover. They suggested that heterosis in red clover was probably caused by a high level of heterozygosity, and the difference among heterozygous genotypes resulted mainly from average or additive effects of the alleles present. Maximum expression of heterosis will be found when inbred lines of red clover are hybridized. However, heterosis can also be obtained by crossing noninbred cultivars or clones. Higher green matter yields

Table 5 Range and mean of dry weight of single-cross progenies in each group

group	N	dry weight (g $plant^{-1}$)			
		root	top	total	mean
high group (S^{+})	1572	1-69	5-345	10-371	137 a
low group (S^{-})	565	1-57	7-316	10-349	120 b
SI group	72	3-47	31-259	36-279	132 a
parents	39	3-41	19-169	22-209	95 c

Means within a column with the same letter were not significantly different at the 5% level

than both the maternal parent and the parental means have been obtained from bulk crosses (Taylor and Smith, 1979).

Root dry weight was not significantly different among the groups. The majority of the biomass production of the progeny plants was found in the top growth (figure 1). Production of top growth was 6-7 times greater than root production.

Mean root dry weight of the progenies of each clones in the high biomass producing group ranged from 9 g $plant^{-1}$ (clone 11-3-2) to 25 g $plant^{-1}$ (clone 26-3-1), and top growth dry weight varied from 64 g $plant^{-1}$ (clone 11-3-2) to 157 g $plant^{-1}$ (clone 40-2-3) (appendix II). There was significant variation ($p<0.05$) for these variables among these clones. However, the determination of root dry weight is very difficult since individual roots have to be dug out.

In the low biomass producing group, root dry weight of the progenies of the clones varied from 8 g plant^{-1} (clone 36-3-3) to 24 g plant^{-1} (clone 3-1-1), and top growth dry weights were between 64 g plant^{-1} (clone 36-3-3) and 133 g plant^{-1} (clone 40-4-2) (appendix II).

Dry weight of plants selected from the progenies for high biomass production was 193% and 263% of the progeny population and parental cultivars, respectively (table 6). Root dry weight of plants selected for the heavy root production was 48 grams per plant which is 263% of parental cultivars and 278% of the progeny population.

4.2.2 Shoot:root ratio

Mean shoot to root ratios of progeny plants were considerably higher than for parental cultivars from which they were selected (figure 2). It appears that selection for improved biomass production increases top growth but has little or no effect on root size.

Shoot:root ratio of clones in the high group varied from 4.43 (clone 7-2-2) to 8.75 (clone 33-2-1) (appendix II). Generally, high yielding clones had shoot:root ratio between 5.5 and 7.8.

