ECOLOGICAL BIOCHEMISTRY OF SECONDARY PLANT COMPOUNDS IN HERBIVORE NUTRITION

J. D. Reed
Department of Animal Sciences, University of Wisconsin-Madison, Madison WI

ABSTRACT
There are over 24,000 known secondary plant compounds including the alkaloids, non-protein amino acids, cyanogenic glycosides, terpenoids, saponins and flavonoids. Research has concentrated on toxicity and nutritional limitations of plants that result from the presence of secondary compounds. Secondary plant compounds have both anti-nutritional and toxic effects on mammalian herbivores. They also affect methods for determining the nutritive value. Plants seldom contain a single class of secondary plant compound and interactions between two or more compounds in the same plant are possible. Dominant range plants contain high amounts of these compounds and manipulating vegetation so that dominant plants contain less inhibitory compounds is a challenge to range and grassland management. More research is required to take advantage of the beneficial effects of secondary plant compounds in plant protection and animal health while minimizing their potentially toxic or anti-nutritional effects.

KEYWORDS
alkaloids, non-protein amino acids, cyanogenic glycosides, terpenoids, saponins, flavonoids, toxicity, anti-nutritional effects

INTRODUCTION
Secondary plant compounds are a large and diverse group of molecules that have a key role in the interactions between herbivores and plants in both natural and agricultural ecosystems. There are over 24,000 known structures that include classes of compounds that are known to have anti-nutritional and toxic effects on mammals (Harborne, 1993), including the alkaloids, non-protein amino acids, cyanogenic glycosides, terpenoids, saponins and flavonoids. Ecological biochemistry is the study of the chemical interactions among organisms in plant and animal communities and the chemical interactions of these communities with their environment (Harborne, 1993). The field of ecological biochemistry was developed from studies on the role of secondary compounds in interactions between invertebrate animals and their food plants and focused on compounds that plants produce as a defense against herbivores and/or pathogenic organisms. Secondary plant compounds are also involved in mutualism, such as in flower pollination by insects and birds and in host recognition by symbiotic bacteria and fungi.

Research on mammalian herbivores has concentrated on toxicity and nutritional limitations of plants that result from the presence of secondary compounds (Cheeke and Shull, 1985). These effects can be classified in two groups:

1. Toxic compounds that are present in plants at low concentrations (generally less than 2% of the dry matter) and have negative physiological effects when absorbed; such as neurological problems, reproductive failure, goiter, gangrene, and death. These secondary plant compounds include alkaloids, cyanogenic glycosides, toxic amino acids, saponins, isoflavonoids and many others.

2. Non-toxic compounds that lower the digestibility and palatability of plants. These compounds generally need to be present at concentrations at over 2% of the dry matter in order to have negative effects. Their primary site of inhibitory activity is in the digestive tract or through sensory organs associated with feeding behavior. Some of these compounds have a structural role; such as lignin, silica and cutin. The role of tannins and volatile-essential-oils (terpenoids) may be in plant defense against predators.

This paper will review some of the major types of secondary plant compounds and their anti-nutritional and toxic effects on mammalian herbivores in order to illustrate some of their potential problems. A comprehensive review is not attempted. Instead, secondary plant compounds from plants with potential forage use and their toxicity problems are discussed. The paper also discusses the implications for determining the nutritive value of indigenous and newly introduced forage species.

TOXIC COMPOUNDS
Alkaloids: Alkaloids are a diverse group of compounds that cause physiological and neurological problems in mammals. Alkaloids are organic bases that contain secondary, tertiary or cyclic amines. Analysis and isolation of alkaloids rely on the difference in solubility of the free base form and the acid form. The free base is usually very soluble in hydrophilic solvents.

Phalaris species including *P. arundinaciae* (Reed Canary grass), are grasses that are adapted to poorly drained soils (Froman and Persson, 1974). *Phalaris* species contain several alkaloids of the tryptamine class of which gramine is representative (Williams et al., 1971; Culvenor, 1973; Corcuera, 1989). These alkaloids are the cause of *Phalaris* staggers and deaths of cattle and sheep grazing *Phalaris* pastures (Marten and Heath, 1973). Variation in alkaloid content and toxicity is large, and is caused by differences between species, genetic differences within species, edaphic and climatic factors.

Datura stramonium is a common weed in cultivated areas and disturbed rangelands. *D. stromonium*, and other members of the Solanaceae, contain tropane alkaloids such as hyoscyamine. Symptoms of poisoning in cattle are paralysis, suspension of secretion and rapid heart action leading to death by asphyxia. The seeds contain the highest content of alkaloids. Cases of poisoning are rare. Greatest danger is when the plant has been mixed in hay (Verdcourt and Trump, 1969).

