TEMPERATE FORAGE GRASS-LEGUME MIXTURES: ADVANCES AND PERSPECTIVES

A.S. Laidlaw[1] and N. Teuber[2]
[1]Department of Applied Plant Science, Queen's University of Belfast, Plant Testing Station, 50 Houston Road, Crossnacreevy, Belfast BT6 9SH, Northern Ireland and
[2]Centro Regional de Investigación Remehue, Instituto de Investigaciones Agropecuarias, Casilla 24-0, Osorno, Chile.

Abstract

The paper summarises some of the advances which have been made a) in increasing understanding of the grass-legume association, especially grass-white clover, so that the association can be more predictably exploited and b) in overcoming limitations in the use of such mixtures. The contribution which forage legumes make to the N economy of mixtures is reviewed with estimates approaching 400 kg N ha^{-1} for some. Uptake by grass of legume-derived N (N transfer) reduces soil mineral N levels and increases the proportion of fixed N in the total legume N relative to legume monoculture. Although N transfer also causes inconsistent contribution of legume to mixed swards, models of the effect of legume derived N on the interaction between grass and legume are helping to predict likely grass-legume balance, even when grazed.

The higher nutritive value and intake of legumes relative to grass is due to a range of factors including faster rate of particle breakdown, faster digestion in the rumen, more non-ammonium N reaching the small intestine and higher efficiency of energy utilization although efficiency of N utilization is lower. Poor utilization is not an issue with birdsfoot trefoil and sainfoin due to their herbage having a high content of condensed tannins which protect protein from degradation in the rumen. Breeding programmes using conventional and biotechnological methods are aiming to improve nutritive value such as increasing protein quality and introducing condensed tannins into clovers and lucerne. Breeding of legumes to reduce antiquality factors, such as bloat, is underway. Breeding to reduce oestrogenic effects has been successful in red clover and subclover.

Advances are leading to improved legume consistency in mixture including improvement in tolerance to biotic and environmental stress by breeding and increased understanding of the role of companion grasses. Research which underpins management techniques to improve predictability of grass-legume balance is also discussed, including the positive and negative role of the grazing animal.

The potential and limitations of grass-legume swards to reduce N loss, including NO_3 leaching, in whole farm systems is evaluated where grass/white clover can reduce leaching by 50% compared with a high fertilizer N system at only 20% reduction in output. Other factors which may result in increased reliance on forage legumes, in addition to the improvements in forage legumes resulting from research, include de-intensification policy decisions to reduce stocking rates, increased uptake of organic farming, increased cost of N fertilizer relative to commodity prices. Shared research effort between countries is advocated to supply adequate resources to solve some of the remaining problems in grass/legume associations and effective technology transfer should include development of decision support systems due to the complexity of the association.

Introduction

Two attributes of temperate forage legumes which are exploited in modern agriculture are their contribution of nitrogen via atmospheric N_2-fixation to grassland and subsequent crops, and their high nutritive value and livestock intake characteristics compared with most temperate grasses at similar stages of development. In contrast to these advantages, unreliability and potential antiquality problems are renowned disadvantages although in mixture with grass, while the former is exacerbated the latter is usually alleviated. Nevertheless, both are viewed as potential limitations to reliance on forage legumes.

This paper will deal with recent studies which have either underpinned the benefits of grass-legume associations or increased their predictability and will refer to work targetted at overcoming their limitations. Consideration is also given to possible developments in the use of grass/legume mixtures throughout temperate agriculture.

Of the temperate forage legumes usually grown in association with grass, white clover (*Trifolium repens*) is the most widely used geographically. Estimates of pasture with white clover include 15M ha in Australasia, and 5M ha in the USA (Marten *et al*., 1989) with about 3-4M ha sown annually throughout the world (Mather *et al*., 1996). The area of lucerne (*Medicago sativa*) in the world is in excess of 30 M ha, the area sown annually estimated to be more than double the area of white clover (Mather *et al*., 1996). However due to the limited duration of lucerne stands and the contribution of ‘indigenous’ white clover to permanent pasture, these figures belie the area of white clover which is exploited relative to lucerne. Estimates of annual sowing of red clover (*Trifolium pratense*), are in the region of about 1.5 M ha with about 4-5 M ha being grown in the US alone (Taylor and Smith, 1995). Subclover (*Trifolium subterraneum*), is mainly grown in Australia having reputedly colonised more than 15 M ha. However it is also used in the USA and the Mediterranean-type climatic regions of the world. About 1.5 M ha of birdsfoot trefoil (*Lotus corniculatus*) is grown in the US, and annual sowing in southern Latin America is approximately 0.25 M ha (Asuaga, 1994). Greater trefoil (*Lotus uliginosus*), alsike clover (*Trifolium hybridum*) and sainfoin (*Onobrychis viciifolia*) have niche uses in temperate grassland.

Many of the principles governing grass-legume mixtures have been established from studies of white clover growing with grass. So while this paper is concerned with temperate grass/legume associations in general, many of the references are drawn from studies on grass-white clover mixtures. Nevertheless, where available and relevant, examples will be cited from studies with other legumes, particularly for contrast. Monographs on white clover (Baker and Williams, 1987), lucerne (Hanson *et al*., 1988) and red clover (Taylor and Quesenberry, 1996) have been published in the recent past and so reference will be made mainly to studies published within the past decade, or so. Other papers presented at this Congress will deal with advances in lucerne technology and in improvement of temperate forage legumes by breeding and so care will be taken not to overlap with these areas.

Nitrogen economy of grass-legume associations

Nitrogen fixation

Estimates of nitrogen fixation have been summarised in Frame *et al.* (1998). Pure lucerne swards and white clover growing with grass in New Zealand have the highest maximum annual estimates of almost 400 kg N ha^{-1} while the recorded maxima for red clover and subclover are about 220 and 210 kg N ha^{-1}, respectively. Birdsfoot trefoil has a lower maximum of about 140 kg N ha^{-1}. While these estimates are drawn mainly from experimental plots, more recently N fixation has been measured in grass-white clover swards on dairy farmlets in New Zealand where in a 0N treatment N_2-fixation ranged over 3 years from 99 to 231 kg N ha^{-1} $annum^{-1}$ (Ledgard *et al*., 1999).

The amount of N fixed is influenced by the amount of legume present. For example, in southern Chile where white clover content is limited by soils with high P fixation, high free Al and low pH and may account for 10% or less of total herbage, estimated annual fixation rates are of the order 70-75 kg N ha^{-1} $annum^{-1}$ when grazed with cattle (Teuber *et al*., 1996). Estimates relating N_2-fixation to the amount of legume biomass for white clover have ranged from 27 to 112 kg N t^{-1} DM clover. While soil mineral N level contributes to this variation it is not the only factor. Even when soil mineral N levels are high white clover relies heavily on N fixation in mixed swards. Grazing reduces the contribution which fixation makes towards total legume N e.g. 0.64 compared with 0.79 in ungrazed swards (Eriksen and Høgh Jensen, 1998). In grazed dairy swards in Victoria (Australia) white clover has been found to fix only about 23 kg N t^{-1} DM (Riffkin *et al*., 1999). Mineralisation of urine will contribute to the lower contribution of fixed N to total legume N (Marriott *et al*., 1991), although differences in technique to measure N_2-fixation may also contribute to variation in estimates. In subclover swards estimates of N fixed per tonne clover biomass are of the order 23-34 kg N (Dear *et al*., 1999).

As with white clover, fixed N accounts for much of the total N in most of the temperate forage legumes growing with grass e.g. in red clover (Mallarino *et al*., 1990), subclover (Unkovitch *et al*., 1996) and birdsfoot trefoil (Farnham and George, 1994). In Italian ryegrass-subclover mixtures, the grass is considered to be twice as efficient at taking up mineral N than clover, even when root volume and weight are similar. This minimises impact on the accompanying subclover's fixation but increases competitiveness of grass towards the legume.

Prospects for improving N_2-fixation include either selection and exploitation of naturally occurring highly effective strains of the appropriate rhizobium or producing new strains by biotechnology. Evaluation of strains of *Rhizobium loti* in Uruguay has shown wide variation in effectiveness and persistence, especially at low pH (Baraibar *et al*., 1999). Such studies provide support for increased effort in selecting strains as inoculants. Another approach using biotechnology is to improve efficiency in energy utilization by nitrogenase e.g. by increasing hydrogenase activity in nodulated birdsfoot trefoil by transconjugation in *Rhizobium loti* (Monza *et al*., 1997). Other possibilities discussed by Chapman *et al.* (1996) include increasing sensitivity of nitrogenase activity to mineral N thereby reducing the requirement for energy and reducing the penalty on DM production. However, reinstatement of the process when soil mineral N becomes depleted may be too slow and could put the legume at a long-term disadvantage.