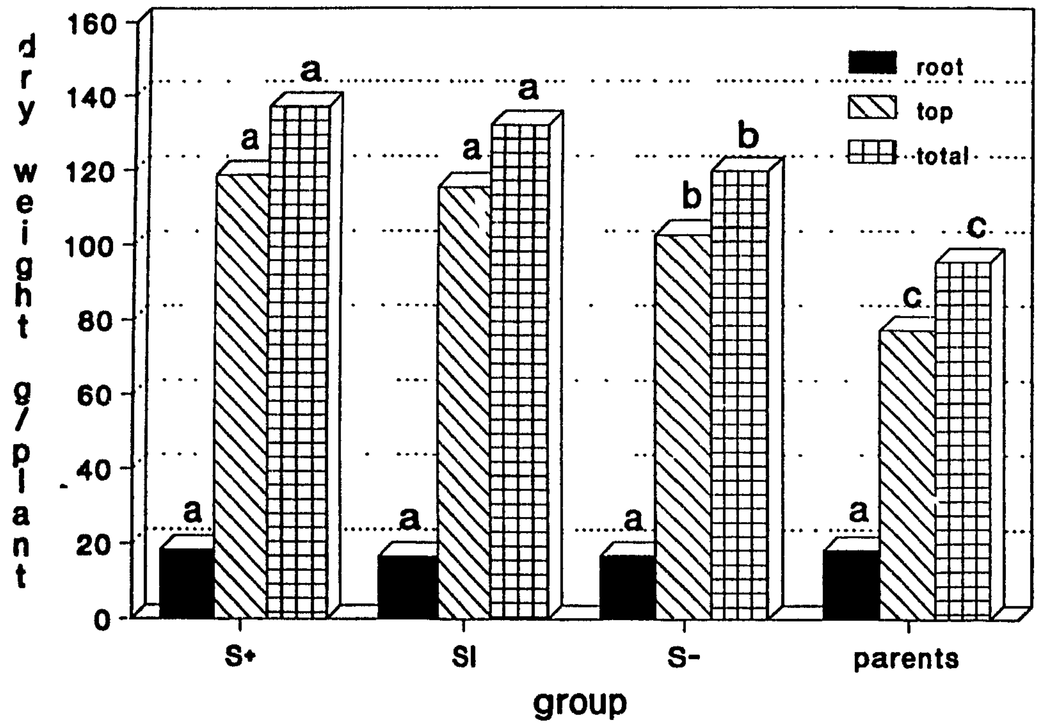

Figure 1 Dry weight of tops, root and total biomass for progenies of three groups of crosses and parental checks

This ratio may be suitable for red clover to produce high biomass.

In the low biomass producing group, shoot:root ratio varied from 3.95 (clone 18-1-2) to 8.11 (clone 39-1-3) (appendix II). In general, the low biomass producing group had lower shoot:root ratios than the high group.

Shoot:root ratio of progenies selected for high biomass plants was about eight which is about two times that of parental cultivars and 2.4 times that of plants selected for heavy root production (table 6).

The shoot-root ratios in both groups were much higher than those were found by Fribourg and Johnson (1954). They found shoot-root ratios of medium red clover ranging from 1.8 to 3.8 (with mean of 2.6) which were higher than those of alfalfas (with mean of 1.8) in the fall of seeding year. They also found that nitrogen content of top growth was 3.02% which was higher than that of root (2.47%). Most of the cultivars that were used in our experiment were productive and well adapted. These modern cultivars with a higher yield potential produced more top growth than those used in the above mentioned study. In the present experiment, plants were space-planted while they were broadcasted in the study of Fribourg and Johnson (1954). This probably also affects the shoot:root ratio, as plants with more space will have more access to resources and thus will produce higher above ground biomass.

The slightly higher shoot:root ratios for plants in the high one-year biomass producing group compared to the low group may indicate that selection for high biomass may select for a more

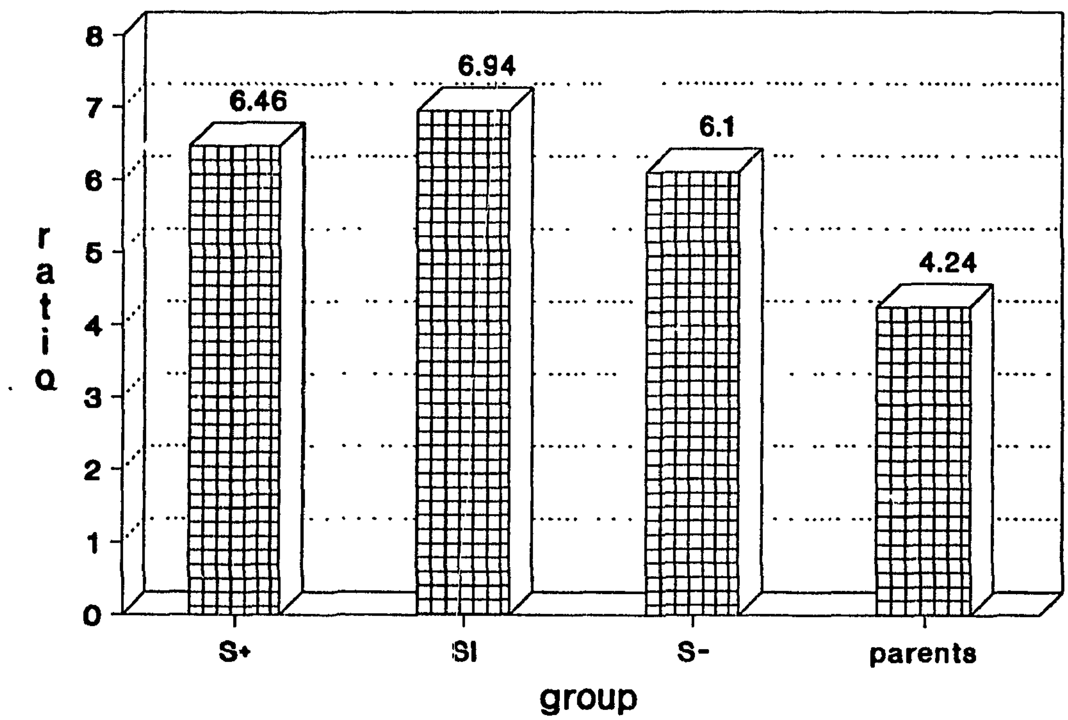

Figure 2 Shoot:root ratio of progenies of the three groups of crosses and parental checks