Ryegrass staggers is a neurological disorder of sheep and cattle grazing perennial ryegrass (*Lolium perenne* L.) that is infected by the endophytic fungus, *Acremonium lolii*. The fungus produces alkaloid mycotoxins that are termed tremogens. Lolitrem B is the main alkaloid but several other tremogens are present (Gallagher et al., 1981; Munday-Finch et al., 1995). The symptoms include incoordination, staggering gate, and sudden collapse (Cheeke, 1995). The endophyte is also associated with reduced liveweight gains and serum prolactin levels in sheep grazing ryegrass pastures (Fletcher and Barrell, 1984). There is considerable seasonal, locational, and varietal variation in the concentration of lolitrem B (Di Menna et al., 1992), as well as variation in distribution within plant parts (Keogh et al., 1996). However, the fungus also produces another alkaloid, peramine, that is a feeding deterrent to the Argentine stem weevil, a major pest of ryegrass (Rowan and Gaynor, 1986). Peramine is not a tremogen and the different alkaloids suggest the possibility that strains of *Acremonium lolii* could be developed that impart weevil resistance without producing ryegrass staggers.

Cyanogenic glycosides: Cyanogenic glycosides are present in many plant families. They liberate cyanide upon enzymatic or acid hydrolysis (Jones, 1972; Conn, 1973. Tapper and Reay, 1973; Tewe and Iyayi, 1989). Cyanogenic glycosides contain a mono or disaccharide β-linked nitrile moiety. Liberation of the nitrile group and generation of cyanide in crushed plant tissues depends on the presence of a β- glucosidase. Some plants contain the cyanogenic glycoside but not the enzyme (Daday, 1954). Toxicity problems are highly variable and depend on the rate and amount of cyanogenic glycoside consumed, the presence of the β-glucosidase, and detoxification of cyanide. Poisoning and death may occur when an uninitiated animal rapidly consumes a plant with moderate to high levels of cyanogenic glycoside. Chronic problems such as goiter may be caused by long term consumption of lower levels. The enzyme rhodanase, present in animal tissues, detoxifies cyanide by conjugation with sulfur to form thiocyanate (Williams, 1959; Scheline, 1978). This thiocyanate may cause goiter (Tapper and Reay, 1973). Some rumen micro-organisms may be able to utilize cyanide as a source of NPN (Van Soest, 1994).

Linamarin and lotaustralin are the cyanogenic glycosides present in the tubers and aerial parts of cassava (*Manihot esculenta*). Acute and chronic toxicity occurs in humans and livestock when varieties that contain high levels are consumed (Hill, 1973). Some cassava varieties are capable of producing over 1000 mg HCN/kg of fresh tissue (de Bruijn, 1973).

Dhurrin is the cyanogenic glycoside that occurs in *Sorghum vulgare*, sudangrass, and sudangrass-sorghum hybrids. Livestock poisoning may occur when young plants and new growth are grazed (Reid and Jung, 1973).

Toxic Amino Acids: There are hundreds of amino acids produced by plants that do not occur in proteins. Some of these are toxic to both livestock and man and occur in agronomically important legumes (Hegarty, 1973). Mimosine is the toxic amino acid present in *Leucaena leucocephala* and other members of the Leguminosae, subfamily Mimosoideae. When infused intravenously in sheep mimosine causes shedding of the fleece. When *L. leucocephala* is fed to sheep, defleecing does not occur because mimosine is metabolized to 3-hydroxy-4(1H)-pyridone (3,4-DHP) in the rumen (Hegarty et al., 1964; Reis et al., 1975; Romeo, 1989). This metabolite is excreted in the urine and is goitergenic (Hegarty et al., 1976). Many negative effects have occurred when cattle, sheep and goats consume high amounts of *L. leucocephala*. These included low liveweight gains, low weight of calves at birth, excessive salivation, hair loss, and enlarged thyroid glands (Jones et al., 1976; Blunt and Jones, 1977; Hamilton et al., 1971). However, ruminal bacteria that completely metabolize mimosine and 3,4-DHP were found in goats from Hawaii and Indonesia (Jones and Lowry, 1982). These bacteria were successfully transferred to cattle in Australia and have largely eliminated the toxicity problem (Jones and Megarrity, 1986).

Indospicine occurs in *Indigofera spicata* and other *Indigofera* species. These legumes are important range plants in Africa and have potential as tropical pasture legumes. Toxicity problems include weight loss, liver and kidney damage, and abortions (Verdcourt and Trump, 1969). Indospicine induced liver lesions, infertility and teratogenic effects in rats and mice that were similar to the effects in livestock (Hegarty, 1973).

Lathyrus species contain toxic amino acids that cause neurological and skeletal diseases called lathyrisms. *Lathyrus sativus*, a widely grown tropical legume, contains the neurotoxin, β-N-oxalyl-Lα,β-diaminopropionic acid (Roy and Spencer, 1989). This toxic amino acid is also present in *Crotalaria* and *Acacia* species. In man, ingestion of *L. sativus* seeds results in weakness, spastic leg muscles, convulsions, and in extreme cases death (Misra et al., 1981). Long term consumption at low levels can also result in neurological symptoms.

Saponins: Saponins are triterpene or steroid glycosides. The carbohydrate moiety consists of pentoses, hexoses, or uronic acids. The terpenoid moiety is called a sapogenin. Saponins form stable foams in low concentrations. This property has been associated with ruminant bloat because saponins are present in many herbaceous legumes (Bondi et al., 1973). Some saponins cause severe physiological disorders such as haemolysis, gastro-enteritis, paralysis and death. The cardiac glycosides are also saponins.

