ABSTRACT

SNYDER, LORI JUNE UNRUH. Evaluation of *Robinia pseudoacacia* L. as Browse for Meat Goat Production in the Southeastern USA. (Under the direction of J. PAUL MUELLER).

Demand for goat meat in the southeastern USA is steadily increasing as a result of preferences exhibited by expanding ethnic communities. Feeding systems can be developed to take advantage of the natural preference of goats for browse. A field study was undertaken in Raleigh, NC to measure the effects of spacing (1.0 or 0.5 m) and coppice height (0.25 or 0.5 m) of a 5-year old stand of black locust (BL; *Robinia pseudoacacia* L.) on growth characteristics such as herbage mass (HM), canopy height (H) and width, number and size of main branches, above ground woody biomass, and root collar diameter. A second objective was through regression analysis to identify one or more of the previously mentioned characteristics as a rapid method to estimate HM. The third objective was to determine the relationship between growing-degree-days (GDD) and HM, H, herbage quality indicators (N, in vitro true dry matter disappearance, neutral and acid detergent fiber (NDF and ADF), cellulose, and 72% sulfuric acid lignin) and anti-quality indicators (Folin-reactive phenolics, condensed and hydrolyzable tannins) of BL. The final objective was to evaluate the N metabolism of goats fed BL foliage. Results indicated that coppicing BL trees at 0.5 m and planting at the widest spacing (1.0 m) produced the greatest plant growth. Average HM (2,600 kg ha^{-1}) was observed for the highest coppice height (0.5 m). The character most closely related to HM was size of main branches. In 1999, a dry year, there was a significant relationship between GDD and NDF, ADF (r^2 =0.90 and 0.80, respectively). In 2000, a wet year, GDD was a poor predictor of NDF and ADF. For 1999 and 2000, GDD was a poor predictor of BL tannin concentrations. From the conclusions of the N metabolism trial, goats

consuming BL had lower digestibilities and higher content of N in the feces. Overall, BL contributes well to a silvopastoral system.

EVALUATION OF ROBINIA PSEUDOACACIA L. AS BROWSE FOR MEAT GOAT PRODUCTION IN THE SOUTHEASTERN USA

By

LORI JUNE UNRUH SNYDER

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

DEPARTMENT OF CROP SCIENCE

Raleigh

2003

APPROVED BY:

_____ _____

_____ _____

_____

Chair of Advisory Committee

DEDICATION

To my Father and Mother for their devotion to dairy farming and to the preservation of farmland

AND

To my beloved Grandfather, William G. Unruh, for showing me that farming is one of the greatest joys a family can share

AND

To my husband, Justin P. Snyder for his faith in me

BIOGRAPHY

Lori June Unruh Snyder was born in Dover, Delaware on December 15, 1973. She was raised in Townsend, Delaware on a dairy farm, where nature and agriculture became her passion. She moved away from the home in 1986 to attended boarding school at St. Andrew's School in Middletown, Delaware and began her college career at the University of Delaware in 1992. In her junior year, she transferred to Cornell University in Ithaca, New York, where she studied Entomology and International Agriculture. She received her Bachelor of Science in Entomology in 1996. She continued at Cornell University for her Master of Science in Crop Science (Concentration in Pasture Ecology), graduating in 1998. Her passion for research and teaching led her to a research assistantship in Crop Science at North Carolina State University.

She was married on August 3, 2002 to Justin P. Snyder from Washington, North Carolina and currently residues in Wake Forest, NC. Her ultimate dream is to return to Delaware to continue the tradition of farming at Taylor's Bridge, where her parents currently reside. If her dream comes true, she would like to add goats to the beef farm and increase the acres of pastureland in the farming system.

ACKNOWLEDGEMENTS

Sincere appreciation to members of my committee: Dr. Paul Mueller, Dr. Jean Marie Luginbuhl, Dr. Cavell Brownie, Dr. Larry King and Dr. George L. Wilson. I want to give a special thanks to Dr. Mueller and Dr. Luginbuhl for their time and effort to make my time spent here an experience worthwhile. I want to thank Amy Conrad, Vicki Fouts, Lucie Smith and Ellen Leanard for their help in the laboratory. Dr. Joe Burns and Dr. Kent Turner, both with the USDA, were also very helpful in preparation of forage testing materials. I would like to thank Brian Fouts for his help with fieldwork during the summers. I was very fortunate to have wonderful summer help: John Mueller, Lisa Benton, Arial Thompson, Jordon Joyce, and Holly Murray. I want to thank Dr. Jim Green for being an excellent mentor in the Forage teaching program and helping with the final preparation of my dissertation. Also, I want to give a special thanks to Dr. Bob Patterson for his support and mentoring in the Agronomy teaching program. I also want to thank the following former Master of Science graduate students: Mary Thurman Wilks and Mike Scott. I am forever grateful that I met Kelly Kuches Palaisa at NCSU. Without a doubt, Kelly will always there for me and I will be there for her. I would like to thank my Mother and Father for their continual support and love. My parents are and will always be my inspiration. I want to give a special thanks to my Grandmother Hickman for her love and prayers. Also, I want to thank the Farmers and the Snyders for their support. Finally I thank my husband, Justin P. Snyder, for his loving care and for his time with me when collecting data from the goats. Justin's support throughout my dissertation has been essential and I thank him for his nurturing love.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

APPENDIX

LIST OF FIGURES

CHAPTER ONE

CHAPTER TWO

Chapter One

Literature Review: Black Locust and Tannins

1.0 Introduction

Black locust (BL; *Robinia pseudoacacia* L.) is a nitrogen-fixing leguminous tree with alternate, pinnately compound leaves native to southeastern North America and naturalized in the temperate regions of Europe and Asia. Black locust is a multi-purpose species often used as a source of timber for fence posts, mine timbers, poles, railroad ties, insulator pins, construction for wooden ships, boxes, crates, stakes, paneling and construction boards (Huntley, 1990; Keresztesi, 1980). Due to its susceptibility to locust borer (*Megacyllene robiniae* [Forst.]) infestations, BL is not an important commercial timber species in the USA (Huntley, 1990). Nevertheless, BL has several other favorable environmental attributes. It is a source of biological nitrogen (N), producing 75 to 150 kg N ha^{-1} $year^{-1}$ (Boring et al., 1981) that can enhance N-poor ecosystems. It has also been used for erosion control (Huntley, 1990) and to scavenge excess nutrients excreted by livestock on steep hillside pastures (Barrett et al., 1990; Feldhake and Schumann, 1997).

Black locust has been used for livestock feed in countries around the world, including Korea and Bulgaria (Keresztesi, 1983 and 1988). It has been documented in the highlands of Nepal and northern India, where BL is naturalized, branches above the reach of livestock are cut when other green forages are scarce, the foliage is fed to livestock, and the wood is used for fuel (Keresztesi 1980; 1983; and 1988). Black locust has been grown successfully in dry Mediterranean summer climates, where the introduction of a woody plant is an effective solution to satisfying feed demand when grasses and legumes become dormant in the summer (Ainalis and Tsiouvaras, 1998; Papachristou and Papanastasis, 1994; Papachristou,

1999; Papachristou et al., 1999). According to Papachristou (1999) and Papachristou et al. (1999), supplementation of BL is an effective goat (*Capra hircus hircus*) feed, producing live-weight gains comparable to alfalfa (*Medicago sativa* L.). Furthermore, researchers have reported high preference of BL as browse for goats (Addlestone et al., 1999; Lambert et al., 1989; Papachristou and Papanastasis, 1994)

Addlestone et al. (1999) concluded that BL had potential as a browse species due to its high herbage mass production (leaves and non-woody material), averaging 2,400 dry matter (DM) kg ha $^{-1}$, relative to other browse species tested. Papanastasis et al. (1997) reported a mean total biomass of 2,280 DM kg ha $^{-1}$ and 1,026 DM kg ha^{-1} of grazeable leaves and twigs.

Black locust leaf crude protein (CP) levels were similar to those of alfalfa, ranging from 20 to 25% (Addlestone, 1996; Baertsche et al., 1986; Boring and Swank, 1984; Papchristou et al., 1999; Unruh et al., 2001). Research also showed that BL accumulates moderately high tissue concentrations of macronutrients (1.27% Ca; 1.72% K; 0.18% P) and produces large quantities of foliage with high leaf surface area (Borning et al., 1981; Addlestone et al., 1999), rendering this species an excellent browse source for cattle or meat goat production. Cheeke (1998) reported that the plant contains substances i.e. that are potentially toxic to livestock. Black locust chemical composition includes not only primary plant metabolites such as N, but also secondary plant metabolites such as phenolics (Cheeke, 1998).

2.0 General Background of Black Locust

2.1 Habitat of Black Locust

Black locust occurs naturally in a wide range of locations and soils. It is indigenous to the Appalachian Mountains in southeastern North America, but is now naturalized throughout temperate regions of eastern North America and parts of Europe and Asia (Barrett et al., 1990). It has been planted in almost every state within the continental USA (Collingwood, 1937) and is commonly found on moist slopes of the eastern mountains below 1,034 m elevation (Harlow et al., 1979). Nevertheless, in the Great Smoky Mountains National Park, BL can be found as high as 1,612 m (Whittaker, 1956). The acceptable soil pH for growth is from 4.6 to 8.2 (Huntley, 1990). It grows best on moist, rich, loamy soils or those of limestone origin. One of the advantages of BL over other trees is that it establishes easily on a wide array of disturbed sites such as old fields or cleared areas, where there is no competing over-story vegetation. It has also been used to control erosion along roadsides (Huntley, 1990).

2.2 Botany of Black Locust

Black locust is in the botanical family of Leguminosae or Fabaceace. It sprouts readily from both stump and roots, especially after being cut or damaged. Its main mode of vegetative reproduction is by root sprouts. At maturity, BL is a medium-sized, oval shaped deciduous tree that stands at a maximum of 14 m high and spreads at least 8 m wide. Black loucst is characterized by rot-resistant wood and N-fixing root nodules (Keresztesi, 1980).

The leaves are alternate and pinnately compound with 7 to 19 oval dark blue-green leaflets with smooth margins that are 2 to 5 cm long. The twigs are angled, somewhat zigzag, with prominent stipular spines 1 to 2.5 cm long. The bark is reddish-brown to nearly black, rough, and is deeply furrowed. Normally, BL does not produce seed until approximately age six (Olson, 1974). The perfect white flowers are very fragrant and

arranged in drooping clusters (racemes), appearing during April and May in North Carolina. Insects, primarily bees (*Apis mellifera*), pollinate the flowers. The fruit is a thin, flat pod, 5 to 10 cm long, containing 4 to 8 seeds and splitting into halves when ripe. In Hungary, BL flowers are vital to commercial honey production, and are noted for producing honey with outstanding taste and color (Keresztesi, 1983).

Black locust is moderately frost tolerant, although cold weather damage has occurred in the colder parts of its range (Roach, 1965). Compared with other eastern hardwood species, it is more susceptible to damage by insects and diseases. The two pest species that make growing BL for timber impractical are the locust borer and heart rot fungi (*Phellinus rimosus* [Berkeley] Pilát and *Polyporus robiniophilus*) (Huntley, 1990). Locust borer larvae and the heart rot fungi attack BL in a synchronous fashion. Locust borer larvae construct feeding tunnels throughout the wood, which serve as entry points for heart rot fungi that cause extensive wood decay. When trees are young, locust borer damage can be so extensive that infested trees are not suitable for fence posts (Roach, 1965). In addition, locust leaf miner (*Odontota dorsalis* [Thunb.]) outbreaks occur almost yearly. The locust twig borer (*Ecdytolopha insiticiana* [Zeller]) attacks over a wide area of the USA and, in heavily infested areas, BL seedling mortality may be high (Huntley, 1990). Witches' broom (*Chlorogenus robiniae* [Holmes]) disease, a virus, affects most large trees in the southern Appalachians and causes extensive decay of trunk wood (Baker, 1972).

2.3 Black Locust as Food and Shelter for Wildlife

As wildlife food, BL seeds are rated low for wildlife intake, but northern bobwhites (*Colinus virginianus*) and other game birds and squirrels (*Sciurus carolinensis*) do consume them at a minimal level. In Illinois, BL provides food for northern bobwhites (Ellis et al.,

1969). Georgia researchers (Harlow et al., 1975) noted that BL is a browse choice for white-tailed deer (*Odocoileus virginianus*), which often browse the young plant growth. Woodpeckers often find homes in older BL trees infected with heart rot. Nest cavities of the downy woodpecker (*Picoides pubescens*), hairy woodpecker (*Picoides villosus*), and common flicker (*Colaptes auratus*) have all been found in BL (Conner et al., 1975).

2.4 Secondary Metabolites of Black Locust and Effect on Animals

As mentioned previously, the heartwood of BL is primarily used for fence posts, poles, railroad ties, and mine timbers and can endure for over 100 years in the soil. Its remarkable decay resistance is due to high flavonoid concentrations (6% of dry weight), specifically the constituents, robtin (15,000 ppm), robinetin (20,000 to 80,000 ppm) and dihydrorobinetin (53,000 to 176,000 ppm) (Smith et al., 1989). Other, flavonoids present in the heartwood are butein, butin, fisetin, fustin, and liquiritigenin (Duke, 2000).

The flowers contain trace amounts of robinin; however, no authors cited flowers as a toxicity concern to livestock (Duke, 2000).

The bark and root are reported to be the most toxic to livestock due to the presence of the secondary metabolite robin, (16,000 ppm) a protein belonging to a group of compounds known as toxalbumins (Cheeke, 1998). Robin is considered a glycoprotein (a lectin) that agglutinates red blood cells (phytohemagglutinins) (Cooper and Johnson, 1984). The bark also contains a glucoside robinitin (30,000 ppm), syringin, a glucoside found in trace amounts, and tannins (33,000 to 70,000 ppm). The inner bark is reported to contain amygdalin and urease (Duke, 2000).

Experimental feeding of the bark to horses (*Equus caballus)* produced toxicity when ingested as an aqueous extract at 0.1% of body weight (BW) and as powdered bark at 0.04%

of BW (Kingsbury, 1964; Cooper and Johnson, 1984). Horses that ingested BL leaves, sprouts and bark showed clinical signs of toxicity as soon as one hour after consumption and required medical attention due to the effect of toxins on the gastrointestinal tract as well as the nervous system. Toxicity symptoms include anorexia, depression, posterior paralysis, abdominal pain, nausea, diarrhea (which may be bloody) and abnormalities in heart rate and/or rhythm. Symptoms indicating colic can also occur. Though death occurs in several cases of colic, BL is not always lethal. Some horses recover despite showing clinical signs, an indication of the dose-dependent nature of the toxin. Postmortem findings have shown mucous inflammation of the gastrointestinal tract and occasional severe gastroenteritis. In some cases, a yellowish pigmentation of the membranes has occurred (Hansen, 1924; Kingsbury, 1964).

Experiments have shown that cattle (*Bos taurus*) are less sensitive to the toxins in BL bark than horses. Toxicity symptoms for cattle include anorexia, weakness, posterior paralysis, nausea, coldness of the extremities, and dilation of the pupils. Death sometimes occurs (Hansen, 1924; Kingsbury, 1964). For cattle, the poisonous substances appear to be about one-tenth as toxic as compared with horses.

Horses and cattle are most commonly poisoned from eating foliage from sprouting BL stumps. As a result, they consume the soft bark containing the deleterious secondary metabolite called robin (a lectin) (Cheeke, 1998). Extreme caution is necessary if horses are confined in the vicinity of BL trees.

No information was reported on the effects of sheep (*Ovis aries*) and goats to BL bark ingestion. Although, ingested seedpods have caused minor illness in sheep (Kingsbury, 1964) due to trace amounts of toxic agents found in the seeds. These agents included the

carbohydrates, sucrose and raffinose, and the non-nutrient amino acid, canavanine (Duke, 2000; Brown, 1998). In general, the seed contains: moisture, 10.3 to 11.5 %; CP, 38.8 to 39.5%; fat, 10.2 to 11.0%; N-free extract, 20.4 to 23.0%; crude fiber, 12.9 to 13.6%; ash, 4.0 to 4.7%; calcium (CaO), 0.19%; and phosphorous (P_20_5), 1.65 % (Duke, 2000).

The leaves are extremely poisonous to chickens (*Gallus domesticus)* and can cause degenerative changes in the liver and kidney (Cooper and Johnson, 1984). More importantly, when ruminants consume the leaves, they may exhibit anti-quality responses affecting their forage intake (Van Soest, 1982). The main toxic factors that have been reported in ruminant species are tannins.

3.0 Tannins: An Overview of Phenolics and Their Role in Livestock Nutrition

3.1 The History of Tannins and their Distribution in Plants

Tannins are widely distributed in vascular plants. They are considered a secondary metabolite belonging to the phenolic class. Phenolic compounds are formed via the shikimic acid pathway. This same pathway also forms isoflavones, coumarins, lignins and the aromatic amino acids trytophan, phenylalanine and tyrosine (Cannas, 2001; Waterman and Mole, 1994). Historically, the tanning process, converting animal hides into leather, has been a part of many cultures for hundreds of years. The word tanning often refers to the action of preserving or waterproofing. Also, tannins are used to flavor many foods and drinks, especially tea and wine (Bernays et al., 1989).

Tannins are present both in Gymnosperms and Angiosperms. However, within Angiosperms, tannins are more prevalent in dicotyledons than in monocotyledons. Dicotyledonous families rich in tannins include Fabaceae, Anacardiaceae, Combretaceae, Rhizophoraceae, Myrtaceae, and Polygonaceae. Certain plant species commonly used to

provide tannins for tanning purposes are wattle (*Acacia* sp.), oak (*Quercus* sp.), eucalyptus (*Eucalyptus* sp.), birch (*Betula* sp.), willow (*Salix caprea*), pine (*Pinus* sp.) and quebracho (*Schinopsis balansae*). Monocotyledons rich in tannins are Sorghum species (Cannas, 2001; Van Soest, 1982), which concentrate tannins in the seeds. Ingredients commonly used in the tanning process are plant extracts from bark, wood, fruit, fruitpods, leaves, roots, and/or plant galls (Cannas, 2001). Although tannins may be present in any or all plant parts, the highest concentrations are found in the woody, lignified tissues (Bernays et al., 1989).

Tannin concentrations are known to fluctuate with age and can change in response to biotic and abiotic stresses (Appel et al., 2001;Waterman and Mole, 1994). Feeney and Bostock (1969) and Becker and Martin (1982) noted that the age of the leaf was important when documenting concentration of phenolics. The protein-precipitating capacity of an immature leaf extract was higher than a mature leaf extract when working with Red Meranti (*Shorea* spp.). In addition, Feeney and Bostock (1969) noted an increase from July to September in tannin concentrations of oak leaves. Waterman and McKey (1989) reported similar finding where younger leaves had higher concentrations of phenolics than mature leaves. Horner (1988) reported that non-linear trends might exist when dilution effects caused by leaf expansion occur. There are, however, contradicting reports from Parker (1977) and Glyphis and Puttick (1988) who found that as the season progressed and or the leaves aged, levels of phenolics increased or at least plateaued.

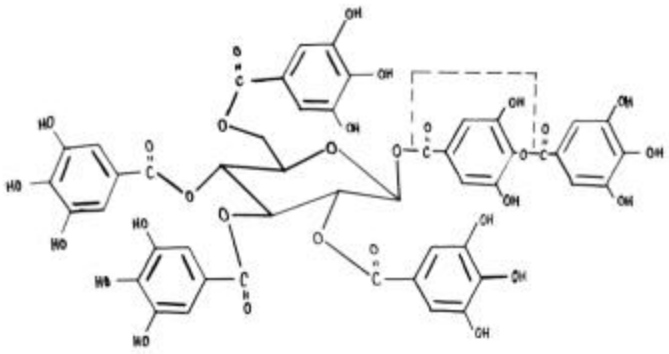
Historically, tannins have been reported to play a role as defensive chemicals that protect plant tissue from herbivore attacks. Though tannins do not have a specific metabolic function, they can have adverse effects upon the following organisms: viruses, bacteria, fungi, insects, reptiles, birds and mammals (Feeny, 1976). Tannins are located in the

vacuoles or surface wax of plants and do not interfere with plants metabolism. They are generally characterized by their ability to form insoluble complexes with protein, starch, cellulose, and minerals.

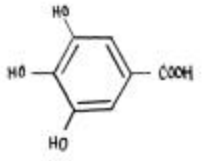
3.2 Chemical Structure of Tannins: Hydrolyzable and Condensed

Tannins are described as oligomeric compounds containing multiple structure units with free phenolic groups that range in molecular weight (MW) from 500 to > 20,000. They are soluble in water, with exception of some high MW structures, and have the ability to bind proteins and form insoluble or soluble tannin-protein complexes (Cannas, 2001; Kumar and Vaithiyanathan, 1990; Kumar and Singh, 1984). Generally, tree leaves and browse contain two types of tannins: hydrolyzable and condensed (Kumar and Vaithiyanathan, 1990). Hydrolyzable tannins (HT; gallotannins and ellagitannins) are polyesters of gallic acid or ellagic acid, respectively, and other phenolic acids derived from them, with a sugar (normally D- glucose) as the central core (Figure 1). The typical M.W. is 900 for gallotannins and 2000 to 5000 for ellagitannins (Bernays et al., 1989). The most notable source of gallotannins is tannic acid that is obtained from the twig galls of Sumac tree (*Rhus semialata)* (Cannas, 2001; Perchellet et al., 1994.). Hydrolyzable tannins are readily hydrolyzed by acid and are present in low amounts in plants. Also, it is known that HT is restricted to dicotyeledons (Bernays et al., 1989). There are two additional classes of HT: taragallotannins (gallic acid and quinic acid as the core) and caffetannins (caffeic acid and quinic acid) (Cannas, 2001; Van Soest, 1982).

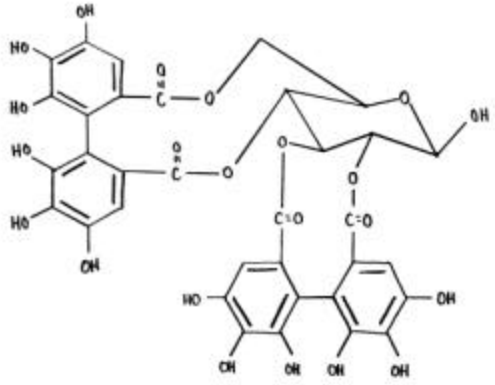
Condensed tannins (CT; proanthocyanidins) are more widely distributed than HT. They are oligomers or polymers (MW= 1,000 to > 20,000) of catechins (flavonoid phenols linked by carbon-carbon bonds), which are resistant to hydrolytic degradation (Figure 2).



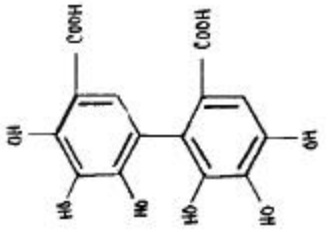
1: Gallotannin



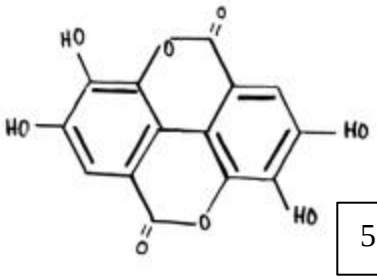
2: Gallic acid



3: Ellagitannin



4: Hexahydroxydiphenic acid



5: Ellagic acid

Figure 1.1: Examples of hydrolyzable tannins: (1) gallotannin and (3) ellagitannin and their hydrolysis products. In the case of gallotannin the dotted line indicates the repeating gallic acid unit (2). Hexahydroxydiphenic acid (4) spontaneously dehydrates to the lactone form as ellagic acid (5) (Van Soest, 1982; Hagerman et al., 1998).

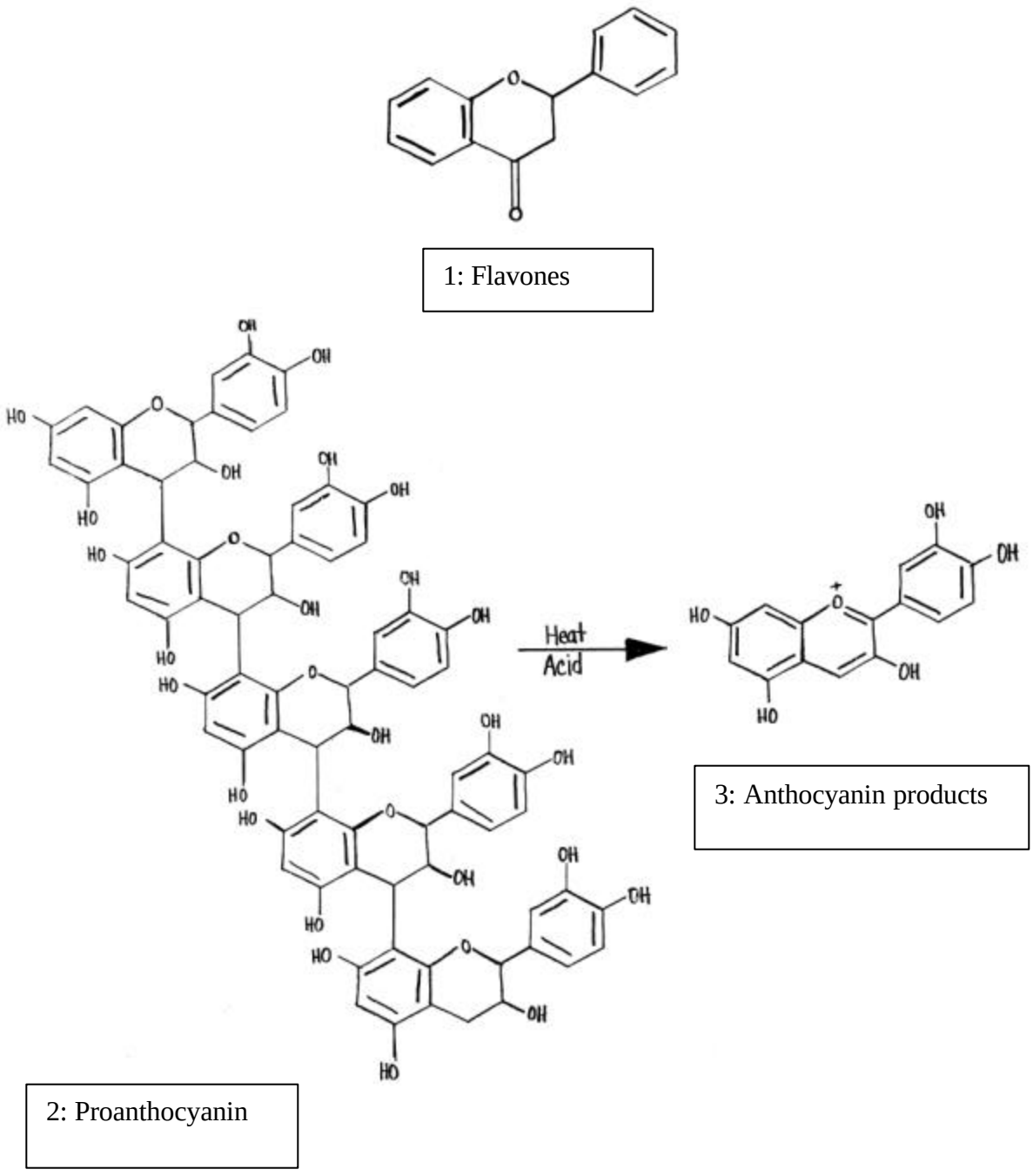


Figure 1.2: Examples of condensed tannins that are polymers of flavo nes: (1). The structure of a typical condensed (proanthocyanidins) tannin (2). When heated with strong acids it depolymerizes to form colored anthocyanin products (3) (Van Soest, 1982; Cheeke, 1998).

The linkage between the monomers, typically a carbon condensation, is relatively stable under the conditions that cleave linkages in HT. Condensed tannins produce red anthocyanins (glycosides of anthocyanidins) upon heating of proanthocyanidins in acidic alcohol solutions. The anthocyanidins produced give the astringent taste of fruit and wines (Van Soest, 1982).

3.3 Anti-quality Factors of Tannins

Several animal scientists explored the nutritional effects of tannins in detail (Kumar and Singh; Robbins et al., 1987; Van Soest, 1982). As expected from their strong efficacy as plant defense chemicals, tannins express a variety of toxic or anti-quality effects when presented in the diet of ruminant animals (Van Soest, 1982).

Animals most commonly consume leaf tissue during browsing. More specifically, it is reported that during chewing, plant tissues containing tannins coat the proteinaceous mucous membranes of the mouth and result in an astringent sensation similar to that experienced by human beings while drinking tea and presumably by large herbivores as well (Van Soest, 1982). This astringency is reported to deter animals from grazing plants containing tannins, leading to a reduction of voluntary intake. Nevertheless, trees and shrubs are components of most natural pastures in many regions of the world (Silanikove et al, 1996). Therefore, it is inevitable that some animals will consume some tannin-rich forage in those situations.

The presence of high tannin levels in forage has been shown to affect palatability, voluntary intake, digestibility, and N retention, leading to decreased animal productivity (Silanikove et al. 1996, Kumar and Vaithiyanathan, 1990; Van Soest, 1982). The reduction of digestibility negatively influences intake because of the filling effect associated with

undigested feedstuff (Cannas, 2001). Many animals exhibit a protective response that acts as a first defense against ingested tannins by secreting proline-rich proteins (PRP) (Mehansho et al, 1987). Browsers such as mule deer (*Odocoilieus heminous heminous*) or goats tend to be more tolerant of tannins in their diets because of their higher production of PRP than other animals. Proline-rich proteins produced in the parotid salivary glands of ruminants are three times greater in browsers than in grazers, while they are intermediate in mixed feeders (Bernays et al, 1989). Deer are more tolerant to tannins than are goats, sheep and cattle (Cannas, 2001). Of significance, PRP have a high affinity for tannins and tend to complex tannins before feed enters the rumen (Kumar and Vaithiyanathan, 1990; Silanikove et al., 1996; Robbins et al., 1987). Provenza and Malechek (1984) noted that perhaps as much as 50 % of the dietary tannins are bound during digestion by the PRP, leading to reduced fecal N losses. Robbins et al. (1987) reported that goats are considered to be mixed grazers and usually consume significant amounts of tanniferous forages that stimulate the salivary glands to permit more PRP production to effectively bind tannins. This phenomenon is more prominent in goats than with sheep or cattle. Also, Silanikove et al. (1996) suggested that exposing goats to tannins enhanced the secretion of proteins in the parotid saliva.

Horton and Christensen (1981) and Ayers et al. (1996) examined the metabolism of sheep and goats fed BL as dried material. Both concluded that BL digestibility was lower than alfalfa meal. They also reported the probable cause of depressed digestibility was tannin-protein complexes formed in the gut of the sheep and goats, although they did not analyze BL for presence of phenolic compounds. Nevertheless, under pasture conditions, animals browse fresh herbage with a high moisture content that may tend to dilute potential anti-quality components. Additionally, herbage fed as meal has a rapid passage rate through

the rumen because of reduced particle size, where fresh whole leaves would tend to change the rate of passage and influence digestibility (Van Soest, 1982).

Papachristou (1999) assessed the value of BL browse using male goats by comparing a diet of 100% BL to a diet of 100% alfalfa pellets. Branches of BL were cut daily and offered within the metabolism crates; however tannin analysis was not correlated with the DM intake. Papachristou (1999) concluded that intake of BL feed was comparable to that of alfalfa pellets. Papachristou et al. (1999) also ran an experiment with BL browse; however, BL was only provided as a supplement (2 kg fresh branches/animal/ day) to goats that were grazing on kermes oak (*Quercus coccifera* L.) shrubland. The results of this study supported the observation that BL was comparable to alfalfa. For a complete research evaluation of BL, concentrations of tannins need to be quantified and the effects that tannins have on animal performance need to be explained.

Kumar and Horigome (1986) purified BL CT and found that their protein-precipitating capacity increased with molecular size. It has not been reported if HT is present in BL and if so, what concentrations will affect animal performance.

Hagerman et al. (1992) performed an experiment with mule deer and Suffolk sheep comparing the effects of digestion with tannic acid (a gallotannin- HT) and quebracho tannin (CT). The results found that CT diminished protein digestibility and HT do not affect protein digestibility. Hagerman et al. (1992) reported that none of the ingested (HT) tannic acid was excreted in the feces because the HT was absorbed in the gastric acid environment beyond the rumen, releasing protein, and passing into the urine.