Nitrogen transfer

In addition to the direct benefit of N_2-fixation to the legume, the grass profits from the legume-derived N. This occurs either via release of N due to decay of subterranean parts of the plant and decomposition of the N-rich litter or via the grazing animal, especially as urine. Transfer by hyphal connections between legume and grass roots *via* arbuscular mycorrhizal fungi is likely to be of minimal ecological or agronomic significance (Rogers *et al*., 2000). Excretion of N from nodules is also not considered to be an important route for N transfer (Russelle *et al*., 1994). Transfer of N from white clover to wheat via subterranean grazing by root feeding larvae has been demonstrated (Murray and Clements, 1998)

In grass-white clover swards estimates vary for underground transfer from about 25% in a grazed sward in New Zealand (Ledgard, 1991) to almost 50% in an upland sward in Wales. Transfer is usually low in the first year of production. In cut plots in Denmark 3% of fixed N in year 1 was transferred whereas this increased to 22% in year 3 (Høgh-Jensen and Schjoerring, 1997). Estimates of the proportion of fixed N_2 transferred from legume to grass vary widely. For example N transferred from red clover to associated grass have been found to range from 13% to 34% (Heichel and Henjum, 1991). Although on an annual basis N transfer may be considered to be relatively low, a ploughed red clover sward can supply 140 to 160 kg N ha^{-1} to the following maize crop (Collins, 1993).

The lower the proportion of biomass harvested the more N is likely to be transferred in cut swards. In general perennial stoloniferous forage legumes transfer more N than erect types; proportionately more N is transferred by small-leaved than larged-leaved white clovers (Laidlaw *et al*., 1996). However it is not clear to what extent the amount transferred is a function of the amount of N available or the accompanying grass plant's ability to take up the transferable N due to large-leaved clovers being aggressive towards grass.

The amount of fixed N transferred via the grazing animal has been estimated to be about 20% in a study with grazing dairy cows in New Zealand (Ledgard, 1991). Due to the complex relationship between the legume and grass and the additional role of the grazing animal, the dynamics of N in the pasture/animal ecosystem prevent an easily predicted equilibrium between sward components being established (Schwinning and Parsons, 1996a). The implications of this complex relationship in determining the content of legume in a mixture in long term swards is discussed later.

The process of death and decay of underground organs in forage legumes is not fully understood. Recent studies have shown that environment has a strong impact on the likelihood of roots of white clover or perennial ryegrass surviving beyond 28 days, warm dry conditions having a strongly adverse effect on survival (Watson *et al*. 2000). In a continuously stocked perennial ryegrass sward in autumn, when white clover root and stolon biomass have been known to decline, white clover roots were estimated to turn over approximately every 3 months, in autumn (Laidlaw *et al*., 1996). This is less than half the lifetime estimated for lucerne fine roots (Dubach and Russelle, 1994).

Improving nitrogen economy of grass/legume swards

Contribution which forage legumes make to DM in mixed swards is not consistent, growth in spring, for example, usually being slower than that of the accompanying grass. This may result in the amount of herbage available being lower than that required either for grazing stock or cutting for conservation. Consequently strategic N application may be necessary. Although the legume is more likely to be adversely affected by spring application of N compared to autumn application, total DM response is higher with spring applications. Organic manures are potential substitutes for fertilizers. Although scorching of legume leaves is a risk (Wightman *et al*., 1997) dilute cattle slurry has a more positive effect on legumes with grass than its nutrient N content would suggest (Nesheim *et al*., 1990).

Applying additional N to a grass/legume sward has the potential to provide excessive N and disturb the balance between the two components, for example, in long-term grass-white clover swards. Applying additional N might not only supply mineral N to patches which are already high in soil mineral N (and hence low in clover), but also increase soil N in patches which are building up clover and so prevent clover completing its recolonisation in those areas. The sequence of oscillations in clover content would be disturbed and adversely affect the average legume content in the long term (Chapman *et al*., 1996).

Nutritive value, intake and animal production

Nutritive value

Forage legumes are generally higher in crude protein, pectin, lignin and minerals than grass while being lower in cellulose, hemicellulose and water soluble carbohydrates. However these differences in nutrient concentration between legumes and grass cannot explain entirely the nutritional superiority and higher intake characteristics associated with forage legumes. Beever and Thorp (1996) have recently reviewed this topic and consider that the following are implicated, albeit not with equal importance:

a) Higher rate of particle breakdown. The reticulum veination in legume leaves is more resistant to breakdown than the parallel veination of grass leaves (Wilman *et al*., 1996).
b) Enhanced rate of ruminal digestion. Legumes are digested more rapidly in the rumen than grass e.g. lucerne compared with grass (Waghorn *et al*., 1989)
c) Higher amount of non ammonium nitrogen (NAN) to the small intestine (due to net synthesis of microbial protein). This is most likely due to the amount and balance of nutrient supply within the rumen e.g. higher protein levels in the legume herbage.
d) Higher efficiency of energy utilization. This is only apparent when ME intake is high due to the rate of decrease in efficiency being higher in grass than in legume based diets.

While the consequence of these attributes is to increase nutrient intake and animal performance compared with grass diets, a disadvantage is the potential for proportionately higher N wastage when legume diets are fed and so potentially more N could be lost from the system to the detriment of the environment. Increasing the amount of readily fermentable carbohydrate in the diet improves efficiency in N utilization within the whole farming system (Ledgard *et al*., 1999).

The disadvantage of relatively low efficiency in utilization of N from forage legumes does not apply to species such as birdsfoot trefoil, greater trefoil and sainfoin as they contain appreciable concentration of condensed tannins (proanthocyanidins). It is well documented that condensed tannins are involved in the higher supply of non-ammonium nitrogen to the small intestine in those species than with clover and lucerne diets. Only recently has it been proved that condensed tannins reduce the degradation of the principal protein in leaves (Rubisco, i.e. ribulose 1,5-bisphosphate carboxylase/oxygenase) but also to a lesser extent reduce its solublisation (Min *et al*., 2000). They increase efficency in use of dietary N when present at 20-40 g kg^{-1} DM, prevent rumen frothy bloat (Barry and McNabb, 1999) and reduce impact of intestinal parasites in sheep (Niezen *et al.* 1995). Lambs infested with parasitic nematodes fed sulla (*Hedysarum coronarium*), a legume with a high condensed tannin content, performed better than when fed lucerne. However, condensed tannins at concentrations greater than 60 g kg^{-1}DM depress intake, digestibility and animal performance.

While legumes laminae are usually of high digestibility, laminae of subclover are highly lignified and so *in vitro* dry matter digestibility (IVDMD) is lower than that of stems. This has implications for management of subclover swards. The diet of grazing animals under a lax grazing regime may contain a high proportion of laminae DM and so have an adverse effect on the digestibility of the diet. Increasing the ratio of petiole to lamina by management, e.g. lengthening regrowth interval, should increase efficiency of utilization of nutrients in subclover diets (Mulholland *et al*., 1996)

Legume rich silages usually have nutritional advantages over grass silages, including efficiency of energy utilization. For example, comparing lucerne and grass silages at equal ME and DM intakes, cattle gained more tissue energy and a similar amount of tissue protein to cattle fed grass silage (Tyrrell *et al*., 1992).

Attempts to improve nutritive value by breeding lucerne for lower lignin content usually results in reduced DM yield, as does selecting for increased protein content (Rotili, 1993). Transgenic lucerne containing seed albumen genes from sunflower to increase methionine and cysteine content, limiting for wool growth in conventional lucerne, have been produced (Taube *et al*., 1995). Transformed white clover with sulphur-rich seed storage protein, delta zein, has also been produced offering the possibility for a white clover cultivar with more highly protected S-rich protein (Sharma *et al*., 1998).

Intake

Heikkila *et al*. (1992) found that 13% more red clover/grass silage than grass silage alone at similar digestibility was consumed by dairy cows. The higher level of digestible NDF in lucerne than grass increases intake of the former (Dado and Allen, 1996).

Although direct comparison between subclover and grass are lacking, Stockdale (1992) has recorded the high figure of 22 kg DM ha^{-1} intake by dairy cows from subclover pasture. Cultivars of subclover differ in shear and compression energy (Ru and Fortune, 1997). While it is inferred that these differences will influence intake, the relationship between textural properties and intake rate has not been established. In birdsfoot trefoil, excessively high condensed tannin concentrations, especially more than 60 g kg^{-1}, reduce intake.