Medicago sativa contains many saponins. Medicagenic acid is unique to alfalfa. These saponins were implicated in causing bloat on alfalfa pastures (Bondi et al., 1973). However, research on alfalfa cultivars with a high and low concentration of saponin indicated that there was no difference between the cultivars in the incidence of bloat (Majak et al, 1995). Lucerne saponins may lower growth rate in chicks and egg production of hens when included in poultry diets above 5%.

Digitalin is the cardiac glycoside in *Digitalis* species and other Scrophulariaceae. The closely related cardiac glycoside, strophanthin, is found in the Apocynaceae. Livestock poisoning caused by strophanthin has occurred in Kenya when sheep and cattle consumed leaves of *Acokanthera longiflora* and *A. schimperi* (Verdcourt and Trump, 1969). Death is caused by heart failure. Extracts of these plants are used as arrow poisons.

Isoflavonoids: Isoflavonoids are common phenolic constituents in pasture legumes. Isoflavonoids consist of two phenolic rings. One is derived from the deamination of tyrosine or phenylalanine to derivatives of cinnamic acid. The other is derived through the conjugation of two carbon units from the acetate - malonate pathway (Wong, 1973; Dewick, 1994). Isoflavonoids are estrogenic and may cause reproductive problems in livestock grazing legume pastures.

Formononetin is an isoflavone that occurs in subterranean clover, *Trifolium subterraneum*, and red clover, *T. pratense*. Although formononetin is not estrogenic, it is metabolized in the rumen to equol, a potent estrogen (Braden and Shutt, 1970). The estrogenic effects of equol include dystocia, failure of ewes to conceive, prolapse uterus, lamb mortality, and permanent infertility problems. There is a large variation in concentration of formononetin that is related to cultivar, season, soil fertility, and plant diseases. Sheep are more susceptible than cattle to the estrogenic effects of equol, although the reason for this difference is unknown (Lundh, 1990). The ruminal metabolism of formononetin to equol was similar in sheep and cattle, as were the levels of equol in blood. Cultivars of *T. subterranean* and *T. pratense* that have low concentrations of formononetin have been developed.

Coumestans are estrogenic isoflavonoids that occur in alfalfa and white clover (*Trifolium repens*) (Wong, 1973). Coumestral has estrogenic activity and has been implicated in reproductive problems in dairy cattle and ewes. Again, there is large variation in concentration that is related to cultivar, season, soil fertility, and plant stress and disease (Reid and Jung, 1973).

COMPOUNDS THAT LOWER DIGESTIBILITY AND/OR PALATABILITY

Lignin: Lignin is a phenylpropenoid polymer associated with structural carbohydrates in the cell walls of higher plants (Harkin, 1967; Harkin, 1973). Lignin is a highly crosslinked amorphous compound that gives rigidity and strength to the otherwise flexible carbohydrate in plant cell walls. This association lowers the digestibility of cell wall carbohydrates, and is an important factor in determining digestibility of forages (Van Soest, 1994). The cell wall carbohydrates and lignin are the most abundant organic molecules on land (Gordon, 1978). These molecules may comprise over 60% of the organic matter of tropical grasses, even in actively growing tissues. It is impossible for grazing or browsing livestock in the tropics to avoid relatively high intake of lignin.

Grass cell walls contain lignin monomeric phenolic acids that are susceptible to saponification by dilute alkaline reagents. Esters of the phenolic acids, ferulic acid and p - coumaric acid, are esterified to arabino-xylans which are saponified by treatment with NaOH (Hartley et al., 1976). These monomeric units are not lignin (Harris and Hartley, 1976), but grass lignin may consist of higher oligomer derived from these phenolic acids. Diferulic acid has been isolated from lignin-carbohydrate complexes (Hartley and Jones, 1976). Saponification of grass lignin increases the digestibility of cell wall carbohydrates.

Legume lignin is less susceptible to saponification and contains fewer phenolic acids than grass lignin (Hartley and Jones, 1977). Ether and carbon-carbon bonds may be more important than ester bonds. Coniferyl alcohol and p-coumaryl alcohol may be the monomeric units of legume lignin (Gordon, 1978). The greater density and lower digestibility of legume cell wall compared to grass cell wall is caused by higher lignin to carbohydrate ratios and greater crosslinking between cell wall polymers.

Condensed Tannins: The nutritional effects of condensed tannins (CT) has received much research and their effects are discussed in the chapter by Waghorn et al. in these proceedings.

Condensed tannins (CT) are flavonoid polymers based on repeating catechin (20) units and other closely related favan-3-ols (Porter, 1994). Condensed tannins form red pigments, anthocyanidins, when heated in acid solutions. Each type of CT (proanthocyanidin) is named after the anthocyanidin produced. Condensed tannins that produce cyanidin are named procyanidins and those that produce delphinidin are named prodelphinidins. These two types are the most common proanthocyanidins. CT cause bitter and astringent tastes and lower the digestibility of proteins and carbohydrates through enzyme inhibition or substrate binding. Important forage legumes that contain CT are *Desmondium intortum* (Ford, 1978), *Indigofera spicata* (Reed, 1983), *Lespedeza cuneata*, *Onobyrychis viciifolia*, *Lotus corniculatus* (Bate-Smith, 1973), and *Coronilla varia* (Burns and Cope, 1974). Condensed tannins are commonly found in most woody plants that are browsed by ruminants. The positive effects of low to moderate concentrations of CT in forage legumes (bloat protection and lower ruminal protein degradation) has led to considerable interest in developing cultivars of alfalfa and white clover that contain CT. The absence of CT in these forages could be considered the major cause of their nutritional problems. Ruminants in natural plant communities normally consume a diet rich in CT.