Horigome et al. (1988) reported that the main phenolic compounds in BL were CT. Kumar and Horigome (1986) purified CT from BL leaves in five fractions and discovered

that their protein-precipitating capacity increased with molecular size. It was noted that when the M. W. was greater than 5,000, the CT became rather insoluble in physiological solutions and lost their protein-precipitating capacity. Singh (1982) reported that the palatability of BL improved with a decrease in tannin content.

Condensed tannins are known to have both positive and negative effects on nutritional quality of legumes. At low levels, they can prevent bloat and increase the level of by-pass protein and some essential amino acids to the small intestine. At high levels, CT may over protect protein resulting in reduced activity of the rumen bacteria, impaired production of gut enzymes (proteases, pectinases, amylases, cellulases and lipases) and loss of protein in the feces (Jackson et al., 1996; Reed, 2001; Sandusky et al., 1977). Continued consumption of CT ultimately leads to gastritis, intestinal irritation, and oedema of the intestine of cattle and sheep (Sandusky et al., 1977).

Some reports have also suggested that tannins, in general, may act directly as anthelmintics against parasitic nematodes as well as indirectly by improving N supply (Butter et al., 2000; Min and Hart, 2003). Recent experiments in New Zealand with dairy cows have attributed a large increase in milk production to the protein-binding properties of tannins in the rumen (Woodward et al. 1999).

One troubling aspect of high-tannin forage is its effect on N metabolism. With ruminants, low N levels can suppress microbial activity in the rumen, thereby decreasing fiber digestibility and lowering forage intake. It is also known that high tannin levels may depress intake by decreasing palatability and suppressing protein digestion if the total tannin concentration is above 5% DM intake (McLeod, 1974). Total phenolics above 9% DM intake can be lethal to an animal that has no other feed (Kumar, 1983). Total phenolics

concentrations of 2% of the feed DM have been reported to reduce food intake due to a decreased DM digestion of the forage (Kumar and Vaithiyanathan, 1990). McSweeney et al. (1988) reported that two of four sheep that ingested 0.9 g HT kg^{-1} BW of yellow-wood foliage (*Terminalia oblongata*) showed signs of toxicity in 15 days.

Reed et al. (1985) reported that sheep adapt slowly to tannins when fed a diet of *Acacia* spp. leaves, suggesting that ruminal organisms in some way detoxify the tannins. Brooker et al. (1994) isolated a tannin-degrading bacteria (*Streptococcus capriunus*) from feral goats that were grazing on *Acacia* spp.; the same bacteria could not be isolated from domestic sheep or goats (Cheeke, 1998). Scientific evidence shows that in general tannins may indirectly affect ruminal function by reducing ruminal ammonia levels through decreased protein degradation in the rumen.

I*n vitro* studies performed by Forwood and Owensby (1985) found estimated digestibility of tree leaves declined with an increase in tannin content. For example, the concentration of total phenolics was 15.3% and digestibility was 42.6% as an example for Bur Oak (*Quercus macrocarpa)*. It is recommended that if tannins are present in forages, the diet should be supplemented with a non-protein nitrogen source such as urea (Van Soest, 1982).

A preventative measure to increase intake in animal diets rich in tannins is to supplement the diet with a compound that has a high affinity for tannins, such as polyethylene glycol-4000 (PEG-4000) (Horigome et al, 1988). Polyethylene glycol-4000 has a higher affinity to tannins than do proteins. It is a water-soluble solid that is used extensively in the cosmetic and toiletry industry. This compound can be sprayed on the forages or added in the diet and is fairly inexpensive (Silanikove et al., 1994). Polyethylene

glycol-4000 often increases feed palatability and digestibility and results in higher animal productivity. Polyethylene glycol-4000 has the ability to inactivate tannins if used with the feed source. Horigome et al. (1988) observed that in rats the protein digestibility of BL leaves increased from 49.1 to 70.7% with the addition of PEG-4000. These results were due to the binding of dietary tannins by PEG-4000. This experiment may help researchers outline future assays, which could be performed on other ruminants such as goats to further understand the effect of tannin-rich browse (Horigome et al., 1998). Nevertheless, PEG-4000 seems to be a useful experimental tool for understanding the effect of tannins on forage utilization; though it seems too impractical for farmers (Cheeke, 1998). Kabasa (2000) reported that PEG-4000 deactivates CT, and it was concluded that CT play a significant role in reducing the negative effects of gastrointestinal worms burdens in the natural free-range feeding systems of the Ankole rangeland in Uganda.

4.0 Summary

Black locust grows very rapidly, survives droughts and severe winters, tolerates infertile and acidic soils, provides an excellent source of N and has been documented to be nutritionally equivalent to alfalfa. Thus, this species holds much potential for agronomists, animal scientists, and soil conservationists.

The role of BL foliage can be seen in the same light as a forage legume. According to Barrett et al. (1990), at high plant densities, the new growth of BL has the potential to be harvested with conventional farm machinery to make hay or silage, because the new stems and stipular spines are soft. Black locust wood is quite useful for lumber, poles, wood fiber, land reclamation, bee keeping, and fuel. In the southeastern USA, BL is common in well-drained woods, thickets and waste areas. They are often planted along highways and

fencerows as ornamentals and for erosion control. The compound leaves should be considered a valuable feed resource for the southeastern region, despite the presence of anti-quality factors in the foliage. Animals such as goats, sheep and cattle can utilize BL foliage if slowly adapted to feeds high in tannins, especially if used as part of a grazing system or if supplemented with other high quality feed.

5.0 References

1. Addlestone, B.J. 1996. Density and cutting height effects on the herbage mass of three tree legumes grown for meat goat production. M.S. thesis. North Carolina State Univ., Raleigh.

2. Addlestone, B.J., J.P. Mueller, and J-M. Luginbuhl. 1999. The establishment and early growth of three leguminous tree species for use in silvopastoral systems of the southeastern USA. Agroforestry Syst. 44:253-265.

3. Ainalis, A.B., and C.N. Tsiouvaras. 1998. Forage production of woody fodder species and herbaceous vegetation in a silvopastoral system in Northern Greece. Agroforestry Syst. 42:1-11.

4. Appel, H.M., H.L. Govenor, M. D'ascenzo, E. Siska, and J.C. Shultz. 2001. Limitations of folin assays of foliar phenolics in ecological studies. J. Chem. Ecol. 27(4):761-778.

5. Ayers, A.C., R.P. Barrett, and P.R. Cheeke. 1996. Feeding value of tree leaves (hybrid popular and black locust) evaluated with sheep, goats, and rabbits. Anim. Feed Sci. Technol. 57:51-62.

6. Baertsche, S.R., M.T. Yokoyama, and J.W. Hanover. 1986. Short rotation, hardwood tree biomass as potential ruminant feed-chemical composition, nylon bag ruminal degradation and ensilement of selected species. J. Anim. Sci. 63:2028-2043.

7. Baker, W.L. 1972. Eastern Forest Insects. USDA Agric. Handb. 1175. U.S. Gov. Print Office, Washington, DC.

8. Barrett, R.P., T. Mebrahtu, and J.W. Hanover. 1990. Black locust: A multi-purpose tree species for temperate climates. p. 278-283. *In*: J. Janick, and J.E. Simon (ed.) Advances in New Crops. Timber Press, Portland, OR.

9. Becker, P., and J.S. Martin. 1982. Protein-binding capacity of tannins in *Shorea* (Dipterocarpaceae) seedling leaves. J. Chem. Ecol. 8:1353-1367.

10. Bernays, E.A., G. Cooper-Driver, and M. Bilgener. 1989. Herbivores and plant tannins. Adv. Ecol. Res. 19:263-291.

11. Boring, L.R., C.D. Monk, and W.T. Swank. 1981. Early regeneration of a clear-cut southern Appalachian forest. J. Ecol. 62:1244-1253.

12. Boring, L.R., and W.T. Swank. 1984. The role of black locust (*Robinia pseudoacacia*) in forest succession. J. Ecol. 72(3):749-766.

13. Brooker, J.D., L.A. O'Donovan, I. Skene, K. Clarke, L. Blackall, and P. Muslera. 1994. *Streptococcus caprinus* sp. nov., a tannin-resistant ruminal bacterium from feral goats. Lett. Appl. Microbiol. 18:313-318.

14. Brown, D. 1998. Toxic Agents in Plants. [Online] Available at http://www.ansci.cornell.edu/plants/toxicplants/toxagent.html (posted 28 July 1998).

15. Butter, N.L., J.M. Dawson, D. Wakelin, and P.J. Buttery. 2000. Effect of dietary tannin and protein concentration on nematode infection (*T. colubriformis*) in lambs. J. Agric. Sci. 134:89-99.

16. Cannas, A. 2001. Tannins: Fascinating but Sometimes Dangerous Molecules. [Online] Available at http://www.ansci.cornell.edu/toxicagents/tannin/tannin.htm (posted16 September 2001).

17. Cheeke, P.R. 1998. Natural Toxicants in Feeds, Forages and Poisonous Plants. Interstate Publishers Inc., Danville, IL.

18. Collingwood, G.H. 1937. Knowing Your Trees. The American Forestry Association. Washington, DC.

19. Conner, R.N., R.G. Hooper, H.S. Crawford, and H.S. Mosby. 1975. Woodpecker nesting habitat in cut and uncut woodlands in Virginia. J. Wildl. Manage. 39(1):144-150.

20. Cooper, M.R., and A.W., Johnson. 1984. Poisonous plants in Britain and their effects on animals and man. Her Majesty's Stationary Office, London, England.

21. Duke, J. A. 2000. Dr. Duke's phytochemical and ethnobotanical databases. [Online] Available at (http://www.ars-grin.gov/duke/) (posted 10 March 1998).

22. Ellis, J.A.; W.R. Edwards, and K.P Thomas. 1969. Response of bobwhites to management in Illinois. J. Wildl. Manage. 33(4):749-762.

23. Feeny, P. 1976. Plant apparency and chemical defense. Rec. Adv. Phytochem. 10:1-40.

24. Feeney, P.P, and H. Bostock. 1969. Seasonal changes in the tannin content of oak leaves. Phytochem. 7:871-880.

25. Feldhake, C.M., and C.M. Schumann. 1997. Establishment of Black Locust in Hill Pastures of Its Native Appalachian Range. *In*: L.E. Buck and J.P. Lassoie (ed.) Proc. 5th North American Agroforestry Conf., Ithaca, New York, Aug. 3-6, 1997.

26. Forwood, J.R., and C.E. Owensby. 1985. Nutritive value of tree leaves. J. Range Manage. 38: 61-64.

27. Glyphis, J.P., and G.M Puttick. 1988. Phenolics in some southern African Mediterranean shrubland plants. Phytochem. 27:743-752.

28. Hagerman, A.E., K.M. Riedl, G.A. Jones, K.N. Sovik, N.T. Ritchard, P.W. Hartzfeld, and T.L. Riechel. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Chem. 46:1887-1892.

29. Hagerman, A.E., C.T. Robbins, Y. Weerasuriya, T. C. Wilson, and C. McCarthur. 1992. Tannin chemistry in relation to digestion. J. Range Manage. 45: 57-62.

30. Hansen, A.A. 1924. Robitin-a potent plant poison. Better Crops. 22(2): 22-23,44.

31. Harlow, W.M., E.S. Harrar, and F.M. White. 1979. Textbook of dendrology. (6th Ed.) McGraw-Hill, New York.

32. Harlow, R.F., P.A. Shrauder, P.A., M.E. Seehorn. 1975. Deer browse resources of the Oconee National Forest. Res. Pap. SE-137. USDA, Forest Service. Southeastern Forest Exp. St. Ashville, NC.

33. Horigome, T., R. Kumar, and K. Okamoto. 1988. Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes in vitro and in the intestine of rats. Br. J. Nutr. 60:275-285.

34. Horner, J.D. 1988. Astringency in douglas fir foliage in relation to phenology and xylem pressure potential. J. Chem. Ecol. 14:1227-1237.

35. Horton, G. M. J., and D.A. Christensen. 1981. Nutritional value of black locust tree leaf meal (*Robinia psudeoacacia*) and alfalfa meal. Can. J. Anim. Sci. 61(2):503-506.

36. Huntley, J.C. 1990. *Robinia pseudoacacia* L., black locust. p.755-761. *In*: Burns, R. M. and Honkala, B. H. (ed). Silvics of North America, Volume 2, Hardwoods. USDA-Forest Service. Agric. Handb. 654. U.S. Gov. Print Office, Washington, DC.

37. Jackson, F.S., T.N. Barry, C. Lascano, and B. Palmer. 1996. The extractable and bound condensed tannin content of leaves from tropical tree, shrub and forage legumes. J. Sci. Food Agric. 71:103-110.

38. Kabasa, J.D. J. Opuda-Asibo, U. ter. Muelen. 2000. The effect of oral administration of polyethylene glycol on faecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. Trop. Anim. Health Prod. 32: (2):73-86.

39. Keresztesi, B. 1988. Black locust: the tree of agriculture. Outlook Agric. 17:77-85.

40. Keresztesi, B. 1983. Breeding and cultivation of black locust, *Robinia psuedoacacia*, in Hungary. For. Ecol. Manage. 6:217-244.

41. Keresztesi, B. 1980. The black locust. Unasylva. 32:23-33.

42. Kingsbury, J.M. 1964. Poisonous Plants of the United States and Canada. Prentice-Hall Inc. Englewood Cliffs, NJ.

43. Kumar, R. 1983. Chemical and biochemical nature of fodder tree leaf tannin. J. Agric. Food Chem. 31:1361-1364.

44. Kumar, R., and M. Singh. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447-453.

45. Kumar, R., and T. Horigome. 1986. Fractionation, characterization and protein precipitating capacity of the condensed tannins from *Robinia psuedoacacia* leaves. J. Agric. Food Chem. 34:487-489.

46. Kumar, R.A., and S. Vaithiyanathan. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30:21-38.

47. Lambert, M.G., G.A. Jung, R.H. Fletcher, P.J. Budding, and D.A. Costall. 1989. Forage Shrubs in North Island New Zealand Hill Country 2, Sheep and Goat Preferences. N.Z. J. Agric. Res. 32:485-490.

48. McLeod, M.N. 1974. Plant tannins: their role in forage quality. Nutr. Abstr. Rev. 44(11):803-815.

49. McSweeney, C.S., P.M. Kennedy, and A. John. 1988. Effect of ingestion of hydrolysable tannins in *Terminalia oblongata* on digestion in sheep fed *Stylosanthes hamata.* Aust. J. Agric. Res. 39: 235-244.

50. Mehansho, H., L.G. Butler, and D.M. Carlson. 1987. Dietary tannins and salivary proline rich proteins: interactions, induction and defense mechanisms. Annu. Rev. Nutr. 7: 423-440.

51. Min, B.R., and S.P. Hart. Tannins for suppression of internal parasites. 2003. J. Anim. Sci. 81 (E. Suppl. 2):E102-E109.

52. Olson, D.F. 1974. *Robinia* L., locust. p. 728-731. *In* C. S. Schopmeyer (ed.). Seeds of Woody Plants in the United States. USDA. Agric. Handb. 450. U.S. Gov. Print Office Washington, DC.

53. Papachristou, T. G. 1999. Assessing the value of black locust (*Robinia pseudoacacia* L.) browse for animal feeding. p.99-103. *In* V. P. Papanastasis, J. Frame and A.S. Nastis (ed.) Grassland and Woody Plants in Europe. Volume 4. Proc. Int. Occasional Symposium of the European Grassland Fed. Thessaloniki, Greece, 27-29 May, 1999.

54. Papachristou, T.G., and V.P. Papanastasis. 1994. Forage value of Mediterranean deciduous woody fodder species and its implication to management of silvo-pastoral systems for goats. Agroforestry Syst. 27:269-282.

55. Papanastasis, V.P., P.D. Platis, and O. Dini-Papanastasi. 1997. Productivity of deciduous woody and fodder species in relation to air-temperature and precipitation in a Mediterranean environment. Agroforestry Syst. 37:187-198.

56. Papachristou, T.G., P.D. Platis, V.P. Papanastasis, and C.N. Tsiouvaris. 1999. Use of deciduous woody species as a diet supplement for goat grazing Mediterranean shrublands during the dry season. Anim. Feed Sci. Technol. 80:267-279.

57. Parker, J. 1977. Phenolics in black oak bark and leaves. J. Chem. Ecol. 3: 489-496.

58. Perchellet, J.P., H.U. Gali, E.M., Perchellet, P.E. Laks, V. Bottari, R.W. Hemingway, and A. Scalbert. 1994. Antitumor-promoting effects of gallotannins, ellagitannins, and flavonoids in mouse skin in vivo. p. 303-327. ACS-symp-ser. American Chemical Society Washington, D.C.

59. Provenza, F.D., and J.C. Malechek. 1984. Diet selection by domestic goats in relation to blackbrush twig chemistry. J App. Eco. 21:831-841.

60. Reed, J.D. 2001. Effects of proanthocyanidins on the digestive and analysis of fiber in forages. J. Range Mange. 54:466-473.

61. Reed, J.D., P.J. Horvath, M.S. Allen, and P.J. Van Soest. 1985. Gravimetric determination of soluble phenolics including tannins from leaves by precipitation with trivalent ytterbium. J. Sci. Food Agric. 36:255-261.

62. Roach, B.A. 1965. Black locust (*Robinia pseudoacacia* L.). p.642-648. *In* H. A. Fowells (ed.). Silvics of Forest Trees of the United States. USDA Agric. Handb. 271. U.S. Gov. Print Office, Washington, D.C.

63. Robbins, C.T., S. Mole, A.E. Hargerman, and T.A. Hanley. 1987. Role of tannins in defending plants against ruminants: reduction in dry matter digestion. Ecology 68:1606-1615.

64. Sandusky, G.E., J.M. Fosnaugh, J.B. Smith, and R. Mohan. 1977. Oak poisoning of cattle in Ohio. J. Am. Vet. Med. Assoc. 171:627-629.

65. Silanikove, N., N. Gilboa, A. Perevolotsky, and Z. Nitsan. 1996. Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Ruminant Res. 21:195-201.

66. Silanikove, N., Z. Nitsan and A. Perevolotsky. 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. J. Agric. Food Chem. 21:195-201.

67. Singh, R.V. 1982. *Robinia psuedoacacia* Linn. Oxford and I.B.H., New Delhi.

68. Smith, A.L., C.L. Campbell, M.P. Diwakar, J.W. Hanover, and R.O. Miller. 1989. Extracts from black locust as wood preservatives: a comparison of the methanol extract with pentachlorophenol and chromated copper arsenate. Holzforschung 43:293-296.

69. Unruh, L.J. J-M. Luginbuhl, and J.P. Mueller. 2001. Intake and digestibility of black locust foliage fed to growing goat wethers. p.413-414. *In* J.A. Gomide and W.R.S. Mattos (ed.) Grassland Ecosystems: and Outlook into the 21st Century. XIX International Grassland Congress. 10-21 February 2001. San Paulo, Brazil.

70. Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Durham and Downey, Inc. Portland, OR.

71. Waterman, P.G., and D.B. McKey. 1989. Herbivory and secondary compounds in rain forest plants. p. 513-536. *In* Leith, H., and M.J.A. Werger (eds.) Tropical Rainforest Ecosystems. Elsevier, Amsterdam.

72. Waterman, P.G., and S. Mole. 1994. Methods in Ecology: Analysis of phenolic plant metabolites. Blackwell Scientific Pubs. Cambridge MA.

73. Whittaker, R.H. 1956. Vegetation of the Great Smoky Mountains. Ecol. Monogr. 26:1-80.

74. Woodward, S.L., M.J Auldist, P.J. Laboyrie and E.B.L. Jansen. 1999. Effect of *L. corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. Proc. N. Z. Soc. of Anim. Prod. 59:152-155.

Chapter Two

The Influence of Spacing and Coppice Height on Herbage Mass and other Growth Characteristics of Black Locust in a Southeastern USA Silvopastoral System

L.J. Unruh Snyder*[1], J.P. Mueller[1], J-M. Luginbuhl[1, 2], and C. Brownie[3.]

Departments of Crop Science[1], Animal Science [2], and Statistics [3]

North Carolina State University, Raleigh, NC 27695-7620.

*Corresponding author (prepared for submission for Agroforestry Systems)

Abstract:

Demand for goat (*Capra hircus hircus*) meat in the southeastern USA is steadily increasing as a result of preferences exhibited by ethnic communities. Feeding systems that include fodder trees can be developed to take advantage of the natural preference of goats for browse. Data were collected for two years (1999 and 2000) to evaluate growth characteristics of a 5-year old stand of black locust (BL; *Robinia pseudoacacia* L.). The experimental design was a randomized complete block arranged as a complete factorial (intra-row spacing 0.5 or 1.0 m and coppice height 0.25 or 0.5 m) replicated six times. Biomass samples were hand-separated into herbage and woody biomass. The size of the main branches was not affected by spacing when trees were coppiced at 0.25 m; however, when coppiced at 0.5 m; trees spaced at 1.0 m had larger branches ($P<0.05$) than trees spaced at 0.5 m. Above ground woody biomass and herbage mass followed similar trends. Spacing did not influence biomass when trees were coppiced at 0.5 m; however, trees spaced at 1.0 m produced less woody and herbaceous biomass than trees spaced at 0.5 m when coppiced at 0.25 m ($P< 0.05$). A strong relationship ($P<0.0001$) was found between herbage mass and main branch size ($r^2=0.80$). The ability to estimate herbage mass from an easily measured variable such as branch size could serve as a valuable tool for researchers, extension

personnel and farmers when developing feed budgets or estimating carrying capacity of BL browse paddocks.

Keywords: black locust, herbage mass, goats, prediction equation, plant population

Abbreviations: **A**; area; **ADF**, acid detergent fiber; **ADL**, acid detergent lignin; **AGWB**, above ground woody biomass; **BL**, black locust; **BW**; body weight; **CP**, crude protein; **DM**; dry matter; **H**, canopy height; **HM,** herbage mass; **IVOMD**; *in vitro* organic matter disappearance; **IVTDMD**; *in vitro* true dry matter disappearance; **LSD**, least significant difference; **N**, nitrogen; **NDF**, neutral detergent fiber; **NMB**, number of main branches with a diameter greater than 10 mm; **RCD**, root collar diameter (at 30 mm above soil surface); **SMBD**, sum of the diameters of the main branches; **V**, Volume; and **W**, tree canopy width.

Introduction:

Interest in goat (*Capra hircus hircus*) meat production in the eastern USA is expanding as a result of increased demand from several ethnic communities, primarily European, Middle Eastern, and Hispanic immigrants (Pinkerton et al., 1994). Including browse (woody brush and tree foliage) as a component of a grazing system takes advantage of the natural tendency of goats to select as much as 50% of their diet from browse sources (Luginbuhl et al., 1996). The incorporation of a browse species, such as black locust (BL; *Robinia pseudoacacia* L.) could fill a feed gap that occurs when cool-season grasses and legumes are semi-dormant in the hot summer months. The decline in quality and productivity of cool-season forages in the summer usually occurs when demand for quality forage by growing and lactating animals is high. It follows that pasture-based meat goat production systems could benefit from browse species capable of biological nitrogen (N)

fixation, and capable of accessing moisture and nutrients deep into the soil profile as a complement to cool-season forages. In North Carolina and much of the southeast region, BL is a native tree that possesses the potential to contribute such a complement. The incorporation of BL as a system component may be beneficial to farmers seeking alternative feed resources for their livestock.

Black locust is drought tolerant, exhibits rapid growth and produces ample herbage (edible leaves and shoots). Addlestone et al. (1999) concluded that BL had potential as a browse species due to its high herbage mass (HM) production (leaves and non-woody material), averaging 2,400 kg dry matter (DM) ha^{-1}, relative to other browse species tested. Papanastasis et al. (1997) reported a mean total biomass of 2,280 kg DM ha^{-1} and 1,026 kg DM ha^{-1} of grazeable leaves and twigs (respectively) for BL grown under a spacing of 1.0 x 1.5 m and cut annually at 10 cm. Black locust appears to be a desirable candidate for a silvopastoral system, due to its ability to fix atmospheric N, 75 to 150 kg N ha^{-1} $year^{-1}$, (Boring et al., 1981) and supply forage high in crude protein (CP), ranging from 20 to 24% (Addlestone et al., 1999).

One of the most important goals when growing BL is to optimize biomass production per unit area. Studying factors such as coppicing and spacing (planting density) of trees helps in understanding the conditions under which optimal production per unit area might occur. These factors may also have direct impact on persistence of the BL stand and the species biodiversity of the silvopastoral system. These and other factors ultimately lead to the economic success or failure of the agroecosystem.

Papanastasis et al. (1998), working in Macedonia, Greece compared 1-year old seedlings of several species (including BL) planted at a spacing of 1.0 x 1. 5 m. Trees were

left uncoppiced or coppiced (at 0.1 m) annually (August to September) for 1, 2, 3, 4, 5, 6, 7, or 8 years from (1987 to 1994). In 1994, the 8-year old trees were coppiced only once as a control treatment for comparison with other coppice regimes. The biomass samples were separated into two categories: grazeable material (leaves and twigs of up to 2.0 mm diameter) and woody branches. Samples were weighed and reweighed after oven drying at 70°C. It was concluded that BL was one of the most productive species, reaching 3.0 m in tree canopy height (H). Biomass ranged from 4,174 g DM plant^{-1} (including grazeable material and woody branches) for trees coppiced once (the 'control' treatment) to 526 to 101 g DM plant^{-1} for trees coppiced 2 to 8 times, respectively. No differences in HM were reported for trees coppiced once annually for 2 to 8 years. It was recommended that trees should be coppiced at a minimum H of 0.5 m although this variable was not studied in the experiment. Trees that were coppiced annually for 8 years survived, thus indicating that BL can withstand annual coppicing.

Ainalis and Tsiouvaras (1998) studied BL and other species in relation to plant spacing under sheep grazing in Macedonia, Northern Greece. Black locust was planted as one-year old seedlings on 1.5, 2.5 or 3.5 m centers corresponding to three different plant densities (4,440, 1,600 and 810 plants ha^{-1}) and coppiced annually at 0.5 m above ground level to keep plants in shrubby form. Sheep grazed the trees at a stocking rate of 1.1 sheep ha^{-1} year in early June and late August of 1992, 1993, and 1994. Tree H was not measured. Black locust, regardless of spacing, produced the most forage among the species tested (700 kg DM ha $^{-1}$). Ainalis and Tsiouvaras (1998) concluded that the close spacing (1.5 x 1.5 m) produced the highest production per unit area and the widest spacing (3.5 x 3.5 m) produced the highest production per plant.

We hypothesized that, due to compensatory effects, variables such as HM and morphology of trees managed under two different population densities (intra-row spacings of 0.5 and 1.0 m) and two different coppice heights (0.25 and 0.5 m) would not differ. A further hypothesis was that a strong and statistically significant relationship exists between HM and readily measurable morphological features such as foliage area and volume, tree canopy H and width (W), root collar diameter at 30 mm above soil surface (RCD), and degree of branching (number of main branches greater than 10 mm [NMB] and or sum of the diameters of the main branches greater than 10 mm [SMBD]).

Previous research has reported predication equations for yields of woody biomass for short rotations of BL tree plantations (Converse and Betters, 1995). These equations did not predict edible foliar biomass that could be used in animal grazing systems. The ability to estimate HM of BL based on easily measured variables may be a valuable aid to researchers, extension personnel and farmers when attempting to develop feed budgets or estimate carrying capacity of BL browse paddocks.

Materials and Methods:

Background:

The study was conducted at the North Carolina State University Meat Goat and Forage Educational Unit in Raleigh, NC at approximately 35.75° N lat and 78.75° W long. The climate is temperate with 1,191 mm long-term mean annual precipitation and average annual maximum and minimum temperatures of 21.1 and 10.5ºC, respectively. Soils of the study area were Cecil series (fine, kaolinitic, thermic, Typic Kanhapludults) on slopes ranging from 6 to 10% (Soil Survey Div., 2001). Soils are deep and well drained with a firm red clay sub-soil and a sandy clay loam surface horizon. Parent material consisted of gneiss,

schist, and other acidic rocks. In November of 1999, one soil sample was taken (15 cm depth) for each plot (72 total) and pooled by replication. Soil samples from the sites were analyzed by North Carolina Department of Agriculture Soil Testing Lab to derive soil chemistry data. Soil fertility at the experimental site was characterized by high base saturation (82%) and a pH of 6.1. Due to adequate P and K levels, soil base saturation, and the ability of BL to fix N, no fertilizer or lime were applied during 1997 to 2000.

Site Establishment:

The field study was established by planting bare-root seedlings from a nursery stock in March 1995 (as reported by Addlestone, 1996). Plots were 9 m by 3 m with 3 m between rows and 3 m alleys between blocks (Addlestone, 1996). Experimental design was a randomized complete block (six replications) with a 2 x 2 factorial treatment set; the factors were plant spacings of 0.5 m (17 trees row^{-1}) or 1.0 m (9 trees row^{-1}) and coppice heights of 0.25 or 0.5 m above ground level. Tree densities were equivalent to 3,333 and 6,296 trees ha^{-1}, respectively. The replications were laid out in two adjacent field areas, one containing four replications with a southern aspect and the other two replications with a northeastern aspect. Each season from 1995 through 2000, the site was fenced (3-wire fence) using a temporary electric wire 1.5 m high (with a 0.75 m high offset wire placed in front of the main fence). The fencing allowed goats to browse and prevented herbivory by white-tailed deer (*Odocoileus virginianus*). Mowing and hand weeding were performed to control aggressive broadleaf weed species during early establishment. The site was not irrigated. From 1995 through 1998 the plots were maintained by annual browsing with goats and by coppicing the trees each winter (February) with pruning shears to original treatment heights (either 0.25 or

0.5 m). Results for the establishment phase (1995 to 1996) were reported by Addlestone et al. (1999). Data collection for this study began on June 1999.

Field Measurements:

The H (measured at the canopy's average tallest branches) was determined using a tree-measuring pole manufactured by Craine Enterprise, Inc (Mound City, IL). The W was measured with a tape at the widest point of an individual tree. The primary branches with a diameter greater than 10 mm were counted and measured with a caliper at the coppice height.

Destructive sampling of the BL trees was performed to determine biomass. Two trees were randomly sampled per plot for the 0.5 m row spacing and one tree for the 1.0 m row spacing. Herbage was stripped by hand from the coppiced material and was weighed in the field in cloth bags on an electronic digital scale. The herbage was a composite sample of leaflets, petiolules, and herbaceous stem tips (soft tissue petioles). The remaining coppiced material (above ground woody biomass [AGWB]) was weighed in the field on a strain gauge load cell. Sub-samples of herbage and AGWB were collected and the material dried for 48 and 120 h, respectively, at 60°C in forced air ovens, then reweighed. All trees within each plot where measured for: HM, H, W, SMBD, NMB, and AGWB.

In February 2000, the five-year-old BL trees were coppiced to 5 cm using a chain saw. This procedure allowed residual effects of coppice height (of 5-yrs of coppicing at 0.25 or 0.5 m) to be measured in the stump biomass and in the ensuing regrowth during the 2000 growing season (April through July). Following the 5 cm coppicing, RCD at 30 mm above soil surface was measured with a caliper, and woody stump biomass (kg) was determined with a strain-gauge load cell in the field. In June of 2000, the same variables measured in 1999 were again measured (HM, H, W, SMBD, NMB and AGWB) and samples were

weighed and dried by the same procedures used previously. All BL measurements were taken on 19 July 1999 and 2000, except for RCD and stump biomass, which were measured on 13 and 14 February 2000.

Lab Measurements: Estimation of Forage Quality

Composite samples of dried herbage were chemically analyzed for Kjeldahl N according to AOAC (1999). Kjeldahl N was then multiplied by 6.25 to estimate CP. The samples were also analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and 72 % sulfuric acid lignin (ADL) sequentially according to Van Soest et al. (1991) as modified by Komarek et al. (1994). Acid detergent lignin was corrected for mineral matter by ashing the ADL residue in a muffle furnace at 500 °C. *In vitro* true DM disappearance (IVTDMD) was determined using ruminal contents collected from a ruminally-cannulated Hereford steer maintained on high-quality alfalfa (*Medicago sativa* L.) hay (Goering and Van Soest, 1970). The steer was fed at 08:00 and 16:00; ruminal contents were collected before the morning feeding. Ruminal fluid was passed through four layers of cheesecloth before being processed for use in the IVTDMD bioassay. After 48 h of incubation with ruminal inoculum in a batch processor (Ankom Technology Corp., Fairport, NY), samples were extracted with neutral detergent solution for IVTDMD estimation.

