FORAGE QUALITY AND THE ENVIRONMENT

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Abstract

The influence of environmental factors on forage quality of temperate and tropical grasses has been reviewed by several authors, who summarized how light, temperature, drought and soil nutrients influence chemical composition, and digestibility of forages grown in contrasting areas of the world. The effects of season of the year on forage growth, grazing behavior and animal performance have also been the subject of numerous papers and reviews. However, there are few recent reviews that summarize how changes in climatic and edaphic factors influence forage quality of legumes with variable levels of condensed tannins (CT), which are important secondary compounds in some temperate and tropical legume species adapted to acid infertile soils. In this paper we summarize properties of CT and their positive and negative effects on forage quality of legumes. We also review published work on the effect of temperature, drought, CO2 concentration, season of the year and soil fertility on the accumulation of CT in temperate and tropical legumes. Results from experiments under controlled conditions indicate that high temperature alone can significantly increase the accumulation of CT in some temperate legume species (i.e. *Lotus pedunculatus*) but not in others (i.e. *L. corniculatus*). However, the effect of low or high temperature on accumulation of CT is considerably greater when accompanied with other environmental factors such as drought, high CO2 concentration and soil nutrient deficiencies. Soil nutrient deficiencies can have a major effect on elevation of CT concentration and overall feed value of temperate and tropical legumes, but only when deficiencies are such that they affect plant growth. Soil fertility and climatic conditions affect not only the concentration of CT but also their monomer composition and MW (molecular weight), as was observed in a tropical legume species well adapted to acid infertile soils. The nutritional significance of these findings are not all that well understood, but it would seem that CT in forage legumes are not a uniform chemical entity given that they can change with edaphic and climatic factors. Finally we suggest that there is a need to investigate alternatives to enhance the feed value of legumes with tannins adapted to acid soils through selection of genotypes with less CT and /or through manipulation of environmental factors such as soil fertility. For this we need to better understand how edaphic and climatic factors affect not only accumulation of CT but also their chemical structure and biological activity and relate these changes to forage intake, digestibility, N utilization, and, ultimately, to performance of ruminant animals.

Keywords: Legumes, condensed tannins, temperate, tropical, acid soils, temperature, drought, soil fertility

Introduction

Environmental factors are well known to have a significant impact on the quality of forage plants, particularly those grown in environments with varying degrees of different stresses. These stresses can result in large variations, between seasons and between years, in forage yield and quality. Stress occurs when an environmental factor is not ideal for plant growth, for example, too low or too high a temperature, water logging, drought, shading, or a soil nutrient deficiency.

The influence of environmental factors on forage quality has been the subject of several reviews, most of which have emphasized how temperature, water stress, light, and soil nutrients influence growth and quality of forage grasses and, to a lesser extent, legumes (Wilson, 1982; Buxton and Casler, 1993; Buxton and Fales, 1994). An important conclusion of studies reviewed is that temperature usually has a greater effect on the digestibility of grasses than do other environmental factors, mainly through its effect on leaf-to-stem ratios, increases in the indigestible cell-wall fraction and concurrent reduction in nonstructural carbohydrates. In contrast, while shading reduces yields, in most cases, in grasses and legumes, it has small and variable effects on forage quality. The effect of drought on forage quality is usually low or even positive, particularly if the stress on leaf mass is not severe. Finally, the effect of soil nutrients on the forage quality of mainly grasses has been found to be relatively small. The application of N fertilizer results in higher yield and increased crude protein. Application of S and Ca to soils with deficiencies of these minerals may enhance forage digestibility through different mechanisms. Alleviating S deficiency through either fertilization or feed supplements for animals may increase forage digestibility through improved rumen fermentation. In the case of Ca applied as fertilizer, digestibility seems to improve through changes in plant cell-wall composition.

Several environmental factors have also been shown to affect quality attributes of forage legumes with variable levels of condensed tannins (CTs), which are important secondary metabolites in some temperate and tropical legume species. High concentration of CT in forage legumes can affect their intake and overall nutritional quality, thus limiting the forage's potential to enhance animal productivity. However, few recent studies have been published on how specific climatic and edaphic factors influence yield and quality of legumes with tannins.

In this paper, we summarize the positive and negative effects of CTs on ruminant nutrition, and examine the response of temperate and tropical legumes to environmental stresses in terms of forage yield and quality attributes. New evidence on the effect of environmental factors on the chemical properties of CTs in tropical legumes has led us to suggest foci for future research.

Limitations of some temperate and tropical forage legumes

Leguminous plants are used in temperate and tropical regions as feed resources in different production systems to improve the performance of ruminant animals and to improve soil nutrient cycling. Several herbaceous and woody legumes are potentially valuable as animal feed resources in regions characterized by acid low-fertility soils. In temperate regions, legumes such as *Lespedeza cuneata*, *Lotus pedunculatus*, and *Lotus* *corniculatus* have been shown to be well adapted to acid soils but also to accumulate the unpalatable condensed tannins (Armstrong 1974; Lowther, 1980; Donnelly, 1981; Scott and Mills, 1981; Scott and Charlton, 1983).