Volatile terpenoids (essential oils): Volatile terpenoids are fat soluble, oxygenated hydrocarbons based on repeating isoprene units. Monoterpenes are based on two isoprene units and are responsible for the characteristic odors of many plants. These compounds are anti-microbial and may lower palatability and digestibility.

Geranial and neral are monoterpenes present in *Cymbopogon* species, Gramineae. These grasses are present in many parts of Africa. The volatile terpenoids are believed to lower their palatability (Field, 1976). The grass genus *Bothriochloa* also includes species containing volatile terpenoids.

Camphor is a cyclic monoterpene and is the major constituent in the volatile oil of *Artemesia* species, Compositae (Welch and McArthur, 1981). *Artemesia* monoterpenes are believed to inhibit rumen micro-organisms and lower palatability. Common African range plants that contain volatile terpenoids are often members of the compositae or labiatae and create problems when rangelands are overgrazed, such as *Tarchonanthus camphoratus*, a shrub that dominates large areas of heavily disturbed rangelands in Kenya.

Silica: Silica is absorbed from the soil solution of silicic acid and deposited in the cell wall. Most grasses accumulate silica, but silica accumulation is uncommon in legumes and other dicots. Cell wall silica is in the opaline form. Silica increases insect and disease resistance of cereals such as rice, oats, wheat and corn (Yoshida et al., 1962; Jones and Handreck, 1967). However, silica lowers the digestibility of grasses (Van Soest and Jones, 1968). Silica is also involved in the formation of urinary calculi (Jones and Handreck, 1965).

Rice is the best known example of cereal crop that accumulates silica (Yoshida et al., 1962). Rice straw has 8 to 15 percent silica on a dry matter basis. Rice hulls may have over 20% making them indigestible and potentially harmful if fed at a high level (Van Soest, 1970).

Aspilia mossambicensis (Compositae) accumulates over 10% of the dry matter of leaves as biogenic silica (Reed, 1983). This dicot is a dominant plant in disturbed grasslands on vertisols in East Africa. The silica is deposited in stylus like trichomes covering the leaf surface and may be an important deterrent to herbivores.

SECONDARY PLANT COMPOUNDS AND THE ANALYSIS OF NUTRITIVE VALUE

The nutrients in feeds consist of energy, protein, vitamins, and minerals. Much laboratory research in developing countries is devoted to estimating the content and availability of these nutrients in indigenous and introduced forages. It is beyond the scope of this section to review this research. However, many of the simpler laboratory methods for estimating nutrient content and availability are very inaccurate when applied to tropical forages because of the lack of understanding of the effects of secondary plant compounds and other feed components on intake and digestibility. Routine methods for the analysis of nutritive value do not include secondary plant compounds. Many introduced forage species and multi-purpose trees are unpalatable or limited in utilization because they contain secondary plant compounds.

Factors that determine the nutritional value of forages are complex. All available information, both qualitative and quantitative, should be used in making judgments about the value of a particular plant species. The fact that a plant is eaten by one type of animal or another is only part of the picture. Too much emphasis has been put on the cursory analysis of crude protein and fiber as indicators, especially in tropical legumes. The presence of secondary plant compounds will interfere with the concept of high crude protein and low fiber indicating high nutritional value.

Qualitative information can be very helpful in assessing the nutritional limitations of potential forage species and in detecting secondary plant compounds. Taste and smell are probably the most informative and can be used with caution to detect the presence of secondary plant compounds such as alkaloids (bitter taste), tannins (bitter and/or astringent), and essential oils (strong smell and oily, turpentine like taste). Information collected from consulting local farmers or pastoralists may be used, especially if a plant is used medicinally or has poisoned livestock. Animal performance is the deciding factor in determining nutritional value. When parameters such as intake digestibility, or productivity fall short of what would be predicted from estimates, an inhibitory secondary plant compound could be the cause.

Toxic Compounds: The presence of alkaloids, cyanogenic glycosides, toxic amino acids, saponins and other toxic secondary plant compounds presents many problems in predicting the nutritive value of forages from routine laboratory analysis. For instance, tropical legumes, both herbaceous and woody, appear to have high nutritional value based on their content of crude protein, fiber, and minerals. Measurements of in vitro digestibility give contradictory results. Actual animal trials have shown that intake and performance is below what would be predicted. In many cases the difference is substantial. Therefore, methods of screening for toxic secondary plant compounds should be introduced into forage and browse selection studies. However, the diversity of these plant compounds makes routine analysis difficult. For instance, there are many colorimeteric methods for determining alkaloid content, but not all alkaloids will react with each colorimetric test. Therefore, several different methods should be used just for screening this single class of secondary compound. The problem will also arise where a potential forage plant can contain a high content of a class of a secondary plant compound that reacts in the analytical method but has no detrimental effect on the herbivore. Screening for toxicity with laboratory animals, such as rats or voles, is another alternative. However, the situation will exist where a toxin is poisonous to the laboratory animal but not at all harmful to ruminants. The opposite situation also is possible. A combination of in vivo and laboratory methods should be adapted for screening, but this is a difficult task.