Statistical Analyses:

Statistical analyses of data were conducted using procedures of SAS (2002). Analysis of variance (ANOVA) was carried out on data combined over years for each variable. Year was treated as a subplot factor. Tests for spacing and coppice effects averaged over years were carried out using whole plot error; tests for years and interactions were carried out using sub plot error. The least significant difference (LSD) was used to

determine significant differences among treatment means, either averaged over years or within years, where appropriate. A significance level of 0.05 was used in all LSD analyses.

Simple and multiple linear regressions were used to determine a prediction equation for estimating HM. The general form for a two variable model is:

$Y = a + b_1x_1 + b_2x_2$

Where

Y= dry HM (g/tree)

x_1=First explanatory variable

x_2=Second explanatory variable

a= Y intercept

b_1= Slope coefficient for x_1

b_2= Slope coefficient for x_2

The correlation coefficient matrix was examined to determine the order to insert variables into the equations. All possible single, two, three and four variable models were examined. The following explanatory variables were used: H, W, NMB, and SMBD. Root collar diameter was added for the year 2000. The variables area (A) and volume (V) were calculated as follows:

Area (m^2) =H x W

Volume (m^3) of a cone= 1/3 * p (radius ^{2})*(H)

In addition, because V is not a linear measure, two transformations of V: the square root of V ($v m^3$) and cube root of V (m) were evaluated.

The variable most correlated to herbage mass was entered first into the regression equation. The remaining variables were then added to determine if they improved the

equation. The r^2 value and the F-statistic (for the new variable) were used to indicate whether a particular variable improved the equation. Additionally, three and four variable models were examined. Finally, residual analysis was used to examine the data set for outliers and to determine if the form of the model was appropriate. Prediction equations were first analyzed by year and then for combined years (1999 and 2000). Year effect and interactions with year were examined to determine whether a single model could be used to predict biomass for one growing season (April through July) for more than one year.

Results and Discussion:

For each experimental period (April through July) for years 1999 and 2000 the average rainfall was 221.0 and 529.3 mm, respectively (NCSU, 2003)

Foliar Analysis of Herbage Quality:

Based on analysis of foliar samples collected in June 1999 and 2000, herbage quality estimates of composite samples of leaflets, petiolules and herbaceous stem tips (soft tissue petioles) was high. Foliar CP of BL averaged 25% (data not shown). Black locust CP levels have been reported to be similar to those of alfalfa, ranging from 20 to 25% (Addlestone, 1996; Baertsche et al., 1986; Boring and Swank 1984; Papchristou et al., 1999; Unruh et al., 2001). Assuming adequate intake (>3% body weight (BW)) and no anti-quality factors, this level of CP exceeds the nutritional requirements (12 to 14% CP) of a 20 kg goat kid gaining 150 g/d by approximately 13 percentage units (NRC, 1981).

The average foliar NDF and ADF values were low, (38 and 21%, respectively), indicating high digestibility. Lignin concentration averaged 11%. Horton and Christensen (1981) reported higher values for ADF (28.5%) and NDF (53.6%), and higher ADL values (13.5 %) of BL, indicating herbage of more advanced maturity was analyzed compared to

that used in our study. For this study IVTDMD concentration averaged 68%, whereas Papachristou et al. (1999) reported *in vitro* organic dry disappearance (IVOMD) as 52%, which is roughly equivalent to 64% IVTDMD (Holden, 1999). Based on the observed NDF, ADF and CP values one would expect IVTDMD to be considerably higher than that observed. This observation suggests the possible digestive interference of anti-quality constituents such as tannins.

Tree Survival:

After 5 years, no significant differences in survival rates were noted for spacing and coppice height treatments (P=0.63). The mean survival rate was 81% for trees spaced at 1.0 m and 88% for trees spaced at 0.5 m. The mean survival rate for trees coppiced at 0.5 m was 86% and for trees coppiced at 0.25 m was 83%. Overall, the highest survival rate (89%) was for trees spaced at 0.5 m spacing and coppiced at 0.5 m, whereas the lowest survival rate (80%) was for trees spaced at 1.0 m and coppiced at 0.25 m. Addlestone et al. (1999) reported that within the first year of establishment of BL the survival rate was 98.1%. The trees were able to withstand repetitive annual browsing, winter coppicing and coppicing to ground level. Papanastasis et al. (1998) reported similar results in that BL was able to withstand annual coppicing over 8 years.

Tree Canopy Height:

Tree H reflects overall vigor, growth rate and ability to recover from the effects of browsing pressure and winter coppice. The mean initial H of the BL trees at planting in March of 1995 was 0.72 m and by the end of June of 1996 the trees averaged 2.98 m (Addlestone, 1996). Comparing the H from 1996 (2.98 m) to the mean H for 1999 (3.31 m) and 2000 (2.91 m) revealed that regrowth following winter coppice was similar for all years.

Because there were no interactions with years for spacing or coppice height, treatment means were averaged over years (Table A.1). The only treatment effect on H was that of coppice height. Trees coppiced at 0.5 m were slightly taller than trees coppiced at 0.25 m (P=0.06) (Table 2.1). This suggests that the lowest coppice height of 0.25 m slightly reduced the subsequent regrowth. Coppicing at 0.25 m most likely reduced the number of meristematic buds available for regrowth and could have decreased the photosynthetic leaf area compared with trees coppiced at 0.5 m.

Tree Canopy Width:

Tree canopy width, like H, reflects the overall vigor of regrowth produced in spring and early summer following a winter coppice. Because there were no interactions with years for spacing or coppice height, treatment effects were averaged over years (Table A.2). There was an interaction effect (Figure 2.1) between coppice and spacing treatments (P=0.039). Trees spaced at 1.0 m and coppiced at 0.5 m had significantly wider canopies (2.21 m) than those in other treatment combinations (Figure 2.1). Trees coppiced at 0.25 m had similar canopy widths regardless of spacing, but for trees coppiced at 0.5 m, those spaced at 1.0 m had significant wider canopies than trees spaced at 0.5 m. Coppicing at 0.5 m and spacing at 1.0 m allowed for the branches to expand in response to the effects of increased meristematic tissue and photosynthetic leaf area as mentioned for H.

Number of Main Branches Greater than 10 mm Diameter:

Number of main branches greater than 10 mm diameter reflects the vigor of regrowth produced in spring and early summer following a winter coppice. Because there were no interactions with years for spacing or coppice height, treatment effects were averaged over years (Table A.3). Coppicing at 0.5 m (Table 2.1) produced slightly more main branches

with diameters greater than 10 mm (5.97) compared with coppicing at 0.25 m (4.82; P=0.08). Spacing had a large effect on the number of branches with diameters greater than 10 mm. Trees spaced at 1.0 m produced more branches greater than 10 mm (P=0.02) than trees spaced at 0.5 m. These results are consistent with results reported by Addlestone (1996) in the establishment phase, where coppicing at 0.5 m produced more main branches (9.2) compared to coppicing at 0.25 m (7.2), and trees spaced at 1.0 m produced more branches (8.7) than trees spaced at 0.5 m (7.7). Addlestone counted all main branches regardless of diameter. These results seem to indicate that wide spacing and high coppicing are stimulatory factors for branching, and that narrow intra-row spacing reduces branching of individual trees due to competition for sunlight and nutrients.

Sum of Main Branch Diameters Greater than 10 mm:

Because there were no interactions with years for spacing or coppice height, treatment means were averaged over years (Table A.4). There was an interaction (Figure 2.2) for the effects of spacing and coppice height (P=0.0049). There was no effect of spacing on the branch size of trees coppiced at 0.25 m, whereas trees spaced at 1.0 m had a significantly greater branch diameter sum than trees spaced at 0.5 m. Addlestone (1996) reported a similar trend for trees with these spacing and coppice height treatments during the establishment phase. This branching response follows much the same pattern reported previously for H and W. Low coppicing reduced meristematic bud tissue available for regrowth and wide spacing decreased intra-row competition for sunlight and nutrients.

Observed values for SMBD in 2000 were noticeably less than in 1999, which most likely was due to the impact of coppicing to 5 cm in February 2000. Subsequently, in 2000, the BL sprouts appeared to be fewer and less vigorous due to the massive loss of

meristematic and vascular tissue compared to 1999 when BL coppice height levels were 0.25 and 0.5 m.

Above Ground Woody Biomass:

Above ground woody biomass produced as a result of the treatments imposed is another indication of overall plant productivity and vigor. Above ground woody biomass also is an important variable where fuel wood is a valued economic product, as is the case in many developing countries. Addlestone (1996) reported an overall mean for AGWB of 2,069.8 kg DM ha^{-1}. The overall mean for AGWB, averaged over years 1999 and 2000, was 3,817.7 kg DM ha^{-1} (1999, 4,686.6 kg DM ha^{-1} and 2000, 2,948.8 kg DM ha^{-1}). It was expected that in the 4 to 5 years following Addlestone's observations the trees would have gradually increased AGWB production despite annual coppicing in late winter. In year 2000, AGWB was noticeably less than that in 1999, which most likely was due to coppicing to 5 cm in February 2000.

Due to no interactions with years for spacing or coppice height, treatment effects were averaged over years (Table A.5 and Table A.8). There was an interaction (Figure 2.3 and Figure 2.4) between effects of spacing and coppice height (P=0.001). At the 1.0 m spacing AGWB was greater for trees coppiced at 0.5 m than for trees coppiced at 0.25 m, whereas there was no difference between AGWB for the two coppice heights at the 0.5 m spacing. The same relationships were seen on per plant and per unit area basis. The wide spacing treatment (1.0 m) and low coppice height (0.25 m) produced the smallest dry weight (2,209.9 kg DM ha^{-1}) on a per unit area basis and was different from that of all other treatments (Figure 2.4). On a per plant basis the wide spacing (1.0 m) and low coppice height (0.25 m) produced similar yield as trees spaced at 0.5 m and coppiced at 0.5m. These

results are similar to responses previously mentioned for H, W and SMBD. Biomass production from trees spaced at 1.0 m was much more sensitive to low coppicing (0.25 m) than production from trees at the narrow 0.5 m spacing.

Stump Biomass When Coppiced to 5 cm (2000):

This procedure allowed for another method to examine the residual effects of the spacing and coppice treatments. The amount of woody biomass accumulated below the imposed coppice regimes (stumps) to a large degree reflects previous tree management conditions. In February 2000, trees were first coppiced to their respective treatment heights and then cut to 5 cm, after which each tree stump was weighed. No interaction effects were evident, but main effects of coppice height and tree spacing were significant (Table A.7). Trees coppiced yearly at 0.5 m had higher stump biomass than trees coppiced annually at 0.25 m (Table 2.1). The wide spacing (1.0 m) also produced more stump biomass compared with narrow spacing (0.5 m). These results seem logical when one considers that low coppicing removed annually more of the woody biomass than coppicing at 0.5 m. It also supports previous observations that wide spacing resulted in reduced intra-row competition and larger, heavier stumps.

Root Collar Diameter Growth (2000):

No significant differences were noted in RCD due to spacing and coppice height. For year 2000, the average RCD was 70 mm. In 1996, average RCD was 6.1 mm at initial planting and increased 430% to an average of 26.3 mm after one season of growth, 01 March 1 to 01 October 1996 (Addlestone, 1996). Addlestone (1996) reported no significant differences between spacing and coppice height with regard to RCD.

Herbage Mass (Edible Biomass):

Mean HM averaged over 1999 and 2000 was 2,141.9 kg DM ha^{-1} (1999, 2,286.3 kg DM ha^{-1} and 2000, 1,997.5 kg DM ha^{-1}). Herbage mass was very similar to that (2,390 kg DM ha^{-1}) reported by Addlestone (1996). Papanastasis et al. (1997) reported a mean total biomass of 2,280 kg DM ha^{-1} and 1,026 kg DM ha^{-1} of grazeable leaves and twigs, respectively. In year 2000, HM was noticeably less (8.7%) than 1999 and was most likely due to coppicing to 5 cm in February 2000, which reduced meristematic tissue compared with 1999, when BL coppice height levels were 0.25 and 0.5 m.

Because we formed no significant interactions with years for spacing or coppice height, treatment effects were averaged across years (Table A.6 and Table A.9). There was an interaction (Figure 2.5 and Figure 2.6) between levels of spacing and coppice treatments ($P<0.01$). As reported for the AGWB, at the 1.0 m spacing there was a large difference in the HM resulting from the different coppicing height treatments whereas HM resulting from coppice height treatments did not differ at the 0.5 m spacing. As with AGWB, low coppicing at 0.25 m had a weakening effect on individual trees at both spacings, but on a per area basis, production was greater at the narrow tree spacing due to increased number of trees per unit area compared with the wide spacing (1.0 m). The wide spacing treatment (1.0 m) and lowest coppice height (0.25 m) produced the least HM (1,311.3 kg DM ha^{-1}) and was different from that in than all other treatments ($P=0.05$). As with AGWB, the same relationships were seen on a per plant basis. The highest per plant HM was found with spacing at 1.0 m and coppicing at 0.5 m (757.3 g $plant^{-1}$). Overall, the highest coppice height (0.5 m) appeared to be less stressful to the tree and provided a healthy base.

In this study HM was measured from a single harvest in both years. It is possible that appreciably higher seasonal yields (perhaps double) could be obtained from a multiple harvest system where trees were cut or browsed two or three times per year.

Prediction of Foliar Biomass:

Correlation analyses were performed separately for each year to determine the strength of the relationship between total accumulated HM (per tree basis) and measurements of: H, W, NMB, SMBD, RCD (2000 only), V and A. Using V always resulted in a higher r^2 value (0.36) than the r^2 values (both 0.33) for the two transformed variables (the square root of V and cube root of V). Linear regressions were calculated for all possible one and two variable models (Table A.10 to Table A.46). The best models involved SMBD, and SMBD and H, as predictors (Table 2.2). The best three and four variable models had only slightly greater r^2 values (data not shown) than the best one and two variable models.

For both 1999 and 2000, treatment effects were added to the model; however, they were not significant and did not affect the model. When years 1999 and 2000 were combined, the best single predictor variable was SMBD, with an r^2 comparable to that for the separate regressions by year. Including year and year by SMBD increased the r^2 to only 0.81 from 0.80. Table A.47 to Table A.63 display correlation and regression analyses for 1999 and 2000 combined data. Figure 2.7 and Table 2.2 display the best single variable prediction equation for data from combined years of 1999 and 2000. Including H as a second predictor increased r^2 from 0.80 to 0.81.

One can obtain an estimate of herbage production ha^{-1} by using the equation from Figure 2.7, converting g $tree^{-1}$ to kg $tree^{-1}$ and then multiplying by the appropriate stand density, which in this case is 3,333 (1.0 m spacing) or 6,296 (0.5 m spacing) trees ha^{-1}.

Conclusions and Recommendations:

After 5 years of continuous production, the data collected in years 1999 and 2000 had many similarities to the data reported by Addlestone (1996 and 1999) during the establishment phase of this experiment (1995 to 1996). Tree survival for all treatments was good, averaging greater than 80%. Data indicated that BL has good potential as a browse species due to its relatively high HM production and its high preference by goats (Addlestone, 1999; Papanastasis et al., 1998). The CP levels of BL were comparable to alfalfa quality. Variables measured in this study (H, W, AGWB, stump biomass, NMB and SMBD) illustrated the effect of intra-row spacing (1.0 and 0.5 m) and coppice heights (0.25 and 0.5 m) on herbage production.

Coppicing trees spaced at 0.5 m had little impact on resulting HM on a per area basis. There was only a difference of 115 kg DM ha^{-1} for HM between trees coppiced at 0.25 m versus those coppiced at 0.5 m. However, the influence of coppice height on the HM from trees more widely spaced (1.0 m) was quite large. A large positive response (1,213 kg DM ha^{-1}) to the higher coppice height was observed. These responses seem to suggest that relatively high coppice (0.5 m) and wide spacing (1.0 m) were desirable. An intra-row planting density of 1.0 m as opposed to 0.5 m would result in considerable economic savings in planting costs (estimated difference of \$652.00 ha^{-1}). Furthermore, if plants are coppiced at 0.5 m, no loss in herbage production can be expected compared with narrower spacing (0.5 m). Ainalis and Tsiouvaras (1998) recommended a density of 4,400 plants ha^{-1} as being the most productive, which is very similar to our estimated density of 3,333 plants ha^{-1} at the 1.0 m spacing.

As mentioned by Papanastasis et al. (1998), coppicing is not identical to grazing or browsing because it removes total biomass whereas animals under proper stocking densities remove only the grazeable material. Consequently, one could argue that the results of this study relate only to those livestock production systems where biomass of fodder trees can be cut and carried to animals in the barn or stall as a supplemental feed. Nevertheless, it should be also noted that if animals cannot reduce the height of trees by grazing, trees will soon grow out of their reach resulting in the need for periodic coppicing in some cases.

A model for estimating HM appears to be feasible and reasonably accurate. The ability to make relatively rapid field estimates of standing HM would seem to be a very useful tool for scientists and graziers interested in accurately estimating stocking rates and in feed budgeting. Measuring the SMBD greater than 10 mm appears to be a relatively simple method of predicting BL dry HM. The SMBD seems to be closely related to the foliar biomass; however, validation of the regression model should be performed as future BL yield information becomes available.

The observations from this study indicate that 5-year old BL trees have the ability to withstand coppicing to 5 cm with little effect on tree survival. The trees recovered by producing basal and root sprouts and produced nearly (8.7% less) the same HM as the previous year when the trees were coppiced to 0.25 or 0.5 m. Further investigation is needed with multiple cut systems to determine optimum harvest regimes for yield and tree survival. Also, there is a need to investigate whether machine coppicing could be just as effective as hand coppicing in terms of tree survival and herbage production. Overall, this research suggests that BL would be an excellent candidate as a silvopastoral component in the southeastern USA based on estimated BL herbage production and estimated quality.

Acknowledgements:

The author would like to thank Brian Fouts, Amy Conrad, and John Mueller and those who volunteered to endure the endless summer days of hand defoliating BL.

References:

1. Addlestone, B.J. 1996. Density and cutting height effects on the herbage mass of three tree legumes grown for meat goat production. M.S. thesis. North Carolina State Univ., Raleigh.

2. Addlestone, B.J., J.P. Mueller, and J-M. Luginbuhl. 1999. The establishment and early growth of three leguminous tree species for use in silvo-pastoral systems of the southeastern USA. Agroforestry Syst. 44:253-265.

3. Ainalis, A.B., and C.N. Tsiouvaras. 1998. Forage production of woody fodder species and herbaceous vegetation in a silvopastoral system in northern Greece. Agroforestry Syst. 42:1-11.

4. AOAC. 1999. International Official Methods of Analysis. (16th Ed.) Assoc. Offic. Anal. Chem., Arlington, VA.

5. Baertsche, S.R., M.T. Yokoyama, and J.W. Hanover. 1986. Short rotation, hardwood tree biomass as potential ruminant feed-chemical composition, nylon bag ruminal degradation and ensilement of selected species. J. Anim. Sci. 63:2028-2043.

6. Boring, L.R., C.D. Monk, and W.T. Swank. 1981. Early regeneration of a clear-coppiced southern Appalachian forest. J. Ecol. 62:1244-1253.

7. Boring, L.R., and W.T. Swank. 1984. The role of black locust (*Robinia pseudoacacia*) in forest succession. J. Ecol. 72(3):749-766.

8. Converse, T.E., and D.R. Betters. 1995. Biomass Yield Equations for Short Rotation Black Locust Plantations in the Central Great Plains. Biomass and Bioenergy 8(4):251-254.

9. Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analyses (apparatus reagents, procedures and some applications). Agric. Handb. 379. U.S. Gov. Print Office, Washington, DC.

10. Holden, L.A. 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci. 82:1791-1794.

11. Horton, G.M.J., and D.A. Christensen. 1981. Nutritional value of black locust tree leaf meal (*Robinia psudeoacacia*) and alfalfa meal. Can. J. Anim. Sci. 61(2):503-506.

12. Komerek, A.R., J.B., Robertson, and J.B, Van Soest. 1994. Comparison of the filter bag technique to conventional filtration in the Van Soest Analysis of 21 feeds. *In*: Proc. Natl. Conf. on Forage Quality, Evaluation and Utilization, Lincoln, NE.

13. Luginhbuhl, J.M., J.T. Green, J. P. Mueller, and M. H. Poore. 1996. Meat goats in land and forage management. In Proceedings of the Southeast Regional Meat Goat Production Symposium. "Meat Goat Production in the Southeast- Today and Tomorrow". February 21-24, 1996. Florida A&M University, Tallahassee.

14. National Research Council (NRC). 1981. Nutrient requirements for goats. National Academy Press, Washington, DC.

15. North Carolina State University. 2003. State Climate Office of NC. [Online]. Available at http://www.nc-climate.ncsu.edu (posted 20 March 2003).

16. Papachristou, T.G., P.D. Platis, V.P. Papanastasis, and C.N. Tsiouvaris. 1999. Use of deciduous woody species as a diet supplement for goat grazing Mediterranean shrublands during the dry season. Anim. Feed Sci. Technol. 80:267-279.

17. Papanastasis, V.P., D.P. Panagiotis, and O. Dini-Papanastasi. 1998. Effects of age and frequency of coppice on productivity of Mediterranean deciduous fodder tree and shrub plantations. For. Ecol. Manage. 110: 283-292.

18. Papanastasis, V.P., P.D.Platis, and O. Dini-Papanastasi. 1997. Productivity of deciduous woody and fodder species in relation to air temperature and precipitation in a Mediterranean environment. Agroforestry Syst. 37:187-198.

19. Pinkerton F., N. Escobar, L. Harwell, and W. Drinkwater. 1994. A survey of prevalent production and marketing practices in meat goats of southern origin. SRDC Publication No. 182. Southern Rural Dev. Center, Mississippi State, MS.

20. SAS. 2002. The SAS System for Windows. Release 8.03. SAS Institute, Cary NC.

21. Soil Survey Division, Natural Resources Conversation Service, United States Dept. of Agric. Official Soil Series Descriptions [Online WWW]. Available URL: "http://ortho.ftw.nrcs.usda.gov/osd/" [Accessed 23 Mar 2001].

22. Unruh, L. J. J-M. Luginbuhl and J. P. Mueller. 2001. Intake and digestibility of black locust foliage fed to growing goat wethers. p.413-414. *In* J. A. Gomide and W. R. S. Mattos (ed.) Grassland Ecosystems: and Outlook into the 21st Century. Proc. XIX Int. Grass. Congress. San Paulo, Brazil.

23. Van Soest, P.J., J.B Robertson, and B.A Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

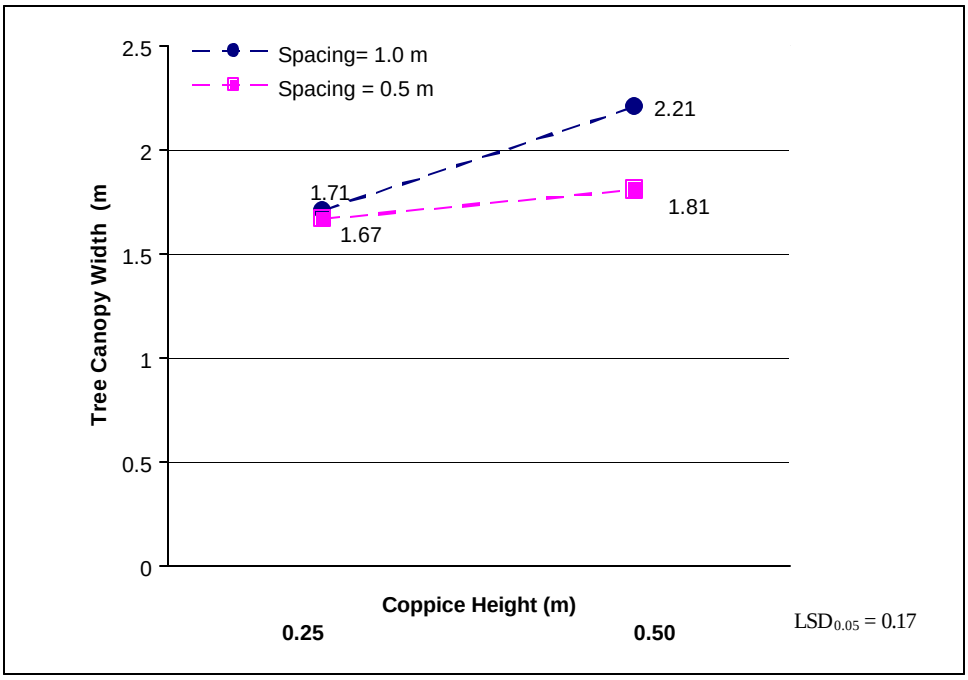


Figure 2.1: Mean tree canopy width (m) per plant of black locust averaged over years (1999 and 2000) in Wake County, North Carolina.

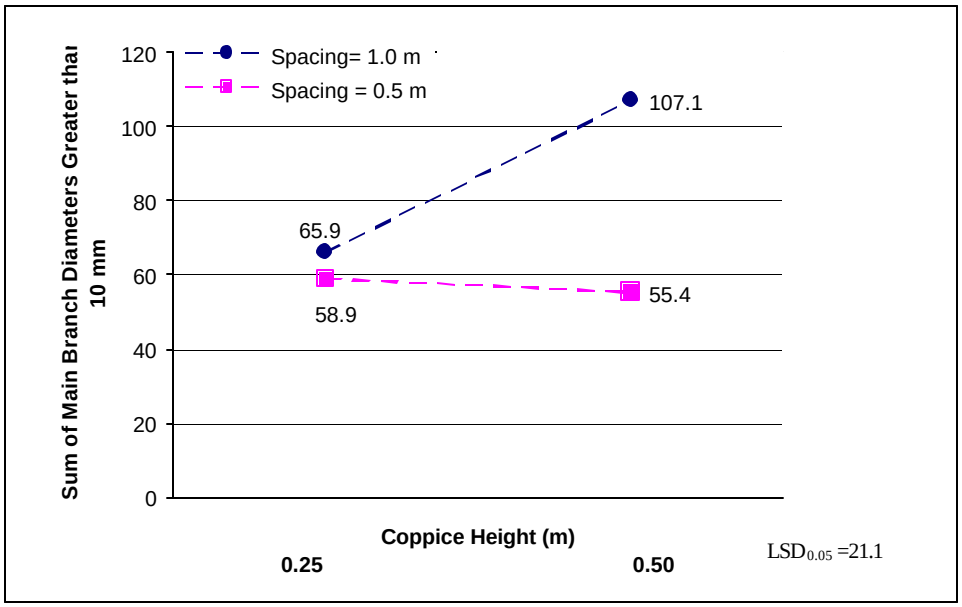


Figure 2.2: Mean sum of main branch diameters greater than 10 mm per plant of black locust averaged over years (1999 and 2000) in Wake County, North Carolina.

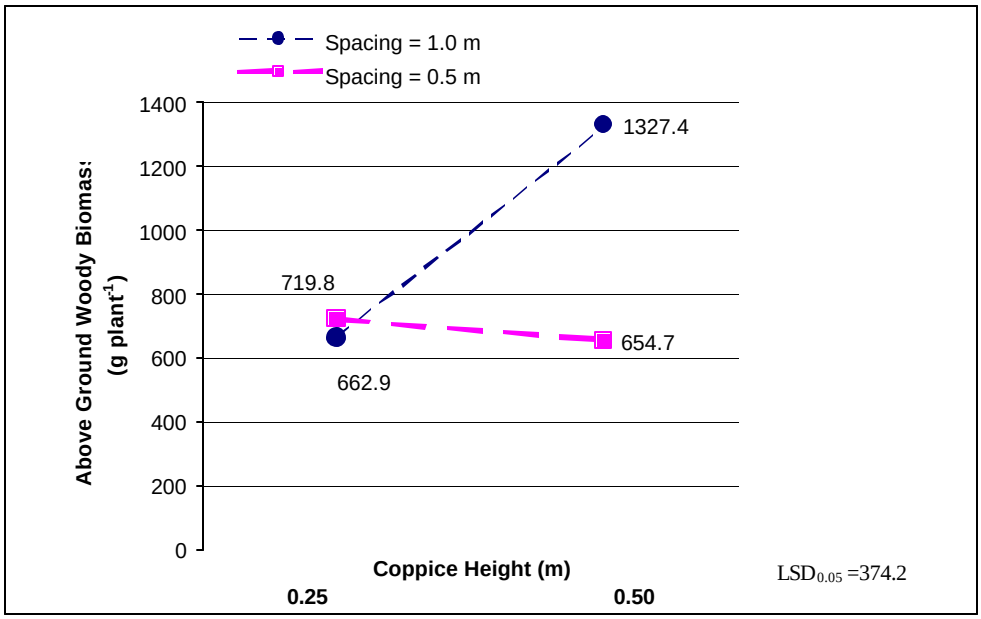


Figure 2.3: Mean above ground woody biomass per plant of black locust averaged over years (1999 and 2000) in Wake County, North Carolina.

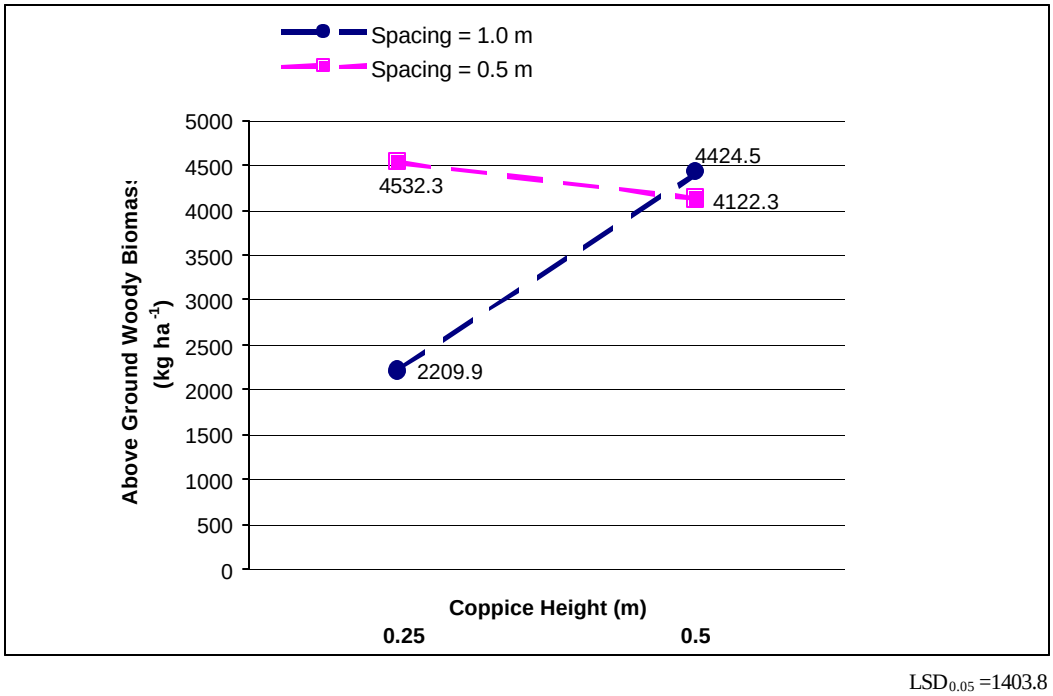


Figure 2.4: Mean above ground woody biomass per unit area of black locust averaged over years (1999 and 2000) in Wake County, North Carolina

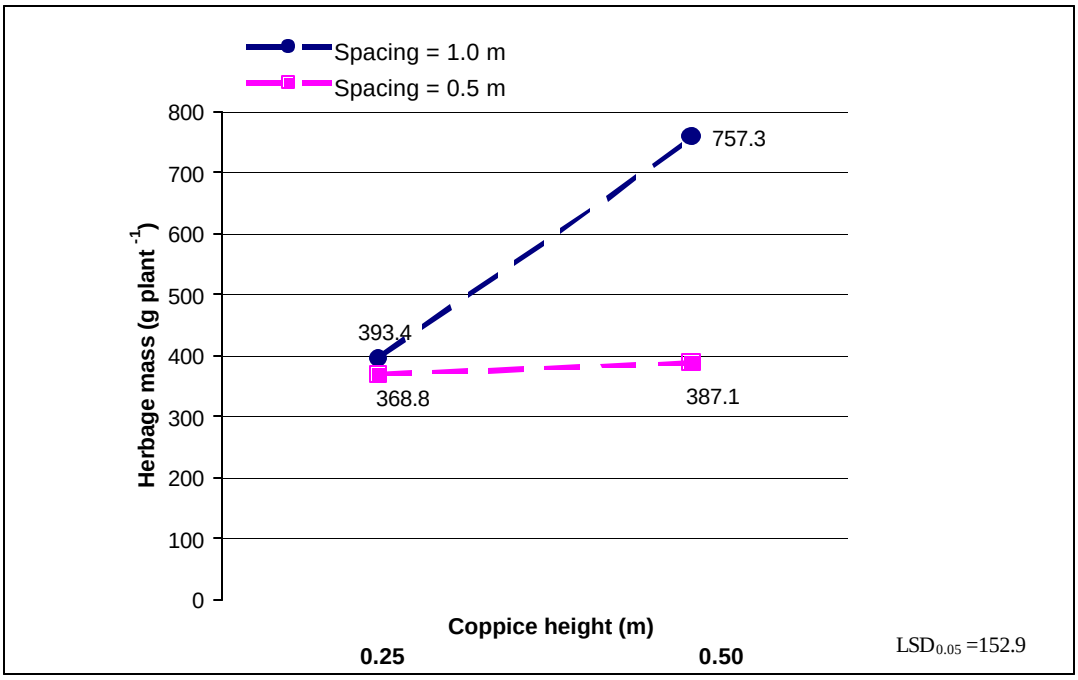


Figure 2.5: Mean herbage mass of black locust per plant averaged over years (1999 and 2000) in Wake County, North Carolina.