Sheep and cattle have a preference for clover over grass, but not exclusively so. Sheep prefer about 70% of their diet to be clover, as found in preference studies in which the animals have access to strips of both monocultures (Penning *et al*., 1997). Potential benefits to intake may not be fully expressed by grazing animals. White clover requires less processing time when a bite is prehended i.e. fewer chews per g DM intake, and intake per bite may be higher than for a grass sward. However, especially if the animal is not under strong physiological demand to maintain a high intake rate, the amount of time spent grazing can be less than for a pure grass sward (Penning *et al*., 1998). Preference for clover has implications for the recovery of white clover under grazing, especially continuous stocking as the clover will be subjected to proportionately higher grazing pressure than grass and so place it at a competitive disadvantage.

Animal production

Harris *et al*. (1998) conclude that the optimum content of white clover in the diet in stall-fed animals is 70-80% for dairy cows. With 50% or more white clover in the diet, higher intakes and, to a lesser extent, higher nutritive value resulted in 16 – 33% more milk than from cows fed 20% white clover.

Reviewing animal production from large scale animal or systems experiments in the UK, Ireland and the Netherlands, Davies and Hopkins (1996) showed that output from grass/white clover swards was about 80% or more of the output from grass receiving N fertilizer in the range 250 – 360 kg N ha^{-1}. Comparisons are variable, however. Sheep production from grass/clover swards has been less than 75% that of grass swards receiving 400 kg N ha^{-1} (Orr *et al*., 1990).

Performance of red clover either grazed or as silage with perennial ryegrass has been reviewed by Frame *et al*. (1998), with many of the references dating from the 1970s and 80s since there is a paucity of recent work. Comparing well made red clover silage with grass silage at similar digestibility, the former would be expected to produce up to 30% more liveweight gain in beef cattle and over 10% increase in milk production over the latter. Production of Red deer grazing red clover have shown benefits of 10-30% over grass and grass-white clover swards.

The earlier estimates of animal production from legumes being higher than more recent studies are probably related to lack of account being taken of reduced gut fill. This has been overcome by taking account of increased daily intake when measuring animal performance based on liveweight.

Antiquality factors

Bloat (tympanites) is a potential hazard in grazed swards containing clovers or lucerne. In reality it is not as prevalent as it is perceived to be. This is enforced by the absence of any cases of bloat in published systems trials with sheep, beef cattle or dairy cows or in monitored on-farm studies. Remedies have been developed to counter bloat (e.g. adopting management which does not involve rapid rise in legume in the diet, ensuring sufficient fibre content is maintained, administering antifoaming agents or lipid based chemicals). Condensed tannins are known to reduce bloat in grazing ruminants and so while the clovers are essentially free from condensed tannins birdsfoot trefoil and sainfoin have high concentrations. The prospect of selecting for high condensed tannin content in the clovers and lucerne is remote and so means of introducing the capability into these species to synthesise condensed tannins are being explored (Morris and Robbins, 1997). Although various forms of hybridisation between tannin-containing and tannin-free species have been proposed, and some attempted, production of transgenic clovers and lucerne seems the most promising route.

An alternative to the introduction of condensed tannin synthesis as a means of reducing the bloating factor in legumes is selection for low rates of cell wall digestion. However this has not resulted in obvious improvement (Hall *et al*., 1994). Irrespective of the pathway to success, removing the bloating factor in legumes, such as white clover, will undoubtedly increase confidence in their use.

Isoflavones may be in sufficiently high concentrations in leaves of some forage legumes (e.g. lucerne, red clover, subclover) to interfere with the reproductive cycle of grazing sheep. Cultivars low in formononetin, the principal cause of the effect, have been bred e.g. red clover in Australia and New Zealand. Other antiquality problems include high levels of saponins in lucerne, and of cyanogenesis in some cultivars of white clover. However, they also play a role in the plant’s resistance to pest attack.

For all of these antiquality problems, the presence of grass in mixed swards potentially dilutes the effect and reduces the risk.

Predictability of the grass-legume association

The grass-legume association is particularly complex as not only do the components interact competitively but the transfer of N from the legume to grass contributes to the complex interaction between the two. Further, the environmental optima of both may vary and the *Rhizobium*-legume association also has particular requirements. Management, especially the grazing behaviour of animals, may also influence the association and in turn the composition of the sward may influence the animal in terms of amount of nutritive value and antiquality factors.

Persistence of forage legumes in mixture is dependent on a number of strategies. In the case of white clover production of stolons ensures the survival of the legume in the long term, while subclover’s persistence is directly related to production of seed. However seed quality is also important in the latter, soft seed increasing the potential for undesirable premature germination. Proportion of soft seed is a function of early maturity warm moist conditions during seed set (Fairbrother and Lowe, 1995) and is cultivar dependent (Blumenthal and Ison, 1994). Red clover's persistence is related to late flowering, wide crowns, high stem population density, adventitious root formation and high autumn production of crown buds for spring stem production (Montpetit and Coulman, 1991). A red clover variety, Astred, capable of producing rhizomes and daughter plants has been developed in Australia as suitable for close grazing.

While competition from grasses will have a strong influence on a legume's persistence, stress independent of competition will also affect its longevity. Legumes are renowned generally for their susceptibility to pests and diseases, including viruses, bacterial and fungal diseases, to heavy metal excess in soil, to low pH and to trace and essential mineral deficiencies. Some forage legume species are excluded from specific areas due to their poor tolerance to drought or to low temperatures. Some are less susceptible to given stress factors than others, e.g. birdsfoot trefoil is more tolerant of acid, low mineral soils than white clover, and through successful breeding programmes some varieties within a species are more tolerant of selected stresses than others.

Breeding for increased tolerance to biotic and environmental stress

Lucerne cultivars have been bred with resistance to most of the commonly occurring diseases although success has been limited for resistance to spring black stem (*Phoma medicaginis* var. *medicaginis*), sclerotinia crown and stem rot (*Sclerotinia trifoliorum*) and *Rhizoctonia* crown and bud rot (Barnes, 1992). Modern cultivars are resistant to nematodes, and resistance to viruses is currently being developed using biotechnology. To ensure winter hardiness in areas with hard winters dormancy has been a major priority in breeding although combining winter hardiness with autumn growth is an on-going challenge. Progress towards breeding tolerance to high free Al has been slow despite considerable effort (Frame *et al*., 1998).

Due to the activity devoted to breeding white clover, 319 known cultivars were identified in the mid 1990s (Caradus and Woodfield, 1996). Past breeding programmes have concentrated on the cultivar's ability to withstand a given management regime although existing cultivars also vary, for example, in their susceptibility to clover rot and to stem nematode. Ongoing breeding using conventional methods, hybridisation and transgenic technology is aimed at increasing resistance to drought, root-invading nematodes, and increasing tolerance to acid and mineral deficient conditions. Breeding for cold hardiness and spring growth, until recently considered to be mutually exclusive, has resulted in a cultivar which combines both attributes (Fothergill *et al*., 1997).

Companion grasses

Compatibility of grasses with legumes depends on the morphology and physiological characteristics of the grass and legume, in combination with the response of each to management imposed and the climate, and soil and biotic conditions under which the crop is growing. The temperate grasses known to be less aggressive are the most compatible with the forage legumes. Hence meadow fescue (*Festuca pratensis*) is the least competitive while cocksfoot (*Dactylis glomerata*) and tall fescue (*Festuca arundinacea*) are the most aggressive (Frame *et al*., 1998). In practice, where forage grass-legume mixtures are used widely in agriculture perennial ryegrass (*Lolium perenne*) is grown with white clover, for example in

New Zealand, and meadow fescue and timothy (*Phleum pratense*) are sown with red clover, as in Sweden. Open-structured swards generally are more compatible with white clover than dense swards, tetraploid perennial ryegrass being less aggressive than diploid types (Gooding and Frame, 1997). Density of the above ground canopy, especially at the base of the sward, may also explain aggressiveness of *Holcus* and *Agrostis* towards white clover.

Ecologically, grasses grown with white clover are more compatible with the ecotypes of white clover with which they were growing naturally than with other types of white clover and, indeed *Rhizobium* (Expert *et al*., 1997). The basis of compatibility between grass and legume may be spatial or temporal and has been exploited in the white clover breeding programme at IGER at Aberystwyth (Rhodes and Ortega, 1997).