In tropical regions, woody and herbaceous legume species that are well adapted to acid low-fertility soils have also been shown to accumulate variable levels of CTs (Lascano *et al*., 1995; Jackson *et al*., 1996). Well-known woody legumes such as *Leucaena leucocephala*, *Gliricidia sepium*, and *Erythrina* spp. can significantly improve liveweight gains and milk yield when used as protein supplements in cut-and-carry or grazing systems (Saucedo *et al*., 1980; Izham *et al*., 1982; Suárez *et al*., 1987; Vargas *et al*., 1988). However, these species are not well adapted to acid soils with high levels of Al, found throughout large areas of tropical America (Argel and Maass, 1995).

Overviews of condensed tannins in forage legumes

Secondary metabolites, known as condensed tannins (CTs), are widely distributed in nature, as indicated by a survey, cited by Muller-Harvey and McAllan (1992), which listed about 80% of woody perennial dicotyledons and 15% of annual and herbaceous dicotyledons as containing tannins. These secondary compounds are polyphenols that are produced by plants as part of the shikimic acid pathway and which can form complexes with proteins, polysaccharides, nucleic acids, steroids, alkaloids, and saponins (Muller-Harvey and McAllan, 1992). Tannins are separated into two groups according to their chemical origin: hydrolyzable and condensed. Hydrolyzable tannins are polymers of phenolic acids (gallic acid, hexahydroxydiphenic acid, and/or derivatives), whereas condensed tannins (CTs) are polymers of falvan-3-ol that produce anthocyanidins on acid degradation (Fahey and Jung, 1989).

Complexes formed between CTs and protein are thought to be either reversible (hydrophobic and/or hydrogen bonds) or irreversible via oxidation of the phenolic compounds into reactive quinones (Fahey and Jung, 1989). The reversible complexes of CTs are stable and insoluble at pH 3 to 7, but are released at pH lower than 3 or higher than 8 (Jones and Mangan, 1977). This property of CTs to bind proteins at neutral pH and release them at low pH values has led many researchers to think that this could be a useful tool for reducing protein degradation in the rumen, thus promoting increased flow and absorption of non-ammonia N in the lower gut, and improving the animals' nutrition. This has been shown experimentally to occur when *Lotus corniculatus* was fed to ruminants (Waghorn *et a1*., 1987). In addition, some properties of CTs present in some legumes have been associated with bloat prevention in ruminants (Gutek *et al*., 1974; Ross and Jones, 1974; Chiquette *et al*., 1989). However, experimental evidence indicates that high concentrations of CTs present in some temperate and tropical legumes can negatively affect digestibility and intake of edible forage (Donnelly and Anthony, 1983; Barry and Duncan, 1984; Barry and Manley, 1984; Terrill *et al*., 1989; Barahona *et al*., 1997).

The positive and negative effects of CTs in forage legumes have been related to their concentration in edible leaves and stems, which concentration varies according to species (Lowther *et al*., 1987; Jackson *et al*., 1996), plant part (Foo *et al*., 1982; Barahona *et al*., 1997), genotype within species (John and Lancashire, 1981; Schultze-Kraft and Benavides, 1988), plant maturity (Lees *et al*., 1995), and environmental factors (Barry and Forss, 1983; Fales, 1984; Anuraga *et al*., 1993). The variation of CT levels with plant genotype and environmental conditions suggests that CT accumulation can be regulated through selection or breeding, and through targeting legumes to specific environmental “niches” that favor low CT concentration in edible forage.

In the next section, we will examine experimental findings on the effect of environmental factors on CT accumulation in temperate and tropical forage legumes.

Effect of environment on the quality of forage legumes with tannins

Three features characterize research published on the effect of environment on the quality of forage legumes with CTs: (1) work was limited to only a few genera and species grown in temperate zones, (2) work on tropical legumes is minimal, and (3) response variables were mostly limited to measurements of CT concentration, N, and *in vitro* digestibility and, in a few cases, to forage intake and/or animal performance.

Research on the influence of ambient temperature, soil moisture, and soil fertility on the forage quality of temperate legumes with CTs has been mainly done with *Lotus corniculatus*, *Lotus pedunculatus*, *Onobrychis vicifolia*, and *Lespedeza cuneata.* In the case of tropical legumes with CTs, the little work done examined the effects of environment on the forage quality of *Desmodium heterocarpon* subsp. *ovalifolium* (herbaceous legume, and better known under its earlier name of *D. ovalifolium*), and, to a lesser extent, *Calliandra calothyrsus* (a shrub legume).