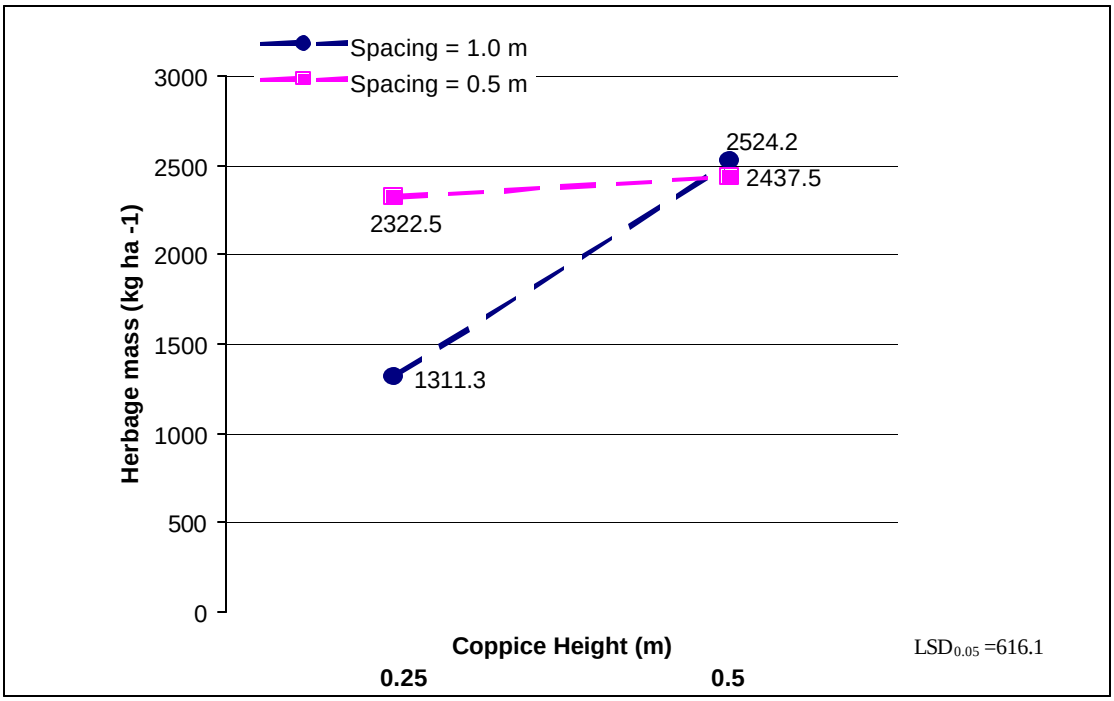


Figure 2.6: Mean herbage mass of black locust per unit area averaged over years (1999 and 2000) in Wake County, North Carolina.

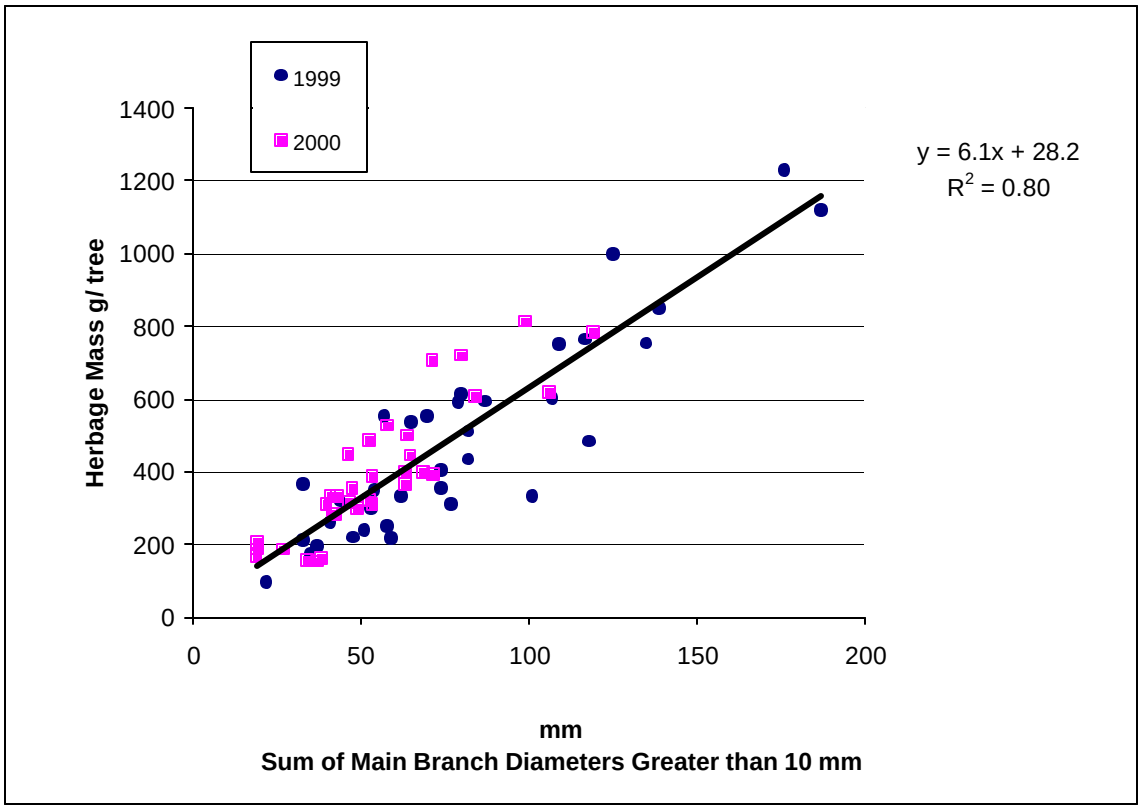


Figure 2.7: Influence of the sum of main branch diameters greater than 10 mm on tree herbage mass of black locust averaged over years (1999 and 2000) in Wake County, North Carolina.

Table 2.1: Influence of coppice height and intra-row tree spacing on several measured variables averaged over years (1999-2000), Wake County, North Carolina.

Treatments (Main Effects)	Canopy Tree Height (m)	Pr>F	Number of Branches > 10 mm	Pr>F	Stump Biomass (kg)	Pr>F
Coppice HT						
0.5 m	3.21	0.06	5.97	0.08	1.89	<0.0001
0.25 m	2.99		4.82		1.21	
Spacing						
1.0 m	3.10	NS	6.2	0.02	1.85	<0.0001
0.5 m	3.10		4.59		1.26	

Table 2.2: Prediction equations for herbage mass for black locust in 1999 and 2000, Wake County, North Carolina.

Year	One or Two Variable Models:	n	R^2	P-value
1999	HM[†] = - 12.9 + 6.2 (SMBD)[‡]	35	0.82	<0.0001
1999	HM= - 490.2 + 5.9 (SMBD) + 150.1 (H)[§]	35	0.87	<0.0001
2000	HM= 12.9 + 6.9 (SMBD)	33	0.79	<0.0001
2000	HM= - 220.2 + 6.4 (SMBD) + 90.7 (H)	33	0.82	<0.0001
1999 & 2000	HM= - 170.0 + 5.7 (SMBD) + 71.2 (H)[§]	68	0.81	<0.0001
1999 & 2000	HM= 28.2 + 6.1(SMBD)	68	0.80	<0.0001

[†]HM=Herbage mass (g tree^{-1}).
[‡]SMBD= Sum of main branch diameters greater than 10 mm.
[§] H= Tree canopy height (m).

Chapter Three

Influence of Growing Degree Days on Browse Quality and Productivity of Black Locust in Southeastern USA

L.J. Unruh Snyder*[1], J.P. Mueller[1], J-M. Luginbuhl[1,2], K. Turner [4], and C. Brownie[3.]

Departments of Crop Science[1], Animal Science [2], and Statistics [3]

North Carolina State University, Raleigh, NC 27695-7620.

USDA ARS, Beaver, WV 25813[4].

*Corresponding author (prepared for submission for Agroforestry Systems)

Abstract:

Demand for goat (*Capra hircus hircus*) meat in southeastern USA is steadily increasing as a result of dietary preferences from growing ethnic communities. Feeding systems can be developed to take advantage of the natural preference of goats for browse. In the southeastern USA, black locust (BL; *Robinia pseudoacacia* L.) trees can contribute to a silvopastoral system through biological nitrogen (N) fixation and by supplying quality forage when other forages are limited. Data were collected for two years (1999 and 2000) in Wake County, North Carolina to determine the relationship between heat units, represented by growing degree-days (GDD) and herbage mass (HM; kg ha^{-1}), tree canopy height (H; m), estimates of herbage quality indicators (crude protein [CP], *in vitro* true dry matter disappearance [IVTDMD], neutral detergent fiber [NDF], acid detergent fiber [ADF], cellulose [CELL], and 72% sulfuric acid lignin [ADL]), and estimates of anti-quality indicators (Folin-reactive phenolics [FR-phenol], condensed tannins [CT], and hydrolyzable tannins [HT]) of BL. Herbage mass samples harvested by hand plucking were used to determine HM and to evaluate browse quality variables. With the exception of IVTDMD (58%), the two-year means of BL herbage quality estimates were high (26% CP, 37% NDF,

and 26% ADF). The two-year means for CELL and ADL were 8 and 16%, respectively. Concentrations of FR-phenol, CT, and HT averaged over years were 7.9, 7.6, and 8.0% dry matter (DM), respectively. Years were different and regression analyses were performed separately using GDD as the independent variable. In 1999, there was a significant relationship between GDD and HM, H, NDF, ADF, CELL, ADL, CP, and IVTDMD (r^2=0.77, 0.58, 0.93, 0.85, 0.92, 0.77, 0.65, and 0.82, respectively). In 2000, the only significant relationships with GDD were CP, ADF, and CELL (r^2=0.58, 0.32, and 0.26, respectively). In 2000, trees were harvested later in the season than in 1999. Furthermore, high rainfall (RF) in 2000 caused a flushing effect of new herbage growth that most likely masked the maturity effects demonstrated in the previous year (1999). In years with moderate to low RF, when multiple growth flushes are absent, GDD appears to be closely related to HM, NDF, ADF, CELL, ADL, CP and IVTDMD. Under these conditions GDD could serve as a useful predictor of HM and quality. In both years GDD was a poor predictor of BL tannin concentrations.

Keywords: black locust, herbage mass, quality, goats, tannins, heat units

Abbreviations: **ADF**, acid detergent fiber; **ADL,** acid lignin; **BL,** black locust; **CELL**, cellulose; **CP,** crude protein; **CT,** condensed tannins; **DM,** dry matter; **DOY**, day of year; **FA**, Folin Denis Assay; **FR-phenol**, Folin-reactive phenolics; **GDD**, growing degree-days; **H,** tree canopy height; **HM,** herbage mass; **HT**, hydrolyzable tannins; **IVTDMD**; *in vitro* true dry matter disappearance; **LSD**, Least significant difference; **N**, nitrogen; **NDF**, neutral detergent fiber; **RF**; rainfall.

Introduction:

Demand for goat (*Capra hircus hircus*) meat in eastern USA is expanding as a result of increased populations in several ethnic communities composed of European, Middle Eastern, and Hispanic immigrants (Pinkerton et al., 1994). Thus, interest in goat production has grown in southeastern USA. Including a browse species as a component of a grazing system takes advantage of the natural tendency for goats to select as much as 50% of their diet from browse (woody brush and tree foliage) (Luginbuhl et al., 1996). A woody browse species, such as black locust (BL; *Robinia pseudoacacia* L.) could fill a feed gap that occurs in the southeast when cool-season grasses and legumes are semi-dormant in the hot summer months. Quality and productivity of cool-season forages often declines in the summer usually when lactating animals demand high quality forage. It follows that pasture-based meat goat production systems can benefit from browse species capable of accessing moisture and nutrients deep in the soil profile. In North Carolina and much of the southeast region, BL is a native tree that has the potential to supply such a complement. Incorporating BL as a system component may be beneficial to livestock/goat farmers seeking sustainable alternative grazing resources.

Black locust is drought tolerant, exhibits rapid growth, and produces ample herbage in the form of edible leaves and shoots. It is a desirable candidate for a pasture system due to its ability to fix 75 to 150 kg atmospheric nitrogen (N) ha^{-1} $year^{-1}$ (Boring et al., 1981) and supply forage high in crude protein (CP), ranging from 20 to 24% (Addlestone et al., 1999); however, BL is known to contain phenolics (tannins) that are high molecular weight (Kumar and Vaithiyananthan, 1990).

Plants can produce a diverse mixture of tannins. Grasses, for example, only produce condensed tannins (CT), whereas some species, such as geranium (*Pelargonium* spp.) only produce hydrolyzable tannins (HT) with varying concentration levels dependent on species. Some species such as oaks (*Quercus* spp.), maples (*Acer* spp.), elms (*Ulmus* spp.), birches (*Betula* spp.), raspberries (*Rubus* spp.) produce both CT and HT (Appel et al., 2001; Cannas, 2001; Van Soest, 1982). Tannin concentrations are known to fluctuate with age and can change in response to biotic and abiotic stresses (Appel et al., 2001; Waterman and Mole, 1994).

High tannin levels, if the total (Folin-reactive; FR) phenolics concentration is above 5% of the diet dry matter (DM) (McLeod, 1974), have been shown to depress voluntary intake by decreasing palatability, digestibility, and N retention, sometimes leading to decreased animal productivity (Silanikove et al., 1996; Kumar and Vaithiyanathan, 1990; Van Soest, 1982).

The ability to predict BL quality or anti-quality (tannins) constituents over a growing season can potentially help with management feedings decisions. Black locust has been reported to supply ample biomass. The ability to predict HM during the peak-growing season could help in grazing management decisions and in determining stocking rates and densities. Addlestone et al. (1999) concluded that BL had potential as a browse species due to its high HM production (leaves and non-woody material), averaging 2,400 kg DM ha $^{-1}$ relative to other browse species tested. Papanastasis et al. (1997) reported a mean total biomass of 2,280 kg DM ha $^{-1}$ and 1,026 kg DM ha^{-1} for grazeable leaves and twigs, respectively.

Reid et al. (1959) used days after April 30 (calendar date) to predict forage quality in the Northeast USA. Onstad and Fick (1983) used accumulated air temperature heat units (or growing degree days; GDD) above a 5°C base to predict several forage quality constituents for alfalfa (*Medicago sativa* L) species, and concluded that predicting standing alfalfa quality from heat units is a promising approach. A relationship between GDD and forage quality and phenolic concentrations has not been reported for BL.

According to Buxton and Fales (1994) plant maturity integrates cumulative effects of physiological processes that are expressed in yield and herbage quality. Thus, information provided by GDD may reflect changes in BL quality factors resulting from the cumulative effects of physiological processes that impact plant maturity. A model that closely describes the relationship between GDD and herbage quality variables would be useful for devising BL utilization strategies.

Our hypothesis was that GDD have a strong influence on plant growth factors such as herbage mass (HM), tree canopy height (H), herbage quality and on anti-quality constituent trends of BL herbage during the peak-growing season (May to July). Variables measured were HM, H, herbage quality constituents (CP, *in vitro* true dry matter disappearance [IVTDMD], neutral detergent fiber [NDF], acid detergent fiber [ADF], cellulose [CELL], and 72% sulfuric acid lignin [ADL], and estimates of anti-quality constituents (FR-phenol, CT, and HT).

Materials and Methods:

Background:

The study was conducted at North Carolina State University Meat Goat and Forage Educational Unit, Wake County, North Carolina (35.75° N lat and 78.75° W long). The climate is temperate with 1,191 mm long-term mean annual precipitation and average annual maximum and minimum temperatures of 21.1 and 10.5ºC, respectively. Soils of the study area were of the Cecil series (fine, kaolinitic, thermic, Typic Kanhapludults) on slopes ranging from 6 to 10% (Soil Survey Div., 2001). These soils are deep and well drained with a firm red clay sub-soil and sandy clay loam surface soil. Parent material consisted of gneiss, schist, and other acidic rocks. In November of 1998, one soil sample was taken (15 cm depth) for each plot (72 total) and pooled by replication. Soil samples from the sites were analyzed by North Carolina Department of Agriculture Soil Testing Lab to derive soil chemistry data. Soil fertility at the experimental site was characterized by high base saturation (82%) and a pH of 6.1. Due to adequate P and K levels, soil base saturation, and the ability of BL to fix N, no fertilizer or lime were applied during 1997 to 2000.

Site Establishment:

The field study was established by planting bare-root seedlings from a general nursery stock in March 1995 (as reported by Addlestone, 1996). Plots, replicated four times, were 9 m by 3 m with 3 m alleys between blocks (Addlestone, 1996). Each plot contained a single row of trees spaced 1 m apart. The first and last tree in each plot served as "border" trees and a single row of trees bordered each side of the experimental area. The trees were coppiced each year in late winter at 0.5 m from the soil surface. Each season from 1995 through 2000, the site was fenced (3-wire fence) using a temporary electric wire 1.5 m high (with a 0.75 m high offset wire placed in front of the main fence). The fencing allowed goats

to browse and prevented herbivory by white-tailed deer (*Odocoileus virginianus*). Mowing and hand weeding were performed to control aggressive broadleaf weed species during early establishment. The site was not irrigated. Data collection for the phase of the study reported here began in May 1999; however, in 1997 and 1998 the plots were maintained by annual browsing with goats and by coppicing the trees with pruning shears to their original heights of 0.5 m in February.

Field Measurements:

Two trees in each plot were randomly selected for each sampling date (1999: 13 May; 28 May; 11 June; and 29 June and 2000: 14 June, 28 June, 17 July and 27 July) so that the same tree was never sampled twice in one growing season. The randomly selected trees were first measured for H using a tree-measuring pole (Craine Enterprise, Inc. Mound City, IL). Destructive sampling was performed by coppicing the trees to 0.50 m and removing all green herbage by hand to determine HM for each tree. The stripped leaves were weighed in the field in cloth bags on a digital electronic balance. Two sub-samples were collected. The first sub sample was dried for 48 h at 60°C in a forced-air oven, and then reweighed for DM calculations. The second sub sample was collected for tannin analysis. The leaf tissues for tannin analysis were immediately frozen in liquid N in the field, transported to the lab and freeze-dried. Upon drying, each sample was ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) using a 1 mm screen and stored in an airtight container until analyzed.

Measurement of Forage Quality:

Dried herbage was chemically analyzed for Kjeldahl N according to AOAC (1999). Crude protein concentration was calculated as percentage N multiplied by 6.25. The samples

were also analyzed for NDF, ADF, and ADL sequentially according to Van Soest et al. (1991) as modified by Komarek et al. (1994). Acid detergent lignin was corrected for mineral matter by ashing the ADL residue in a muffle furnace at 500°C. Cellulose concentration was calculated as the difference between ADF and ADL plus ash. *In vitro* true DM disappearance was determined using ruminal contents collected from a ruminally-cannulated Hereford steer maintained on high-quality alfalfa hay (Goering and Van Soest, 1970). The steer was fed at 08:00 and 16:00; ruminal contents were collected before the morning feeding. Ruminal fluid was passed through four layers of cheesecloth before being processed for use in the IVTDMD bioassay. After 48 h of incubation with ruminal inoculum in a batch processor (Ankom Technology Corp., Fairport, NY), samples were extracted with neutral detergent solution for IVTDMD estimation.

Measurements of Tannins:

Samples were sent to the Penn State Pesticide Research Laboratory, University Park, PA for FR-phenol, CT and HT determination according to procedures reported by Appel et al. (2001).

Each sample (FR-phenol, CT and HT) used BL as a self- standard. Based on the work reported by Hagerman and Butler (1989) and Martin and Martin (1982), purified phenolics from the plant tissue being studied should be used as standards (self-standards), because the relationship between the Folin-Denis Assay (FA; used to determine total phenolics) absorbance and the standard differs significantly with sample source. Appel et al. (2001) confirmed that using commercial standards either under or over estimated actual polyphenols, leading to quantitatively deceptive conclusions.

In this study, BL self-standards were produced by crudely purifying phenolics using silica gel (LH20) columns, which produced a powder consisting of methanol or acetone-soluble phenolics (J.C. Shultz, personal communication, 2002). Standard curves were constructed by measuring the absorbance of a dilution series. For BL samples, extraction was focused on larger molecular weight polymers (tannins). Hence, the standards and data were for acetone-extracted phenolics, which favors large molecular weight polyphenols (J.C. Shultz, personal communication, 2002).

Folin-reactive phenolics were measured using a FA reagent, a redox indicator that turns blue when reduced. According to Appel et al. (2001), phenolics are excellent reducing agents with reducing power (reactivity) that varies with structure and concentration.

Condensed tannins were analyzed using the butanol-HCL assay, which quantifies hydrolyzed proanthocyanidin residues to their anthocyanin, monomer components. Anthocyanin was measured at 550 nm (Appel et al., 2001). A self-standard for BL was used instead of the commercial standard quebracho, a condensed tannin mixture from the leguminous tree shrub, *Schinopsis balansae* (Appel et al., 2001).

Hydrolyzable tannins were analyzed with self-standards using a potassium iodate method modified for quantitative use (quantified galloyl esters), following the procedures of Shultz and Baldwin (1982).

Statistical Analyses:

Statistical analyses of data were conducted using procedures of SAS (2002). Analysis of variance (ANOVA) of data combined over years showed significant year effects, and further analyses were carried out separately by year. The trees were sampled four times

annually (every two weeks) to monitor changes in HM, quality constituents (NDF, ADF, ADL, CELL, IVTDMD, and CP) and estimates of anti-quality indicators (FR-phenol, CT, and HT) throughout the season. The least significant difference (LSD) test was used to determine significant differences among treatment means. A significant level of 0.05 was used in all LSD analyses.

Simple linear regression was used to determine a statistically reliable function for predicting H, HM, NDF, ADF, ADL, CELL, IVTDMD, CP, FR-phenol, CT and HT from GDD.

The general form for a single variable model is:

$Y = a + bx$

Where

Y= quality/anti-quality variable (concentration)

x= explanatory variable

a= Y intercept

b= Slope coefficient for x

A correlation coefficient matrix was examined to determine the strength of relationships between all variables (Table A.64: A.67). Regression analysis was used to determine whether a particular variable had a significant linear relationship with GDD.

Growing Degree-Days Calculation:

Growing degree-days are a temperature-derived index representative of the amount of heat that the plants are exposed to, which in turn is directly related to the plant's rate of growth and development (Onstead and Fick, 1983). Growing degree-days may be calculated

several ways. Different base temperatures used for different crops, i.e. wheat (*Triticum* spp.) corn (*Zea mays*) and cotton (*Gossypium* spp.) are 5, 10, and 15°C, respectively (Acquaah, 2002). Two GDD formulas are commonly used. One formula calculates the number of GDD for a given day by averaging the maximum and minimum air temperature (°C) within a 24-h period then subtracting the appropriate base temperature for the organism being monitored. Another method, proposed by the National Oceanic and Atmospheric Administration in 1969 (NCSU, 2003) and referred to as the 'Modified Growing Degree Day' formula, is used in the United States to monitor corn development. The modified GDD formula places an upper limit of 30°C on the daily maximum air temperature and the average temperature less than base temperature (i.e., 10°C) is set equal to zero.

For this study, we accepted the general GDD formula, although, for comparison, the 'Modified Growing Degree Day' formula was also analyzed. A base temperature of 10°C was selected for the general GDD formula, because BL growth appears to initiate when daily temperatures average 10°C. A season total was obtained by summing the daily growing degrees from 01 March through the sampling date of interest, because during March bud emergence is usually observed (Mueller, personal communication, 2003).

The formula used in this study was:

GDD = ? {[(T air_{max} + T air_{min}) / 2] - 10 °C}, where T air_{max} and T air_{min} are the daily maximum and minimum air temperatures.

The temperature data for 1999 and 2000 were recorded at the Reedy Creek Station, in Wake County, North Carolina. This site was located approximately 0.5 km from the experimental area.

Results and Discussion:

Foliar Estimates of Forage Quality:

With the possible exception of IVTDMD, herbage quality estimates of BL foliar samples collected in 1999 and 2000 were high (Table 3.1). Crude protein values for BL ranged from 31.5 to 27.5% (N concentration ranged from 5.2 to 3.5%). Assuming adequate intake (>3% body weight [BW]) and no anti-quality factors, the observed CP concentration was well above the nutritional requirements (14 % CP) of a 20 kg goat kid gaining 150 g d^{-1} (NRC, 1981).

The relatively low foliar NDF and ADF values observed for BL ranging from 26.2 to 45.1% and 17.0 to 29.9%, respectively would indicate that the BL herbage has potential for high digestibility. Yet values observed for IVTDMD suggested a tannin induced depression of digestibility.

The observed ADL values for this study ranged 10.1 to 20.5%, which was within the range for a leguminous browse species previously reported by Short et al. (1974). Papachristou and Papanastasis (1994) reported BL ADL values ranging from 5.6 to 12 %. In general, as herbage ages, the percentage of soluble material within the cells decreases and the concentrations of ADL and CELL increase (Pearson and Ison, 1987). In 1999, CP decreased and ADL increased during the growing season resulting in reduced foliage digestibility as the season progressed. The CELL values observed in this experiment ranged from 6.8 to 9.5%, which appears low compared to Addlestone et al. (1999) who reported CELL values of 17% from plants harvested on 26 August. The CELL levels reported in this study came from BL

harvested from May-July indicating less mature plants were harvested compared with the values reported by Addlestone et al. (1999).

In vitro true dry matter disappearance averaged over both years was 58%, but in 1999 at the first two sampling dates, (13 and 28 May) IVTDMD values were high (73.7 and 70.3%, respectively). In May of 1999, the elevated IVTDMD levels are most likely the result of highly digestible new leaf growth. Sampling in May of 1999 began when GDD were 375 and 515 for 13 and 28 May, respectively, compared to later sampling dates in 1999, where GDD were 726 and 950 for 11 and 29 June, respectively, and IVTDMD levels were 56.6 and 52.9%, respectively. The IVTDMD values were generally lower than expected considering the concentrations of NDF, ADF, and CP observed. This would suggest that anti-quality constituents such as tannins were interfering with normal digestive processes (Van Soest, 1982). When comparing cell wall contents (NDF, ADF and CELL) for 1999, there was an increase in concentrations over time. This result would be expected as plant tissues mature. Nevertheless this was not the case in 2000 when no significant relationships were detected.

Concentrations of FR-phenol, CT and HT decreased over time in year 1999. This suggests a decline in tannin concentrations in leaf tissue with increasing tree maturity. Feeney and Bostock (1969) noted an increase from July to September in the CT concentrations of oak leaves. Waterman and McKey (1989) reported a similar finding where younger leaves had higher concentrations of phenolics than mature leaves. Horner (1988) reported that non-linear trends might exist when dilution effects caused by leaf expansion occur. This was the case for 2000, when linear trends were not evident. There are, however,

contradicting reports from Parker (1977) and Glyphis and Puttick (1988) who found that as the season progressed and or the leaves aged, levels of phenolics increased or at least plateaued.

The FR-phenol concentrations ranged from 5.3 to 11.9%. According to McLeod (1974) concentrations of FR-phenol above 5% DM intake could reduce digestion and above 9% DM intake could be lethal to an animal that has no other feed (Kumar 1983). Makkar and Becker (1998) reported levels of FR-phenol in BL foliage of 6.6%, which was within the range reported in this study (Table 3.2).

Although HT have been identified in leaf tissue of species other than BL (Kumar and Vaithiyanathan 1990); HT was not believed to be as important as the CT concentrations because HT, once ingested by the animal, readily hydrolyze under mild acid or alkaline conditions into sugars and phenolic carboxylic acids (Van Soest, 1982). Nevertheless, McSweeney et al. (1988) reported that two of four sheep that ingested 0.9 g HT kg^{-1} BW from yellow-wood foliage (*Terminalia oblongata*) showed signs of toxicity in 15 days.

Condensed tannins have been reported to depress voluntary intake, rates of body growth, and ruminal fiber digestion (Terrill et al., 1992). Nevertheless, moderate to low levels of CT (2 to 4% DM) are now believed to be beneficial to ruminant diets, helping to increase absorption of essential amino acids from the small intestine up to 62% (Waghorn et al., 1987), acting directly as anthelmintics against parasitic nematodes and indirectly improving N supply to animals (Butter et al., 2000; Min and Hart, 2003). In this study, CT concentrations of BL foliage ranged from 6.3 to 12.6%. No other information regarding the tannin concentrations of BL has been reported with the exception of Kumar and Horigome,

1986 who purified CT in five fractions and concluded that BL protein-precipitating capacity increased with molecular size. Manuscripts documenting concentrations of FR-phenol, HT, and CT of BL in the same study have not been reported in the literature.

Trends in 1999 and 2000:

Across dates in 1999 significant relationships were evident; however, in 2000 trends across sampling dates were not well defined and few significant relationships were present (Table 3.1 and Table 3.2).

The only significant relationships with GDD in 2000 were N ($p=0.0001$), ADF ($p=0.05$) and CELL ($p=0.05$) (Table 3.1). Concentrations of NDF and ADL would normally be expected to increase throughout the growing season; however, in 2000 the means for both NDF and ADL showed no clear trend. This was also the case for ADF, CELL and IVTDMD. *In vitro* true DM disappearance would normally be expected to decrease over the growing season; however, in 2000 no trend was present (Table 3.1). Also, no trends were seen in 2000 for FR-phenol, CT and HT as compared with 1999, when there was a significant decrease in concentration throughout the growing season (Table 3.2).

Differences in measured variables between 1999 and 2000 were most likely caused by the flush of new growth that was observed during the sampling period in 2000. The flush of new growth during the 2000 season most likely obscured the normal maturation process of leaves produced because samples collected involved the removal of all leaves on every tree. New leaf growth was mixed with the older leaves and probably masked the normal maturation process, resulting in little or no difference in quality variables measured across the sampling period in 2000.

To help explain the differences observed between years, rainfall (RF) amounts were examined in two ways. First, RF was reported by summing from 01 March to each sampling date **(**Table 3.1). Second, RF was reported as a cumulative monthly total and as a total for each growing season (Table 3.3). In 2000 there was a much higher volume of RF (529.3 mm) compared with 1999 (221 mm). The high RF during the 2000-growing season supports the visual observation of growth flushes that produced dilution effects and no clear herbage quality trends over the 2000 sampling period.