Table 6 Root (plus crown), top growth, and total dry weight (g $plant^{-1}$) and shoot:root ratio of progeny population, parental cultivars, plants selected for high biomass and heavy root production

group	root	top	total	shoot:root
progeny population	17 c	112 c	130 c	6.50
parental cultivars	18 c	77 d	95 d	4.24
high biomass progeny selections	28 b	223 a	251 a	8.07
heavy root progeny selections	48 a	161 b	209 b	3.36

Mean within a column with the same letter were not significantly different at the 5% level

annual growth habit at the same time. As was found by Fribourg and Johnson (1954), sweet clover which is a biennial legume, had a much higher shoot:root ratio (about 9) than the perennial legumes (alfalfa and red clover with the ratios about 1.5). This suggests that more attention should be given to top growth when selecting for improved biomass. The effect of this change on the storage of root reserves and subsequent persistence should be examined.

4.2.3 Single-cross progenies

Table 7 and table 8 give dry weight (g plant^{-1}) of the single-cross progenies of all clones in the high and low biomass producing groups. The dry weights under the column 'single-cross' are the average of the dry weight of all single crosses in which that clone was one of the parents. The reciprocal difference among clones of total dry weight of progenies was not significant ($F = 0.77$ with 40 df, $p > 0.8217$). This suggests that there is no maternal effect for this character.

4.2.4 Combining ability

Certain genotypes produced high yielding progeny in crosses with all other genotypes indicating high general combining ability. All clones, except clone 11-3-2, in the high biomass producing group had higher total dry weight than parental check cultivars. They ranged from 104% to 193% of check cultivars (table 7). In the low producing parental group, only five clones produced less total biomass in progenies than check cultivars (table 8). They yielded 74% to 159% of check cultivars.

The largest general combining ability effects for one-year total dry weight production of red clover were observed in clones

40-2-3, 19-2-5, 26-3-1,32-2-1, and 6-2-1. These clones had general combining abilities of 52, 45, 37, and 35 g plant^{-1}, respectively. These clones and other high general combining ability clones should be useful for the development of synthetic cultivars for short-term (one year) biomass production or plowdown purposes.

4.2.5 Heritability estimates

The estimates of narrow-sense heritability for single-year biomass production were 0.22 and 0.23 from parent-progeny regression and parent-progeny correlation, respectively (table 9). The realized heritability estimate for population selected for high biomass was 0.15. Since the biomass of the population selected for low biomass was much higher than that of the parental population, the realized heritability estimate for this selection would be negative and thus would be considered to be 0. These relatively low heritability estimates suggest that progeny testing will have to be carried out to effectively select for this trait.

Variable heritability estimates have been obtained for forage yield and other characters in previous studies on red clover. Anderson *et al.* (1974) also found low heritability estimates for forage yield (20.7%), persistence, and dates of first bloom. They concluded that response to individual plant selection would be low. However, high heritability values for crude protein content, dry

Table 7 Total dry weight (g plant^{-1}) of single-cross progenies of clones in the high biomass producing group and their general combining ability (GCA)

clone	single-cross dry weight	single-cross rank	GCA
40-2-3	180	1	52
19-2-5	173	2	45
32-2-1	165	3	37
26-3-1	163	4	35
9-3-2	161	5	33
6-2-1	154	6	26
18-2-4	152	7	24
27-2-2	151	8	23
13-2-5	151	8	23
28-1-5	149	10	21
7-2-1	147	11	19
19-2-3	146	12	18
19-1-5	145	13	17
37-3-2	143	14	15

clone	single-cross		GCA
	dry weight	rank	
24-2-2	143	14	15
37-4-4	142	16	14
27-3-5	141	17	13
5-3-4	140	18	12
26-4-1	139	19	11
18-3-5	138	20	10
34-3-1	138	20	10
18-2-3	137	22	9
10-3-3	134	23	6
39-2-4	134	23	6
12-4-2	133	25	5
38-1-1	133	25	5
10-3-4	131	27	3
20-1-4	130	28	2
37-4-3	128	29	0
33-2-1	128	29	0

clone	single-cross		GCA
	dry weight	rank	
15-3-2	126	31	-2
9-3-4	124	32	-4
37-4-2	122	33	-6
9-3-3	122	33	-6
19-4-3	118	35	-10
3-4-1	117	36	-11
38-1-2	115	37	-13
13-3-2	114	38	-14
39-2-5	112	39	-16
7-1-5	111	40	-17
9-1-5	110	41	-18
40-1-4	109	42	-19
7-2-2	108	43	-20
40-2-5	99	44	-29
parents	95	45	-
11-3-2	73	46	-55