Compounds that lower Digestibility or Palatability: These secondary plant compounds are present in large enough quantities to have a large influence on analytical methods for determining nutritive value. Condensed tannins, like lignin, are phenolic polymers, but are generally soluble in aqueous and organic solvents. Sample preparation has an important effect on CT determination. Oven drying decreases CT solubility and leads to increases in total fiber-bound nitrogen, acid-detergent-fiber, and lignin. Soluble CT interfere with the interpretation of cell contents (neutral-detergent-solubles) as being highly digestible. Insoluble CT increase lignin and other fiber fractions, but may have very different nutritional effects than these fiber fractions.

Volatile-essential-oils also lead to errors of interpreting analytical methods for determining nutritional value. Since these terpenoids are hydrocarbons, they contain much higher gross energy values per unit weight than carbohydrates and protein. These volatile oils are lost in oven drying and solubility measurements. They will also substantially increase the ether extract. These effects will lead to an over-estimation of the nutritional value of plants that contain large amounts of volatile oils.

Interactions between two or more secondary plant compounds: Plant species seldom contain a single class of secondary plant compound. Interactions between two or more compounds in the same plant are possible. For instance, CT lower the absorption of methionine. This amino acid is the sulfur donor for the detoxification of cyanide by rhodanase to form thiocyanate. Therefore, CT may increase the toxicity of a plant that contains cyanogenic glycosides and CT, as in the case of casava forage (Reed et al., 1982).

Research carried out in a collaborative project between the International Livestock Research Institute (ILRI) and the University of Wisconsin-Madison demonstrated that sheep fed on diets based on cereal crop residues supplemented with accessions varying in content of proanthocyanidins showed different growth rates (Wiegand et al., 1995). Sheep fed on accessions that had a high content of CT had the lowest intake of cereal crop residue and the lowest digestibility of protein, nitrogen retention and growth rate. However, sheep fed on the accession with a moderate content of CT had a higher growth rate and nitrogen retention than the sheep fed on the accession with the lowest content. These results suggest that the variation in CT in *Sesbania* could be used to select accessions with a level of CT that does not have a detrimental effect on protein digestion but does improve nitrogen metabolism and growth.

However, when *Sesbania* was fed to day old chicks the results indicated that some accessions contain a toxic secondary plant compound (Brown et al., 1987; Shqueir et al., 1989a and 1989b; Reed and Aleemudin, 1995). Researchers in Kenya and Malawi have reported sporadic toxicity in ruminants (Semenye et al., 1987). Research carried out at the University of Wisconsin-Madison in collaboration with ILRI indicated that accessions with a low content of CT were the most toxic to day old chicks (Reed and Aleemudin, 1995). The toxicity study ranked the accessions in the inverse order to their ranking by sheep growth rate and content of CT. Preliminary results from thin layer chromatography (TLC) and qualitative analysis indicates that the accession most toxic to chicks contained the highest levels of saponins that may be responsible for the toxicity. The major saponins in *S. sesban* belong to a class called the oleanane saponins (Dorsaz et al., 1988). At least 6 different glycosides of oleanolic acid are present. Our results using TLC show at least 7 different compounds. *S. sesban* is richer in saponins than the closely related species, *S. goetzii* and *S. keniensis*. *S. goetzii* is practically devoid of saponins. There is also the possibility that interactions between CT and saponins in accessions with higher levels of CT reduce the toxicity of saponin (Freeland et al., 1985).

CONCLUSION

Natural vegetation utilized as forage may contain high levels of secondary plant compounds. Poisoning is uncommon because livestock avoid toxic plants but it is impossible for livestock to avoid consuming compounds such as lignin, tannins, volatile oils, silica and cutin. Plants that dominate vegetation contain high amounts of these compounds which lower their nutritive value. Understanding factors that control the dominance of these plants and ways to manipulate vegetation so that dominant plants contain less inhibitory compounds are important challenges in range and grassland management research.

The successful manipulation of the ruminal microflora to eliminate mimosine toxicity suggests that this approach could be used to solve toxicity problems in other forage species. Naturally occurring microbial species that can detoxify secondary plant compounds may exist in the ruminal populations of hosts that regularly consume the toxic plant. Strains of ruminal bacteria that degrade secondary plant compounds also could be developed through the use of microbial selection and molecular genetics. Although this approach to solving toxicity problems is an excellent research theme with considerable practical impact, there are several problems with implementing the research. Obtaining sustainable funding to support the interdisciplinary teams that are required to carry out research on tropical forages is difficult. The large diversity of secondary plant compounds within and among plant species necessitates a highly focused approach to research on solving a problem on a single combination of toxin and plant species. There is a scarcity of highly trained plant and animal scientists, phytochemists and microbiologist who are willing to devote careers to solving problems related to the effects of secondary plant compounds on the nutritive value of forages.