Temperate legumes

Temperature. Results from early studies had shown that CT concentration in temperate legumes increased as the growing season advanced and that this was associated with increased mean day temperature and decreases in rainfall (Donnelly, 1959). However, under field conditions, isolating the confounding effects of day temperature and precipitation during the growing season from the concurrent aging of plants was impossible. In other studies where temperature was associated with changes in CT concentration in legumes, the observed responses were confounded with light intensity. Thus, to avoid these confounding effects, some studies were carried out under conditions of the controlled growth chamber or greenhouse.

The effects of low and high temperatures on quality attributes were tested in *Lespedeza cuneata* genotypes with low or normal tannin contents (Fales, 1984) and in *Lotus pedunculatus* clones with low, medium, or high CT contents (Lees *et al*., 1994), all under conditions of controlled environment. Results (Table 1) showed that CT concentration in foliar tissue of both legume species increased with temperature. In the leaves of the normal- and low-tannin *L. cuneata* genotypes, CT concentration was 10% and 2% units higher, respectively, at the high temperature than at the low temperature. Increases in CT concentration in *L. pedunculatus* as a result of temperature ranged from 3% to 5% units.

In experiments with *L. pedunculatus* (Lees *et al*., 1994), CT concentration increased over time in plants subjected to two temperature regimes (20^{o}C and 30^{o}C), but the increment was considerably faster and stayed higher in plants grown at 30^{o}C (Table 1). However, regrowth in plants grown at the lower temperature appeared healthy, whereas plants grown at the higher temperature exhibited signs of heat stress (senescent lower leaves, small leaf area, elongated stems, and abundant inflorescence) and soil nutrient deficiencies. Limited growth after cutting in plants exposed to the high temperature regime was not accompanied by an increase in CT concentration, which in fact, was lower than in the regrowth of plants exposed to the low temperature, suggesting low levels of storage carbohydrates in roots before cutting.

Temperature and soil moisture. In many regions of the world, low soil moisture is a major stress that limits biomass yield of forage plants. However, drought in tropical regions is usually associated with relatively high ambient temperatures. In Australia, Anuraga *et al*. (1993) conducted a growth chamber experiment to examine the combined effects of temperature and soil moisture on growth and CT accumulation of *L. corniculatus* and *L. pedunculatus*. Results (Table 1) indicated that high temperatures increased CT concentration in *L. pedunculatus*, but had only a small effect on CT levels in *L. corniculatus*. Moisture stress induced a larger accumulation of CTs in leaves of *L. pedunculatus* as temperature increased, accompanied by reduced forage yield.

Temperature, carbon dioxide, and soil moisture. The effect of contrasting temperatures in combination with different CO_2 concentrations and watering regimes on CT accumulation and other quality parameters was examined in three genotypes of *L. corniculatus* (Carter *et al*., 1999). Results (Table 1) showed that, with all genotypes, doubling CO_2 concentration increased CT levels, but that increasing growth temperature reduced CT levels. Drought had a smaller effect than temperature on CT accumulation, but caused CT levels in leaf tissues to drop. Combining the effects of high temperature, low CO_2, and drought resulted in the lowest accumulation of CTs in *L. corniculatus*.

As previously discussed, exposure of *L. pedunculatus* to high temperatures resulted in a higher accumulation of CTs than at low temperatures (Anuraga *et al*., 1993; Lees *et al*., 1994). This is contrary to what was found with *L. corniculatus* by Carter *et al*., (1999), who showed that accumulation of CTs in this species increased in response to the combined effects of low temperature and high CO_2 concentration, regardless of soil moisture. Other studies have shown that (1) low temperature and moisture stress had limited effect on CT accumulation in *L. corniculatus* (Anuraga *et al*., 1993), (2) CT levels were inherently higher in *L. pedunculatus* than in *L. corniculatus* (Lowther *et al*., 1987), and (3) the chemical structure of CTs differed between the two *Lotus* species (McNabb *et al*., 1997).

Thus, the accumulation of CTs in temperate legumes in response to temperature is probably variable, depending on both species and genotype, at least in the case of the genus *Lotus*. Species with inherently low levels of CTs (e.g., *L. corniculatus*) seem to respond either not at all or by only slightly accumulating CTs when grown under low temperatures. In contrast, species with inherently high levels of CTs (*L. pedunculatus* and *Les. cuneata*) seem invariably to accumulate more tannins when grown under high temperatures. The combined effect of environmental factors such as temperature, CO_2, soil moisture, and soil nutrient deficiencies is also larger on CT accumulation of temperate legumes than any single factor alone. The severity of the climatic stress imposed may also have a significant effect on biomass yield and on CT accumulation in edible tissue of temperate legumes.