Subclover is at a competitive disadvantage when grown with aggressive perennial grasses whereas *Phalaris aquatica* allows subclover seedlings to develop relatively freely during the early stages in a sward's development. Less moisture is lost from the upper strata of the soil profile when companions of low green biomass are grown, resulting in a higher proportion of seeds germinating and more seedlings developing in dry environments e.g. the wheat belt of eastern Australia. However, prolonged association with *Phalaris* can outcompete subclover and so management strategies have to be implemented to curb *Phalaris*' aggression (Dear *et al.*, 2000).

White clover has poor radiation use efficiency due to grass overtopping the clover canopy in spring, the higher proportion of light absorbed by clover than its contribution to leaf area would suggest, higher specific shoot and root respiration in clover than grass and the additional energy cost due to N_2-fixation (reviewed by Nassiri, 1998). While potential to improve the first of these by management or breeding is discussed later, more fundamental breeding criteria need to be introduced to deal with the other limitations. However an understanding of these factors provides the necessary insight to devise breeding strategies either conventionally or by resort to molecular techniques.

Legumes have some competitive advantages over companion grasses including:

a) the N_2-fixing ability in N deficient soils,
b) occupancy in the upper horizons of the canopy at some stages in the mixture's growth cycle,
c) capacity to outcompete grasses for some nutrients e.g. divalent Ca and Mg, due to higher cation exchange capacity in red clover.
d) ability in some instances to acidify rhizosphere to solublise P more efficiently than grasses
e) in some instances (lucerne and, to a lesser extent, red clover) are deep rooting
f) more highly mycotrophic and so can exploit association with arbuscular mycorrhiza more easily than grasses and benefit, for example, in P deficient soils.

However due to the 'altruistic' characteristic of legumes to provide their accompanying grass with nitrogen, the more the legume flourishes, the greater is the potential for the accompanying grass to be competitive (Chapman *et al*., 1996). Therefore, the challenge in managing long-term grass-legume swards is to maintain a consistent balance between legume and grass by controlling the advantages of each of the components.

Management to control grass-legume balance

While inclusion of a companion grass will invariably increase the total annual yield of swards containing white clover and red clover, only exceptionally will it increase yield of swards containing lucerne. Management of mixtures containing lucerne are difficult to maintain as a cutting frequency which suits one is detrimental to the other (Jung *et al*., 1996). White clover contribution, on the other hand, is relatively insensitive to cutting interval over the cutting interval range 7 to 42 days (Fisher and Wilman, 1995) suggesting that perennial ryegrass and white clover's response to reduced cutting frequency is similar, albeit within the range in this experiment. Lucerne contribution declined markedly when cutting interval was reduced from 6 to 3 weeks. As the erect legumes are grown primarily for conservation, this is not a serious problem. However it limits their flexibility in grassland systems as it reduces their use as an alternative pasture for grazing. Some advances are being made in the development of lucerne and red clover cultivars to withstand grazing.

The scope for manipulating the content of legume in grass-legume mixtures by sowing rate or method of sowing is limited. Despite marked differences in the canopy of forage legumes, e.g. comparing white clover and lucerne, they respond similarly to varying companion grass and legume seeding rate or spacial arrangement of the two i.e. any effect is short term. This demonstrates the close interaction between the two components and the robustness of an equilibrium which is essentially independent of their previous status, environmental conditions being more influential.

In direct drilling (no-till) situations establishing legumes are at a disadvantage when at the seedling stage, being more prone to pests or less able to compete physiologically. Allelopathy from sown grasses has also been shown to be involved e.g. tall fescue and cocksfoot against lucerne (Chung and Miller, 1995). Protocols for introducing the stoloniferous white clover into existing swards have been developed, the principal management procedures being designed to reduce grass competitiveness. Although direct drilling is a recommended method for reintroducing some legumes into existing swards risk of failure is high, especially in difficult soils unless specific guidelines are followed.

Reducing competitiveness of grass by avoiding addition of mineral N to the soil is of major benefit to the legume. This applies generally to forage legumes. In grass/white clover swards, application of only 66 kg N ha^{-1} reduced white clover contribution, even in autumn when clover contribution is only half that of the 0 N treatment, the increased competition resulting in fewer stolon growing points than leaf production per stolon (Fisher and Wilman, 1995)

White clover is particularly sensitive to grass competition in spring as it is less able to extend its petioles into the upper stratum of the canopy at that time of year. Also at the end of winter, fewer branches appear at nodes on stolons which have been produced over autumn and winter than those of comparable position on stolons in summer, even when cultured under optimum conditions (Teuber and Laidlaw, 1998). Potential photosynthesis of white clover increases due to early spring defoliation, which also results in an increase in growing point density and clover contribution later in the season. Removing competition from grass by application of grass suppressants in late autumn results in branches being produced at recently produced nodes, if temperatures allow, increasing white clover growth in spring (Patterson *et al*., 1995). By implementing management which reduces aggression of grass over mild winters in temperate maritime regions, attainment of very low clover levels may be avoided, even at stages in the cycle in long term swards when low clover contribution would be expected.

Change in the balance between grass and legume when N fertilizer is applied is well documented and the underlying causes of the change have implications not only for mixed swards receiving fertilizer but also for grazed swards in which mineralised N in excreta will influence the sward similarly. The change in balance brought about by increased mineral N application may be due to:

a) reduced radiation use efficiency (RUE) of clover
b) additional energy requirement to raise leaf laminae to the top of the canopy
c) reduced branching due to lower R:FR at the base of the sward

d) increased death of young branches.

Cycles in the relative contribution of grass and legume, especially in grazed swards have been observed over periods of years. Such cycles seem to be prevalent in pasture maintained under relatively constant management conditions and in which the legume would otherwise thrive if competition from grass was absent. The cycle is initiated due to the legume providing N, especially under conditions of low soil mineral N, to the benefit of grass and the detriment of the legume. However the reduction in the legume gradually causes the input of N to decline due to N leaching, volatilization, denitrification and offtake exceeding N supply. Grass growth is reduced and the remnants of legume in the pasture are able to re-establish a strong legume component in the sward. Such cycles may be partly responsible for the reported 'clover crashes' which have been extreme cases of white clover decline in recent decades although other factors, such as intraspecific competition are also implicated (Fothergill *et al*., 1996).

Schwinning and Parsons (1996a,b) have developed a model to provide further understanding of the dynamics of nitrogen in grazed grass-clover swards. While at extreme soil mineral N concentrations one or other of the components is excluded, at fixed intermediate soil N concentrations the model does not predict coexistence. To achieve this, soil N levels have to vary, the variability having to be influenced by the plants. This results in cycles as described above but lags develop due to the slow response of soil N levels to the sources of supply. Due to these lags, cycles have a periodicity of at least 4 years. Patches develop in the field varying in the stages of the cycle and possibly with different periodicities. Thus the average legume content over the field eventually becomes stable although the content of legume in the patches is constantly changing.

Grazing effects

Grazing effects on grass/white clover have been recently reviewed by Frame and Laidlaw (1998). The impact of grazing may be immediate, due to direct impact of defoliation and deposition of excreta, and longer term due to the changes invoked by the change in environment and removal of foliage either due to passive or active selection. Legumes tend to make a higher contribution to the upper layers in the canopy compared with the basal layers e.g. in grass-white clover swards. Consequently the legume content in the layer of herbage removed by a grazing animal from the top of the canopy will contain more legume than the content of the sward above ground level. The legume content in the diet of the animal may be increased further by active selection, the legume being intentionally selected within the sward canopy. Alternatively, if the area is heterogeneous with regard to legume, patches of pasture may be chosen to provide the animal with its desired legume content.

The growth of white clover relative to its companion grass is generally lower under grazing than cutting. The optimum system under which white clover should be managed depends on the definition of optimum. For example, white clover may contribute more under rotational grazing than continuous grazing (e.g. Reyneri *et al*., 1996) but intermittent grazing may result in white clover being less able to withstand stress, such as drought (Brock and Hay, 1993). There may also be valid environmental reasons for maintaining white clover content below a given level as well as benefits to animal health, such as minimising the risk of bloat.