However, as we obtain more knowledge of the role of secondary compounds in the interactions between mammalian herbivores and their food plants, more research will be devoted to manipulating the secondary chemistry of plants to take advantage of their beneficial effects in plant protection and animal health while minimizing their potentially toxic or anti-nutritional effects. This research will require an ecological approach to the study of these fascinating plant compounds and their effects in grassland ecosystems.

REFERENCES

Bate-Smith, E.C. 1973. Tannins of herbaceous leguminosae. Phytochemistry **12**: 1809 - 1812.

Blunt, C.G. and R.J. Jones. 1977. Steer liveweight gains in relation to the production and time on *Leucaena leucocephala* pastures. Tropical Grasslands **11**: 159 - 164.

Bondi, A., Y. Birk and B. Gestetner. 1973. Forage saponnins. Pages 511-528 *in* G.W. Butler and R.W. Bailey, eds. Chemistry and Biochemistry of Forage, Academic Press, London.

Braden, A.W.H. and D.A. Shutt. 1970. The metabolism of pasture oestrogens in ruminants. Pages 770-773 *in* Proc. 11th Int. Grassl. Congr.

Brown, D. L., D.A. Barnes, S.A. Rezende and K.C Klasing. 1987. Yield, composition and feeding value of irrigated *Sesbania sesban* var. nubica leaves harvested at latitude 38 N during a Mediterranean summer. Animal Feed Science and Technology **18**: 247-255.

Burns, J.C. and W.A. Cope. 1974. Nutritive value of crownvetch forage as influenced by structural constituents and phenolic and tannin compounds. Agronomy J. **66**: 195-200.

Cheeke, P.R. 1995. Endogenous toxins and mycotoxins in forage grasses and their effects on livestock. J. Anim. Sci. **73**: 909-918.

Cheeke, P.R., and L.R. Shull. 1985. Natural toxicants in feeds and plants, AVI Publishing Company,Westport, Connecticut.

Conn, E.E. 1973. Cyanogenic glycosides. Pages 299-308 *in* Toxicants occuring naturally in foods. National Academy of Sciences, Washington D C.

Corcuera, L.J. 1989. Indole alkaloids from *Phalaris* and other gramineae. Pages 169-177 *in* P.R.Cheeke, ed. Toxicants of plant origin volume I alkaloids, CRC Press, Boca Raton, Florida.

Culvenor, C.C. 1973. Alkaloids. Pages 375-446 *in* G.W. Butler and R.W. Bailey, eds. Chemistry and Biochemistry of Forage, Academic Press, London.

Daday, H. 1954. Gene frequencies in wild population of *Trifolium repens* 1: distribution by latitude. Heredity **8**: 61 - 78.

de Bruijin, G.H. 1973. The cyanogenic character of cassava (*Manihot esculenta*). Pages 43-48 *in* Chronic cassava toxicity, proceedings of an interdisciplinary workshop. Int. Develop. Res. Centre Monogr. IDRC-010e. IDRC, Ottawa.

Dewick, P.M. 1994. Isoflavonoids. Pages 117-238 *in* J.B. Harborne, ed. The flavonoids: advances in research since 1986, Chapman and Hall, London.

Di Menna, M.E., P. H. Mortimer, R.A. Prestidge, A.D. Hawkes and J.M. Sprosen. 1992. Lolitrem B concentrations, counts of *Acremonium lolii* hyphae, and the incidence of ryegrass staggers in lambs on plots of A. lolii-infected perennial ryegrass. N. Z. J. Agric. Res. **35**: 211-217.

Dorsaz, A., M. Hostettmann, and K. Hostettmann. 1988. Molluscicidal saponins from *Sesbania sesban*. Planta Medica 225-227.

Field, C.R. 1976. Palatability factors and nutritive values of the food of buffaloes (*Syncerus caffer*) in Uganda. E. Africa Wild. J. **14**: 181-201.

Fletcher, L.R. and Barrell. 1984. Reduced liveweight gain and serum prolactin levels in hoggets grazing ryegrasses containing *Lolium* endophyte. N. Z. Vet. J. **32**: 139-140.

Ford, C.W. 1978. In vitro digestibility and chemical composition of three tropical pasture legumes, *Desmodium intortum* cv. Greenleaf, *D. Tortuosum* and *Macroptilium atropurpureum* cv. Siratro. Aust. J. Agric. Res. **29**: 963-974.

Freeland, W.J., P.H. Calcott and L.R. Anderson. 1985. Tannins and saponin: Interaction in herbivore diets. Biochemical Systematics and Ecology **13**: 189-193.

Froman, B. and S. Persson. 1974. An illustrated guide to the grasses of Ethiopia. Chilalo Agricultural Development Unit, Asella, Ethiopia.

Gallagher, R.T., E.P. White and P.H. Mortimer. 1981. Ryegrass staggers: isolation of potent neurotoxins lolitremA and lolitrem B from staggers-producing pastures. N. Z. Vet. J. **29**: 189-190.