The impact of changing climatic factors on forage quality parameters other than CT accumulation has been studied in some cases. A negative relationship was observed between CT concentration of more than 2.5% to 3.0% of dry matter (DM) in *L. corniculatus* and initial rate of gas production during *in vitro* fermentation with rumen microorganisms (Carter *et al*., 1999). Digestibility of *L. corniculatus* was also found to increase with drought and high temperature, but to decrease with increased CO_2 concentration (Carter *et al*., 1999). The changes, induced by the combination of temperature and moisture stress, in CT levels in forage legumes can also affect forage intake (Barry and Duncan, 1984; Terrill *et al*., 1989) and N use by ruminants. Low CT concentration in *L. corniculatus* has been associated with decreased N digestibility, but with increased apparent absorption of essential amino acids in sheep (Waghorn *et al*., 1987). In contrast, higher levels of CTs in *L. pedunculatus* have also been shown to result in reduced N digestibility, but with no effect on amino acids absorption in the lower gut of sheep (Waghorn *et al*., 1994).

Soil fertility. A major constraint to growing well-known commercial forage legume cultivars is low soil fertility, particularly in tropical regions, which are characterized by large areas of acid low-fertility soils (Houston, 1993). Thus, heavy emphasis has been placed on evaluating and selecting legumes adapted to acid soils (Argel and Maass, 1995). Acid soils are also common in certain regions of temperate-climate zones and, as a result, researchers in southern USA, New Zealand, and Australia have been interested in selecting legumes that perform well in these soils (Donnelly and Anthony, 1970; Scott and Charlton, 1983).

Farmers often comment that they see cattle preferring legumes with CTs when the forages are well fertilized or when they are grown in sites with high soil fertility. This anecdotal evidence prompted researchers to design experiments to assess the effect of fertilizer on CT concentration and on the palatability of temperate and tropical forage legumes with varying levels of tannins. The effect of fertilizer level on CT concentration and, to a lesser extent, on forage acceptability has been limited to only a few temperate (*L. cuneata* and *Lotus* spp.) and tropical (*D. heterocarpon* subsp. *ovalifolium* and *C. calothyrsus*) legumes, as pointed out earlier.

Early work in southern USA indicated that CT concentration in *Les. cuneata* decreased when plants were grown in greenhouse pots containing a loamy soil, fertilized with K. However, the reduced CT levels due to K fertilization relative to CT levels in unfertilized plants was observed only after 3 years, when the control plants were showing symptoms of extreme deficiency and significantly reduced yields (Wilson, 1955).

New Zealand researchers have also been interested in determining how soil fertility and fertilizer applications influence CT levels in *L. pedunculatus* and *L. corniculatus* (Barry and Forss, 1983; Lowther *et al*., 1987). Field studies showed that CT levels in *L. pedunculatus* cv. Grasslands Maku declined significantly (from 8%-11% of DM to 2%-3% of DM) when the legume was grown in a high-fertility soil as opposed to an acid soil with no fertilizer application (Barry and Forss, 1983). However, applying a combined P and S fertilizer to *L. pedunculatus* grown in acid soils reduced CT concentration to 4%-5% of DM and increased biomass yield. The negative relationship between CT level and plant yield was evident only during the 15 months following fertilizer application, after which subsequent measurements showed increases in plant yield due to residual fertilizer, but no changes in CT levels, for reasons not yet understood.

The effect of S fertilization on CT concentration in different genotypes of *L. corniculatus* and on *L. pedunculatus* cv. Grassland Maku grown under low and high S conditions were examined in a field trial by Lowther *et al*. (1987) in New Zealand. Results indicated that, as expected, CT concentration was higher in *L. pedunculatus* (6% to 10% of DM) than in *L. corniculatus* (0.1% to 4% of DM), regardless of S fertilization. Increasing the level of S fertilization (from 20 to 50 kg/ha) resulted in a three-fold increase in yield of *L. pedunculatus*, but had no consistent effect on CT accumulation, as would have been expected from previous observations (Barry and Forss, 1983). This was probably associated with a suboptimal application of S, given that S concentration in the fertilized plants was considered borderline (0.12%) of deficiency, even at the higher rate of S application. In the same study, variation in CT levels in *L. corniculatus* was more associated with plant type than with level of S fertilization. The highest level (4%) of CTs was measured in an erect genotype of *L. corniculatus*, and this was below the range (8% to 11%) at which CTs affect voluntary intake, digestibility, and N use by ruminants (Barry and Duncan, 1984).

From the evidence reviewed, elevation of CT concentration in temperate legumes occurs only when soil nutrient deficiencies are such that they affect plant growth. In addition, soil fertility or fertilizer application has a minimal effect on CT concentration in legumes that have inherently low levels of tannins, as was shown with genotypes of *L. corniculatus* evaluated in New Zealand.