Tree Canopy Height and Herbage Mass:

Mean tree canopy height and HM of BL for 1999 and 2000 are reported in Table 3.4. In 1999, an increase in H occurred across sampling dates, with H ranging from 1.4 m in May to 2.9 m by the end of June. In 2000, measurement began one month later than in 1999, and mean H was similar at all sampling dates (mean of 2.9 m). This suggests that virtually all of the growth in tree height observed during 2000 took place before sampling began. As observed for H, HM in 1999 exhibited an increase over time ranging from 336 kg DM ha^{-1} in May to 1,609 kg DM ha^{-1} by the end of June. Herbage mass in 2000 did not differ significantly across sampling dates with an overall mean of 2,011 kg DM ha^{-1}. The differences between years for H and HM can be partially explained by differences in sampling dates, and correspondingly, GDD. In 2000 trees were first measured after accumulating more than twice the GDD that were observed at the first measurement in 1999 (Table 3.4). The GDD for 1999 ranged from 375 to 950 GDD while for 2000, the range was 827 to 1,471. This difference is primarily due to the shift in sampling dates between the two years. In 1999, sampling was initiated on 13 May and ended on 29 June. In 2000, sampling

did not begin until 14 June and ended on 27 July, about 1 month later than 1999. As previously mentioned the amount of RF accumulated during the sampling periods and the primary growing season in both years was very different (Table 3.3). Total seasonal RF was 3.5 times greater in 2000 than in 1999.

The Relationship between GDD and Herbage Constituents:

Cumulative (GDD) heat units during spring growth may vary greatly from year to year, resulting in large quality differences on the same day of year (DOY). For example, in 1999 and 2000 on the same DOY (June; DOY 180), leaf concentration of quality variables differed greatly (Table 3.1). Even though the DOY were the same, GDD were quite different. In 2000 there were more than 100 GDD on the same DOY as in 1999. These differences in DOY and GDD are further illustrated when one considers the differences between the first sampling date in 1999 and 2000. The difference accounted for between years using DOY represents a 25% increase while that using GDD represents a 121% increase.

Each of the quality and anti-quality variables was regressed on GDD (calculated from March 01, 1999 and 2000), using the general and modified GDD formulas (Table 3.5). The general GDD formula produced the highest r^2 values.

For 1999, significant GDD regressions were observed for H and HM with r^2 values of 0.58 and 0.77, respectively; however in 2000, no relationships were evident.

In 1999, GDD was a strong predictor of NDF (r^2=0.93). According to Van Soest (1982), NDF is a helpful indicator of plant cell wall concentration and is negatively related to animal intake (Van Soest, 1982). Thus, based on this relationship if BL averaged 30% NDF

at 515 GDD, an additional 211 GDD would be needed to reach 40% NDF. Therefore, beginning harvest or grazing of BL at 30% NDF would have given about a 14 day window to complete grazing before a value of 40% NDF was reached. To graze herbage with a NDF value of 26%, grazing would need to be initiated when 375 GDD have accumulated.

Conclusions and Recommendations:

Forage quality is a key determinant of farm profitability. Maturity at harvest is the most important factor affecting forage quality. Poor quality forages increase livestock feed costs per unit of production and limit milk production and weight gain. As BL foliage matures DM digestibility usually decreases. The ability to predict herbage quality factors such as CP or NDF *in situ* is important to graziers attempting to provide adequate nutrition to achieve desired levels of animal productivity. Data from this research confirms that under a certain set of environmental conditions NDF, ADF, IVTDMD, CELL and ADL can be predicted using GDD. Furthermore during seasons when high RF causes repeated flushes of new growth, prediction of herbage quality from GDD is unlikely. Research reported here provides a glimpse of the possibilities for future studies. There is a need to build an understanding of specifically how changes in BL herbage quality are influenced by environmental conditions. Growing degree-days show promise as a predictor of nutritive value providing seasonal climatic effects are considered. Nevertheless, GDD models do not appear appropriate to predict the concentration of various tannin fractions in BL herbage. Tannin data reported in this study (FR-phenol, CT, and HT) adds considerably to the existing body of scientific literature on BL herbage and provides the first complete set of data for the most important tannin fractions for a growing season. The specific impacts of these phenolic

compounds on goat production are yet to be determined. Black locust provides the potential for adequate summer forage for goats. Nevertheless, further research is needed to confirm and expand GDD relationships presented here and to obtain a better understanding of seasonal fluctuations of tannin fractions. Using the GDD model in planning grazing and herbage harvest appears to hold promise as a practical management approach. According to Fick et al., (1994) we should keep in mind that the goal is to be able to predict forage quality as an alternative when forage testing is not possible.

Acknowledgements:

The author would like to thank Amy Conrad and the students who endured the endless summer days of hand defoliating black locust.

References:

1. Acquaah, G. 2002. Principles of crop production: theories, techniques and technology. Prentice Hall, Upper Saddle River, NJ.

2. Addlestone, B.J. 1996. Density and cutting height effects on the herbage mass of three tree legumes grown for meat goat production. M.S. thesis. North Carolina State Univ., Raleigh.

3. Addlestone, B. J., J.P. Mueller, and J-M. Luginbuhl. 1999. The establishment and early growth of three leguminous tree species for use in silvo-pastoral systems of the southeastern USA. Agroforestry Syst. 44:253-265.

4. AOAC. 1999. International Official Methods of Analysis. (16th Ed.) Assoc. Offic. Anal. Chem., Arlington, VA.

5. Appel, H.M., H.L. Govenor, M. D'ascenzo, E. Siska, and J.C. Shultz. 2001. Limitations of folin assays of foliar phenolics in ecological studies. J. Chem. Ecol. 27(4):761-778.

6. Boring, L.R., C.D. Monk, and W.T. Swank. 1981. Early regeneration of a clear-cut southern Appalachian forest. J. Ecol. 62:1244-1253.

7. Butter, N.L., J.M. Dawson, D. Wakelin, and P.J. Buttery. 2000. Effect of dietary tannin and protein concentration on nematode infection (*T. colubriformis*) in lambs. J. Agric. Sci. 134:89-99.

8. Buxton, D.R., and S.L. Fales. 1994. Plant environment and quality. p. 155-199. *In* G.C. Fahey, Jr. et al. (ed.) Forage quality, evaluation and utilization. ASA, CSSA and SSSA, Madison, WI.

9. Cannas, A. 2001. Tannins: Fascinating but Sometimes Dangerous Molecules. [Online] Available at http://www.ansci.cornell.edu/toxicagents/tannin/tannin.htm (posted16 September 2001).

10. Feeney, P.P, and H. Bostock. 1969. Seasonal changes in the tannin content of oak leaves. Phytochem. 7:871-880.

11. Fick, G.W., P.W. Wilkens, and J. H. Cherney. 1994. Modeling forage quality changes in the growing crop. p. 757-795. *In* G.C. Fahey, Jr. et al. (ed.) Forage quality, evaluation and utilization. ASA, CSSA and SSSA, Madison, WI.

12. Glyphis, J.P., and G.M Puttick. 1988. Phenolics in some southern African Mediterranean shrubland plants. Phytochem. 27:743-752.

13. Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures and some applications). USDA, ARS. Agric. Handb. 379. U.S. Gov. Print Office, Washington, DC.

14. Hagerman, A.E., and L.G. Butler. 1989. Choosing appropriate methods and standards for assaying tannins. J. Chem. Ecol. 15:1795-1810.

15. Horner, J.D. 1988. Astringency in douglas fir foliage in relation to phenology and xylem pressure potential. J. Chem. Ecol. 14:1227-1237.

16. Komarek, A.R., J.B. Robertson, and J.B. Van Soest. 1994. Comparison of the filter bag technique to conventional filtration in the Van Soest Analysis of 21 feeds. *In*: Proc. Natl. Conf. on Forage Quality, Evaluation and Utilization, Lincoln, NE.

17. Kumar, R. 1983. Chemical and biochemical nature of fodder tree leaf tannin. J. Agric. Food Chem. 31:1361-1364.

18. Kumar, R., and T. Horigome. 1986. Fractionation, characterization and protein precipitating capacity of the condensed tannins from *Robinia psuedoacacia* leaves. J. Agric. Food Chem. 34:487-489.

19. Kumar, R.A., and S. Vaithiyanathan. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30:21-38.

20. Luginhbuhl, J.M., J.T. Green, J. P. Mueller, and M. H. Poore. 1996. Meat goats in land and forage management. In Proceedings of the Southeast Regional Meat Goat Production Symposium. "Meat Goat Production in the Southeast- Today and Tomorrow". February 21-24, 1996. Florida A&M University, Tallahassee.

21. Makkar, H.P.S., and K. Becker. 1998. Do tannins in leaves for trees and shrubs from African and Himalayan regions differ in level and activity? Agroforestry Syst. 40:59-68.

22. Martin, M.M., and J.L. Martin. 1982. Tannin assays in ecological studies: Lack of correlation between phenolics, proanthocyanidins and protein-precipitating constituents in mature foliage of six oak species. Oecologia 54: 205-211.

23. McLeod, M.N. 1974. Plant tannins: their role in forage quality. Nutr. Abstr. Rev. 44(11):803-815.

24. McSweeney, C.S., P.M. Kennedy, and A. John. 1988. Effect of ingestion of hydrolyzable tannins in *Terminalia oblongata* on digestion in sheep fed *Stylosanthes hamata.* Aust. J. Agric. Res. 39: 235-244.

25. Min, B.R., and S.P. Hart. 2003 Tannins for suppression of internal parasites. J. Anim. Sci. 81 (E. Suppl. 2):E102-E109.

26. National Research Council (NRC). 1981. Nutrient requirements for goats. National Academy Press, Washington, DC.

27. North Carolina State University. 2003. State Climate Office of NC. [Online]. Available at http://www.nc-climate.ncsu.edu (posted 20 March 2003).

28. Onstad, D.W., and G.W. Fick. 1983. Predicting crude protein, in vitro true digestibility, and leaf proportion in alfalfa herbage. Crop. Sci. 23:961-964.

29. Papachristou, T.G., and V.P. Papanastasis. 1994. Forage value of Mediterranean deciduous woody fodder species and its implication to management of silvo-pastoral systems for goats. Agroforestry Syst. 27:269-282.

30. Papanastasis, V.P., P.D. Platis, and O. Dini-Papanastasi. 1997. Productivity of deciduous woody and fodder species in relation to air-temperature and precipitation in a Mediterranean environment. Agroforestry Syst. 37:187-198.

31. Parker, J. 1977. Phenolics in black oak bark and leaves. J. Chem. Ecol. 3: 489-496.

32. Pearson, C.P., and R.L. Ison. 1987. Agronomy of Grassland Systems. Cambridge Univ. Press. New York, NY.

33. Pinkerton F, N. Escobar, L. Harwell, and W. Drinkwater. 1994. A survey of prevalent production and marketing practices in meat goats of southern origin. SRDC Publication No. 182. Southern Rural Dev. Center, Mississippi State, MS.

34. Reid, J.T., W.K. Kennedy, K.L. Turk, S.T. Slack, G.W. Trimberger, and R.P. Murphy. 1959. Effect of growth stage, chemical composition, and physical properties upon the nutritive value of forages. J. Dairy Sci. 42:567-571.

35. Shultz, J.C., and I.T. Baldwin. 1982. Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217:148-151.

36. Short H.L., R.M. Blair, and C.A. Segelquist. 1974. Fiber composition and forage digestibility by small ruminants. J. Wildl. Manage. 38:197-202.

37. Silanikove, N., N. Gilboa, A. Perevolotsky, and Z. Nitsan. 1996. Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Ruminant Res. 21:195-201.

38. SAS. 2002. The SAS System for Windows. Release 8.03. SAS Institute, Cary NC.

39. Soil Survey Division, Natural Resources Conversation Service, United States Dept. of Agric. Official Soil Series Descriptions [Online WWW]. Available URL: "http://ortho.ftw.nrcs.usda.gov/osd/" [Accessed 23 Mar 2001].

40. Terrill, T.H., G.B. Douglas, A.G. Foote, R.W. Purchas, G.F. Wilson, and T. N. Barry. 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. J. Ag. Sci. 119:265-273.

41. Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Durham and Downey, Inc. Portland, OR.

42. Van Soest, P.J., J.B Robertson, and B.A Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

43. Waghorn, G.C., M.J. Ulyatt, A. John, and M.T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Br. J. Nutr. 57: 115-126.

44. Waterman, P.G., and D.B. McKey. 1989. Herbivory and secondary compounds in rain forest plants. p. 513-536. *In* Leith, H. and M.J.A. Werger (eds.) Tropical Rainforest Ecosystems. Elsevier; Amsterdam.

45. Waterman, P.G., and S. Mole. 1994. Methods in Ecology: Analysis of phenolic plant metabolites. Blackwell Scientific Pubs. Cambridge MA.

Table 3.1: NDF, ADF, CELL, LIG, N, and IVTDMD concentrations of black locust herbage in 1999 and 2000, Wake County, North Carolina. [†]

Sampling date	NDF	ADF	CELL	ADL	N	IVTDMD	RF[‡]	GDD[§]
	----------g kg $^{-1}$ DM[¶]----------						mm	
1999								
13-May	262 c[#]	170 b	68 d	101 b	52 a	737 a	60.5	375
28-May	302 c	189 b	77 c	112 b	50 a	703 a	85.3	515
11-June	394 b	268 a	85 b	183 a	44 b	566 b	85.3	726
29-June	451 a	299 a	95 a	205 a	44 b	529 b	91.4	950
2000								
14-June	407 a	320 a	89 a	209 a	40 a	495 b	202.2	827
28-June	381 a	283 ab	92 a	182 a	38 b	553 a	227.8	1054
17-July	386 a	284 ab	88 a	189 a	35 c	552 a	306.1	1339
27-July	368 a	263 b	78 b	168 a	35 c	510 ab	395.7	1471

[†]NDF=neutral detergent fiber; ADF= acid detergent fiber; CELL= cellulose; LIG=acid detergent lignin; N= nitrogen; IVTDMD= *in vitro* true dry matter disappearance.

[‡] RF=Rainfall; summed from 01 March to sampling date.

[§] GDD= growing degree-days=? {[(T air_{max} + T air_{min}) / 2] - 10 °C}, from 01 March.

[¶] DM= dry matter.

[#] Within columns and for each year individually, means followed by the same letters are not significantly different according to LSD (0.05).

Table 3.2: FA-phenol, CT, and HT concentrations of black locust herbage in 1999 and 2000, Wake County, North Carolina. [†]

Sampling date	FR-phenol	CT	HT	GDD[‡]
	----------% g/kg DM[§]----------			
1999				
13-May	7.5 a[¶]	8.3 a	8.4 a	375
28-May	6.7 a	7.5 ab	7.1 ab	515
11-June	6.2 a	7.1 ab	6.5 b	726
29-June	5.7 a	6.3 b	6.4 b	950
2000				
14-June	7.9 b	8.8 ab	11.0 a	827
28-June	9.2 ab	10.5 ab	8.5 ab	1054
17-July	7.9 b	7.9 b	6.4 b	1339
27-July	11.9 a	12.6 a	10.0 ab	1471

[†]FR-phenol= Folin-reactive phenolics; CT= condensed tannins; HT= hydrolyzable tannins.
[‡] GDD=growing degree-days=? {[($Tair_{max}$ + $Tair_{min}$) / 2] - 10 °C}, from 01 March.
[§] DM=dry matter.
[¶] Within columns and for each year individually, means followed by the same letters are not significantly different according to LSD (0.05).

1

Table 3.3: Total and monthly rainfall at the experimental site for sampling periods in 1999 and 2000, Wake County, North Carolina.

Month	Rainfall	
	1999	2000
	----------mm----------	
March	99.1	87.4
April	55.4	131.3
May	30.0	24.4
June	6.4	74.7
July	30.5	211.6
Total	**221.4**	**529.3**

1

Table 3.4: Black locust mean canopy height and dry herbage mass in 1999 and 2000, Wake County, North Carolina.

Sampling date	Canopy Height	Herbage Mass	GDD[†]
	m	kg ha^{-1}	
1999			
13-May	1.4 c[‡]	336 c	375
28-May	1.8 c	735 bc	515
11-June	2.4 b	1164 ab	726
29-June	2.9 a	1609 a	950
2000			
14-June	3.2 a	1458 a	827
28-June	2.8 a	2253 a	1054
17-July	3.1 a	2554 a	1339
27-July	2.8 a	1780 a	1471

[†] GDD= growing degree-days=? {[($Tair_{max}$ + T air_{min}) / 2] - 10 °C}, from 01 March.

[‡] Within columns and for each year individually, means followed by the same letters are not significantly different according to LSD (0.05).

Table 3.5: Linear regression equations for NDF, ADF, CELL, ADL, N, IVTDMD, FA-phenol, CT, HT, H, and HM as a function of GDD from 01 March 1999 and 2000, Wake County, North Carolina. †

Regression Equations	SE‡	r^{2}§
1999		
NDF=134.4 +0.34(GDD)	23.7	0.93***
ADF=76.6 +0.24(GDD)	24.9	0.85***
CELL=52.5+0.04(GDD)	3.3	0.92***
ADL=23.8+0.19(GDD)	26.1	0.77**
N=58.2-0.02(GDD)	2.9	0.65***
IVTDMD=885.7 -0.38(GDD)	45.4	0.82***
FR-phenol=8.4 -0.003(GDD)	1.3	0.23
CT=9.3 -0.003(GDD)	1.1	0.34*
HT=9.2 -0.003(GDD)	1.4	0.25
H=0.84 +0.002(GDD)	0.39	0.58**
HM=-434.7 +2.2(GDD)	293.7	0.77***
2000		
NDF=441.5 -0.05(GDD)	26.5	0.19
ADF=373.2 -0.07(GDD)	28.3	0.32*
CELL=104.9 -0.01(GDD)	6.8	0.26*
ADL=245.6 -0.05(GDD)	27.9	0.19
N=46.8 -0.008(GDD)	1.9	0.58***
IVTDMD=496.9+ 0.03(GDD)	39.2	0.03
FR-phenol=4.5 +0.004(GDD)	2.2	0.19
CT=6.3+-0.003(GDD)	2.6	0.08
HT=12.4 -0.003(GDD)	2.9	0.07
H=3.4-0.0004(GDD)	0.51	0.05
HM=1151.3 + 0.73 (GDD)	836.2	0.05

† NDF, neutral detergent fiber; ADF, acid detergent fiber; CELL, cellulose; ADL, acid lignin; N, nitrogen; IVTDMD; *in vitro* true dry matter disappearance; FR-phenol, Folin-reactive phenolics; CT, condensed tannins; HT, hydrolyzable tannins; H, canopy height; HM, herbage mass; GDD, growing degree days =?{[(T air_{max} + T air_{min}) / 2] - 10 °C} (from 01 March).

‡SE= Standard error of mean

§ r^2 = coefficient of simple determination.

*, **, *** Significant at P= 0.05, 0.01, and 0.001 levels, respectively.

Chapter Four

Intake, Digestibility, and Nitrogen Utilization of Black Locust Foliage Fed to Growing Goat Wethers

L.J. Unruh Snyder*[1], J-M. Luginbuhl[1,2], J.P. Mueller[1] , A. Conrad[2], and K. Turner[3]

Departments of Crop Science[1] and Animal Science [2],

North Carolina State University, Raleigh, NC 27695-7620;USA;

USDA ARS, Beaver, WV 25813; USA[3]

*Corresponding author (prepared for submission for Animal Science)

Abstract:

Black locust (BL; *Robinia pseudoacacia*), a native tree of southeastern USA, known to contain substantial levels of condensed tannins (CT), was fed to sixteen, four month old (20.4 kg body weight [BW]) Boer cross wether goats (*Capra hircus hircus*). Four diets were stall fed in a randomized complete block design (RCBD) with four replications in each of two years. The objective of this research was to study the effect of BL tannins on intake, digestibility of DM, and N metabolism. First year (1999) diets were H_E (100% Eastern gamagrass [EGH]; *Tripsacum dactyloides* hay), H_EG (70% EGH and 30% mixture of 59% ground corn [GC; *Zea mays*] and 36% soybean meal [SBM; *Glycine max*], and 5% minerals), 25BL99 (75% EGH and 25% BL leaves), and 50BL99 (50% EGH and 50% BL leaves). Second year (2000) diets were H_O (100% orchardgrass [OGH; *Dactylis glomerata L.*] hay), H_OG (70% OGH and 30% mixture of 63% GC and 37% SBM), 50BL00 (50% OGH and 50 % BL leaves), and 75BL00 (25% OGH and 75% BL leaves). In 1999, diet apparent digestibilities of the diets in order listed above for dry matter (DM) were (62.4, 68.2, 58.0, and 60.6% [P=0.001]) and crude protein (CP) were (62.8, 72.5, 56.0, and 59.1% [P=0.001]). In 1999, goats that consumed diets H_E and H_EG had higher digestibilities (P=0.0001) of

neutral detergent fiber (NDF) and acid detergent fiber (ADF) than did goats consuming diets 25BL99 and 50BL99. Acid detergent lignin (ADL) digestibilities for diets 25BL99 (-56.7%) and 50BL99 (-49.3%) were negative due to the probable complexing of tannins with the lignin and nitrogen fraction. Intake of DM was similar across diets. For 2000, diet apparent digestibilities for DM [64.4, 71.7, 64.8 and 65.4%] and CP [70.0, 76.0, 66.6, 66.5%] were not significantly different. Goats that consumed diets 50BL00 and 75BL00 had lower digestibilities (P=0.0004) of NDF and ADF than did goats consuming diets H_O and H_OG. Lignin digestibility for diets 50BL00 (9.4%) and 75BL00 (29.6%) were positive compared to those for year 1999. Overall, BL had 10% DM as CT and 18 to 34% DM as hydrolyzable tannins (HT). Total volatile fatty acid (VFA) concentrations were not depressed in 1999, but in year 2000, VFA concentrations were lower in diets 50BL00 and 75BL00, perhaps because of the increased tannin concentrations within those diets. The N balance, in 1999, showed that diet H_EG was higher (P=0.01) than diet H_E in both N intake and urinary N. Fecal N for diets 25BL99 and 50BL99 was similar to that for diet H_EG. In 1999, fecal N excretion as a percentage of N intake was significantly higher (P <0.02) in BL diets, although urinary N as a percentage of N intake did not differ among diets. In 2000, N intake and fecal N output for BL diets were about 6% higher compared to diet H_O (P=0.01) and H_OG (P=0.02). There were no significant differences in urinary N output among diets. Nitrogen retained as a percentage of N intake was not significantly different among diets. Fecal N as a percentage of N intake was significantly lower (P=0.01) for diet H_OG (24.0%) than for diets 50BL00 and 75BL00 (33.4 and 33.5%, respectively). Urinary N as a percentage of N intake was significantly higher for diets H_O and H_OG compared to the BL diets (P=0.02). Increased

levels of BL in the diets increased fecal N, which suggested that tannins formed dietary protein complexes, and hindered digestibility of cell wall constituents.

Keywords: *Robinia pseudoacacia*, Boer goats, intake, digestibility, tannins

Abbreviations: **ADF**, acid detergent fiber; **ADL**, acid detergent lignin; **BL**, black locust; **BW**, body weight; **CELL**, cellulose; **CP,** crude protein; **CT,** condensed tannins; **DM,** dry matter; **DMI**, dry matter intake; **EGH,** eastern gamagrass hay; **FA**, Folin-Denis assay; **GC**, ground corn; $\mathbf{H_E}$; Diet 1 1999; $\mathbf{H_EG}$; Diet 2 1999; $\mathbf{H_O}$; Diet 1 2000; $\mathbf{H_OG}$; Diet 2 2000; **HM,** herbage mass; **HT**, hydrolyzable tannins; **IVTDMD**; *in vitro* true dry matter disappearance; **MW**, molecular weight; **N**, nitrogen; **NDF**, neutral detergent fiber; **OGH**, orchardgrass hay; **PRP**, proline-rich proteins; **PUN**, plasma urea nitrogen; **RCBD**, randomized complete block design; **SBM**, soybean meal; **FR-phenol**, Folin-reactive phenolics; **VFA**, volatile fatty acids; **25BL99**; Diet 3 1999; **50BL99**; Diet 4 1999; **50BL00**; Diet 3 2000; **75BL00**; Diet 4 2000.

Introduction:

In southeastern USA, goats (*Capra hircus hircus*) are becoming an important livestock species due to increased demand for goat meat by various ethnic groups. Goat meat also fills a gap in some high-value niche markets for people who prefer low levels of total and saturated fat (Pinkerton et al., 1994). For the past decade, the domestic market has not been able to supply these increasing demands.

An important consideration in increasing meat goat production is the availability of environmentally-sustainable production systems. Black locust (BL; *Robinia pseudoacacia* L.) is indigenous to Southern Appalachian deciduous forests. It is an appropriate source of

biological nitrogen (N) that can be used to enhance N-poor ecosystems, control erosion, and provide potential browse for livestock (Keresztesi, 1980).

Black locust leaves have been used for livestock feed around the world, including Korea and Bulgaria (Keresztesi, 1983 and 1988). In the highlands of Nepal and northern India, where BL is naturalized, it has been documented that branches above the reach of livestock are cut and fed to livestock when other green forages are scarce, and the wood is used for fuel (Keresztesi 1980, 1983, and 1988). Black locust has been grown successfully in dry Mediterranean summer climates to effectively satisfy feed demand when grasses and legumes became dormant in the summer (Ainalis and Tsiouvaras, 1998; Papachristou and Papanastasis, 1994; Papachristou, 1999; Papachristou et al., 1999).

Horton and Christensen (1981) and Ayers et al. (1996) examined the metabolism of sheep and goats fed artificially dried BL foliage. They concluded that BL digestibility was lower than that of alfalfa meal. They also reported the probable cause of depressed digestibility was tannin-protein complexes formed in the gut of the sheep and goats although they did not analyze BL for presence of phenolic compounds (tannins). According to Papachristou (1999) and Papachristou et al. (1999), BL supplementation was an effective livestock feed, producing live-weight gains comparable to alfalfa (*Medicago sativa* L.). Furthermore, researchers have reported that goats have a high preference for BL as browse (Addlestone et al., 1999; Lambert et al., 1989; Papachristou and Papanastasis, 1994)

Black locust has been reported to supply ample biomass. Addlestone et al. (1999) concluded that BL had potential as a browse species due to its high herbage mass (HM) production (leaves and non-woody material), averaging 2,400 kg dry matter (DM) ha $^{-1}$ relative to other browse species tested. Papanastasis et al. (1997) reported a mean total

biomass and a mean total grazeable leaves and twigs of 2,280 and 1,026 kg DM ha^{-1}, respectively.

Black locust leaf crude protein (CP) levels have been reported to be similar to those of alfalfa, ranging from 20 and 25% (Addlestone et al., 1999; Baertsche et al., 1986; Boring and Swank, 1984; Papachristou et al., 1999; Unruh et al., 2001). Research also showed that BL accumulates moderately high macronutrient tissue concentrations (1.27% Ca, 1.72% K, and 0.18% P) and produces large foliage quantities having high leaf surface area (Borning et al., 1981; Addlestone et al., 1999), rendering this species an excellent browse source for meat goat production.

Phenolic compounds (e.g. tannins) occur in BL (Kumar and Horigome, 1986), acting as chemical defenses against herbivory. The presence of tannins is hypothesized to negatively affect intake (Horigome et al., 1988; Kumar and Vaithiyanathan, 1990; Van Soest, 1982) and digestibility of foliage (Robbins et al., 1987; Hagerman et al., 1992). High tannin concentrations fed to grazing animals can have adverse effects on nutrition. The digestive tract reacts by producing an insoluble protein-tannin precipitate that is poorly digestible in the rumen and the lower digestive tract and is lost in the feces (Kumar and Singh, 1984). Tannins also can inhibit microbial enzymes used in fiber breakdown (Kumar and Singh, 1984). Nonetheless, dietary tannins have some benefits such as acting directly as anthelmintics against parasitic nematodes, indirectly improving N supply (Butter et al., 2000) and preventing bloat (Van Soest, 1982).

Kumar and Horigome (1986) purified BL CT and found that their protein-precipitating capacity increased with molecular size. It has not been reported if hydrolyzable

tannins (HT) are present in BL, and, if so, what concentrations will affect animal performance.

Hydrolyzable tannins (gallotannins and ellagitannins), polyesters of gallic acid or ellagic acid, respectively, and other phenolic acids derived from them, also contain a sugar (normally D-glucose) as the central core. The typical molecular weight (MW) for gallotannins is 900 and 2,000 to 5,000 for ellagitannins (Bernays et al., 1989). Hydrolyzable tannins, readily hydrolyzed by acid, are usually present in low-molecular mass restricted to only dicotyledonous plants (Bernays et al., 1989). Two additional classes of HT are taragallotannins (gallic acid and quinic acid as the core) and caffetannins (caffeic acid and quinic acid) (Cannas, 2001; Van Soest, 1982).

Condensed tannins (proanthocyanidins) are more widely distributed than HT in plants. They are oligomers or polymers (MW= 1,000 to > 20,000) of catechins (flavonoid phenols linked by carbon-carbon bonds), which are resistant to hydrolytic degradation. The linkage between the monomers, typically a carbon condensation, is relatively stable under the conditions that cleave linkages in HT. Condensed tannins produce red anthocyanins (glycosides of anthocyanidins) upon heating proanthocyanidins in acidic alcohol solutions and give an astringent taste to fruit and wines (Van Soest, 1982).

Some animals have the ability to secrete proline-rich proteins (PRP), which exhibit a protective response that acts as a first defense against ingested tannins (Mehansho et al., 1987). Browsers such as mule deer (*Odocoilieus heminous heminous*) or goats tend to be more tolerant of tannins in their diets because of their higher PRP production than other animals. Proline-rich proteins produced in the ruminant's parotid salivary glands are three times greater per unit body mass in browsers than in grazers, whereas they are intermediate

in mixed feeders (Bernays et al., 1989). Deer are more tolerant to tannins than are goats, sheep (*Ovis aries*) and cattle (*Bos taurus*) (Cannas, 2001). Proline rich proteins have a high affinity for tannins and tend to complex tannins before feed enters the rumen (Kumar and Vaithiyanathan, 1990; Silanikove et al., 1996; Robbins et al., 1987). Provenza and Malechek (1984) noted that perhaps as much as 50% of dietary tannins are bound during digestion by PRP leading to reduced fecal N losses. Robbins et al. (1987) reported that goats are considered to be mixed grazers and usually consume significant amounts of tanniferous forages that stimulate the salivary glands to allow more PRP production to effectively bind tannins. This phenomenon is more prominent in goats than with sheep or cattle. Also, Silanikove et al. (1996) suggested that exposing goats to tannins enhances the secretion of PRP in the parotid saliva.

The objectives of this study were to evaluate the intake, digestibility and N balance of fresh BL foliage by goats and to determine how the presence of tannins in the BL foliage interacts with those factors.

Materials and Methods:

General Experimental Procedures:

In 1999 and 2000, two randomized complete block design (RCBD) stall-feeding trials with four replications were conducted at North Carolina State University's Metabolism Educational Unit in Raleigh, NC (35.75° N lat and 78.75° W long). In both years, sixteen, four-month-old (averaging 20.4 kg body weight [BW]) crossbred Boer goat wethers were used. Prior to the experiments, goats were grazed on pasture that was predominantly tall fescue (*Festuca arundinacea* Schreb.). All goats were weighed and treated for elimination of gastrointestinal parasites (Ivermectin, Marial, Division of Merek and Co., Rahway, NJ) prior

to the initiation of the experiment. Goats were divided into four groups of similar BW and then randomly assigned to individual stalls (metabolism crates that were raised and slotted).

On Day 1, prior to the start of the adjustment period, the sixteen goats were shrunk weighed and then were placed in metabolism crates and fed a standard diet of 70% hay (1999: Eastern gamagrass [EGH; *Tripsacum dactyloides* L.] and 2000:orchardgrass [OGH; *Dactylis glomerata* L.]) and 30% grain mix (59%; ground corn [GC; *Zea mays* L.] and 36%; soybean [SBM; *Glycine max* L.], and 5% dolomitic limestone and goat minerals). All goats were fed trace-mineralized salt blocks (sodium chloride) free choice and had free access to water.

On Day 2, before the AM feeding, blood, urine, and feces samples were collected from all goats for baseline data. The experimental diets were fed starting on Day 2 and the intake phase began on Day 7. The goats were offered feed at 3% of BW d^{-1} with allowance made for 15% feed refusals. Daily DM intake (DMI) potential was estimated at 600 g DM d^{-1}. The correct proportions of BL leaves to be fed daily were calculated using previously determined barn dry DM concentrations of BL leaf samples. When intakes had stabilized, the digestion phase began (Day 24). The digestion phase included a 5-day total fecal and urine collection with weights taken on Day 6 of the digestion phase.