Gordon, A.J. 1978. The chemical structure of lignin and quantitative and qualitative methods of analysis in foodstuffs. Pages 59-103 *in* G.A. Spiller, ed. Topics in Dietary Fiber Research, Pleum Press, New York.

Hamilton, R.J., L.E. Donaldson and L.J. Lambourne. 1971. *Leucaena leucocephala* as a feed for dairy cows: direct effects on reproduction and residual effect on the calf and lactation. Aust. J Agric. Res. **22**: 681-692.

Harborne, J.B. 1993. Introduction to Ecological Biochemistry. Academic Press Limited, London. p. 318.

Harkin, J.M. 1967. Lignin-a natural polymeric product of phenol oxidation. Pages 243-321*in* W.I. Taylor and A.R. Battersby, eds. Oxidative coupling of phenols, Marcel Dekker, New York.

Harkin, J.M. 1973. Lignin. Pages 323-373 *in* G.W. Butler and R.W. Bailey, eds. Chemistry and Biochemistry of Forage, Academic Press, London.

Harris, P.J. and R.D. Hartley. 1976. Detection of bound ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy. Nature 259: 508-510.

Hartley, R.D. and E.C. Jones. 1976. Diferulic acid as a component of cell walls of *Lolium multiflorum*. Phytochemistry **15**: 1157-1160.

Hartley, R.D. and E.C. Jones. 1977. Phenolic components and degradability of cell walls of grass and legume species. Phytochemistry **16**: 1531-1534.

Hartley, R.D., E.C. Jones and J.M. Wood. 1976. Carbohydrates and carbohydrate esters of ferulic acid released from cell walls of *Lolium multiflorum* by treatment with cellulolytic enzymes. Phytochemistry **15**: 305-307.

Hegarty, M.P. 1973. Free amino acids, bound amino acids, amines and ureides. Pages 1-62 *in* G.W. Butler and R.W. Bailey, eds. Chemistry and Biochemistry of Forage, Academic Press, London.

Hegarty, M.P., P.G. Schinckel and R.D. Court. 1964. Reaction of sheep to the consumption of *Leucaena glauca* Benth and to its toxic principle mimosine. Aust. J. Agric. Res. **15**: 153-167.

Hegarty, M.P., R.D. Court, G.S. Christie and C.P. Lee. 1976. Mimosine in *Leucaena leucocephala* is metabolised to a goitrogen in ruminants. Aust. vet. J. **52**: 490.

Hill, D.C. 1973. Chronic cyanide toxicity in domestic animals. Pages 105-111 *in* Chronic cassava toxicity, proceedings of an interdisciplinary workshop. Int. Develop. Res. Centre Monogr. IDRC-010e. IDRC, Ottawa.