Tropical legumes

As stated before, little research has been conducted on defining how environmental factors influence the concentration of CTs and other quality parameters of tropical legumes. This is probably because of the low use of legumes by farmers in tropical regions. What follows is a summary of results obtained by researchers at the Centro Internacional de Agricultura Tropical (CIAT), Colombia, who have been mainly interested in defining how soil fertility and fertilizer application affects CT levels and other quality parameters of *D. ovalifolium* and, more recently, of *C. calothyrsus*. Both these legumes are very well adapted to acid soils and have high CT levels (Jackson *et al*., 1996).

Fertilization. In the early 1980s, cattle grazing a pure stand of *D. ovalifolium* in the Colombian Eastern Plains were seen to markedly prefer forage where relatively large quantities of fertilizer had been accidentally dropped. This observation led to the setting-up of a field experiment with the objective of comparing quality parameters and acceptability of forage to cattle as a function of fertilizer application (treatments = [1] P + Ca; [2] P + Ca + K; [3] P + Ca + K + Mg + S; and [4] control). Results showed small differences in forage yield with the fertilizer combination P + Ca + K, but an almost two-fold increase when S was added to the mixture (Lascano and Salinas, 1982). Increased biomass production with the combined fertilizer was associated with a 9% unit reduction in CT concentration and a 0.5% unit increase in N in leaf tissue. Large increases in S (from 0.09% to 0.15%), K (0.62% to 0.75%), and, to a lesser extent, P (0.12% to 0.15%) also occurred in leaf tissue. The most striking finding was that cattle preferred to graze the area where S had been applied, and that only when forage-on-offer in the S-fertilized plots was limited did the animals graze the area fertilized with P + Ca + K (Figure 1). Very little grazing was observed in the control plot and the areas fertilized only with P + Ca.

To further test the hypothesis that S was involved in the nutritive quality and acceptability of *D. ovalifolium*, a second experiment was carried out in the Colombian Eastern Plains (Salinas and Lascano, 1983). Fertilizer combinations with and without S were applied and measurements on forage yield, forage quality, and grazing preference were carried out during 8 months, which included a rainy (6 months) and dry season (2 months). Results confirmed previous findings that forage yield was higher when S was applied in combination with other nutrients (P + Ca + K + Mg) and that this was associated with fewer CTs and more N and S in leaf tissue (Figure 2). Observations on grazing frequency also indicated that, in both seasons of the year, animals spent more time in the plots where S had been added (86% of active eating time) than where no S was applied (14% of active eating time). Fertilization with S was subsequently shown to improve the liveweight gain of cattle grazing a mixture of *Brachiaria decumbens* and *D. ovalifolium* in the Eastern Plains, presumably due to improved intake and quality of the legume (Pérez, 1997).

The positive effect of S fertilization in combination with other nutrients in reducing CT levels and enhancing the overall forage value of a tropical legume with tannins closely agrees with what was found in New Zealand with *L. pedunculatus* grown in an acid soil (Barry and Forss, 1983). Both legumes have inherently high CT levels, and the reduced tannins, as a result of fertilization, were associated with increased forage yield and N content in leaf tissue. Thus, the feed value of legumes with tannins when grown in acid soils is probably and largely a function of deficiencies of key minerals such as S and P in the soil.

Soil fertility and season. To further investigate the effect of soil fertility on CT concentration and other quality parameters of *D. ovalifolium*, a large collaborative project was carried out in contrasting sites of Colombia. The project was funded by the BMZ (Germany) and DFID (UK), and involved the University of Hohenheim (Germany), IGER (UK), and CIAT (Colombia) (Schmidt *et al*., 1997). A core collection of 18 *D. ovalifolium* accessions was planted with two levels of fertilizer in six sites that represented savannas, humid forest margins, and humid and subhumid hillsides. One major finding was that the combined effects of soil fertility and climate had a greater effect on forage quality than did plant genotype. In this paper, we only summarize results obtained at two savanna sites, characterized by having very acid low-fertility soils and contrasting textures (clay loam and sandy loam).

Forage yield and quality of *D. ovalifolium* grown in savannas varied according to fertilizer and season, but the effect of fertilizer was considerably greater (Table 2). In the wet season, forage biomass increased by as much as seven times with fertilizer application and was associated with reduced CT concentration, increased levels of N in leaves, and improved digestibility. Similarly, forage yield of the legume during the dry season was also higher in the fertilized plots and again was associated with lower CT levels, more leaf N, and higher digestibility.

In the other agroecological sites included in the G × E study, differences in legume forage yield due to fertilizer were less dramatic than in the savanna sites. Neither were they associated with major changes in CT levels, leaf N, or digestibility. The reasons may have been, possibly, the better natural fertility of the soils at these sites and the less pronounced dry seasons. However, results from the more humid sites (forest margins), characterized by high rainfall throughout the year, indicated a marked effect of soil physical properties on CT accumulation (A. Salamanca *et al*., 2000, unpublished results). Lack of oxygen in the soil, resulting from poor internal drainage, was associated with poor root development, which, in turn, presumably affected uptake of nutrients and plant development. Thus, once again, for both temperate and tropical legumes, changes in CT accumulation as a result of environmental factors are evident only when the stress imposed greatly affects plant growth.