The stability between grass and clover in association in grazed swards is important in the context of nitrogen dynamics within mixed grazed swards. In addition to the changes which take place in soil N levels within patches and the corresponding effect on white clover growth, rejected areas in continuously grazed grass/white clover swards may be havens of uninterrupted growth for white clover. These may allow white clover to recover more rapidly from severe defoliation than grass and so increase its contribution to herbage (Teuber and Laidlaw, 1996). Consequently, in swards continuously grazed by cattle, such areas may buffer decline in clover content and maintain reasonably constant annual levels or impose only gradual decline from very high levels. In contrast to cattle- grazed swards, reduction in white clover content in sheep grazed swards can be rapid under continuous grazing (Orr *et al*., 1990). This is partly accounted for by rapid branching in the early stages of sheep grazing. However, repeated defoliation at short intervals reduces stolon branching, a reduction in water soluble carbohydrates, especially in stolons. Removal of stolon apices when defoliation is severe reduces branching (Brink, 1996). Incorporation of a 'rest period', possibly for conservation, into a continuous stocking system can improve white clover clover's subsequent contribution to the sward (Gooding *et al*., 1996).

Continuously grazed swards with cattle at low stocking rates is detrimental to white clover as sustained tall swards will deprive the base of the sward with light (especially with a high red:far red ratio) (Teuber and Laidlaw, 1995) and high energy expenditure in petioles extending to the top of the tall sward (Nassiri, 1998). So underutilized swards may exhibit higher clover contribution in the early stages of the sward's life but the decline may be rapid, relative to more highly stocked swards (Steen and Laidlaw, 1995). White clover content also fluctuates from year to year in rotationally grazed swards. Where very low white clover levels are due to periods of aggressive grass growth caused by increasing soil nitrogen, reducing grass competitiveness in spring by close grazing provides clover stolons with an opportunity to branch (Laidlaw *et al*., 1992).

Sheep grazing generally results in stolons with thin short internodes and small leaves. Cattle grazed swards have higher clover content than sheep grazed swards and so lambs grazing after cattle have higher liveweight gains than when following sheep (Wright *et al*., 1992). Goats also consume proportionately less clover than sheep when grazing to similar sward heights and again lambs following goats have higher liveweight gains than when following sheep grazing white clover swards (del Pozo *et al*., 1996). In studies of the preference of sheep and goats for grass and clover in a free choice situation, sheep and cattle choose about 70% of their diet as clover while goats choose about 50% (Penning *et al*. 1997).

Excretal deposition will influence the legume content due to the high concentration of mineral N applied in readily mineralisable urine. Defoliation and excreta deposition create disturbance to a uniform sward and change the cycle periodicity for that patch i.e. change the oscillation rate (Schwinning and Parsons, 1996a). Localised deposition of N increases soil mineral N content disturbing the progress towards equilibrium between species in the patches but providing sites for clover in the longer term as N declines in these patches and clover from adjacent patches can encroach.

Environmental issues involving forage legumes

Increasingly in some countries, simple cost-benefit considerations are not the principal and only criterion for choice of forage or pasture type as environmental considerations have to be taken into account. In such areas where high fertilizer use, especially N fertilizers, is prohibited to protect water supplies from excessive nitrate content, the prospect for increased legume use is good. However in other regions where grass-legume associations are still viewed as being unstable and short-term, the dependability of grass species adapted to that area is likely to continue to be a major factor in determining the type of forage used. The challenge is to translate the advances which have been made into easily adopted systems of management which will provide long term stable mixtures allowing the benefits of legumes to the N economy and to animal production to be fully exploited.

As already discussed, efficiency of dietary N usage in legumes is lower than grass, mainly as a consequence of low ruminal fermentation caused by low available C. In general, however, the amount of N likely to be lost from a production system is most dependent on the amount of N within the system rather than whether it has been derived from fertilizer, organic manures or fertilizer, although the routes of loss may vary. A series of scenarios has been considered for a typical dairy farm in the S W of England (Jarvis *et al*., 1996). Comparing a farm dependent on white clover contributing 144 kg N ha^{-1} as fixation with one receiving 250 kg N ha^{-1} from N fertilizer, they estimated that loss from the former (in leaching, denitrification and volatilization) was only 56% that from the latter, albeit that the stocking rate of the clover-based farm was only 80% of the fertilizer N system. Leaching of N from the clover system was half that from the grass/N system. Maintaining a relatively low clover content to fix just over 70 kg N ha^{-1} was enough to sustain milk production equivalent to 80% of a system receiving 250 kg N ha^{-1} and reduced N losses by almost 60%. However, while the benefit of the legume to the N economy of the sward is exploited highly efficiently where clover content is low, the requirement for rapid N cycling may not be met and the animal does not derive much benefit from the potential nutritive value of the legume at such low levels in the sward. Introducing maize into the high clover system resulted in a reduction in N loss of 65% compared with the fertilizer N system, NH_3 volatilization in particular being reduced most.

In an actual comparison of N inputs and outputs in a range of systems in New Zealand Ledgard *et al*.(1999) found the grass/clover system to be the least wasteful of N, the mean N loss being on average over 3 years 55% that of a similar system receiving 200 kg N ha^{-1} and stocked at a similar rate to that of the clover system. Leaching in the clover system relative to the 200N system varied from less than 30% to almost 50%. The reduction in loss is of a similar order to that estimated by Jarvis *et al*. (1996) and demonstrate the robustness of the estimates.

Losses of N, especially as leachate, depend on the weather. Losses from grass-legume swards due to leaching in NE USA have been found to be higher subsequent to a drought than in a ‘normal’ year (Stout *et al*., 2000) due, among other factors, to a higher amount of senescent plant residue from N-rich legume plants.

Efficiency of processes rendering N available from subclover in a ley-wheat system in Australia has been shown to be low with less than 1/3 of N in clover roots and shoots being mineralised within 6 months of incorporation into the soil. In contrast urine N was both readily lost and taken up by the subsequent wheat (each about 20-25% of which 10% was lost to leaching) (Thompson and Fillery, 1997).

Grass-legume swards are potential sources of high loss when ploughed and cultivated as in excess of 200 kgN ha^{-1} can be lost by leaching. Reduction in loss of N_2O and in mineralisation following ploughing might be reduced by either cutting and avoiding grazing for a long enough period prior to ploughing to reduce organic matter degradability and reduce the amount of plant residue with mineralisable N. Ploughing in spring also releases less mineral N from grass/white clover compared with autumn ploughing (Høgh-Jensen, 1996).

In the UK, attempts have been made to validate a model of N flow in a grass/white clover system. Shortcomings in prediction of N_2-fixation and drainage rates as well as possible oversimplification of the grass-legume interaction contribute to inaccuracies in the simulation of N flow in grass/clover systems have become apparent (Wu and McGechan,1999). Nevertheless such models provide feasible explanations for the sustainability of grass-legume systems, conditional on return of nutrients, e.g. slurry, and quantify the build-up of organic N in the soil in N balances in grassland systems.

Prospects for forage legumes

Currently the contribution which legumes in mixed swards make to forage production varies widely from country to country. New Zealand relies heavily on white clover as a source of N for grassland, although the average content in swards is about 20% of herbage DM (Clark and Harris, 1996). In Sweden and Canada, for example, where conserved herbage is important in ruminant production, red clover and lucerne make significant contributions to herbage production. While seed sales of forage legumes, especially white clover, would suggest that much of the reseeded grassland in the UK is rich in white clover initially, uptake of legume based systems is slow despite favourable production and economic comparisons with fertilizer based systems (reviewed by Doyle and Bevan, 1996). However, The economic argument for increased reliance on forage legumes is dependent on the cost of nitrogen fertilizer relative to other costs and the price of the commodity produced. Even in a country such as New Zealand where nitrogen fertilizer is relatively expensive and milk prices are dictated by world markets the economic optimum in dairying is to apply about 100 kg N ha^{-1} to grass/white clover swards (Clark and Harris, 1996).

In addition to the potential role which forage legumes can play in alleviating nitrogen loss from ruminant production systems, already discussed, and in rural environmental enhancement programmes (such as the Rural Environmental Protection Scheme in Ireland), premiums paid for organic produce also offer incentive for increased reliance on forage legumes. Estimates of demand for organic produce vary but currently demand well outstrips supply in many countries. Sweden, for example, (a country in which most of the grassland has been sown with either red or white clover) has set targets for a proportion of its agricultural produce to be ‘organic’. The government has encouraged its uptake by appointing specialist advisers, subsidising organic farming and channelling research effort into organic farming. In Japan, where organically produced milk can be double the price of conventionally produced milk, the prospect of white clover or birdsfoot trefoil being integrated into viable systems is promising. Therefore forage legumes have a major role to play in such systems not only as a forage but also as a valuable source of nitrogen and a means of preventing build-up of weeds and soil-borne diseases in the following arable crop (Younie and Hermansen, 2000).