Animal Diets and Feeding Procedures:

First year (1999) diets were the negative control, H_E (100% EGH), the positive control, $H_E G$ (70% EGH and 30% mixture of GC [59%], SBM [36%], and 5% mineral mixture), 25BL99 (75% EGH and 25% BL leaves), and 50BL99 (50% EGH and 50% BL leaves). Second year (2000) diets were the negative control, H_O (100% OGH), the positive control, $H_O G$ (70% OGH and 30% mixture of GC and SBM, and 5% mineral mixture),

50BL00 (50% OGH and 50% BL leaves), and 75BL00 (25% OGH and 75% BL leaves). Goats were fed hay in the AM and BL leaves in the PM. Feed refusals were removed and weighed before each feeding. Each year, the square hay bales of EGH or OGH were passed lengthwise through a hydraulic bale processor (Van Dale 5600; J. Starr Industries, Fort Atkinson, WI) with stationary knives placed 10 cm apart. Cutting the hay to lengths of 7 to 13 cm made it easier to handle and reduced feed wastes.

Black Locust Leaf Collection:

The BL trees used in the study were growing at the North Carolina State University Field Research Station in Raleigh, NC. The climate is temperate with 1,191 mm long-term mean annual rainfall and an average annual maximum and minimum temperatures of 21.1 and 10.5ºC, respectively (NCSU, 2003). Soils at the study area were of the Cecil series (fine, kaolinitic, thermic, Typic Kanhapludults) on slopes ranging from 6 to 10% (Soil Survey Div., 2001). These soils are deep and well drained with a firm red clay sub-soil and a sandy clay loam surface soil. Parent material consisted of gneiss, schist, and other acidic rocks. Soil fertility at the experimental site was characterized by high base saturation (82%) and a pH of 6.1. Due to adequate P and K levels, soil base saturation, and the ability of BL to fix N, no fertilizer or lime were applied during 1999 and 2000.

Black locust leaves used in the study were harvested daily by hand and offered fresh from four-year old BL stands. Harvested BL leaves were stored on large trays in a walk-in cooler (9°C) until feeding time. Leaves were either fed immediately (same day) or stored for a maximum of two days until fed. Sub-samples of BL foliage for chemical analysis were taken on a daily basis and immediately placed in liquid N. Because of limited freeze dryer

facilities all samples were initially stored in a freezer (-20 °C) until they could be further processed.

Measurements and Preparation for Chemical Analyses:

Each experimental period in 1999 and 2000 consisted of a 24 d intake phase followed by a 5 d digestion phase. Adjustments of feed intake were based on the previous day's intake. A daily sample of the "as-fed" treatment was obtained and composites were made on a weekly basis. Feed refusals were weighed daily and saved for each animal then composites made on a weekly basis. Weekly samples for each period were composited, thoroughly mixed, oven dried for 48 h at 60°C and stored at room temperature until analyzed.

Total urinary volume was recorded daily throughout the digestion period and urinary pH was measured prior to the addition of 50 ml of 0.5N HCl (added to each urine bucket to keep urinary pH below 3 to avoid N losses). The urine plus acid was stored in plastic vials and frozen (-20°C) until analyzed. The total fecal output was recorded daily throughout the digestion period by placing fecal-collection bags on each goat. Bags were emptied twice daily and feces were weighed, and a 10% sub-sample was taken daily. Sub-samples were pooled during the 5-day digestion phase and for each day samples were oven dried for 48 h at 55°C, and analyzed after the experiment.

Blood samples to determine plasma urea-N were withdrawn by jugular venipuncture prior to the start of the experiment at the mid-point of the trial, and at the end of the experiment with 20-gauge, 2.54-cm needles and 10-mL vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Vacutainer tubes for processing plasma contained EDTA as an anticoagulant. After blood samples were taken they were immediately chilled in an ice bath and centrifuged at 10,000 x *g* for 10 min. Plasma was decanted to clean tubes

that were then stored at -20°C until analyzed. At the completion of the experiment, ruminal fluid samples were aspirated by stomach tube from all animals on all four diets. Ruminal pH was determined using a Cardy Twin pH meter (Spectrum Technologies, Inc., Plainfield, IL) immediately after ruminal fluid was filtered through 4 layers of cheesecloth. Ruminal fluid samples were then chilled in an ice bath to stop fermentation and then frozen (-20°C) until analyzed. Ruminal fluid samples were thawed, centrifuged at 3,600 x *g* for 10 min and the supernate (8 mL) was mixed with 2 mL of 25% metaphosphoric acid. The mixture was covered, held at room temperature for 30 min, centrifuged again at 3,600 x *g* for 10 min, and analyzed for ammonia and volatile fatty acids (VFA).

Laboratory Analyses of Feeds, Refusals, Urinary and Fecal Fractions:

Feeds, refusals, and fecal samples were dried separately for 48 h at 60°C in a forced-air oven for DM determination (AOAC, 1999). Both the dried herbage and fecal samples were ground through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and analyzed for DM and Kjeldahl-N according to AOAC (1999). Crude protein concentration was calculated as percentage Kjeldahl-N multiplied by 6.25. Urinary samples were analyzed for Kjeldahl-N content (AOAC, 1999). Feed, refusals, and fecal samples were also analyzed for detergent fiber analyses performed sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF), and 72 % sulfuric acid detergent lignin (ADL) concentrations as described by Van Soest et al. (1991) and modified by Komarek et al. (1994) for use in an Ankom fiber apparatus (Ankom Technology, Fairport, NY). Hemicellulose (HEMI) was determined as the difference between NDF and ADF. Cellulose (CELL) concentration was calculated by subtracting the difference between ADF and ADL

plus ash. Acid detergent lignin was corrected for mineral matter by ashing the ADL residue in a muffle furnace at 500°C.

In vitro true DM disappearance (IVTDMD) was determined using ruminal contents collected from a ruminally-cannulated Hereford steer maintained on high-quality alfalfa (*Medicago sativa* L) hay (modified by Goering and Van Soest, 1970). The steer was fed at 08:00 and 16:00, and ruminal contents were collected before the morning feeding. Ruminal fluid was passed through four layers of cheesecloth before being processed for use in the IVTDMD bioassay. After 48 h of incubation with ruminal inoculum in a batch processor (Ankom Technology, Fairport, NY), samples were extracted with neutral detergent solution for IVTDMD estimation.

Ruminal ammonia samples were analyzed using the Kjeldahl system described above and was determined by the colorimetric procedure for Kjeldahl-N (AOAC, 1999). Ruminal VFA concentrations were determined by Varion 3800 gas chromatography (Varian Chromatography Systems, Walnut Creek, CA) with a 15-m (0.53 mm i.d. x 0.5 µm film thickness) Nukol fused-silica capillary (Supelco, Bellefonte, PA). Nitrogen gas was used as a carrier gas for injection. Plasma urea N (PUN) was determined colorimetrically by an automated diacetyl-monoxime method (Marsh et al.; 1965).

Laboratory Analyses of Tannins:

Samples were sent to the Penn State Pesticide Research Laboratory, University Park, PA, for Folin-reactive phenolics (FR-phenol), CT and HT determination according to procedures reported by Appel et al. (2001).

Each sample (FR-phenol, CT and HT) used BL as a self- standard. Based on the work reported by Hagerman and Butler (1989) and Martin and Martin (1982), purified

phenolics from the plant tissue being studied should be used as standards (self-standards), because the relationship between the Folin-Denis Assay (FA; used to determine total phenolics) absorbance and the standard differs significantly with sample source. Appel et al. (2001) confirmed that using commercial standards either under or over estimated actual polyphenols, leading to quantitatively deceptive conclusions.

In this study, BL self-standards were produced by crudely purifying phenolics using silica gel (LH20) columns, which produced a powder consisting of methanol or acetone-soluble phenolics (J.C. Shultz, personal communication, 2002). Standard curves were constructed by measuring the absorbance of a dilution series. For BL samples, extraction was focused on larger molecular weight polymers (tannins). Hence, the standards and data were for acetone-extracted phenolics, which favors large molecular weight polyphenols (J.C. Shultz, personal communication, 2002).

Acetone (70% in water) favors larger polyphenols. "Smaller chain phenolics are not effectively extracted this way and were not of interest because smaller phenolics a) are relatively mobile in water, b) do not bind or chelate proteins or cations very effectively, and c) are unstudied in digestion context." (J.C. Shultz, personal communication, 2002).

Folin-reactive phenolics were measured using a FA reagent, a redox indicator that turns blue when reduced. According to Appel et al. (2001), phenolics are excellent reducing agents with reducing power (reactivity) that varies with structure and concentration.

Condensed tannins were analyzed using the butanol-HCL assay, which quantifies hydrolyzed proanthocyanidin residues to its anthocyanin, monomer components. Anthocyanin was measured at 550 nm (Appel et al., 2001). A self-standard for BL was used

instead of the commercial standard quebracho, a condensed tannin mixture from the leguminous tree shrub *Schinopsis balansae* (Appel et al., 2001).

Hydrolyzable tannins were analyzed with self-standards using a potassium iodate method modified for quantitative use (quantified galloyl esters), following the procedures of Shultz and Baldwin (1982).

Statistical Procedures:

Statistical analyses of data were conducted using SAS (2002). Data were analyzed separately for each year because diets differed in 1999 and 2000. For each year, data were subjected to ANOVA for a RCBD with four replications and subsampling was performed using the GLM procedure of SAS (2002). The model used was $Y = \mu$ + block (df=3) + diet (df=3) + block x diet (df = 9) + residual. The interaction block x diet was used as error term to test for diet effects. Diet means were examined using pre-planned treatment contrasts (Steel and Torrie, 1980). Unless otherwise indicated, significant differences were declared when $P<0.05$. Statistical analysis for PUN was analyzed for sampling date effect. If an interaction existed between sampling dates and treatments, treatment means for each sampling date were reported separately.

Results:

Chemical Composition of Feed Ingredients:

The chemical composition of feed ingredients in 1999 and 2000 is presented in Table 4.1. In 1999 and 2000, CP values of the hays were 13.9 (EGH) and 15.5% (OGH), respectively. This difference was probably due to forage species and to the maturity of the hay in 1999 (EGH) compared to the less mature OGH selected in 2000. In 1999 and 2000, BL samples had lower NDF and ADF values, but higher ADL concentrations compared to

EGH and OGH. The CP levels for the grain mix in 1999 and 2000 were 26.5 and 19.4%, respectively. In 2000, ADF and ADL concentrations of BL leaves were 52 and 60% higher, respectively, than in 1999. Conversely, HT concentration of BL leaves were 83% lower in 2000 than in 1999.

Plant Phenolics (Tannins):

Plant phenolics (tannins) are secondary compounds that function to enable the plant to tolerate soil, water and climatic stresses or may act as deterrents to grazing herbivores. Black locust has been documented to contain CT in its foliage; however, the presence of HT and its concentrations have not been reported for BL in the literature until now (Table 4.1).

The levels of FR-phenol, CT, and HT (Table 4.1) were relatively low (<2.1) for the grain mix, EGH, and OGH in 1999 and 2000. The FR-phenol, CT, and HT concentration found in BL in 1999 were 12.9, 10.4, and 34.2%, respectively. In 2000, FR-phenol, CT and HT concentrations were 12.6, 10.3, and 18.7%, respectively. The elevated concentrations of HT in 1999 was most likely due to relatively immature leaf growth that was harvested one month earlier than in 2000.

Chemical Composition of Diets:

1999:

Chemical composition of diets was based on N, cell-wall constituents and tannin components (Table 4.2). Diet H_E had higher NDF (73.6%) and lower CP (13.6%) values reflecting the advanced maturity of the experimental hay compared with the H_O diet fed in 2000. The CP concentrations of diets H_EG, 25BL99, and 50BL99 were higher (17.7, 18.2, and 20.7%, respectively) than for diet H_E because of the addition of grain concentrate or BL foliage. The CP concentrations were similar for diets H_EG and 25BL99, but diet 50BL99 CP

concentration was 2% higher due to the elevated BL percentage in the diet. The CP requirement of a 20 kg growing meat goat is 14% CP (NRC, 1981). With the exception of diet H_E, which approached this level, all diets contained sufficient amounts of CP.

Plant phenolics have been reported to be beneficial or detrimental to the ruminant, depending on the concentration. Concentrations of 2 to 4 % DM of CT in the diet protect protein from ruminal degradation and increase the absorption of essential amino acids whereas concentrations of 5 to 10% depress voluntary feed intake (Terrill et al., 1992; Barry and McNabb, 1999). Folin-reactive phenolics for all diets ranged from 0.7 to 7.5%. The concentration of CT for diets 50BL99 and 25BL99 were 6 and 3.7 %, respectively, and the concentrations of HT were 23.5 and 14.7 %, respectively. The concentrations of CT and HT for H_E (0.8 and 2.1%, respectively) and for H_EG (0.6 and 1.8%, respectively) were expected to be lower than those of BL diets because the ingredients consisted of low-tannin species (Cannas, 2001).

2000:

In 2000, diet H_O was higher in quality because of the use of less mature hay compared to EGH used in 1999 (Table 4.2). The CP concentrations of diets 50BL00 and 75BL00 were higher (22.6 and 24.7%, respectively) than for control diets H_O and H_OG (15.5 and 17.5%, respectively) due to the contribution of BL foliage. Diet 75BL00 was 2% higher in CP concentration compared to 50BL00 because of the increased percentage of BL in the diet. Diets 50BL00 and 75BL00 used hay to dilute the locust foliage CP concentration. In 2000, all diets contained sufficient CP amounts to satisfy animal requirements (NRC, 1981).

The range of FR-phenol for all diets was 0.9 to 7.1% (Table 4.2). The concentration of CT was 6.4 and 4.9% for diets 75BL00 and 50BL, respectively. The concentration of HT

was 15.6 to 11.7% for diets 75BL00 and 50BL00, respectively. The concentrations of CT and HT for H_O (0.9 and 1.3%, respectively) and for H_OG (0.6 and 0.9%, respectively) were expected to be lower because the ingredients consisted of low tannin fractions present in each species (Cannas, 2001).

Intake and Digestibility:

Understanding the animals' intake of feed is fundamental to nutrition, because it determines the level of nutrients ingested and, therefore, the animal's response and function to its diet. Intake of feed is regulated and restricted by the requirement of the animals' physiology and metabolism, whereas digestibility qualitatively describes the net food intake and its utilization. By definition, apparent digestibility is considered to be the difference between amounts fed and amounts removed in the feces (Van Soest, 1982).

1999:

Goats consumed similar amounts of DM for all diets (Table 4.3) even though H_EG was measurably higher (P=0.05). The average grain consumed for diet H_EG was 136.2 g. The amount of BL consumed for diet 50BL99 (168.8 g) was higher (P=0.004) than for diet 25BL99 (101.0 g) because of the higher percentage offered in the diet.

Apparent digestibilities of DM (62.4, 68.2, 58.0, and 60.6% [P=0.01]) and CP (62.8, 72.4, 56.0, and 59.1% [P=0.001]) differed significantly between diets, being greater for diet H_EG (positive control) and H_E (negative control) than for BL diets. Apparent digestibilities of DM, CP, NDF, HEMI, CELL and ADL were not significantly different for diets 25BL99 and 50BL99 except for ADF (P=0.02). Goats consuming diets H_E and H_EG had much higher NDF, ADF, CELL, LIGN (P<0.02) apparent digestibilities compared with animals consuming diets 25BL99 and 50BL99 with depressed fiber digestion. Acid detergent lignin

digestibility varied widely among diets and was negative only for diets 25BL99 and 50BL99. Closer examination of the data indicated that feeding BL foliage resulted in negative ADL digestibility for every animal with diets 25BL99 and 50BL99. Dry matter intakes were low and only averaged 1.6% BW (Table 4.5). The DM intake requirement of a 20 kg growing meat goat is 2.2% (NRC, 1981). All goats consumed below their recommended DM intake level.

2000:

Goats consumed similar amounts of DM for all diets; however less (P=0.03) was consumed for diet 75 BL00 than for diet 50 BL00 (Table 4.4). The intake of hay for the BL diets was significantly lower (P<0.002) compared to H_O and H_OG. Goats fed diet 75BL00 consumed the least hay (74.9 g) compared to diet 50BL00 (242.4g) because of the increased percentage of BL in the diet. The intake of hay was similar for H_O and H_OG (Table 4.4). The average grain consumed for diet H_OG was 111.4 g, which was 25 g lower than in 1999. The higher quality hay offered in 2000 compared to 1999 could have caused the difference. We observed that the goats did not find the grain as palatable and molasses (1 g) was mixed into the grain to help increase palatability. Intakes of DM averaged 2.2% compared with 1.6% for 1999 (Table 4.5). The increased intake in 2000 could have been partly due to the immature OGH fed (Table 4.4). The intake of DM requirement of a 20 kg growing meat goat is 2.2% (NRC, 1981). With the exception of diet 75BL00 (1.7%), intake levels were sufficient for the other diets.

Black locust diets appeared to depress DM, fiber and CP digestibilities (Table 4.4). Apparent DM digestibilities were not significantly different for diets 50BL00 and 75BL00. There were no significant differences between diets and no significant treatment contrasts for

HEMI, CELL, and ADL values. In 2000, no negative ADL values (such as those reported in 1999) were observed. The lowest ADL digestibility was for Diet 50BL00 (9.4%) compared to diets H_O, H_OG and 75BL00 (averaging 27%).

Nitrogen Balance:

Nitrogen balance builds an understanding of what happens to the nitrogenous components of the diet once ingested. For example, if animals are given excessive protein in high-protein diets, then excess protein can convert to ammonia in the rumen and be absorbed through rumen wall into the blood and excreted as urea in the urine. In contrast, if low-protein diets are fed, than microbial synthesis uses endogenous recycled urea, and therefore very little urea appears in the urinary N fraction (Van Soest, 1982). Fecal N loss is largely indigestible microbial matter, which is related to the intake of DM. Fecal N in ruminants should average about 0.6% of DM intake (Van Soest, 1982). In addition, increased fecal N can also be related to decreased apparent digestibilities of NDF and ADF, because of the loss of fermentable matter from the rumen to the lower tract (Orskov et al. 1972).

1999:

Nitrogen balance is reported in Table 4.6. Nitrogen intake and urinary N output were similar for diets H_EG, 25BL99, and 50BL99. Diet H_EG (positive control) was higher ($P=0.01$) than diet H_E (negative control) in both N intake and urinary N (Table 4.6). Diets 25BL99 and 50BL99 were similar in both N intake and urinary N. Fecal N for diets 25BL99 and 50BL99 were similar only with Diet H_EG.

Retained N as a percentage of N intake was higher for diet H_EG than other diets although not significantly different due to large animal-to-animal variation. Fecal N excretion as a percentage of N intake was significantly higher ($P < 0.02$) in BL diets. Urinary

N as a percentage of N intake did not differ among diets. Total N recovered in feces and urine was similar between diets and ranged from 72 to 92.4% of N intake.

2000:

Nitrogen intake and fecal N output for BL diets were about 6% higher compared to diet H_O (P=0.01) and H_OG (P=0.02). There were no significant differences in urinary N output among diets. The amount of digested N was significantly lower for diets H_O and H_OG compared to the BL diets. Nitrogen retained as a percentage of N intake was similar among diets. Fecal N as a percentage of N intake was significantly lower (P=0.01) for diet H_OG (24.0%) than for diets 50BL00 and 75BL00 (33.4 and 33.5%, respectively). Urinary N as a percentage of N intake was significantly higher for diets H_O and H_OG compared to the BL diets (P=0.02). Between diets, the total N recovered in feces and urine were similar and ranged from 64 to 82.7 % of N intake.

Mean Body Weight and Daily Weight Gains for 1999 and 2000:

Mean BW and average daily gains for 1999 and 2000 are presented in Table 4.7 for 1999 and 2000. There were no significant differences between BW and daily weight gain for 1999. In 2000, animals fed diet H_O (18 kg) averaged slightly lower BW than those fed diets H_OG, 50BL00, and 75BL00 (averaging 20.5 kg). Daily weight gain in 2000 was slightly higher for diets 50BL00 and 75BL00 (averaging 118 g d^{-1}) compared to Diet H_O (50 g d^{-1}).

Urinary and Fecal Output for 1999 and 2000:

Urinary and fecal outputs were measured to record volume differences that may occur between diet treatments. The sub-samples of urine and feces were analyzed for N concentration for N balance calculations. Mean values of urinary and fecal output during the digestion period for years 1999 and 2000 are given in Table 4.8. For 1999, the mean urine

and fecal output were 400 mL d^{-1} and 138 g d^{-1}, respectively. In 2000, the mean urine and fecal output were slightly higher than in 1999, where urine and fecal output were 459 mL d^{-1} and 177 g d^{-1}, respectively. There were no differences between diets for 1999 and 2000.

Ruminal and Urinary pH and Ruminal Ammonia (NH_3-N) for 1999 and 2000:

Urinary and ruminal pH were measured to understand how diet composition changes the pH of the urine and how ruminal fermentation processes change the pH of the rumen. A rumen pH below 6.2 may inhibit the rate of digestion (Grant and Mertens, 1992). Ruminal ammonia (NH_3-N) was measured to see if tannins affected the concentration of ammonia in the rumen.

Ruminal and urinary pH and ruminal NH_3-N values are given in Table 4.9. The average ruminal pH was 6.3 and 6.8 for 1999 and 2000, respectively. Although there were no significant treatment contrasts in 1999 for ruminal pH, in 2000, diets H_O and H_OG had higher (P=<0.002) pH values (pH 7) than diets 50BL00 (6.6) and 75BL00 (6.4).

In 1999, ruminal NH_3-N concentration for diet H_EG was significantly higher (22.2 mg dL^{-1}) than for the other diets (P=0.0001). In 2000, there were no significant differences for ruminal NH_3-N concentrations between diets H_O and H_OG and between diets 50BL00 and 75BL00; however diets H_O and H_OG were higher (P=<0.002) in ruminal NH_3-N concentrations than the BL diets (Table 4.9). Regardless of the diets, ruminal NH_3-N concentrations (ranging from 8.2 to 22.3 mg dL^{-1}) were above the critical level 5 mg dL^{-1} indicated by Satter and Slyter (1974) as being adequate to meet the requirements for N of rumen microbial growth and microbial protein synthesis.

The average urine pH was 7.3 and 8.1 for 1999 and 2000, respectively. There were no significant differences between treatment contrasts for either year for urinary pH. Diet 25BL99 had the lowest observed urinary pH (6.5).

Plasma Urea Nitrogen for 1999 and 2000:

Most N utilized in the rumen is in the form of ammonia; however if it is not utilized in the rumen it is absorbed across the rumen wall into the blood stream. Blood ammonia levels normally remain low because the liver converts ammonia back to urea a detoxified form (Tyrrell et al., 1970).

Mean PUN values are given for 1999 and 2000 in Table 4.10. For 1999, there were no significant differences among the three collection dates (08 and 17 June and 01 July) and data were pooled (date*diet interaction) means are reported averaged over dates. In 1999, Diet H_EG had significantly (P=0.02) higher PUN (15.7 mg dL^{-1}) than for diets H_E, 25BL99, and 50BL99 (11.6, 12.0, and 13.8 mg dL^{-1}, respectively).

For 2000, the three collection dates (06 June and 03 and 14 July) were significantly different (P=0.02) and were considered separately. For most diets there was an increase of PUN levels throughout the experimental trial; however, diet 50BL00 showed no change in PUN concentrations from the first collection date (06 June). Diet 50BL00 had the lowest PUN value (13.2 mg dL^{-1}) compared to other diets (averaging 20 mg dL^{-1}).

Volatile Fatty Acid Concentrations:

Animals depend heavily on the efficiency with which insoluble plant cell-wall carbohydrates in their feed are broken down in the rumen to VFA, which are then absorbed as energy. Ruminal microbial activity is important in this situation. If high concentrations of CT are present in the forage it may limit the extent to which the microbes can degrade the

plant cell polymers in the rumen because of the inhibition of the microbial processes (Bae et al., 1993 and McAllister et al., 1994).

1999:

Mean total VFA and VFA molar proportions are presented in Table 4.11 for 1999 and 2000. In 1999, total VFA ranged from 64.1 to 88.9 mmol L^{-1} for diets H_E (negative control) and H_EG (positive control), respectively, and from 70.6 and 65.1 mmol L^{-1} for diets 25BL99 and 50BL99. For all diet treatment contrasts, total VFA concentrations did not seem to be reduced by the presence of tannins. Acetic acid was highest for 25BL99 diet (82.2 mmol L^{-1}). Propionic acid concentrations were similar for all diets, averaging 13.7 mmol L^{-1}. Butyric acid concentration for diet H_EG (7.5 mmol L^{-1}) was significantly higher than for diets 25BL99 and 50BL99 (3.8 and 4.9 mmol L^{-1}, respectively; P=0.02) and for diet H_E (3.3 mmol L^{-1}; P=0.01). Isobutyric and isovaleric values were similar for all diets. There was a significant difference in valeric acid values for diets H_EG compared to BL diets (P=0.02). The acetic:propionic acid ratio was highest in diet 25BL99 (6.5), however there were no significant differences among diets .

2000:

Total VFA for diets H_O and H_OG were higher (P<0.002) compared to diets 50BL00 and 75BL00. The presences of tannins seemed to decrease the total VFA concentrations by almost 50% compared to the H_O and H_OG diets. Acetic acid was similar among diets averaging 72.5 mmol L^{-1}; however propionic acid was lower for diets 50BL00 and 75BL00 (14.6 and 14.3 mmol L^{-1}, respectively) compared to diets H_O and H_OG (17.5 and 17.0 mmol L^{-1}, respectively). Butyric acid was similar for all diets. The acetic:propionic ratios were highest in diets 50BL00 and 75BL00, averaging 5.1 compared to the other diets ranging from

4.1 to 4.3. Overall, total VFA concentrations appeared similar to other goat data ranging from 57 to 84 mmol L^{-1}, according to Luginbuhl (unpublished data, 2003).

DISCUSSION:

The observed levels of CT and HT in the BL foliage in 1999 were 10.4 and 34.2% DM, respectively and in 2000, 10.3 and 18.7% DM, respectively. Feeney and Bostock (1969) and Becker and Martin (1982) noted that leaf age of *Quercus* and *Shorea* spp. was an important factor influencing phenolic concentrations. They reported that the protein-precipitating capacity of immature leaf extract was higher than that of mature leaves, and that an increase in CT concentrations occurred from July to September. Waterman and McKey (1989) reported a similar finding whereby young leaves had higher concentrations of phenolics than mature leaves. In 1999, our data for HT followed a similar pattern as levels of HT were almost double than those observed in 2000 (Table 4.1). This could be attributed to the age of the leaves, because in 1999 we collected them one month earlier in the season than in 2000. Nevertheless, this was not the case with CT and FR-phenol. The HT could have been more highly concentrated in the plant vacuoles of the immature foliage than FR-phenol or CT, and thus did not change concentrations as drastically as CT and FR-phenol as the plant matured. This could explain why FR-phenol and CT were similar in 1999 and 2000, despite the maturity differences in the foliage. Another explanation could be that a flush of new growth occurred prior to collection in the 2000 season obscuring the normal maturation process of leaves produced early in the season. New leaves from the growth flush were mixed with the older leaves and probably masked the normal maturation process resulting in little or no difference in concentrations of FR-phenol and CT over the 4-week trial period. In

fact, Horner (1988) reported that non-linear trends might exist when dilution effects caused by leaf expansion occur.

The only published data of CT for BL are those reported by Kumar and Horigome (1986). Their work cannot be compared with our data for CT because they purified CT in five fractions and concluded that BL protein-precipitating capacity increased with molecular weight, without providing actual concentrations of CT in the plant tissue. Concentrations of FR-phenol observed in our study were similar to values reported by Makkar and Becker (1998), who reported a FR-phenol concentration at 6.6% for BL foliage. The concentrations of HT reported in this article contribute to the existing body of literature because no other documentation exists for HT in BL.

As mentioned previously, plant phenolics (tannins) can be beneficial or detrimental to the ruminant, depending on the concentration. In ruminants, dietary tannins (2 to 4% DM of CT) have been shown to impart beneficial effects because they reduce wasteful protein degradation in the rumen by the formation of a protein-tannin complex formed by hydrogen bonding at a near neutral pH (pH 6.0 to 7.0). The complex appears to dissociate post-ruminally at a low pH (less than 3.5 in the abomasum) where, presumably, the protein becomes available for digestion (Barry et al., 2001; Butter et al., 2000; Terrill et al., 1992). Thus, CT-containing plants can protect dietary protein against degradation in the rumen and increase the absorption of essential amino acids in the abomasum and small intestine by up to 62%, resulting in an improved nutritional status of the animal (Waghorn et al., 1987). Other reported benefits of low concentrations of CT (2 to 4%) in the diet are their efficiency in controlling parasitic nematodes and in the prevention of bloat (Min and Hart, 2003).

On the other hand, a diet containing 5 to 10% DM of CT can depress voluntary feed intake and digestibility (Barry et al., 2001; Butter et al., 2000; Kumar and Vaithiyanathan, 1990; McLeod, 1974; Terrill et al., 1992). In our study, CT concentrations of BL diets from 3.7 to 6.4 % DM reduced DM intake and digestibilities of fiber fractions (NDF and ADF). The intake of BL only as a percentage of BW for diets 25BL99 and 50BL99 were 0.48 and 0.86%, respectively, and for 50BL00 and 75BL00 were 1.44 and 1.38% (data not shown). In 1999, the lower intake percentage could be attributed to forage species and to the blend of the more mature hay fed; however, these differences could also have been due to elevated HT levels in EHG compared with data from OGH in 2000.

Differences in apparent DM digestibilities were similar to those observed in other studies comparing tannin-rich with tannin-free forages (Barry et al., 2001; Butter et al., 2000; Kumar and Vaithiyanathan, 1990; McLeod, 1974; Terrill et al., 1992). According to McLeod (1974), tannins may bind to macromolecules such as CELL and HEMI. McLeod (1974) reported that decease in apparent digestibilities could be due to the substrate being protected by tannins from hydrolysis and by direct inhibition of digestive enzymes by tannins.

Although relatively high concentrations of HT were observed in our study, HT are not believed to be as important as CT because HT, once ingested by the animal, readily hydrolyze under mild acid or alkaline conditions into sugars and phenolic carboxylic acids (Van Soest, 1982). Nevertheless, McLeod (1974) reported that HT could be degraded to sugars and phenols in the digestive tract with possible toxic effects. McSweeney et al. (1988) reported that two out of four sheep that ingested 0.9 g HT kg^{-1} BW of yellow-wood foliage (*Terminalia oblongata*) showed signs of toxicity in 15 d. The highest concentration

of HT observed in our experiment in 1999 for diet 50BL99 (0.37 g HT kg^{-1} BW) did not produce signs of toxicity, only reduced intakes.

Folin-reactive phenolics above 9 % DM in a diet can be lethal to an animal that has no other feed (Kumar, 1983); however, we reported FR-phenolics of BL diets up to 7.5 % and did not observe any mortalities or sickness in our studies.

There was an increase in fecal N associated with higher levels of dietary tannins. In 1999 and 2000, elevated levels of fecal N could be directly attributable to the ability of tannins to form dietary protein complexes resistant to microbial and enzymatic digestion or to undegradable fiber-bound proteins in BL (Makkar et al., 1987; Jackson et al., 1996; Reed, 2001; Sandusky et al., 1977). The increased fecal N excretion found in diets 25BL99 and 50BL99 was most likely attributable to the higher CP levels in BL diets, indicating that incorporation of BL in the diets lead to the formation of indigestible tannin-N complexes.

In 1999, a positive correlation was observed between CP and ADL digestibilities for diets 25BL99 and 50BL99 ($r = 0.78$; $P = 0.02$) (data not shown), indicating that some lignin and tannins complexed with the CP fraction. Fahey et al. (1980) postulated that ADL and other phenolic complexes such as tannins are degraded as they pass through the gastrointestinal tract and that the resulting phenolic acids form undigestible complexes with N fragments that are excreted in the feces (Mehansho et al. 1987).

High urinary N as a percentage of N intake indicates high protein or inorganic N intake and rapid ruminal digestion, resulting in ammonia production in excess of microbial needs (Merkel et al., 1999; Van Soest, 1982.). In 1999, all diets contained the same amount of urinary N as a percentage of N intake, which was estimated at 45%. In 2000, the highest level of urinary N as a percentage of N intake was found in the negative (H_O) and positive

(H_OG) control diets (52.8 and 53.1%, respectively), and the lowest concentrations were found in diets 50BL00 and 75BL00 (38.5 and 30.5%, respectively). These differences could be attributed to the ingestion of tannin containing-forage (Silvanikove et al., 1996 and Kumar and Singh, 1984).