Another important finding of the studies with *D. ovalifolium* was that fertilization level affected not only CT concentration but also their monomer composition (R. Barahona *et al*., 1999 unpublished results). For example, the CTs of plants grown with low fertilizer in sandy loam savanna soils had a higher cyanidin-to-pelargonidin ratio than did the CTs of plants grown in the same soil receiving high fertilization.

The study also showed that the molecular weights (MWs) of the CTs also varied with environmental conditions and that MW estimates were positively correlated with rate of digestion in the early stages of fermentation (R. Barahona *et al*., 1999, unpublished results). The nutritional significance of these findings are not well understood, but CTs in tropical legumes do not appear to be a uniform chemical entity, given that they can change with edaphic and climatic factors.

In *C. calothyrsus*, we also found that CT accumulation in leaf tissue was affected by genotype and by soil fertility (C. E. Lascano *et al*., 2000, unpublished results). The CT concentration was higher in one genotype, regardless of site but, in both sites, the CT level was higher in the forage harvested in the acid-soil site than in that harvested in the fertile-soil site. The nutritional implications of these changes in CT levels are currently being investigated at CIAT.

In *C. calothyrsus*, differences in monomer composition of CTs were also recorded in contrasting genotypes (J. Stewart *et al.*, 2000, submitted) but, in this case, were not affected by soil fertility (C. E. Lascano *et al*., 2000, unpublished results). The significance of this finding is not completely understood, but the reactivity of CTs with proteins (BSA and rubisco) was observed to increase with an increasing delphinidin-to-cyanidin (PD:PC) ratio (J. Stewart *et al*., 2000, submitted; C. E. Lascano *et al*., 2000, unpublished results). These results agree with findings from earlier work with *Lotus* spp. that showed that the reactivity of CTs also increased with increasing delphinidin-to-cyanidin ratio (Jones *et al*., 1976). Subsequent results showed that CTs in *L. pedunculatus* were more effective in reducing *in vitro* degradation of rubisco by rumen microbes than CTs from *L. corniculatus*. This difference was related to differences in the monomer composition of CTs in the two legume species (McNabb *et al*., 1997).

Thus, the biological significance of differences in monomer composition and MWs of CTs in forage legumes as affected by species and by environmental factors may be related to the way tannins interact with proteins. If this is the case, they would then have a significant impact on N use by ruminants.

Future research challenges

The value of a forage species to a livestock producer depends largely on its capacity to produce animal products, which, in turn, is related to the quantity and quality of the forage offered. In tropical regions, liveweight gain and milk yield can be significantly depressed in pastures based on grass alone (Toledo, 1985). Grass pastures sown in areas with acid low-fertility soils degrade over time if no fertilizer is applied or if the species used are susceptible to biotic constraints. Such degradation is partially reflected in the loss of grass productivity and increased weed invasion, which affect carrying capacity and animal performance.

An alternative to minimize short- and long-term declines in quality and quantity of pasture biomass and thus increase livestock production is to use legumes in pastures (Lascano and Estrada, 1989; Lascano and Avila, 1993). The rationale for this alternative is that most tropical legumes have a higher nutritive value than grasses and, through symbiotic nitrogen fixation, can enhance production and quality of the companion grass and improve soil fertility. However, the incorporation of legumes into livestock systems in marginal areas is slow, partly because some species, well adapted to acid low-fertility soils (e.g., *D. ovalifolium, C. calothyrsus*, and *Flemingia macrophylla*) are perceived as having low utility because of high CT concentrations. Thus, alternatives must be found to enhance the forage value of legumes adapted to acid soils, by either selecting genotypes with fewer CTs and/or by manipulating environmental factors such as soil fertility.

In the next paragraphs, we suggest some future research areas.

Effect of environmental factors on the chemical properties of tannins

The effect of climatic and soil-related factors on the chemical structure of tannins in temperate and tropical legumes is not well documented. Previous work had shown that the MWs and monomer composition of CTs can vary with species and plant organ (Foo and Porter, 1980; Foo *et al*., 1982; Williams *et al*., 1983), and with season, as shown for sorghum (Butler, 1982). Early results also showed that CTs from sainfoin (*Onobrychis vicifolia*) were less effective in binding and precipitating protein than were CTs from *Lotus* spp. (Jones *et al*., 1976). The difference between the two genera was associated with the higher MWs of CTs in sainfoin than in *Lotus* spp. Other researchers had also indicated that CTs with large MWs tend to lose their protein-precipitating capacity (Kumar and Vaithiyanathan, 1990), which could have implications for N use by ruminant animals.