Table 8 Total dry weight (g $plant^{-1}$) of single-cross progenies of clones in the low biomass producing group and their general combining ability (GCA)

clone	single-cross dry weight	single-cross rank	GCA
40-4-2	152	1	24
24-4-5	140	2	12
3-1-1	138	3	10
31-1-2	135	4	7
2-2-2	134	5	6
22-2-5	133	6	5
39-1-3	133	6	5
7-3-3	132	8	4
34-3-3	132	8	4
19-3-4	124	10	-4
1-3-1	121	11	-7
10-4-1	120	12	-8
30-3-4	120	12	-8
12-4-5	118	14	-10
31-4-4	111	15	-17

clone	single-cross		GCA
	dry weight	rank	
26-2-2	111	15	-17
18-1-2	106	17	-22
10-2-3	102	18	-26
parents	95	19	-
3-2-2	91	20	-37
31-2-1	87	21	-41
27-1-3	87	21	-41
14-2-2	79	23	-49
36-3-3	72	24	-56

Table 9 Heritability estimates for one-year biomass production of red clover

method of estimation	h^2
parent-progeny regression	0.23
parent-progeny correlation	0.22
realized heritability	0.15

matter yield, growth habit and corolla tube length of red clover have been reported (Taylor and Smith, 1979). Cornelius *et al.* (1977) also found low heritability estimates for number of plants (persistence) and vigor of red clover obtained from I_1 single crosses.

4.2.6 Cultivar components

Plants were selected from 9 of the 10 cultivars screened to choose the parental plants for the crosses. However, the majority of the plants came from three cultivars, namely Prosper, Lakeland and Atlas. The highest biomass plants selected in the progeny were in a similar proportion according to cultivar as the parental population (figure 3).

Cultivar components of plants selected from the progenies for high biomass production (about 85% came from the cultivars Prosper, Atlas, Lakeland and Arlington) were similar to high biomass producing parents and plants selected for heavy root production (about 86% from Prosper, Atlas, Marathon and Arlington) (table 10).

4.2.7 Growth types

Most progenies within the high biomass producing group (S^+) were in growth types 4 and 3 (74 and 24%), only few had growth types 2 and 1, and none had growth type 0 (table 11). It was the same with crosses within the low biomass producing group (S^-), and crosses between groups (SI). This indicated that this character was not closely related to biomass production. Many workers (Bird, 1948; Smith, 1957; Smith, 1963; Therrien and Smith, 1960; Taylor *et al.*, 1968; Choo, 1984) have found that the persistence of red clover plants was correlated with the first year performance. Plants that have good persistence will usually produce less top growth in the first year. These plants will be a rosette and will not flower in the first year. However, Choo (1984), by using three red clover cultivars (Prosper I, Florex, and Tristan), found that high-yielding plants could be found among non-flowering plants in the same proportion as for flowering plants. These plants also had good regrowth and could produce high seed yield which was different from the mammoth type. He also suggested that flowering habit of red clover plants could be used as a criterion to select plants with good persistence and high yields.

As mentioned previously (section 4.1.2), most parental plants in the high group had growth types 2 and 3, and those in the low group had growth types o and 1. Thus, it might be posssible to conclude that growth types was not a highly heritable character. Taylor *et al.* (1966) also found that parent-progeny correlations