Overall, ruminal pH observed in our studies was similar to that reported with goats fed grass diets (Islam et al., 2000). The ruminal pH values for both diets were in optimal ranges for fiber digestion, above 5.8 (Orskov and Ryle, 1990). However, in 2000, ruminal pH was lowest in BL diets. There was no report in the literature to suggest that tannins affect the pH of the rumen; however CT have been shown to reduce ruminal ammonia levels (Terrill et al., 1992). In 1999 and 2000, ruminal ammonia concentration was significantly lower for animals fed BL diets compared with positive control (Table 4.9). Horton and Christensen (1981) reported depressed levels of ruminal ammonia, averaging 5.2 mg dL^{-1}, when lambs were fed a diet of 100% BL meal compared with 29 mg dL^{-1} for a 100% alfalfa meal diet. For both years ruminal ammonia levels appeared to be in an appropriate range (8.2 to 22.3 mg dL^{-1}) for goats (Luginbuhl, unpublished data, 2003). Horton and Christensen (1981) reported PUN levels of 11.7 mg dL-1 for lambs fed a 100 % BL diet, compared to an alfalfa diet, which averaged 28.9 mg dL^{-1}. According to Luginbuhl (personal communication, 2003) ranges for PUN from 16.4 to 26.9 mg dL^{-1} are common for goats.

Tannins have been found to reduce total VFA *in vivo* (Terrill et al., 1992) as seen in 1999 and 2000. Changes in VFA concentrations of the rumen as a result of the adverse effects of tannins on microbial activity may, however, decrease ruminal pH (as observed in 2000). Ruminal pH values between 5 and 7 favor the interaction of tannins with nutrients and microbes (Marin and Martin, 1983). The ruminal pH levels in our study ranged from 6.1

to 7.0, thus this may have accounted for an anti-microbial effect of tannins on ruminal ammonia and VFA production.

CONCLUSIONS AND RECOMMENDATIONS:

We have reported that BL could possibly depress digestibilities of DM, cell-wall constituents, ruminal pH, and total VFA concentrations; however, the production of PRP may increase the efficiency of BL. Proline rich proteins have a high affinity for tannins and tend to complex tannins before feed enters the rumen (Kumar and Vaithiyanathan, 1990; Silanikove et al., 1996; Robbins et al., 1987). Paradoxically, the production of PRP can be associated with increased fecal N, as observed in this study (Van Soest, 1982).

Additional research is warranted to examine meat goat performance under grazing situations where animals would have free access to a greater variety of herbaceous plants to dilute and possibly counter-balance the negative effects of tannin compounds found in BL, while taking advantage of the low fiber and high CP concentration found in this browse species.

Other recent research has shown that BL browse can be an effective supplement for grazing animals consuming low-quality forage during the summer (Papachristou and Papanastasis, 1994; Papachristou et al., 1999). Black locust browse paddocks could be used to maintain the body condition of goats over the summer as a substitute for expensive commercial supplementary feeds. Additionally, BL has high landscape value in N-poor ecosystems, because of its ability to annually fix 75 to 150 kg atmospheric N ha^{-1} (Boring et al., 1981). The fact that BL can be established easily on a wide range of soils makes it a valuable browse species for incorporation within many suitable pasture systems. It is drought tolerant, exhibits rapid growth, and produces ample herbage in the form of edible

leaves and shoots. It is a desirable candidate for silvopastoral system due to high CP levels, ranging from 20 to 24% (Addlestone et al., 1999) and its high HM production (leaves and non-woody material), ranging from 2,400 to 2,280 kg DM ha^{-1} relative to other browse species tested (Addlestone et al., 1999; Papanastasis et al., 1997).

Data reported in our study will hopefully stimulate further research on the impact of phenolic compounds such as CT and HT on goat intake and digestion of BL herbage.

Acknowledgements:

The author would like to thank Amy Conrad and those who endured the endless summer days of hand defoliating black locust. A special thanks to Dr. Cavell Brownie, Department of Statistics, North Carolina State University, for statistical consultations.

References:

1. Addlestone, B.J., J.P. Mueller, and J-M. Luginbuhl. 1999. The establishment and early growth of three leguminous tree species for use in silvo-pastoral systems of the southeastern USA. Agroforestry Syst. 44:253-265.

2. Ainalis, A.B., and C.N. Tsiouvaras. 1998. Forage production of woody fodder species and herbaceous vegetation in a silvopastoral system in Northern Greece. Agroforestry Syst. 42:1-11.

3. AOAC. 1999. International Official Methods of Analysis. (16th Ed.) Assoc. Offic. Anal. Chem., Arlington, VA.

4. Appel, H.M., H.L. Govenor, M. D'ascenzo, E. Siska, and J. C. Shultz. 2001. Limitations of folin assays of foliar phenolics in ecological studies. J. Chem. Ecol. 27 (4):761-778.

5. Ayers, A.C., R.P. Barrett, and P.R. Cheeke. 1996. Feeding value of tree leaves (hybrid popular and black locust) evaluated with sheep, goats, and rabbits. Anim. Feed Sci. Technol. 57:51-62.

6. Bae, H. D., T. K. McAllister, L. J. Yanke, K.-J. Cheng, and A. Muir. 1993. Effect of condensed tannins on endoglucanase activity and filter paper digestion by *Fibrobacter succinogenes* S85. Appl. Environ. Microbiol. 59:2132-2138.

7. Baertsche, S.R., M.T. Yokoyama, and J.W. Hanover. 1986. Short rotation, hardwood tree biomass as potential ruminant feed-chemical composition, nylon bag ruminal degradation and ensilement of selected species. J. Anim. Sci. 63:2028-2043.

8. Barry, T.N, D.M. McNeil, and W.C. McNabb. 2001. Plant secondary compounds; their impact on nutritive value and upon animal production. p.445-452. *In* Proc. XIX Int. Grass. Cong. Sau Paulo, Brazil.

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

10. Becker, P., and J.S. Martin. 1982. Protein precipitating capacity of tannins in *Shorea* seeding leaves. J. Chem. Ecol. 8:1353-1367.

11. Bernays, E.A., G. Cooper-Driver, and M. Bilgener. 1989. Herbivores and plant tannins. Adv. Ecol. Res. 19:263-291.

12. Boring, L.R., C.D. Monk, and W.T. Swank. 1981. Early regeneration of a clear-cut southern Appalachian forest. J. Ecol. 62:1244-1253.

13. Boring, L.R., and W.T. Swank. 1984. The role of black locust (*Robinia pseudoacacia*) in forest succession. J. Ecol. 72(3):749-766.

14. Butter, N.L., J.M. Dawson, D. Wakelin, and P.J. Buttery. 2000. Effect of dietary tannin and protein concentration on nematode infection (*T. colubriformis*) in lambs. J. Agric. Sci. 134:89-99.

15. Cannas, A. 2001. Tannins: Fascinating but Sometimes Dangerous Molecules. [Online] Available at http://www.ansci.cornell.edu/toxicagents/tannin/tannin.htm (posted16 September 2001)

16. Fahey, Jr., G.C., S.Y., Al-Haydari, F.C. Hinds, and D.E. Short. 1980. Phenolic compounds in roughages and their fate in the digestive system of sheep. J. Anim. Sci. 50:1165-1172.

17. Feeney, P.P, and H. Bostock. 1969. Seasonal changes in the tannin content of oak leaves. Phytochem. 7:871-880.

18. Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures and some applications). Agric. Handb. 379. U.S. Gov. Print Office, Washington, DC.

19. Grant, R.J., and D.R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.

20. Hagerman, A.E., and L.G. Butler. 1989. Choosing appropriate methods and standards for assaying tannins. J. Chem. Ecol. 15:1795-1810.

21. Hagerman, A.E., C.T. Robbins, Y. Weerasuriya, T.C. Wilson, and C. McCarthur. 1992. Tannin chemistry in relation to digestion. J. Range Manage. 45: 57-62.

22. Horigome, T., R. Kumar, and K. Okamoto. 1988. Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes in vitro and in the intestine of rats. Br. J. Nutr. 60:275-285.

23. Horner, J.D. 1988. Astringency in douglas fir foliage in relation to phenology and xylem pressure potential. J. Chem. Ecol. 14:1227-1237.

24. Horton, G.M.J., and D.A. Christensen. 1981. Nutritional value of black locust tree leaf meal (*Robinia psudeoacacia*) and alfalfa meal. Can. J. Anim. Sci. 61(2):503-506.

25. Islam, M., H. Abe, Y. Hayashi, and F. Terada. 2000. Effects of feeding Italian ryegrass with corn on rumen environment, nutrient digestibility, methane emission, and energy and nitrogen utilization at two intake levels of goats. Small Rum. Res. 38: 165-174.

26. Jackson, F.S., T.N. Barry, C. Lascano, and B. Palmer. 1996. The extractable and bound condensed tannin content of leaves from tropical tree, shrub and forage legumes. J. Sci. Food Agric. 71:103-110.

27. Keresztesi, B. 1988. Black locust: the tree of agriculture. Outlook Agric. 17:77-85.

28. Keresztesi, B. 1983. Breeding and cultivation of black locust, *Robinia psuedoacacia*, in Hungary. For. Ecol. Manage. 6:217-244.

29. Keresztesi, B. 1980. The black locust. Unasylva 32:23-33.

30. Komarek, A.R., J.B. Robertson, and J.B. Van Soest. 1994. Comparison of the filter bag technique to conventional filtration in the Van Soest Analysis of 21 feeds. *In*: Proc. Natl. Conf. on Forage Quality, Evaluation and Utilization, Lincoln, NE.

31. Kumar, R. 1983. Chemical and biochemical nature of fodder tree leaf tannin. J. Agric. Food Chem. 31:1361-1364.

32. Kumar, R., and M. Singh. 1984. Tannins: their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447-453.

33. Kumar, R., and T. Horigome. 1986. Fractionation, characterization and protein precipitating capacity of the condensed tannins from *Robinia psuedoacacia* leaves. J. Agric. Food Chem. 34:487-489.

34. Kumar, R.A., and S. Vaithiyanathan. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Anim. Feed Sci. Technol. 30:21-38.

35. Lambert, M.G., G.A. Jung, R.H. Fletcher, P.J. Budding, and D.A. Costall. 1989. Forage Shrubs in North Island New Zealand Hill Country 2, Sheep and Goat Preferences. N.Z. J. Agric. Res. 32:485-490.

36. Makkar, H.P.S., and K. Becker. 1998. Do tannins in leaves of trees and shrubs from African and Himalayan regions differ in level and activity? Agroforestry Syst. 40: 59-68.

37. Makkar, H.P.S., B. Singh, and R.K. Dawra. 1987. Tannin nutrient interactions-a review. International J. Anim. Sci. 2: 127-140.

38. Marsh, W. H., B. Fingerhut, and H. Miller. 1965. Automated and manual direct method for the determination of blood urea. Clin. Chem. 11:624-627.

39. Martin, M.M., and J.L. Martin. 1982. Tannin assays in ecological studies: Lack of correlation between phenolics, proanthocyanidins and protein-precipitating constituents in mature foliage of six oak species. Oecologia 54: 205-211.

40. McAllister, T.A., Bae, H.D., Jones, G.A. & Cheng, K.J. 1994. Microbial attachment and feed digestion in the rumen. J. Anim. Sci. 72(11), 3004-18.

41. McLeod, M.N. 1974. Plant tannins: their role in forage quality. Nutr. Abstr. Rev. 44(11):803-815.

42. McSweeney, C.S., P.M. Kennedy, and A. John. 1988. Effect of ingestion of hydrolyzable tannins in *Terminalia oblongata* on digestion in sheep fed *Stylosanthes hamata.* Aust. J. Agric. Res. 39: 235-244.

43. Mehansho, H., L.G. Butler, and D.M. Carlson. 1987. Dietary tannins and salivary proline rich proteins: interactions, induction and defense mechanisms. Annu. Rev. Nutr., 7: 423-440.

44. Merkel, R.C., K.R. Pond, J.C. Burns, and D.S. Fisher. 1999. Intake, digestibility and nitrogen utilization of three tropical tree legumes. I. As sole feeds compared to *Asystasia intrusa* and *Brachiaria brizantha*. An. Feed Sci. Tech. 82. 91-106.

45. Min, B.R., and S.P. Hart. 2003 Tannins for suppression of internal parasites. J. Anim. Sci. 81 (E. Suppl. 2):E102-E109.

46. National Research Council (NRC). 1981. Nutrient requirements for goats. National Academy Press, Washington, DC.

47. North Carolina State University. 2003. State Climate Office of NC. [Online]. Available at http://www.nc-climate.ncsu.edu (posted 20 March 2003).

48. Orskov, E.R., and M. Ryle. 1990. Energy Nutrition in Ruminants Elsevier Applied Science, Oxford.

49. Papachristou, T. G. 1999. Assessing the value of black locust (*Robinia pseudoacacia* L.) browse for animal feeding. p.99-103. *In* V. P. Papanastasis, J. Frame and A.S. Nastis (ed.) Grassland and Woody Plants in Europe. Volume 4. Proc. Int. Occasional Symposium of the European Grassland Fed. Thessaloniki, Greece, 27-29 May, 1999.

50. Papachristou, T.G., P.D. Platis, V.P. Papanastasis, and C.N. Tsiouvaris. 1999. Use of deciduous woody species as a diet supplement for goat grazing Mediterranean shrublands during the dry season. Anim. Feed Sci. Technol. 80:267-279.

51. Papachristou, T.G., and V.P. Papanastasis. 1994. Forage value of Mediterranean deciduous woody fodder species and its implication to management of silvo-pastoral systems for goats. Agroforestry Syst. 27:269-282.

52. Papanastasis, V.P., P.D. Platis, and O. Dini-Papanastasi. 1997. Productivity of deciduous woody and fodder species in relation to air-temperature and precipitation in a Mediterranean environment. Agroforestry Syst. 37:187-198.

53. Pinkerton, F., N. Escobar, L. Harwell, and W. Drinkwater. 1994. A survey of prevalent production and marketing practices in meat goats of southern origin. SRDC. Publication No. 182. Southern Rural Dev. Center Mississippi State, MS.

54. Provenza, F.D., and J.C. Malechek. 1984. Diet selection by domestic goats in relation to blackbrush twig chemistry. J App. Eco. 21:831-841.

55. Reed, J.D. 2001. Effects of proanthocyanidins on the digestive and analysis of fiber in forages. J. Range Manage. 54:466-473.

56. Robbins, C.T., S. Mole, A.E. Hargerman, and T.A. Hanley. 1987. Role of tannins in defending plants against ruminants: reduction in dry matter digestion. Ecology 68:1606-1615.

57. Sandusky, G.E., J.M. Fosnaugh, J.B. Smith, and R. Mohan. 1977. Oak poisoning of cattle in Ohio. J. Am. Vet. Med. Assoc. 171:627-629.

58. Satter, L.D., and L.L. Slyter. 1974. Effect of rumen ammonia concentration on rumen microbial production in vitro. Br. J. Nutr. 34:199-208.

59. Shultz, J.C., and I.T. Baldwin. 1982. Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217-148:151.

60. Silanikove, N., N. Gilboa, A. Perevolotsky, and Z. Nitsan. 1996. Goats fed tannin-containing leaves do not exhibit toxic syndromes. Small Ruminant Res. 21:195-201.

61. Steel, R.G.D., J.H. Torrie, and D.A Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach (3rd Edition). McGraw-Hill Series in Probability and Statistics, WCB/McGraw-Hill, Co., New York.

62. SAS. 2002. The SAS System for Windows. Release 8.03. SAS Institute, Cary NC.

63. Soil Survey Division, Natural Resources Conversation Service, United States Dept. of Agric. Official Soil Series Descriptions [Online WWW]. Available URL: "http://ortho.ftw.nrcs.usda.gov/osd/" [Accessed 23 Mar 2001].

64. Terrill, T.H., G.B. Douglas, A.G. Foote, R.W. Purchas, G.F. Wilson, and T. N. Barry. 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. J. Ag. Sci. 119:265-273.

65. Tyrrell, H.F., P.W. Moe, and W.P. Flatt. 1970. Influence of excess protein intake on energy metabolism of the dairy cow. Proc. 5th Symp. on Energy Metabolism, Vitznau, Switzerland.

66. Unruh, L. J. J-M. Luginbuhl and J. P. Mueller. 2001. Intake and digestibility of black locust foliage fed to growing goat wethers. p.413-414. *In* J. A. Gomide and W. R. S. Mattos (ed.) Grassland Ecosystems: and Outlook into the 21st Century. Proc. XIX Int. Grass. Cong. Sao Pablo, Brazil.

67. Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Durham and Downey, Inc. Portland, OR.

68. Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

69. Waghorn, G.C., M.J. Ulyatt, A. John, and M.T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Br. J. Nutr. 57: 115-126.

70. Waterman, P.G., and D.B. McKey. 1989. Herbivory and secondary compounds in rain forest plants. p. 513-536. *In* Leith, H. and M.J.A. Werger (eds.) Tropical Rainforest Ecosystems. Elsevier; Amsterdam.

Table 4. 1: Composition of feed ingredients for goats fed different diets in 1999 and 2000, Raleigh, NC, USA.

	CP[a]	NDF[b]	ADF[c]	HEMI[d]	CELL[e]	ADL[f]	IVTDMD[g]	FR-P[h]	CT[i]	HT[j]
1999	----------% DM----------									
Tripsacum dactyloides	13.9	73.7	36.7	37.0	32.2	4.3	68.9	0.90	0.80	2.1
Grain Mix[†]	26.5	10.9	3.5	7.4	2.9	0.8	97.6	0.33	0.15	1.1
Zea mays Meal[‡]	9.8	9.9	2.7	7.2	1.9	0.2	96.4	ND[#]	ND	ND
Glycine max Meal[§]	54.1	9.8	4.7	5.1	3.5	0.4	97.9	ND	ND	ND
Robinia pseudoacacia	26.3	32.9	21.3	11.6	8.9	12.9	55.2	12.9	10.4	34.2
2000										
Dactylis glomerata	15.5	65.3	36.2	29.1	28.3	4.6	71.4	1.1	0.9	1.3
Grain Mix[†]	19.4	8.5	2.7	5.8	1.9	0.4	97.1	0.43	0.10	0.34
Zea mays Meal[‡]	11.0	9.9	2.7	7.2	1.9	0.2	96.4	ND	ND	ND
Glycine max Meal[§]	52.0	9.8	4.7	5.1	3.5	0.4	97.9	ND	ND	ND
Robinia pseudoacacia	24.5	39.5	32.4	7.1	9.8	20.7	56.2	12.6	10.3	18.7

[a] Crude protein.
[b] Neutral-detergent fiber.
[c] Acid-detergent fiber.
[d] Hemicellulose
[e] Cellulose.
[f] Acid-detergent lignin (72% H_2SO_4).
[g] *In vitro* true dry matter disappearance.
[h] Folin-reactive phenolics.
[i] Condensed tannins.
[j] Hydrolyzable tannins.
[†] Grain mix was 59% ground corn[‡] (*Zea mays*), 36% soybean [§] (*Glycine max*) meal, and (5%) mineral mix.
[#] Not determined.

Table 4.2: Composition of diet consumed, 1999 and 2000, Raleigh, NC, USA.*

	Diets							
	1999				2000			
	H_E[a]	$H_E G$[b]	25BL99[c]	50BL99[d]	H_O[e]	$H_O G$[f]	50BL00[g]	75BL00[h]
	% DM							
Crude protein	13.6	17.7	18.2	20.3	15.5	17.5	22.6	24.7
Neutral detergent fiber	73.6	53.4	59.4	51.9	65.3	45.8	51.3	44.9
Acid detergent fiber	36.3	25.8	30.7	28.2	35.8	24.4	34.6	33.3
Hemicellulose	37.3	27.6	28.7	23.7	29.5	21.4	16.7	11.6
Cellulose	32.1	22.8	24.2	19.7	28.1	18.9	17.8	13.5
Lignin[†]	4.0	3.1	6.8	8.7	4.5	3.1	14.2	17.7
Folin-Reactive Phenolics	0.9	0.7	4.9	7.5	1.1	0.9	6.1	7.1
Condensed Tannins	0.8	0.6	3.7	6.0	0.9	0.6	4.9	6.4
Hydrolyzable Tannins	2.1	1.8	14.7	23.5	1.3	0.9	11.7	15.6

*Corrected for feed refusals.

[a]100% Eastern gamagrass hay (EGH; *Tripsacum dactyloides*)

[b]70% EGH and 30% mixture of ground corn (59%) (GC; *Zea mays*), soybean (36%) (SBM; *Glycine max*) meal, and mineral mix (5%).

[c]75% EGH and 25% black locust (BL; *Robinia pseudoacacia*).

[d]50% EGH and 50% BL.

[e]100% Orchardgrass (OGH; *Dactylis glomerata)* hay.

[f]70% OGH and 30 % GC (59%), SBM (36%), and mineral mix (5%).

[g]50% OGH and 50% BL.

[h]25% OGH and 75% BL.

[†] Acid-detergent lignin (72 % H_2SO_4).

Table 4.3: Intake and digestibility of diets fed to goats in 1999, Raleigh, NC, USA.

	----------Diets----------					----------Treatment Contrasts----------			
Item	H_E [†]	H_EG [‡]	25BL99[§]	50BL99[¶]	Diet Effect	H_E vs. H_EG	H_E vs. 25BL99 & 50 BL99	H_EG vs. 25BL99 & 50 BL99	25BL99 vs. 50BL99
					----------P-value----------				
Intake (g/ DM d)									
Hay	283.9	270.9	220.0	144.5	ns [a]	ns	0.05	ns	ns
Grain	0.0	136.2	0.0	0.0	na[b]	na	na	na	na
Black Locust	0.0	0.0	101.0	168.8	0.004	na	na	na	0.004
Total	283.9	407.1	321	313.3	ns	ns	ns	ns	ns
Digestibilities (%)									
DM	62.4	68.2	58.0	60.6	<0.01	0.02	0.08	0.001	ns
Crude Protein	62.8	72.5	56.0	59.1	0.001	0.003	0.02	<0.0001	ns
Neutral Detergent Fiber	70.0	66.4	52.0	47.5	<0.0001	ns	<0.0001	<0.0001	0.07
Acid Detergent Fiber	69.6	65.8	39.0	30.7	<0.0001	ns	<0.0001	<0.0001	0.02
Hemicellulose	70.5	67.0	65.0	67.5	0.05	0.07	0.02	ns	ns
Cellulose	76.9	73.4	69.0	68.4	0.01	ns	0.001	0.02	ns
Lignin[#]	23.5	19.8	-57.0	-49.3	<0.0001	ns	<0.0001	<0.0001	ns

[†] 100% Eastern gamagrass (EGH; *Tripsacum dactyloides)* hay.
[‡] 70% EGH and 30 % mixture of ground corn (59%) (GC; *Zea mays*), soybean (36%) (SBM; *Glycine max*) meal, and (5%) mineral mix.
[§] 75% EGH and 25% black locust (BL; *Robinia pseudoacacia*).
[¶] 50% EGH and 50% BL.
[a] Non-significant.
[b] Not applicable
[#] Acid-detergent lignin (72 % H_2SO_4).

Table 4.4: Intake and digestibility of diets fed to goats in 2000, Raleigh NC, USA.

	Diets					Treatment Contrast			
Item	H_O [†]	$H_O G$ [‡]	50BL00[§]	75BL00[¶]	Diet Effect	H_O vs. $H_O G$.	H_O vs. 50BL00 & 75BL00	$H_O G$ vs. 50BL00 & 75BL00	50BL00 vs. 75BL00
					P-value				
Intake (g/ DM d)									
Hay	397.8	338.9	242.4	74.9	0.0002	ns[a]	<0.0001	0.002	0.001
Grain	0.0	111.4	0.0	0.0	na[b]	na	na	na	na
Black Locust	0.0	0.0	307.1	286.8	ns	na	na	na	ns
Total	397.8	450.3	549.5	361.72	ns	ns	ns	ns	0.03
Digestibilities (%)									
DM	64.4	71.7	64.8	65.4	ns	0.05	ns	0.05	ns
Crude Protein	70	76	66.6	66.5	ns	ns	ns	0.01	ns
Neutral Detergent Fiber	68.3	67.8	52.1	46.7	0.003	ns	0.0004	0.001	ns
Acid Detergent Fiber	64.5	64.8	45	46.2	0.04	ns	0.01	0.01	ns
Hemicellulose	73	71.3	66.9	47.9	ns	ns	ns	ns	ns
Cellulose	71.8	72.7	71.8	67.5	ns	ns	ns	ns	ns
Lignin[#]	26.8	26.7	9.4	29.6	ns	ns	ns	ns	ns

[†] 100% Orchardgrass (OGH; *Dactylis glomerata)* hay.
[‡] 70% OGH and 30 % mixture of ground corn (59%) (*Zea mays*) and soybean (*Glycine max*) meal (36%) and mineral mix (5%).
[§] 50% OGH and 50% black locust (BL; *Robinia pseudoacacia*).
[¶] 25% OGH and 75% BL.
[a] Non-significant.
[b] Not applicable
[#] Acid-detergent lignin (72 % H_2SO_4).

Table 4.5: Dry matter intake as a percentage of body weight during the digestion period and diet treatment contrasts, Raleigh NC, USA, 1999-2000.

	Dry Matter	
	1999	**2000**
Diets	----------%----------	
Hay Only[†]	1.5	2.2
Hay + Grain Mix[‡]	1.9	2.2
25% BL[§] and 75% Hay (1999 only)	1.5	—
50% BL and 50% Hay	1.6	2.6
75% BL and 25% Hay (2000 only)	—	1.8
LSD	0.7	0.7
Treatments Contrasts	----------P-value----------	
Hay vs. (Hay + Grain Mix)	ns[b]	ns
Hay vs. BL Diets	ns	ns
Hay + Grain vs. BL Diets	ns	ns
25% BL vs. 50% BL	ns	—
50% BL vs. 75% BL	—	0.03

[†] Hay (H_E; 1999=*Tripsacum dactyloides*; H_O; 2000=*Dactylis glomerata*).
[‡] Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) Meal, and 5% Mineral Mix. ($H_E G$; 1999 and $H_O G$; 2000)
[§] Black locust (*Robinia pseudoacacia*).
[b] Non-significant.

Table 4.6: Nitrogen balance and diet treatment contrasts, Raleigh, NC, USA, 1999-2000.

	N Intake		Fecal N		Urinary N		Digested N		Retained N		N Retained/ N Intake		Fecal N/ N Intake		Urinary N/ N Intake	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Diets	g d^{-1}										%					
Hay[†]	6.5	11.4	2.5	3.4	3.0	6.0	3.8	7.7	1.0	1.9	13.3	17.3	37.1	29.9	49.6	52.8
Hay + Grain Mix[‡]	12.9	13.1	3.6	3.3	5.7	7.1	9.1	9.6	3.6	2.7	28.0	22.9	27.5	24.0	44.5	53.1
25% BL[§] and 75% Hay (1999 only)	9.2	—	4.1	—	4.5	—	4.8	—	0.6	—	7.6	—	44.1	—	47.9	—
50% BL and 50% Hay	10.1	21.8	4.1	7.3	4.3	8.0	5.7	13.9	1.7	6.5	14.7	28.1	40.9	33.4	44.4	38.5
75% BL and 25% Hay (2000 only)	—	18.5	—	6.3	—	5.6	—	11.7	—	6.6	—	36.0	—	33.5	—	30.5
LSD	4.1	6.5	1.5	2.9	2.2	3.5	2.6	4.0	2.2	4.0	22.0	21.0	5.3	7.7	21.0	17.6
Treatment Contrasts	P-value															
Hay vs. (Hay + Grain Mix)	0.01	ns[b]	ns	ns	0.02	ns	0.001	ns	0.04	ns	ns	ns	0.003	ns	ns	ns
Hay vs. BL Diets	ns	0.01	0.02	0.01	ns	ns	ns	0.01	ns	0.01	ns	ns	0.02	ns	ns	0.02
Hay+ Grain vs. BL Diets	ns	0.02	ns	0.01	ns	ns	0.004	0.07	0.03	0.03	0.08	ns	<.0001	0.01	ns	0.02
25% BL vs. 50% BL	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—
50% BL vs. 75% BL	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns

[†] Hay (H_E; 1999=*Tripsacum dactyloides*; H_O; 2000=*Dactylis glomerata*).
[‡] Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) Meal, and 5% Mineral Mix. (H_EG; 1999 and H_OG; 2000)
[§] Black Locust (*Robinia pseudoacacia*).
[b] Non-significant.

Table 4.7: Mean body weight, average daily gains, and diet treatment contrasts in 1999 and 2000, Raleigh NC, USA.

	Mean Body Weight		Daily Weight Gain	
	1999	**2000**	**1999**	**2000**
Diets	**kg**		**g d^{-1}**	
Hay[†]	19.7	17.9	98.0	50.0
Hay + Grain Mix[‡]	21.6	20.6	187.0	70.0
25% BL[§] and 75% Hay (1999 only)	21.0	—	124.0	—
50% BL and 50% Hay	20.0	21.3	152.0	138.0
75% BL and 25% Hay (2000 only)	—	20.6	—	97.0
LSD	2.4	2.1	112.0	2.2
Treatment Contrasts		P-value		
Hay vs. (Hay + Grain Mix)	ns[b]	0.02	ns	ns
Hay vs. BL Diets	ns	0.004	ns	0.07
Hay+ Grain vs. BL Diets	ns	ns	ns	ns
25% BL vs. 50% BL	ns	—	ns	—
50% BL vs. 75% BL	—	ns	—	ns

[†] Hay (H_E; 1999=*Tripsacum dactyloides*; H_O; 2000=*Dactylis glomerata*).
[‡] Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) Meal, and 5% Mineral Mix. ($H_E G$; 1999 and $H_O G$; 2000)
[§] Black Locust(*Robinia pseudoacacia*).
[b] Non-significant.

Table 4.8:Urinary and fecal output means during the digestion period and treatment contrasts for goats fed different diets, Raleigh, NC, USA, 1999-2000.

	Urine		Feces	
	1999	**2000**	**1999**	**2000**
Diets	mL d^{-1}		g d^{-1}	
Hay[†]	425	418	121	173
Hay + Grain Mix[‡]	391	367	156	147
25% BL[§] and 75% Hay (1999 only)	456	—	144	—
50% BL and 50% Hay	328	597	131	229
75% BL and 25% Hay (2000 only)	—	455	—	177
LSD	185	267	66	93

Treatment Contrasts	**P-value**			
Hay vs. (Hay + Grain Mix)	ns[b]	ns	ns	ns
Hay vs. BL Diets	ns	ns	ns	ns
Hay+ Grain vs. BL Diets	ns	ns	ns	ns
25% BL vs. 50% BL	ns	—	ns	—
50% BL vs. 75% BL	—	ns	—	ns

[†] Hay (H_E; 1999=*Tripsacum dactyloides*; H_O; 2000=*Dactylis glomerata*).
[‡] Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) Meal, and 5% Mineral Mix. (H_EG; 1999 and H_OG; 2000)
[§] Black Locust(*Robinia pseudoacacia*).
[b] Non-significant.

Table 4.9: Rumen pH, urine pH, and ruminal ammonia concentrations means and treatment contrasts for goats fed different diets, Raleigh, NC, USA, 1999-2000.

	Rumen		Urine		Ruminal Ammonia	
	1999	2000	1999	2000	1999	2000
Diets	----------pH----------				-------mg dL^{-1}----------	
Hay[†]	6.4	7.0	7.7	8.1	10.1	19.8
Hay + Grain Mix[‡]	6.1	7.0	7.8	8.1	22.3	22.2
25% BL[§] and 75% Hay (1999 only)	6.2	—	6.5	—	8.4	—
50% BL and 50% Hay	6.5	6.6	7.0	8.1	10.3	9.1
75% BL and 25% Hay (2000 only)	—	6.4	—	8.1	—	8.2
LSD	0.5	0.3	2.0	0.1	3.6	6.7
Treatment Contrasts			P- value			
Hay vs. (Hay + Grain Mix)	ns[b]	ns	ns	ns	<0.0001	ns
Hay vs. BL Diets	ns	0.002	ns	ns	ns	0.002
Hay+ Grain vs. BL Diets	ns	0.0001	ns	ns	<0.0001	0.0001
25% BL vs. 50% BL	ns	—	ns	ns	ns	—
50% BL vs. 75% BL	—	ns	—	—	—	ns

[†] Hay (H_E; 1999=*Tripsacum dactyloides*; H_O; 2000=*Dactylis glomerata*).
[‡] Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) Meal, and 5% Mineral Mix. ($H_E G$; 1999 and $H_O G$; 2000)
[§] Black Locust(*Robinia pseudoacacia*).
[b] Non-significant.