Within the European Union, a commitment to reform includes aims to develop agriculture’s ability to compete in world markets without subsidy, using methods unharmful to the environment to produce high-quality products (European Commission, 1998). Also, it is expected that with removal of livestock headage payments, more emphasis will be placed on production per animal. Forage legumes have the potential to contribute positively to all of these aims

Underlying trends in some countries of reduced fertilizer usage (FAO, 1999) has both positive and negative implications for forage legumes. Reduction in nitrogen fertilizer usage has obvious advantages for developing legume usage, whereas reduced input of other nutrients is likely to hinder legume growth and development. Indeed, the choice of one of the principal legumes traditionally grown in an area may need to be reconsidered, and a species more tolerant of acid, low soil nutrient conditions, e.g. birdsfoot trefoil, may be more appropriate. This also justifies effort in breeding cultivars of the productive forage legumes with improved efficiency in nutrient uptake.

While much of the research on temperate forage legumes is concentrated on white clover and lucerne, this emphasis may preclude consideration of other legumes as more appropriate for given conditions. For example, the role of legumes as pioneers in improving N status of unimproved hill land such as greater lotus in New Zealand may have wider application than hitherto considered. The potential for the use of legumes to improve grassland in areas where the better land is required for crops for direct human consumption, such as in south east Asia, has yet to be fully evaluated. Other niche legumes are important regionally, such as arrow-leaf clover (*Trifolium vesiculosum*) in southern USA. Evaluation of forage legumes for given areas and regions is often restricted to the more common species due to limited resources preventing a more imaginative range of species to be included.

The value of strong regional research and development effort ensures that resource is channelled towards addressing specific problems raised in that region. In Eastern Canada forage legume seed sold accounts for almost 40% of the tonnage of all forage seed sold annually and research findings are channelled to the farmer by well developed programmes run by specialist advisers, although future funding is uncertain. Farmers are well aware of the value of forage legumes, especially for hay and silage. In contrast, in other regions research may be well ahead of the needs of farmers and so technology transfer may be a more urgent priority. An encouraging development in the link between research and extension has been on-farm monitoring of legume performance and output (Bax and Browne, 1995; Frankow-Lindberg *et al*., 1996). Records are collected which would not normally be kept, nor easily gathered, by the farmer and allow analysis of the contribution of the legume to the total farm enterprise. This can be compared to that expected from research findings and to indicate further research, if necessary.

For more fundamental research, which is vital to form the basis of new breeding strategies and appropriate management practices, international cooperation offers an effective means of continuing valuable research particularly in an environment of severe underfunding. This is demonstrated in funding offered by EU either within the major Framework programmes or within COST (European Co-operation in the field of Scientific and Technical Research). The multisite work on overwintering and spring growth of clover cultivars carried out in five cooperating countries in Europe is an example the value of this approach (Fothergill *et al*., 1997).

Risk and complexity are probably the two most serious obstacles to grass/legume uptake in countries which have a history of intensive farming based on grass/N fertilizer. As highlighted in this paper, science has made considerable advances towards reducing risk but there are still uncertainties. Further, many farmers who could derive most benefit from grass-legume swards are likely to be the least skilled in managing them. This raises the need for effective technology transfer. Unfortunately, many countries have either run down or disbanded their state-sponsored extension services and commercial interest is unlikely to provide the necessary advisory support for a product or philosophy which does not require the sale of a specific product. Reduction in extension personnel warrants implementation of computer technology to aid those remaining. Decision support systems for pasture management are currently being developed and used on an increasing scale, especially in Australia and New Zealand. Incorporating predictive functions for herbage growth and interaction between herbage components into them offer a potentially valuable technology transfer tool to reduce risk and increase predictability.

Acknowledgements

We wish to thank Professor J. Hodgson (New Zealand), Drs B.E. Frankow-Lindberg (Sweden), I. Higuchi (Japan), T. Kunelius (Canada), D. McGilloway (Ireland) and M. Zimkova (Slovakia) for supplying information about forage legume use in their respective regions and to Dr John Frame for constructive comments during preparation of the paper.

References

Asuaga, A. (1994). Use and production of Lotus corniculatus in Uruguay. In: Beuselink P.R. and Roberts C.A. (eds) *Proceedings of the First International Lotus Symposium, St Louis, USA*. pp. 134-141.University of Missouri-Columbia Press.

Baker, M.J. and Williams W.M. (1987). *White Clover*. Wallingford:CAB International

Baraibar, A., Frioni L., Guedes M.E. and Ljunggren H. (1999) Symbiotic effectiveness and ecological characterization of indigenous *Rhizobium loti* populations in Uruguay. *Pesquisa Agropecuaria Brasileira*, **34:** 1011-1017.

Barry, T.N. and McNabb W.C. (1999). The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition*, **81:** 263-272.

Bax, J.A. and Browne I. (1995). *The Use of Clover on Dairy Farms*. Research Summary, Milk Development Council, London, 21 pp.

Barnes, D.K. (1992). Forage legume breeding past, present and future. *Proceedings of the 14th General Meeting of the European Grassland Federation, Lahti*, 78-86.

Beever, D.E. and Thorp C. (1996). Advances in the understanding of factors influencing the nutritive value of legumes. In: Younie D. (ed.*) Legumes in Sustainable Farming Systems*. pp. 194-207. Reading: British Grassland Society.

Blumenthal, M. and Ison R. (1994). Plant population dynamics in subterranean clover and murex medic swards. 1. Size and composition of seed bank. *Australian Journal of Agricultural Research*, **45:** 913-928.

Brink, G.E. (1996). White clover response to clover apex removal. In Phillips T. (ed.) *Proceedings of the 14th Trifolium Conference*. p. 31. Lexington, Kentucky.

Brock, J. and Hay R.J.M. (1993). An ecological approach to forage management. In: Baker M.J. (ed.) *Proceedings of the XVII International Grassland Congress.* pp. 837-842. Palmerston North: New Zealand Grassland Association, etc.

Caradus, J.R. and Woodfield D.R. (1996). World checklist of white clover varieties. *New Zealand Journal of Agricultural Research*, **40:** 115-206.

Chapman, D.F., Parsons A.J. and Schwinning S. (1996). Management of clover in grazed pastures: expectations limitations and opportunities. In: Woodfield D.R. (ed.) *White Clover: New Zealand's Competitive Edge*. Grassland Research and Practice Series No. 6. pp. 55-64. Palmerston North: New Zealand Grassland Association.

Chung, I.M. and Miller D.A. (1995). Allelopathic influence of nine forage grass extracts on germination and seedling growth of alfalfa. *Agronomy Journal*, **87:** 767-772.

Clark and Harris (1996). White clover or nitrogen fertilizer for dairying? In: Woodfield D.R. (ed.) *White Clover: New Zealand's Competitive Edge*. Grassland Research and Practice Series No. 6. pp. 107-114. Palmerston North: New Zealand Grassland Association.

Doyle, C.J. and Bevan K. (1996) Economic effects of legume-based grassland. In: Younie D. (ed.) *Legumes in Sustainable Farming Systems*. pp. 247-256. Reading: British Grassland Society

Dado, R.G and Allen M.S. (1996). Enhanced intake and production of cows offered ensiled alfalfa with higher NDF digestibility. *Journal of Dairy Science*, **79:** 418-428.

Davies, D.A. and Hopkins A. (1996). Production benefits of legumes in grassland. In: Younie D. (ed.) *Legumes in Sustainable Farming Systems*. pp. 234-246. Reading: British Grassland Society

Dear, B.S., Cocks P.S., Peoples M.B., Swan A.D. and Smith A.B. (1999). Nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.) growing in pure culture and in mixtures with varying densities of *lucerne (Medicago sativa* L.) or phalaris (*Phalaris aquatica* L.). *Australian Journal of Agricultural Research,* **50:** 1047-1058.

Dear, B.S., Cocks P.S., Swan A.D., Wolfe E.C. and Ayre L.M. (2000). Effect of phalaris (*Phalaris aquatica* L.) and lucerne (*Medicago sativa* L.) density on seed yield and regeneration of subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Agricultural Research*, **51:** 267-278.

Del Pozo, M., Wright I.A., Whyte T.K. and Colgrove P.M. (1996). Effects of grazing by sheep or goats on sward composition in ryegrass/white clover pasture and on subsequent performance of weaned lambs. *Grass and Forage Science*, **51:** 142-154.

Dubach, M. and Russelle M.P. (1994). Forage legume roots and nodules and their role in nitrogen transfer. *Agronomy Journal*, **86:** 259-266.