Jones, D.A. 1972. Cyanogenic glycosides and their function. Pages

103-124 *in* J.B. Harborne, ed. Phytochemical ecology, Academic Press, London.
Jones, L.H.P. and K.A. Handreck. 1965. The relation between the silica content of the diet and the excretion of silica by sheep. J. Agric. Sci. **65**: 129-134.
Jones, L.H.P. and K.A. Handreck. 1967. Silica in soils, plants, and animals. Advances in Agronomy **19**: 107-149.
Jones, R.J. and J.B. Lowery. 1982. Annual Report 1981-82. CSIRO Division of Tropical Crops and Pastures, Brisbane, Australia.
Jones, R.J. and R.G. Megarrity. 1986. Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australia ruminants to overcome the toxicity of leucaena. Aust. Vet J. **63**: 259.
Jones, R.J., C.G. Blunt and J.H.D. Holmes. 1976. Enlarged thyroid glands in cattle grazing Leucaena pasture. Tropical Grasslands **10**: 113-116.
Keogh, R.G., B.A. Tapper and R.H. Fletcher. 1996. Distributions of the fungal endophyte Acremonium lolii, and of the alkaloids lolitrem B and peramine, within perennial ryegrass. N. Z. J. Agric. Res. **39**: 121-127.
Lundh, L. 1990. Uptake, metabolism and biological effects of plant estrogens in sheep and cattle. Swedish University of Agricultural Sciences, Uppsala.
Majak, M., J.W. Hall and W.P. McCaughey. 1995. Pasture management strategies for reducing the risk of legume bloat in cattle. J. Anim. Sci. **73**: 1493-1498.
Marten, G.C. and M.E. Heath. 1973. Reed canarygrass. Pages 263-276 in M.E. Heath, D.S. Metacalfe and R.E. Barnes, eds. Forages, The Iowa State University Press, Ames, Iowa.
Misra, B.K., S.P. Singh and G.K. Barat. 1981. Oxdrapo: the *Lathyrus sativus* neurotoxin. Intal. Plant Foods Hum. Nutr. **30**: 259-270.
Munday-Finch, S.C., C.O. Miles, A.L. Wilkins and A.D. Hawkes. 1995. Isolation and structure elucidation of lolitrem A, a tremorgenic mycotoxin from perrennial ryegrass infected with *Acremonium lolii*. J. Agric. Food Chem. **43**: 1283-1288.
Porter, L.J. 1994. Flavans and proanthocyanidins. Pages 23-55 *in* J.B. Harborne, ed. The flavonoids: advances in research since 1986, Chapman and Hall, London.
Reed, J. D., R. E. McDowell, P. J. Van Soest and P. J. Horvath. 1982. Condensed tannins: a factor limiting the use of cassava forage. J. Sci. Food Agric. **33**: 213- 220.
Reed, J.D. 1983. The nutritional ecology of game and cattle on a Kenyan ranch. PhD Thesis, Cornell University, Ithaca, New York.
Reed, J.D. and Y. Aleemudin. 1995. Toxicity effects of *Sesbania sesban* and *Sesbania goetzei* feed on broiler chicks. Internal Project Report, Department of Meat and Animal Science, University of Wisconsin-Madison, Madison, Wisconsin.
Reid, R.L. and G.A. Jung. 1973. Forage animal stresses. Pages 659-653 *in* M.E. Heath, D.S. Metacalfe and R.E. Barnes, eds. Forages, The Iowa State University Press, Ames, Iowa.
Reis, P.J., D.A. Funks and M.P. Hegarty. 1975. Fate of mimosine administered orally to sheep and its effectiveness as a defleecing agent. Aust. J. Biol. Sci. **28**: 495-501.
Romeo, J.T. 1989. Heterocyclic amino and imino acids, (mimosine, azetidine-2-carboxylic acid, and pipecolic acid). Pages 125-139 *in* P.R.Cheeke, ed. Toxicants of plant origin volume III proteins and amino acids, CRC Press, Boca Raton, Florida.
Rowan, D.D. and D.L. Gaynor. 1986. Isolation of feeding deterrents against Argentine stem weevil from ryegrass infected with endophyte *Acremonium loliae.* J. of Chemical Ecology **12**: 647-658.
Roy, D.N. and P.S. Spencer. 1989. Lathyrogens. Pages 169-201 *in* P.R.Cheeke, ed. Toxicants of plant origin volume III proteins and amino acids, CRC Press, Boca Raton, Florida.
Scheline, R.R. 1978. Mammalian metabolism of plant xenobiotics. Academic Press, New York.
Schqueir, A.A., D.L. Brown and K.C Klassing. 1989a. Canavanine content and toxicity of sesbania leaf meal for growing chicks. Animal Feed Science and Technology **25**: 137-147.
Schqueir, A.A., D.L. Brown, J.S. Taylor, I. Rivkin and K.C. Klasing. 1989b. Effect of solvent extractions, heat treatments and added colesterol on *Sesbania sesban* toxicity in growing chicks. Animal Feed Science and Technology **25**: 127-135.
Semenye, P.P., L. Musalia, J.F.M. Onim and H.A. Fitzhugh. 1987. Toxicity of *Leucaena leucocephala* and *Sesbania sesban* sun dried leaf hays. Pages 93-101 *in* Proceedings of 6th KVA/SR-CRSP Kenya Workshop, Nairobi, Kenya.
Tapper, B.A. and P.F. Reay. 1973. Cyanogenic glycosides and glucosiniates (Mustard oil glucosides). Pages 447-476 *in* G.W. Butler and R.W. Bailey, eds. Chemistry and Biochemistry of Forage, Academic Press, London.
Tewe, O.O. and E.A. Iyayi. 1989. Cyanogenic glycosides. Pages 43-60 *in* P.R.Cheeke, ed. Toxicants of plant origin volume II glycosides, CRC Press, Boca Raton, Florida.
Van Soest, P.J. 1970. The role of silicon in the nutrition of plants and animals. Cornell Nutrition Conference for Feed manufacturers, Buffalo, New York.
Van Soest, P.J. 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, New York.
Van Soest, P.J. and L.H.P. Jones. 1968. Effect of silica in forages upon digestibility. Dairy Sci.
51: 1644-1648.
Verdcourt, B. and E.C. Trump. 1969. Common poisonous plants of East Africa. Collins, London.
Welch, B.L. and E.D. McArthur. 1981. Variation of monoterpenoid content among subspecies and accessions of *Artemesia tridentata* grown in a uniform garden. Journal of Range Management **34**: 380-384.
Wiegand, R. O., J. D. Reed, A. N. Said and V. N. Ummuna. 1995. Proanthocyanidins and the use of *Sesbania sesban* and *Sesbana goetzei* as protein supplements. Animal Feed Science and Technology **56**: 207-216.
Williams, M., R. F. Barnes and J. M. Cassady. 1971. Characterization of alkaloids in palatable and unpalatable clones of *Phalaris arundinacea*. Crop Sci. **11**: 213-217.
Williams, R.F. 1959. Detoxification mechanisms. Chapman and Hall, London.
Wong, E. 1973. Plant Phenolics. Pages 265-322 *in* G.W. Butler and R.W. Bailey, eds. Chemistry and Biochemistry of Forage, Academic Press, London.
Yoshida, S., Y. Ohnishi and K. Kitagishi. 1962. Histochemistry of silicon in rice plant: I. a new method for determining the localization of silicon within plant tissues. Soil Science and Plant Nutrition **8**: 30-41.