Our studies have indicated differences in monomer composition of CTs in two genotypes of the shrub legume *C. calothyrsus* and an effect of environmental factors on monomer composition and MWs of *D. ovalifolium* grown in contrasting sites. In addition, comparison across forage species has shown that the nutritional effects of CTs were also a function of their level of hydroxylation and their association (bound versus soluble) with other plant tissues (R. Barahona *et al.*, 1999, unpublished results). At the same concentration, CTs from *D. ovalifolium* were more effective than tannins from *Leucaena leucocephala* in preventing the degradation of substrates and in reducing the activity of fungal fibrolytic enzymes (R. Barahona *et al*., 1999, unpublished results).

Other factors, such as tannin concentration relative to protein in the forage and their solubility in the rumen fluid, could also affect CT activity.

Thus, future research on the relationship between environmental factors and the feed value of legumes with tannins should emphasize:

1. The better definition of those environmental factors that induce changes in the chemical structure and properties of CTs in legumes,
2. The understanding of how these changes are mediated, and
3. Ultimately, the understanding of how they affect the nutritional quality of legumes with tannins and the resulting performance of animals consuming these legumes.

Effect of environmental factors on the feed value of tropical legumes containing tannins

The evidence reviewed indicated that climatic and soil nutrient deficiencies can have a significant effect on reducing forage yield and increasing CT concentration in the edible material of temperate and tropical legumes. The most striking results on overall enhancement of feed value of temperate (e.g., *L. pedunculatus*) and tropical legumes (e.g., *D. ovalifolium* and *C. calothyrsus*) were recorded for the correction of a soil nutrient deficiency (i.e., of S) and for improving soil fertility (i.e., acid vs. fertile soils). However, limited information exists on the extent to which correcting soil nutrient deficiencies will affect the long-term performance of animals fed with or grazing legumes with tannins. Some evidence suggests that the residual effect of fertilization on the quality of legumes with CTs may be short term. Thus, future research should aim at evaluating the long-term utility for dairy and beef cattle production of legumes with tannins in response to fertilizers when grown in acid low-fertility soils.

Targeting legumes with tannins

A major challenge faced by the forage specialist is to predict the overall performance and feed value of forages when grown in contrasting environments. Consequently, interest is growing in linking information on environmental adaptation of forage species and accessions with GIS. Available evidence suggests that the two major factors affecting the feed value of certain legumes with CTs are soil fertility and rainfall. Thus, future work should attempt to use GIS tools and existing information on environmental adaptation of temperate and tropical forages, as a way of facilitating the targeting of legumes to locations and production systems where soil fertility and rainfall are not constraints, or where farmers can use organic or inorganic fertilizers as part of their forage management strategies.

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Table 1 - Effect of temperature alone (A) and in combination with soil moisture (B) and CO_2 concentration (C) on the accumulation of condensed tannins (CTs) as a percentage of dry matter in temperate legumes.

Description	CTs in leaves	Source
A. Temperature alone		
Lespedeza cuneata		Fales, 1984
Low tannin contents		
22/17°C	14.5	
32/34°C	24.9	
Normal tannin contents		
22/17°C	3.5	
32/34°C	5.5	
Lotus pedunculatus		Lees *et al*., 1994
Low tannin contents		
20°C	8.6	
30°C	12.9	
Medium tannin contents		
20°C	11.2	
30°C	14.8	
High tannin contents		
20°C	12.9	
30°C	17.3	
B. Temperature + soil moisture		
Lotus spp.		Anuraga *et al*., 1993
L. pedunculatus		
14/10°C + low soil moisture	1.8	
32/28°C + low soil moisture	5.0	
L. corniculatus		
14/10°C + low soil moisture	0.3	
32/28°C + low soil moisture	0.3	
C. Temperature + soil moisture + CO_2 concentration		
Lotus corniculatus		Carter *et al*., 1999
18/10°C + high soil moisture + high CO_2	6.5	
18/10°C + low soil moisture + high CO_2	7.0	
25/15°C + high soil moisture + high CO_2	4.5	
25/15°C + low soil moisture + high CO_2	3.2	

Table 2 - Forage yield and quality of accessions of *Desmodium heterocarpon* subsp. o*valifolium* grown in contrasting soils in the Colombian savannas with two levels of fertilizer (CIAT, 1999, unpublished results).

Measurements[a]	Clay loam		Sandy loam	
	Low fertilizer[b]	High fertilizer[c]	Low fertilizer[b]	High fertilizer[c]
Dry season				
Biomass (DM g/m^2)	109.0	199.0	51.0	230.0
Condensed tannins (% of DM)	10.2	7.2	12.0	8.0
CP in leaf tissue (% of DM)	12.4	16.5	11.9	16.2
IVDMD of leaf tissue (%)	37.0	50.4	26.7	50.1
Wet season				
Biomass (DM g/m^2)	240.0	471.0	62.0	423.0
Condensed tannins (% of DM)	9.4	6.0	9.9	7.3
CP in leaf tissue (% of DM)	14.2	18.5	11.4	16.7
IVDMD of leaf tissue (%)	41.5	53.0	40.4	55.2

a. DM = dry matter; CP = crude protein; IVDMD = *in vitro* dry matter digestibility.
b. 10 P + 20 K + 150 Ca + 5 S + 2.5 Zn + 0.3 B (kg/ha).
c. 50 P + 50 K + 500 Ca + 20 S + 2.5 Zn + 0.3 B (kg/ha).