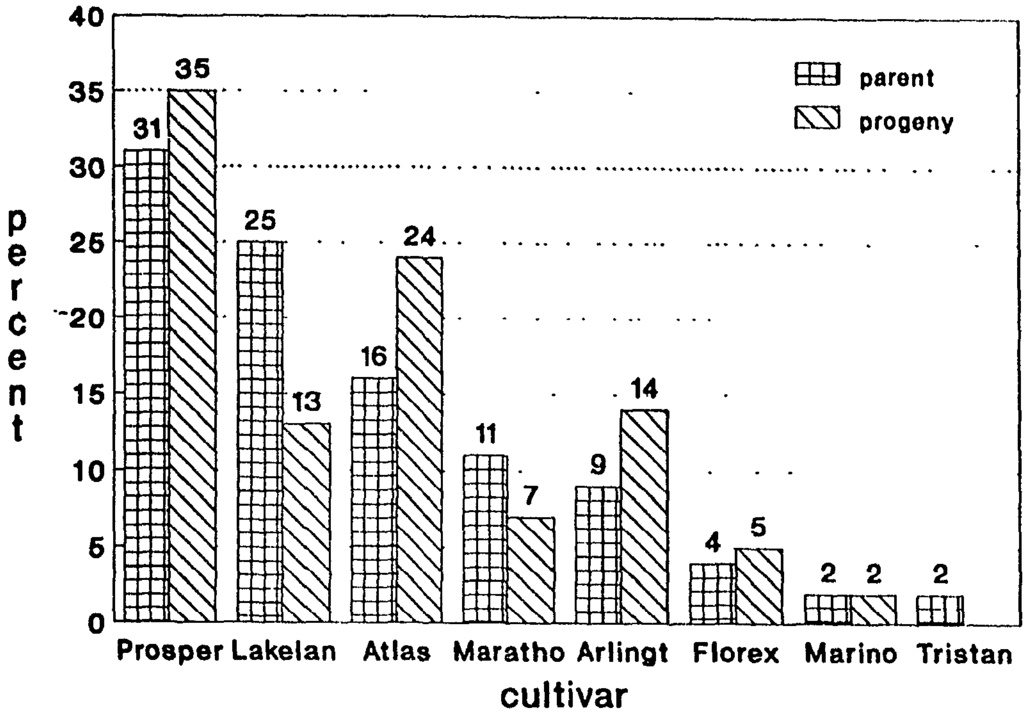

Figure 3 Percentage of high biomass plants selected from parental and progeny populations according to cultivars

Table 10 Cultivar components of plants selected for high biomass and heavy root production

cultivar	high biomass group		heavy root group	
	number	percentage	number	percentage
Arlington	20	14	9	13
Atlas	34	24	12	17
Florex	7	5	1	1
Kuhn	0	0	0	0
Lakeland	21	14	8	11
Marathon	10	7	13	18
Marino	3	2	1	1
Persist	0	0	0	0
Prosper	49	34	28	39
Tristan	0	0	0	0

for days to first bloom were not significant. In another study, Taylor *et al.* (1968) found non-significant correlations between parents and progenies for the number of flowers in the seeding year. An interaction between red clover clones and photoperiod for flowering habit has been found (Ludwig *et al.*, 1953). However, relatively high heritability estimates for growth type have

Table 11 Growth type of progenies within the high biomass producing group (S^+), the low biomass producing group (S^-), and between both groups (SI)

growth type	S^+		SI		S^-	
	number	percent	number	percent	number	percent
4	292	74	11	61	97	68
3	96	24	7	39	30	21
2	4	1	0	0	4	3
1	3	1	0	0	7	5
0	1	0	0	0	5	4
total	396	100	18	100	143	100

previously been found (Coulman and Oakes, 1987). The difference in the present study may result from the fact that in 1990 the red clover was transplanted to the field approximately one week earlier than the parents in 1989. These plants had adequate time to reach a stage of development in which they could respond to the long days of the summer. Thus, most of the plants flowered profusely (Smith, 1957; Therrien and Smith, 1960). The other factor that may have the effect on growth type was the time of measurement. Growth type of the progenies was evaluated about two weeks later than that of the parental population. This character may also have an interaction with other environmental conditions such as soil moisture, and temperature (Kendall, 1958).

5. CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

Concerns for the environment have led to more interest in sustainable agricultural systems. Forage legumes have been used as a renewable, environmentally friendly source of nitrogen for other crops for long period of time. Red clover, with many favorable characters, is one of the best forage legumes that has been used for the purpose of plowdown green manure. Improved red clover bred for plowdown purposes will make a greater contribution to sustainable agriculture.

Red clover genotypes that have much higher yield potential for one-year biomass production have been found from this study. These genotypes should be used to develop the improved synthetic cultivars for plowdown purpose. However, progeny testing has to be carried out in further selection for this trait since low heritability estimates for this character have been found.