Eriksen, J. and Høgh Jensen H. (1998). Variations in the natural abundance of ^{15}N in ryegrass/white clover shoot material as influenced by cattle grazing. Plant and Soil, **205:** 67-76.

European Commission (1998). *The Agricultural Situation in the European Union*. 1998 Report. Brussels: European Commission.

Expert, J.M., Jacquart P., Obaton M. and Luscher A. (1997). Neighbourhood effect of genotypes on *Rhizobium leguminosarum* biovar *trifolii*, *Trifolium repens* and *Lolium perenne*. *Theoretical and Applied Genetics*, **94:** 486-492.

Fairbrother, T.E. and Lowe D.E. (1995). Temperature and soil water effects on dormancy and mortality of subclover seed. *Agronomy Journal*, **87:** 252-257.

FAO (1999) *FAO Yearbook 1998*. Rome: Food and Agriculture Organisation.

Farnham, D.E. and George J.R. (1994). Dinitrogen fixation and nitrogen transfer in birdsfoot trefoil and orchard grass communities. *Agronomy Journal*, **86:** 690-696.

Fisher, A. and Wilman D. (1995). Effect of interval between harvests and spring applied fertilizer N on the growth of white clover in mixed swards. *Grass and Forage Science*, 50, 162-171.

Fothergill, M., Davies D.A., Morgan C.T. and Jones J.R. (1996). White clover crashes. In: Younie D. (ed.) Legumes in Sustainable Farming Systems. pp. 172-176. Reading: British Grassland Society.

Fothergill, M., Collins R.P., Rhodes I., Frankow-Lindberg B.E., Guckert A., Guichard M.P., Robin C., Luscher A., Nosberger J., Staheli B. and Stoffel S. (1997). Overwintering and spring growth of white clover: a European perspective. In: Waddell J.R. (ed.) *Seeds of Progress*. Occasional Symposium No. 31. pp. 160-163. Reading: British Grassland Society

Frame J. (1990). The role of red clover in the United Kingdom pastures. *Outlook on Agriculture*, **19:** 49-55.

Frame, J. and Laidlaw A.S. (1998). Managing white clover in mixed swards; principles and practice. *Pastos*, **28:** 5-13

Frame, J., Charlton J.F.L. and Laidlaw A.S. (1998). *Temperate Forage Legumes*. Wallingford: CAB International

Frankow-Lindberg, B.E., Danielsson D.A. and Moore C.A. (1996). The uptake of white clover technology in farm practice. *FAO,REUR Technical Series No. 42*. pp. 37-43. Rome: FAO.

Gooding, R.F., Frame J. and Thomas C. (1996). Effects of sward type and rest periods from sheep grazing on white clover presence in white clover/perennial ryegrass associations. *Grass and Forage Science*, **51:** 180-189.

Gooding, R.F. and Frame J. (1997). Effects of continuous sheep stocking and strategic rest periods on the sward characteristics of binary perennial ryegrass/white clover associations. *Grass and Forage Science*, **52:** 350-359.

Hall, J.W., Majat W., Stout D.G., Cheng K.J., Golpen B.P. and Howarth R.E. (1994). Bloat in cattle fed alfalfa selected for a low initial rate of digestion. *Canadian Journal of Animal Science,* **74:** 451-456.

Hanson, A.A., Barnes D.K. and Hill R.R. (1988). *Alfalfa and Alfalfa Improvement*. Agronomy Monograph No. 29. Madison, Wisconson: ASA/CSSA/SSSA.

Harris, S.L., Auldist M.J., Clark D.A. and Jansen E.L.B (1998). Effects of white clover content on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. *Journal of Dairy Research*, **65:** 389-400.

Heichel, G.H. and Henjum K.I. (1991). Dinitrogen fixation, nitrogen transfer and productivity of forage legume-grass communities. *Crop Science*, **31:** 202-208.

Heikkila, T., Toivonen V. and Mela T. (1992). Comparison of red clover-grass silage with grass silage for milk production. *Proceedings of the 14th General Meeting of the European Grassland Federation, Lahti, Finland*, 388-391.

Høgh Jensen, H. (1996). Nitrogen recovery after clover/ryegrass leys. In: Younie D. (ed.) *Legumes in Sustainable Farming Systems*. pp. 130-132. Reading: British Grassland Society.

Høgh Jensen, H. and Schjoerring J.K. (1997). Interactions between white clover and ryegrass under contrasting nitrogen availability: N_2 fixation, N fertilizer recovery , N transfer and water use efficiency. *Plant and Soil*, **197:** 187-199.

Jarvis, S.C., Wilkins R.J. and Pain B.F. (1996). Opportunities for reducing the environmental impact of dairy farming managements: a systems approach. *Grass and Forage Science*, **51:** 21-31..

Jung, G.A., Shaffer J.A. and Everhart J.H. (1996). Harvest frequency and cultivar influence on yield and protein of alfalfa-ryegrass mixtures. *Agronomy Journa*l, **88:** 817-822.

Laidlaw, A.S., Teuber N.G. and Withers J.A. (1992). Out-of-season management of grass/clover swards to manipulate clover content. *Grass and Forage Science*, **47:** 220-229.

Laidlaw, A.S., Chrisie P. And Lee H.W. (1996). Effect of white clover cultivar on apparent transfer of nitrogen from clover to grass and estimation of relative turnover rates in roots. *Plant and Soil*, **179:** 243-253.

Ledgard, S.F. (1991). Transfer of fixed nitrogen from white clover to associated grasses in swards grazed by dairy cows estimated using ^{15}N methods. *Plant and Soil*, **131:** 215-223.

Ledgard, S.F., Penno J.W. and Sprosen M.S. (1999). Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer. *Journal of Agricultural Science, Cambridge*, **132:** 215-225.

Mallarino, A.P., Wedin W.F., Perdomo C.H., Goyenola R.S. and West C.P. (1990). Nitrogen transfer from white clover, red clover and birdsfoot trefoil to associated grass. *Agronomy Journal*, **82:** 790-795.

Marriott, C.A., Smith M.A. and Brunton M.A. (1991). Effects of urine in white clover. FAO/REUR Technical Series No. 19. pp. 103-108. Rome:FAO.

Marten, G.C., Matches A.G., Barnes, R.F., Brougham R.W., Clements R.J. and Sheath G.W. (1989). *Persistence of Forage Legumes*. Madison, Wisconson: ASSA/CSSA/SSSA.

Mather, R.D.J., Melhuish D.T. and Herlihy M. (1996). Trends in the global marketing of white clover cultivars. In: Woodfield D.R. (ed.) *White Clover: New Zealand's Competitive Edge*. Grassland Research and Practice Series No. 6. pp. 7-14. Palmerston North: New Zealand Grassland Association.

Min, B.R., McMabb W.C., Barry T.N. and Peters J.S. (2000). Solubilization and degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC4.1.1.39:Rubisco) protein from white clover (*Trifolium repens*) and *Lotus corniculatus* by rumen microorganisms and the effect of condensed tannins on these processes. *Journal of Agricultural Science, Cambridge,* **134:** 305-317.

Monpetit, J.M. and Coulman B.E. (1991). Relationship between spring vigor and the presence of adventitious roots in established stands of red clover. *Canadian Journal of Plant Science*, **71:** 749-754.

Monza, J., Diaz P., Borsani O., Ruiz Argueso T. and Palacios J.M. (1997). Evaluation and improvement of the energy efficiency of nitrogen fixation in *Lotus corniculatus* nodules induced by *Rhizobium loti* strains indigenous to Uruguay. *World Journal of Microbiology and Biotechnology*, **13:** 565-571

Morris, P. and Robbins M.P. (1997). Manipulating condensed tannins in forage legumes. In: McKersie B.D. and Brown D.C.W. (eds) *Biotechnology and the Improvement of Forage Legumes*. pp147-174. Wallingford: CAB International.

Mulholland J.G., Nandra K.S., Scott G.B., Jones A.W. And Coombes N.E. (1996) Nutritive value of subterranean clover in a temperate environment. *Australian Journal of Experimental Agriculture*, **36:** 803-814.

Murray P.J. and Clements R.O. (1998). Transfer of nitrogen between clover and wheat: Effect of root herbivory. *European Journal of Soil Biology*, **34:** 25-30.

Nassiri Mahallati, M. (1998). *Modelling Interactions in Grass-Clover Mixtures*. PhD Thesis, Wageningen Agricultural University.

Nesheim, L., Boller B.C., Lehmann J. and Walther U. (1990). The effect of nitrogen in cattle slurry and mineral fertilizers on nitrogen fixation by white clover. *Grass and Forage Science*, **45:** 91-97.