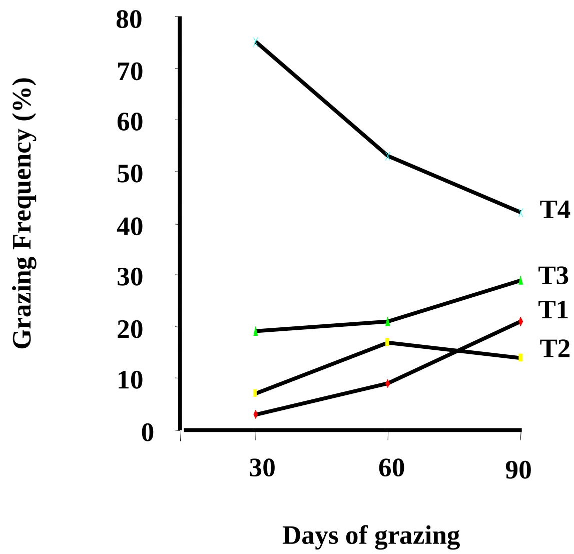

Figure 1 - Effect of fertilizer application on acceptability of *Desmodium heterocarpon* subsp. *ovalifolium* to grazing animals in the Colombian Eastern Plains (Treatments: T1 = control; T2 = 25 P + 117 Ca (kg/ha); T3 = 25 P + 117 Ca + 36 K (kg/ha); T4 = 25 P + 117 Ca + 36 K + 22 Mg + 44 S (kg/ha) (adapted from Lascano and Salinas, 1982).

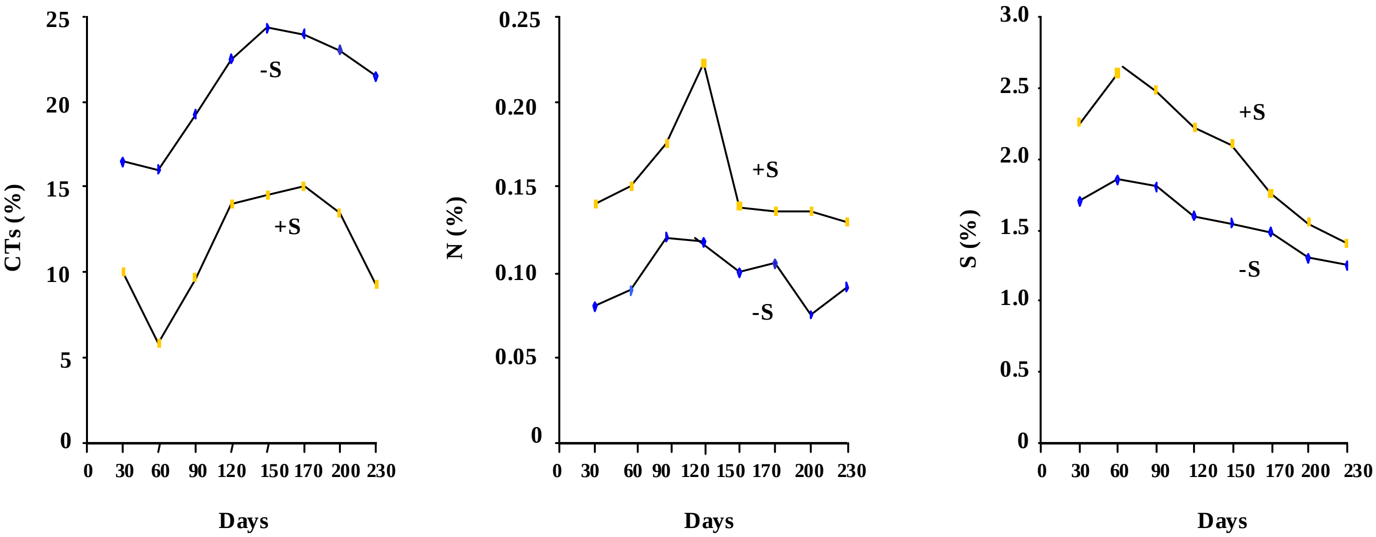

Figure 2 - Effect of applying sulfur fertilizer on the concentration of condensed tannins (CTs), nitrogen (N) and sulfur (S) contents of leaves of *Desmodium heterocarpon* subsp. *ovalifolium* grown in the Colombian Eastern Plains (adapted from Salinas and Lascano, 1983).