In selecting for high short-term biomass production, a high proportion of the selected plants showed a higher shoot:root ratio and flowered profusely in the year of establishment. This type of plant may be less persistent. The nitrogen content and yield of these selected plants and their synthetics should be examined. The effect on root reserves and persistence of these plants should also be evaluated. The change in the plant growth habit may affect the chemical composition of the plants, thus the effect of this change on the decomposition and decomposition rate should be investigated.

Some selected genotypes have given single-cross combinations with very high biomass yield. This indicates high heterosis for this character. To fully use this heterosis, further studies should be carried out on the potential and possibility of hybrid cultivar development.

Normally, tetraploid red clover has higher yield and bigger plants than the diploid. The yield potential of tetraploid red clover for one-year biomass production should be determined. However, the poorer seed production and subsequent higher seed costs of tetraploid red clover may discourage its use as a plowdown crop.

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APPENDIX I

Table 1 Top growth, root (plus crown), and total dry weight (g $plant^{-1}$) and growth type of plants selected for high biomass production

cultivar	clone	top	root	total	growth type
Atlas	19-2-5	361	235	596	3
Atlas	19-2-3	376	174	550	3
Prosper	40-2-3	296	233	529	2
Lakeland	38-1-1	233	292	525	2
Marathon	28-1-5	383	124	507	3
Marathon	26-3-1	221	281	502	3
Lakeland	9-3-3	233	265	498	3
Prosper	10-3-3	327	164	491	3
Lakeland	33-2-1	214	277	491	3
Atlas	39-2-4	326	161	487	2
Lakeland	9-3-4	212	260	472	3
Atlas	39-2-5	251	220	471	2
Marino	5-3-4	219	251	470	3
Prosper	27-2-2	194	273	467	2
Prosper	15-3-2	248	219	467	2

cultivar	clone	top	root	total	growth type
Arlington	18-3-5	159	305	464	3
Prosper	9-1-5	213	246	459	3
Lakeland	26-4-1	178	275	453	2
Prosper	10-3-4	227	204	431	2
Lakeland	27-3-5	290	140	430	3
Florex	12-3-2	252	172	424	3
Atlas	19-2-4	274	150	424	3
Tristan	11-3-2	239	184	423	3
Prosper	40-2-5	188	235	423	2
Arlington	13-2-5	215	207	422	2
Arlington	32-2-1	219	202	421	2
Prosper	3-4-1	213	206	419	3
Lakeland	13-3-2	247	167	414	3
Marathon	24-2-2	229	180	409	3
Marathon	7-2-1	174	230	404	2
Atlas	20-1-4	174	230	404	3
Tristan	23-4-1	169	235	404	2
Lakeland	38-1-2	224	117	401	2

cultivar	clone	top	root	total	growth type
Marathon	7-2-2	171	225	396	3
Lakeland	9-3-2	215	179	394	3
Prosper	18-2-4	274	120	394	3
Prosper	18-2-3	173	220	393	3
Prosper	40-1-4	201	192	393	2
Prosper	37-4-2	236	147	383	2
Atlas	6-2-1	186	189	375	2
Florex	19-1-5	236	133	369	4
Prosper	37-4-3	225	143	368	3
Arlington	12-4-2	217	147	364	3
Florex	34-3-1	204	159	363	3
Atlas	39-2-3	190	170	360	1
Lakeland	7-1-5	172	185	357	3
Prosper	37-4-4	223	131	354	2
Lakeland	19-4-3	226	121	347	3
Atlas	37-3-2	218	129	347	4
Florex	25-4-1	244	101	345	3

Table 2 Top growth dry weight (g plant^{-1}) and growth type of plants selected for low biomass production

cultivar	clone	dry weight	growth type
Lakeland	3-2-2	92	1
Atlas	7-3-3	84	1
Persist	10-4-1	80	1
Marino	14-3-5	70	4
Persist	19-3-4	59	3
Marino	10-2-3	57	1
Marino	27-1-3	52	0
Atlas	22-1-1	52	2
Marino	31-2-1	52	1
Tristan	14-2-2	50	3
Kuhn	37-2-3	42	3
Atlas	2-4-1	42	3
Tristan	36-3-3	27	0
Tristan	20-4-3	26	3
Marino	6-1-4	23	1
Arlington	26-2-2	23	0

cultivar	clone	dry weight	growth type
Kuhn	12-1-4	22	1
Florex	1-3-1	20	1
Persist	18-1-2	18	0
Florex	34-3-3	18	0
Arlington	30-3-4	18	0
Marathon	31-1-2	17	0
Lakeland	22-2-5	17	1
Arlington	2-2-2	17	0
Marino	27-4-3	14	3
Marathon	3-1-1	13	0
Arlington	40-4-2	13	0
Arlington	12-4-5	12	2
Persist	25-3-4	10	0
Florex	31-4-4	10	0
Atlas	39-1-3	8	3
Atlas	24-4-5	5	0