Niezen, J.H., Waghorn T.S., Charleston W.A.G. and Waghorn G.C. (1995) Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *Journal of Agricultural Science, Cambridge*, **125:** 281-289.

Orr, R.J., Parsons A.J., Penning P.D. and Treacher T.T. (1990). Sward composition, animal performance and the potential production of grass/white clover swards continuously stocked with sheep. *Grass and Forage Science*, **45**: 325-336.

Patterson, J.D., Laidlaw A.S. And Mcbride J. (1995). The influence of autumn management and companion grass on the development of white clover overwinter in mixed swards. *Grass and Forage Science*, **50:** 345-352.

Penning, P.D., Johnston R.H. and Orr R.J. (1997). Effects of continuous stocking with sheep or goats on sward composition and animal production from grass and white clover pasture. *Small Ruminant Research*, **21:** 19-29.

Penning, P.D., Gibb M.J., Parsons A.J., Orr R.J. and Harvey A. (1998). Behavioural and physiological factors limiting intake by grazing ruminants. In Keane M.G. and O'Riordan (eds) *Pasture Ecology and Animal Intake*. European Union Concerted Action. AIR3-CT93-0947. Occasional Publication No.3. pp. 10-20. Dublin:Teagasc.

Reyneri, A., Grignani C. and Cavallero A. (1996). The role of white clover in south European grazing systems; the Po Valley situation. *FAO/REU Technical Series No. 42*. pp. 19-27. Rome:FAO.

Rhodes, I. and Ortega F. (1996). Progress in Forage Legume Breeding. In: Younie D. (ed.) *Legumes in Sustainable Farming Systems*. pp. 62-71. Reading: British Grassland Society.

Riffkin, P.A., Quigley P.E., Cameron F.J., Peoples M.B and Theis J.E. (1999). Annual fixation in grazed pastures in south-western Victoria. *Australian Journal of Agricultural Research*, **50:** 273-281.

Rogers, J.B., Laidlaw A.S. and Christie P. (2000). The role of arbuscular mycorrhizal fungi in the transfer of nutrients between white clover and perennial ryegrass. *Chemosphere* ,**42:** 51-57.

Rotili, P. (1993). Methods and procedures in variety constitution. In: Rotili P. and Zannone L. (eds) *The Future of Lucerne Biotechnology, Breeding and Variety Constitution*. Proceedings of the X International Conference of EUCARPIA. Medicago spp. Group. Pp. 499-508. Lodi, Italy: Instituto Sperimentale per le Colture Foraggere.

Ru, Y.J. and Fortune J.A. (1997). Effect of cultivar and grazing intensity in spring on shear and compression energies of dry mature subterranean clover (*Trifolium subterraneum* L.). *Australian Journal of Agricultural Research*, **48:** 1199-1206.

Russelle, M.P., Allan D.L. and Gourley C.J.P. (1994). Direct assessment of symbiotically fixed nitrogen in the rhizosphere of alfalfa. *Plant and Soil*, **159:** 233-243.

Schwinning, S. and Parsons A.J. (1996a). Analysis of the coexistence mechanisms for grasses and legumes in grazed systems. *Journal of Ecology*, **84**: 799-813.

Schwinning, S. And Parsons A.J. (1996b). A spatially explicit population model of stoloniferous N-fixing legumes in mixed pasture with grass. *Journal of Ecology*, **84:** 815-826.

Sharma, S.B., Hancock K.R., Ealing P.M. and White D.W.R. (1998). Expression of a sulphur-rich maize seed storage protein, delta zein, in white clover (*Trifolium repens*) to improve forage quality. *Molecular Breeding*, **4:** 435-448.

Steen, R.W.J. and Laidlaw A.S. (1995). The effect of fertilizer nitrogen on the stock-carrying capacity of ryegrass/white clover swards continuously grazed by beef cattle. *Irish Journal of Agricultural and Food Research*, **34:** 123-132.

Stockdale, C.R. (1992). The productivity of dairy cows fed irrigated subterranean clover herbage. *Australian Journal of Agricultural Research*, **43:** 1281-1295.

Stout, W.L., Fales S.L., Muller L.D., Schnabel R.R. and Weaver S.R. (2000). Water quality implications of nitrate leaching from intensively grazed swards in northeast US. *Agriculture Ecosystems and Environment*, **77:** 203-210.

Taylor, N.L. and Quesenberry K.H. (1996). *Red Clover Science*. Dordrecht: Kluwer Academic Press.

Taylor, N.L. and Smith R.R. (1995). Red clover. In: Barnes R.F., Miller D.A. and Nelson C.J. (eds) *Forages. Vol 1. An Introduction to Grassland Agriculture*. 5th Edition. pp. 217-226.Ames, Iowa: Iowa State University Press.

Teuber, N.G. and Laidlaw A.S. (1995). Effect of herbage rejection by steers on white clover (*Trifolium repens*) branching and development in continuously stocked grass-clover swards. *Journal of Agricultural Science, Cambridge*, **124:** 205-212.

Teuber, N.G. and Laidlaw A.S. (1996). Influence of irradiance on branch growth of white clover stolons in rejected areas within grazed swards. *Grass and Forage Science*, **51:** 73-80.

Teuber, N.G., Barrientos L. And Angulo L. (1996). Evaluación preliminar del aporte de nitrógeno por fijación simbiótica en praderas permanentes bajo pastereo en tres áreas de la Xa Región. *Proceedings of the XXI Congress of the Annual Meeting of the Chilean Society of Animal Production*, 31-32.

Teuber, N.G. and Laidlaw A.S. (1998). Efecto de luz suplementaria en la capacidad de ramification de un nudo en el estolon principal de trebol blanco. (Effect of supplementary light on white clover stolons branching). *Proceedings of the XXIII Annual Meeting of the Chilean Association of Animal Production*, 101-102.

Thompson, R.B. and Fillery I.R.P. (1997). Transformation in soil and turnover to wheat of nitrogen from components of grazed pasture in the south of *Western Australia. Australian Journal of Agricultural Research*, **48:** 1033-1047.

Titchen, N.M. and Philipps L. (1996). Environmental effects of legume based grassland systems. In: Younie D. (ed.) *Legumes in Sustainable Farming Systems*. pp. 257-261. Reading: British Grassland Society.

Tyrrell, H.F., Thomson D.J., Waldo D.R., Goering H.K. and Haaland G.L. (1992). Utilization of energy and nitrogen by yearling Holstein cattle fed direct cut alfalfa or orchardgrass ensiled with formic acid plus formaldehyde. *Journal of Animal Science*, **69:** 3163-3177.

Unkovitch, M.J., Sanford P. And Pate J.S. (1996). Nodulation and nitrogen fixation by subterranean clover in acid soils as influenced by lime application, toxic aluminium, soil mineral N and competition from annual grass. *Soil Biology and Biochemistry*, **28:** 639-648.

Waghorn, G.C., Shelton I.D. and Thomas V.J. (1989). Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. *British Journal of Nutrition*, **61:** 409-423.

Watson, C.A., Ross J.M., Bagnaresi U., Minotti G.F., Roffi F., Atkinson D., Black K.E. and Hooker J.E. (2000). Environment-induced modifications to root longevity in *Lolium perenne* and *Trifolium repens*. *Annals of Botany*, **85:** 397-401

Wightman, P.S., Franklin M.F. And Younie D. (1997). The effect of sward height on responses of grass/white clover to slurry application. *Grass and Forage Science,* **52:** 42-51.

Wilman, D., Mtengeti E.J. and Mosley G. (1996). Physical structure of 12 forage species in relation to rate of intake by sheep. *Journal of Agricultural Science, Cambridge*, **126:** 277-285.

Wright, I.A., Jones J.R and Parsons A.J. (1992). Performance of weaned lambs on grass/clover swards previously grazed by cattle or sheep. *Proceedings of the 3rd Research Conference, British Grassland Society, Greenmount College*, *Antrim*, 61-62.

Wu L. And Mcgechan M.B. (1999). Simulation of nitrogen uptake, fixation and leaching in a grass/white clover mixture. *Grass and Forage Science*, **54:** 30-41.

Younie, D. and Hermansen J. (2000). The role of grassland in organic livestock farming. In: Soegaard K., Ohlsson C., Sehested J., Hutchings N.J. and Kristensen T. (eds) *Grassland Farming: Balancing Environmetal and Economic Demands*. Grassland Science in Europe Vol. 5. pp. 493-513. European Grassland Federation.