APPENDIX II

Table 1 Root (plus crown), top growth, and total dry weight (g/plant) and shoot-root ratios of single-cross progenies of clones in the high biomass producing group

clone	no. of crosses	root (+crown)	top growth	total	shoot: root
40-2-3	39	22	158	180	7.2
19-2-5	27	23	150	173	6.5
32-2-1	24	19	146	165	7.7
26-3-1	9	25	138	163	5.5
9-3-2	4	22	139	161	6.3
6-2-1	4	17	137	154	8.0
18-2-4	18	19	133	152	7.0
27-2-2	5	21	130	151	6.2
13-2-5	31	19	132	151	6.9
7-2-1	18	22	125	147	5.7
19-2-3	19	19	127	146	6.7
28-1-5	5	22	127	149	5.8
19-1-5	27	17	128	145	7.5
37-3-2	29	17	126	143	7.4

clone	no. of crosses	root (+crown)	top growth	total	shoot: root
24-2-2	18	19	124	143	6.5
37-4-4	25	18	124	142	6.9
27-3-5	32	18	123	141	6.8
5-3-4	14	21	119	140	5.7
26-4-1	17	22	117	139	5.3
18-3-5	36	19	119	138	6.3
34-3-1	13	20	118	138	5.9
18-2-3	27	21	116	137	5.5
10-3-3	10	18	116	134	6.4
39-2-4	5	24	110	134	4.6
12-4-2	4	18	115	133	6.4
38-1-1	25	19	114	133	6.0
10-3-4	4	21	110	131	5.2
20-1-4	13	19	111	130	5.8
37-4-3	30	18	110	128	6.1
33-2-1	39	13	115	128	8.8
15-3-2	31	18	108	126	6.0

clone	no. of crosses	root (+crown)	top growth	total	shoot: root
9-3-4	15	18	106	124	5.9
37-4-2	14	15	107	122	7.1
9-3-3	11	17	105	122	6.2
19-4-3	28	17	101	118	5.9
3-4-1	11	12	105	117	8.7
38-1-2	9	17	99	116	5.8
13-3-2	19	16	98	114	6.1
39-2-5	16	15	97	112	6.5
7-1-5	7	16	95	111	5.9
9-1-5	27	18	92	110	5.1
40-1-4	20	16	93	109	5.8
7-2-2	7	20	88	108	4.4
40-2-5	13	15	84	99	5.6
11-3-2	12	9	64	73	7.1
checks	-	18	77	95	4.3
$LSD_{0.05}$		6	21	23	

Table 2 Root (plus crown), top growth, and total dry weight (g/plant) and shoot-root ratios of single-cross progenies of clones in the low biomass producing group

clone	no. of crosses	root (+crown)	top growth	total	shoot: root
40-4-2	20	19	133	152	7.0
24-4-5	29	19	121	140	6.4
3-1-1	6	24	114	138	4.7
31-1-2	17	20	115	135	5.7
2-2-2	10	19	115	134	6.0
22-2-5	4	20	113	133	5.6
39-1-3	20	14	119	133	7.0
7-3-3	16	22	110	132	5.0
34-3-3	14	21	111	132	5.3
19-3-4	3	19	105	124	5.5
1-3-1	12	18	103	121	5.7
30-3-4	17	20	100	120	5.0
10-4-1	4	15	105	120	7.0
12-4-5	13	14	104	118	7.4
31-4-4	18	16	95	111	5.9

clone	no. of crosses	root (+crown)	top growth	total	shoot: root
26-2-2	35	13	98	111	7.5
18-1-2	9	21	85	106	4.0
10-2-3	15	14	88	102	6.3
checks		18	77	95	4.3
3-2-2	6	10	81	91	8.1
31-2-1	8	14	73	87	5.2
27-1-3	6	15	72	87	4.8
14-2-2	15	10	69	79	6.9
36-3-3	8	8	64	72	8.0
$LSD_{0.05}$		5	18	20	