Table 4.10: Plasma urea nitrogen (PUN) concentration means and diet treatment contrasts, Raleigh NC, USA, 1999-2000.

	1999 PUN	2000 PUN		
	Combined Dates [†]	6-June	3-July	14-July
	mg dL^{-1}			
Diets				
Hay[‡]	11.6	14.9	18.8	21.8
Hay + Grain Mix[§]	15.7	13.5	18.5	20.1
25% BL[#] and 75% Hay (1999 only)	12.0	—	—	—
50% BL and 50% Hay	13.8	13.8	16.6	13.2
75% BL and 25% Hay (2000 only)	—	15.6	18.7	19.4
LSD	3.3	3.8	2.9	2.9
Treatment Contrasts	P-value			
Hay vs. (Hay + Grain Mix)	0.02	ns	ns	ns
Hay vs. BL Diets	ns[b]	ns	ns	0.001
Hay+ Grain vs. BL Diets	0.05	ns	ns	0.01
25% BL vs. 50% BL	ns	—	—	—
50% BL vs. 75% BL	—	ns	ns	0.001

[†]Dates (08 June, 17 June and 01 July) were not significantly different (p=0.22).
[‡]Hay (H_E; 1999=*Tripsicum dactyloides*; H_O; 2000=*Dactylis glomerata*).
[§]Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) meal and 5% mineral mix). ($H_E G$; 1999 and $H_O G$; 2000)
[#]Black Locust (*Robinia pseudoacacia*).
[b] Non-significant.

Table 4.11: Volatile fatty acid contents and diet treatment contrasts, Raleigh NC, USA, 1999-2000.

	Total VFA		Acetic		Propionic		Butyric		Iso-butyric		Valeric		Iso-valeric		Acetic:Propionic Ratio	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
	---mmol L^{-1}--		----------		----------		----------		mM/100mM		----------		----------		----------	
Diets																
Hay[†]	64.1	86.1	79.8	71.1	14.2	17.5	3.8	7.3	0.9	1.3	0.4	1.1	0.9	1.7	5.7	4.1
Hay + Grain Mix[‡]	88.9	81.9	75.6	71.3	14.3	17.0	7.5	8.0	0.8	1.2	0.6	1.1	1.2	1.5	5.3	4.3
25% BL[§] and 75% Hay (1999 only)	70.6	—	82.2	—	12.8	—	3.8	—	0.6	—	0.1	—	0.6	—	6.5	—
50% BL and 50% Hay	65.1	48.2	78.7	72.7	13.6	14.6	4.9	8.6	1.2	1.5	0.4	0.9	1.3	1.8	5.8	4.9
75% BL and 25% Hay (2000 only)	—	40.3	—	73.3	—	14.3	—	7.7	—	1.9	—	0.5	—	2.5	—	5.2
LSD	31.4	22.4	5.7	4	1.8	2.3	2.7	2.4	1.0	0.5	0.4	0.6	1.0	0.7	1.1	0.8
Treatment Contrasts																
	P-value															
Hay vs. (Hay + Grain Mix)	ns[b]	ns	ns	ns	ns	ns	0.01	ns	ns	ns	ns	ns	ns	ns	ns	ns
Hay vs. BL Diets	ns	0.001	ns	ns	ns	0.01	ns	ns	ns	0.08	ns	ns	ns	ns	ns	0.01
Hay+ Grain vs. BL Diets	ns	0.002	0.05	ns	ns	0.02	0.02	ns	ns	0.03	0.02	ns	ns	0.04	0.08	0.03
25% BL vs. 50% BL	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—
50% BL vs. 75% BL	—	ns	—	ns	—	ns	—	ns	—	ns	—	ns	—	0.08	—	ns

[†] Hay (H_E; 1999=*Tripsacum dactyloides*; H_O; 2000=*Dactylis glomerata*).

[‡] Grain Mix of 59% Ground Corn (*Zea mays*), 36% Soybean (*Glycine max*) Meal, and 5% Mineral Mix. ($H_E G$; 1999 and $H_O G$; 2000)

[§] Black Locust (*Robinia pseudoacacia*).

[b] Non-significant.

DISSERTATION SUMMARY:

Spacing and Coppicing:

Coppicing trees spaced at 0.5 m had little impact on resulting HM. The influence of coppice height on the HM from trees more widely spaced (1.0 m) was quite large. A large positive response (1,365 kg ha^{-1}) to the highest coppice height was observed. These responses seem to suggest that relatively high coppice (0.5 m) and wide spacing (1.0 m) were desirable. Results of this study relate only to those livestock production systems where biomass of fodder trees can be cut and carried to animals in the barn or stall as a supplemental feed. Measuring the SMBD greater than 10 mm appears to be a relatively simple method of predicting BL dry HM. The SMBD seems to be closely related to the foliar biomass (r^2 value =0.80); however, validation of the regression model should be performed as future BL yield information becomes available.

Growing Degree Days:

Data from this research confirms that under a certain set of environmental conditions NDF, ADF, IVTDMD, CELL and ADL can be predicted using GDD. Furthermore during seasons when high RF causes repeated flushes of new growth, prediction of herbage quality from GDD is unlikely. GDD models do not appear appropriate to predict the concentration of various tannin fractions in BL herbage. Tannin data reported in this study (FR-phenol, HT, and CT) adds considerably to the existing body of scientific literature.

Further research is needed to confirm GDD relationships presented here and to obtain a better understanding of seasonal fluctuations of tannin fractions. Using the GDD model in planning grazing and herbage harvest appears to hold promise as a practical management approach.

INTAKE, DIGESTIBILITY, and N-BALANCE:

In 1999, goats consumed similar amounts of DM for all diets. In 2000, goats consumed similar amounts of DM for all diets; however less was consumed for diet 75 BL00 than for diet 50BL00 (P=0.04). In 1999 and 2000, NDF and ADF digestibilities for BL diets were lower than the control diets.In 2000, total VFA and ruminal NH_3-N concentrations were depressed for goats fed BL diets compared to controls.

The N balance, in 1999, showed that diet H_EG was higher (P=0.01) than diet H_E in both N intake and urinary N. Fecal N for diets 25BL99 and 50BL99 was similar to that for diet H_EG. In 1999, fecal N excretion as a percentage of N intake was significantly higher (P <0.02) in BL diets, although urinary N as a percentage of N intake did not differ among diets.

In 2000, N intake and fecal N output for BL diets were about 6% higher compared to diet H_O (P=0.01) and H_OG (P=0.02). There were no significant differences in urinary N output among diets. Nitrogen retained as a percentage of N intake was not significantly different among diets. Fecal N as a percentage of N intake was significantly lower (P=0.01) for diet H_OG (24.0%) than for diets 50BL00 and 75BL00 (33.4 and 33.5%, respectively). Urinary N as a percentage of N intake was significantly higher for diets H_O and H_OG compared to the BL diets (P=0.02).

Increased levels of BL in the diets increased fecal N, which suggested that tannins formed dietary protein complexes, and hindered digestibility of cell wall constituents.

Additional research is warranted to examine meat goat performance under grazing situations where animals would have free access to a greater variety of herbaceous plants to dilute and possibly counter-balance the negative effects of tannin compounds found in BL, while taking advantage of the low fiber and high CP concentration found in this browse species.

APPENDIX:

Table A.1: Analysis of Variance Procedure for black locust to determine significant differences in tree height (m) among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	6.92574762	0.25650917	2.48	0.0221
Error	19	1.96456833	0.10339833		
Corrected Total	46	8.89031596			

R-Square	Coeff Var	Root MSE	Treehtm Mean
0.779022	10.35891	0.321556	3.104149

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	2.22891708	0.44578342	4.31	0.0086
Coppice	1	0.44653841	0.44653841	4.32	0.0515
Spacing	1	0.00173841	0.00173841	0.02	0.8982
Coppice*Spacing	1	0.22081143	0.22081143	2.14	0.1603
Rep*Coppice*Spacing	15	1.58929363	0.10595291	1.02	0.4727
Year	1	1.88074794	1.88074794	18.19	0.0004
Year*Coppice	1	0.01968286	0.01968286	0.19	0.6675
Year*Spacing	1	0.32641143	0.32641143	3.16	0.0916
Year*Coppice*Spacing	1	0.13786698	0.13786698	1.33	0.2625

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	0.44653841	0.44653841	4.21	0.0580
Spacing	1	0.00173841	0.00173841	0.02	0.8998
Coppice*Spacing	1	0.22081143	0.22081143	2.08	0.1694

Table A.2: Analysis of Variance Procedure for black locust to determine significant differences in tree width (m) among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	12.12501730	0.44907471	2.48	0.0220
Error	19	3.43850825	0.18097412		
Corrected Total	46	15.56352555			

R-Square	Coeff Var	Root MSE	Treewidm Mean
0.779066	22.89711	0.425411	1.857922

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	3.70914510	0.74182902	4.10	0.0108
Coppice	1	1.12489479	1.12489479	6.22	0.0221
Spacing	1	0.48286386	0.48286386	2.67	0.1188
Coppice*Spacing	1	0.40978007	0.40978007	2.26	0.1488
Rep*Coppice*Spacing	15	0.53086187	0.03539079	0.20	0.9988
Year	1	5.26534141	5.26534141	29.09	<.0001
Year*Coppice	1	0.03340729	0.03340729	0.18	0.6723
Year*Spacing	1	0.01535993	0.01535993	0.08	0.7740
Year*Coppice*Spacing	1	0.04991627	0.04991627	0.28	0.6055

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	1.12489479	1.12489479	31.78	<.0001
Spacing	1	0.48286386	0.48286386	13.64	0.0022
Coppice*Spacing	1	0.40978007	0.40978007	11.58	0.0039

Table A. 3: Analyses of Variance Procedure for black locust to determine significant differences in number of branches greater than 10 mm diameter among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	240.1021671	8.8926729	2.50	0.0213
Error	19	67.6129630	3.5585770		
Corrected Total	46	307.7151300			

R-Square	Coeff Var	Root MSE	nobran Mean
0.780274	34.81480	1.886419	5.418440

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	8.67231481	1.73446296	0.49	0.7815
Coppice	1	19.64585538	19.64585538	5.52	0.0298
Spacing	1	35.22257496	35.22257496	9.90	0.0053
Coppice*Spacing	1	13.96860670	13.96860670	3.93	0.0622
Rep*Coppice*Spacing	15	84.17175926	5.61145062	1.58	0.1730
Year	1	81.60881834	81.60881834	22.93	0.0001
Year*Coppice	1	2.20141093	2.20141093	0.62	0.4413
Year*Spacing	1	0.74638448	0.74638448	0.21	0.6522
Year*Coppice*Spacing	1	1.10617284	1.10617284	0.31	0.5837

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	19.64585538	19.64585538	3.50	0.0810
Spacing	1	35.22257496	35.22257496	6.28	0.0242
Coppice*Spacing	1	13.96860670	13.96860670	2.49	0.1355

Table A.4: Analysis of Variance Procedure for black locust to determine significant differences in sum of main branch diameters greater than 10 mm among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	41397.05195	1533.22415	2.26	0.0347
Error	19	12877.74970	677.77630		
Corrected Total	46	54274.80165			

R-Square	Coeff Var	Root MSE	SumBran Mean
0.762731	36.07854	26.03414	72.15961

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	3029.259216	605.851843	0.89	0.5048
Coppice	1	3619.446522	3619.446522	5.34	0.0322
Spacing	1	9143.148699	9143.148699	13.49	0.0016
Coppice*Spacing	1	6223.407464	6223.407464	9.18	0.0069
Rep*Coppice*Spacing	15	8583.087138	572.205809	0.84	0.6259
Year	1	8068.592037	8068.592037	11.90	0.0027
Year*Coppice	1	0.242593	0.242593	0.00	0.9851
Year*Spacing	1	502.259339	502.259339	0.74	0.4001
Year*Coppice*Spacing	1	711.327339	711.327339	1.05	0.3185

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	3619.446522	3619.446522	6.33	0.0238
Spacing	1	9143.148699	9143.148699	15.98	0.0012
Coppice*Spacing	1	6223.407464	6223.407464	10.88	0.0049

Table A.5: Analysis of Variance Procedure for black locust to determine significant differences in mean above ground woody biomass (kg/ha) among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	168889943.9	6255183.1	1.48	0.1884
Error	19	80094609.2	4215505.7		
Corrected Total	46	24898453.0			

R-Square	Coeff Var	Root MSE	agwb Mean
0.678314	53.92983	2053.17	3807.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	42157821.41	8431564.28	2.00	0.1248
Coppice	1	7369354.38	7369354.38	1.75	0.2018
Spacing	1	14066988.24	14066988.24	3.34	0.0835
Coppice*Spacing	1	22772773.52	22772773.52	5.4	0.0314
Rep*Coppice*Spacing	15	38169777.27	2544651.82	0.60	0.8374
Year	1	38956013.21	38956013.21	9.24	0.0067
Year*Coppice	1	867808.95	867808.95	0.21	0.6552
Year*Spacing	1	905275.09	905275.09	0.21	0.6483
Year*Coppice*Spacing	1	7679684.65	7679684.65	1.82	0.1930

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	7369354.38	7369354.38	2.90	0.1094
Spacing	1	14066988.24	14066988.24	5.53	0.0328
Coppice*Spacing	1	22772773.52	22772773.52	8.95	0.0091

Table A.6: Analysis of Variance Procedure for black locust to determine significant differences in herbage mass (kg/ha) among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	26702032.88	988964.18	2.02	0.0584
Error	19	9308009.60	489895.24		
Corrected Total	46	36010042.48			

R-Square	Coeff Var	Root MSE	Herbage mass Mean
0.741515	32.62784	699.9252	2145.77

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	4573904.5	91478.900	1.87	0.1478
Coppice	1	4471917.064	4471917.064	9.13	0.0070
Spacing	1	2865214.674	2865214.674	5.85	0.0258
Coppice*Spacing	1	3943874.681	3943874.681	8.05	0.0105
Rep*Coppice*Spacing	15	7353527.679	490235.179	1.00	0.4917
Year	1	1331547.939	1331547.939	2.72	0.1157
Year*Coppice	1	184061.105	184061.105	0.38	0.5472
Year*Spacing	1	530656.889	530656.889	1.08	0.3110
Year*Coppice*Spacing	1	883773.889	883773.889	1.80	0.1951

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	4471917.064	4471917.064	9.12	0.0086
Spacing	1	2865214.674	2865214.674	5.84	0.0288
Coppice*Spacing	1	3943874.681	3943874.681	8.04	0.0125

Table A.7: Analysis of Variance Procedure for black locust to determine significant differences in mean stump biomass (kg) among treatment spacing and coppice heights for year 2000.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	6.15776703	0.26772900	.	.
Error	0	0.00000000	.		
Corrected Total	23	6.15776703			

R-Square	Coeff Var	Root MSE	Stumpwt (kg) Mean
1.000000	.	.	1.554491

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	2.69952638	2.69952638	36.55	<.0001
Spacing	1	2.07075923	2.07075923	28.04	<.0001
Coppice*Spacing	1	0.13963995	0.13963995	1.89	0.1893

Table A.8: Analysis of Variance Procedure for black locust to determine significant differences in mean above ground woody biomass (g/plant) among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	10790734.78	399656.84	1.50	0.1816
Error	19	5060253.91	2666329.15		
Corrected Total	46	15850988.69			

R-Square	Coeff Var	Root MSE	Agwb (g plant) Mean
0.680761	61.16046	516.0709	843.7983

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	2036568.541	407313.708	1.53	0.2278
Coppice	1	920905.338	920905.338	3.46	0.0785
Spacing	1	975175.336	975175.336	3.66	0.0709
Coppice*Spacing	1	1654839.863	1654839.863	6.21	0.0221
Rep*Coppice*Spacing	15	2711843.326	180789.555	0.68	0.7746
Year	1	1862443.224	1862443.224	6.99	0.0160
Year*Coppice	1	327.344	327.344	0.00	0.9724
Year*Spacing	1	49420.403	49420.403	0.19	0.6715
Year*Coppice*Spacing	1	324896.241	324896.241	1.22	0.2832

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	920905.338	920905.338	5.09	0.0394
Spacing	1	975175.336	975175.336	5.39	0.0347
Coppice*Spacing	1	1654839.863	1654839.863	9.15	0.0085

Table A.9: Analysis of Variance Procedure for black locust to determine significant differences in herbage mass (g/plant) among years (1999 and 2000).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	27	2097360.110	77680.004	2.56	0.0187
Error	19	575766.908	30303.521		
Corrected Total	46	2673127.018			

R-Square	Coeff Var	Root MSE	Herbage mass (g/plant) Mean
0.784609	36.34	174.0791	478.9681

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	5	251831.9613	50366.3923	1.66	0.1922
Coppice	1	391029.6195	391029.6195	12.90	0.0019
Spacing	1	417899.5795	417899.5795	13.79	0.0015
Coppice*Spacing	1	365869.7162	365869.7162	12.07	0.0025
Rep*Coppice*Spacing	15	452850.6278	30190.0419	1.00	0.4953
Year	1	45498.6706	45498.6706	1.50	0.2354
Year*Coppice	1	1028.0182	1028.0182	0.03	0.8558
Year*Spacing	1	7338.2085	7338.2085	0.24	0.6283
Year*Coppice*Spacing	1	34367.5881	34367.5881	1.13	0.3002

Tests of Hypotheses Using the Type III MS for Rep*Coppice*Spacing as an Error Term					
Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Coppice	1	391029.6195	391029.6195	12.95	0.0026
Spacing	1	417899.5795	417899.5795	13.84	0.0021
Coppice*Spacing	1	365869.7162	365869.7162	12.12	0.0034

Table A.10: Simple Statistics for Chapter 2 prediction equation variables for 1999 data.

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DM leafwt	35	479.74619	274.02434	16791	92.50000	1226
Sum Bran	35	79.17143	39.84565	2771	22.00000	187.00000
NoBran	35	3.77143	1.78368	132.00000	1.00000	9.00000
Treewidm	35	2.17787	0.48816	76.22540	1.04140	3.30200
Treehtm	35	3.34743	0.42336	117.16000	2.48000	3.98000
Volume	35	4.43751	2.18677	155.31296	1.08459	11.36072
Area	35	7.35266	2.17588	257.34315	3.78460	13.14196
Variables =	1) DM leafwt = DM leaf weight (g/tree); 2) Sum Bran = Sum of main branch diameters greater than 10 mm; 3) NoBran = Number of branches greater than 10 mm diameter; 4) Treewidm = Tree width (m); 5) Treehtm = Tree height (m); 6) Volume (m^3), V=1/3 * p ((1/2 * tree width) ^2)*tree height; 7) Area (m^2), A=total height (H m) x tree canopy width (W m).					

Table A.11: Pearson Correlation Coefficients, N = 35 Prob > |r| under H0: Rho=0 for measured variables for 1999 compared to DM leaf weight (g/tree).

	DM leafwt	Sum Bran	NoBran	Treewidm	Treehtm
DM leafwt	1.00000	0.90460 <.0001	0.77194 <.0001	0.60394 0.0001	0.40117 0.0169
Sum Bran	0.90460 <.0001	1.00000	0.92672 <.0001	0.52615 0.0012	0.19715 0.2563
NoBran	0.77194 <.0001	0.92672 <.0001	1.00000	0.36802 0.0296	-0.04949 0.7777
Treewidm	0.60394 0.0001	0.52615 0.0012	0.36802 0.0296	1.00000	0.31082 0.0692
Treehtm	0.40117 0.0169	0.19715 0.2563	-0.04949 0.7777	0.31082 0.0692	1.00000
Variables =	1) DM leaf wt = DM leaf weight (g/tree); 2) Sum Bran = Sum of main branch diameters greater than 10 mm ; 3) NoBran = Number of branches greater than 10 mm diameter; 4) Treewidm = Tree width (m)				

Table A.12: Pearson Correlation Coefficients, N = 35 Prob > |r| under H0: Rho=0 for calculated variables for 1999 compared to DM leaf weight (g/tree).

	DM leafwt	Volume	Area	Vol23	Volsqrt	Volcu
DM leafwt	1.00000	0.65357 <.0001	0.65127 <.0001	0.64966 <.0001	0.64524 <.0001	0.63910 <.0001
Volume	0.65357 <.0001	1.00000	0.98110 <.0001	0.99375 <.0001	0.98579 <.0001	0.97458 <.0001
Area	0.65127 <.0001	0.98110 <.0001	1.00000	0.98569 <.0001	0.98302 <.0001	0.97692 <.0001
Variables	1) DM leaf wt = DM leaf weight (g/tree); 2) Volume (m^3), V=1/3 * p ((1/2 * tree width) ^2)*tree height; 3) Area (m^2), A=total height (H m) x tree canopy width (W m).					

Table A.13: General linear model using sum of main branch diameters greater than 10 mm as in dependent variable and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2089146.353	2089146.353	148.62	<.0001
Error	33	463891.102	14057.306		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.818298	24.71380	118.5635	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	148.62	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	148.62	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-12.78366658	45.09912708	-0.28	0.7786
Sum Bran	6.22105563	0.51030603	12.19	<.0001

Table A.14: General linear model using number of branches greater than 10 mm diameter as independent variable and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1521316.605	1521316.605	48.66	<.0001
Error	33	1031720.850	31264.268		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.595885	36.85637	176.8170	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	48.66	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	48.66	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	32.4868665	70.74082836	0.46	0.6491
NoBran	118.5914885	17.00074100	6.98	<.0001

Table A.15: General linear model using volume (m^3) of the tree as independent variable and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1090550.616	1090550.616	24.61	<.0001
Error	33	1462486.839	44317.783		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.427158	43.88110	210.5179	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Volume	1	1090550.616	1090550.616	24.61	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Volume	1	1090550.616	1090550.616	24.61	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	116.3172893	81.44759648	1.43	0.1627
Volume	81.8992309	16.50995626	4.96	<.0001

Table A.16: General linear model using area (m^2) of the tree as independent variable and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1082887.003	1082887.003	24.31	<.0001
Error	33	1470150.451	44550.014		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.424156	43.99592	211.0687	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Area	1	1082887.003	1082887.003	24.31	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Area	1	1082887.003	1082887.003	24.31	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-123.3161621	127.4160115	-0.97	0.3402
Area	82.0196016	16.6360419	4.93	<.0001

Table A.17: General linear model using tree width (m) as independent variable and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	931196.541	931196.541	18.95	0.0001
Error	33	1621840.914	49146.694		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.364741	46.20996	221.6905	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treewidm	1	931196.5412	931196.5412	18.95	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treewidm	1	931196.5412	931196.5412	18.95	0.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-258.5770340	173.7083745	-1.49	0.1461
Treewidm	339.0118386	77.8827618	4.35	0.0001

Table A.18: General linear model using tree height (m) as independent variable and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	410883.171	410883.171	6.33	0.0169
Error	33	2142154.284	64913.766		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.160939	53.10762	254.7818	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treehtm	1	410883.1710	410883.1710	6.33	0.0169

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treehtm	1	410883.1710	410883.1710	6.33	0.0169

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-389.4480205	348.1563398	-1.12	0.2714
Treehtm	259.6602725	103.2083263	2.52	0.0169

Table A.19: General linear model using sum of main branch diameters greater than 10 mm and tree height (m) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2221040.924	1110520.462	107.04	<.0001
Error	32	331996.531	10374.892		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.869960	21.23148	101.8572	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	201.37	<.0001
Treehtm	1	131894.571	131894.571	12.71	0.0012

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	1810157.753	1810157.753	174.47	<.0001
Treehtm	1	131894.571	131894.571	12.71	0.0012

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-490.2160407	139.3955939	-3.52	0.0013
Sum Bran	5.9067185	0.4471774	13.21	<.0001
Treehtm	150.0611250	42.0868596	3.57	0.0012

Table A.20: General linear model using sum of main branch diameters greater than 10 mm and area (m^2) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2219044.500	1109522.250	106.30	<.0001
Error	32	333992.955	10437.280		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.869178	21.29522	102.1630	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	200.16	<.0001
Area	1	129898.147	129898.147	12.45	0.0013

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	1136157.496	1136157.496	108.86	<.0001
Area	1	129898.147	129898.147	12.45	0.0013

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-182.9270080	61.93689869	-2.95	0.0058
Sum Bran	5.3141879	0.50934412	10.43	<.0001
Area	32.9052766	9.32733838	3.53	0.0013

Table A.21: General linear model using sum of main branch diameters greater than 10 mm and volume (m^3) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2192726.591	1096363.296	97.37	<.0001
Error	32	360310.863	11259.714		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.858870	22.11832	106.1118	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	185.54	<.0001
Volume	1	103580.239	103580.239	9.20	0.0048

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	1102175.975	1102175.975	97.89	<.0001
Volume	1	103580.239	103580.239	9.20	0.0048

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-75.96786686	45.42168560	-1.67	0.1042
Sum Bran	5.34552382	0.54029194	9.89	<.0001
Volume	29.85936106	9.84477388	3.03	0.0048

Table A.22: General linear model using sum of main branch diameters greater than 10 mm and number of branches greater than 10 mm diameter as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2168813.709	1084406.854	90.31	<.0001
Error	32	384223.746	12006.992		

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.849503	22.84050	109.5764	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	173.99	<.0001
NoBran	1	79667.356	79667.356	6.64	0.0148

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	647497.1041	647497.1041	53.93	<.0001
NoBran	1	79667.3562	79667.3562	6.64	0.0148

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	22.39315007	43.86079297	0.51	0.6132
Sum Bran	9.21727221	1.25516439	7.34	<.0001
NoBran	-72.22503581	28.03913910	-2.58	0.0148

Table A 23: General linear model using sum of main branch diameters greater than 10 mm and tree width (m) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2146968.948	1073484.474	84.60	<.0001
Error	32	406068.507	12689.641		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.840947	23.48081	112.6483	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	2089146.353	2089146.353	164.63	<.0001
Treewidm	1	57822.595	57822.595	4.56	0.0405

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	1215772.407	1215772.407	95.81	<.0001
Treewidm	1	57822.595	57822.595	4.56	0.0405

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-178.4355287	88.64590392	-2.01	0.0526
Sum Bran	5.5806951	0.57014697	9.79	<.0001
Treewidm	99.3403022	46.53731818	2.13	0.0405

Table A.24: General linear model using number of branches greater than 10 mm diameter and area (m^2) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2052760.704	1026380.352	65.65	<.0001
Error	32	500276.751	15633.648		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.804046	26.06265	125.0346	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	97.31	<.0001
Area	1	531444.100	531444.100	33.99	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	969873.7009	969873.7009	62.04	<.0001
Area	1	531444.0996	531444.0996	33.99	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-331.2304029	79.96246815	-4.14	0.0002
NoBran	98.5019057	12.50596509	7.88	<.0001
Area	59.7720572	10.25178712	5.83	<.0001

Table A.25: General linear model using number of branches greater than 10 mm diameter and tree height (m) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2015386.015	1007693.007	59.98	<.0001
Error	32	537651.440	16801.608		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.789407	27.01866	129.6210	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	90.55	<.0001
Treehtm	1	494069.410	494069.410	29.41	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	1604502.844	1604502.844	95.50	<.0001
Treehtm	1	494069.410	494069.410	29.41	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-934.4401378	185.6978569	-5.03	<.0001
NoBran	121.9400576	12.4781892	9.77	<.0001
Treehtm	285.0839368	52.5719616	5.42	<.0001

Table A.26: General linear model using number of branches greater than 10 mm diameter and volume (m^3) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1981532.295	990766.148	55.48	<.0001
Error	32	571505.160	17859.536		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.776147	27.85631	133.6396	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	85.18	<.0001
Volume	1	460215.690	460215.690	25.77	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	890981.6790	890981.6790	49.89	<.0001
Volume	1	460215.6904	460215.6904	25.77	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-132.2425266	62.54368629	-2.11	0.0424
NoBran	96.0316605	13.59612474	7.06	<.0001
Volume	56.2955355	11.08991794	5.08	<.0001

Table A.27: General linear model using number of branches greater than 10 mm diameter and tree width (m) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1823418.933	911709.466	39.99	<.0001
Error	32	729618.522	22800.579		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.714216	31.47469	150.9986	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	66.72	<.0001
Treewidm	1	302102.328	302102.328	13.25	0.0010

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	892222.3914	892222.3914	39.13	<.0001
Treewidm	1	302102.3278	302102.3278	13.25	0.0010

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-340.9046256	119.0465423	-2.86	0.0073
NoBran	97.6747548	15.6141629	6.26	<.0001
Treewidm	207.6697673	57.0517771	3.64	0.0010

Table A.28: General linear model using number of branches greater than 10 mm diameter tree width (m) and tree height (m) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2118718.137	706239.379	50.41	<.0001
Error	31	434319.318	14010.301		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.829881	24.67244	118.3651	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	108.59	<.0001
Treewidm	1	302102.328	302102.328	21.56	<.0001
Treehtm	1	295299.204	295299.204	21.08	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	1058755.178	1058755.178	75.57	<.0001
Treewidm	1	103332.122	103332.122	7.38	0.0107
Treehtm	1	295299.204	295299.204	21.08	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-1000.425923	171.3042843	-5.84	<.0001
NoBran	108.278234	12.4556706	8.69	<.0001
Treewidm	129.879604	47.8241222	2.72	0.0107
Treehtm	235.687713	51.3368872	4.59	<.0001

Table A 29: General linear model using number of branches greater than 10 mm diameter volume (m^3) and tree height (m) and tree height (m) as independent variables and DM leaf weight (g/tree) as the dependent variable for 1999 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2114701.945	704900.648	49.85	<.0001
Error	31	438335.510	14139.855		
Corrected Total	34	2553037.455			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.828308	24.78626	118.9111	479.7462

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	1521316.605	1521316.605	107.59	<.0001
Volume	1	460215.690	460215.690	32.55	<.0001
Treehtm	1	133169.649	133169.649	9.42	0.0044

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	1019453.264	1019453.264	72.10	<.0001
Volume	1	99315.930	99315.930	7.02	0.0126
Treehtm	1	133169.649	133169.649	9.42	0.0044

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-697.4498900	192.3979298	-3.63	0.0010
NoBran	107.5619849	12.6676959	8.49	<.0001
Volume	32.9964687	12.4503193	2.65	0.0126
Treehtm	186.7437849	60.8507603	3.07	0.0044

Table A.30: Simple Statistics for Chapter 2 predication equation variables for 2000 data.

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DM leafwt	33	391.25636	183.63261	12911	152.38000	811.64000
Sum Bran	33	55.05242	23.78136	1817	18.90000	119.13000
Treewidm	33	1.46396	0.50875	48.31067	0.60800	2.55867
Treehtm	33	2.88485	0.34298	95.20000	2.23000	3.90000
NoBran	33	6.48485	3.09355	214.00000	2.00000	14.00000
RCD	33	6.40303	2.11859	211.30000	3.20000	11.10000
Area	33	4.30941	1.89443	142.21069	1.54432	9.97880
Volume	33	1.88669	1.51164	62.26085	0.24582	6.68437
Variables=	1) DM leafwt = DM leaf weight (g/tree); 2) Sum Bran = Sum of main branch diameters greater than 10 mm; 3) Treewidm = Tree width (m); 4) Treehtm = Tree height (m); 5) NoBran = Number of branches greater than 10 mm diameter; 6) RCD= Root Collar Diameter (mm); 7) Area (m^2), A=total height (H m) x tree canopy width (W m); 8) Volume (m^3), V=1/3 * p ((1/2 * tree width) ^2)*tree height).					

Table A.31: Pearson Correlation Coefficients, N = 33 Prob > |r| under H0: Rho=0 for measured variables for 2000 compared to DM leaf weight (g/tree).

	DM leafwt	Sum Bran	Treewidm	Treehtm	NoBran	RCD
DM leafwt	1.00000	0.88989 <.0001	0.52108 0.0019	0.49575 0.0033	0.45948 0.0071	0.42864 0.0128
Sum Bran	0.88989 <.0001	1.00000	0.51995 0.0019	0.39664 0.0223	0.52509 0.0017	0.47958 0.0047
Treewidm	0.52108 0.0019	0.51995 0.0019	1.00000	0.50893 0.0025	0.36488 0.0368	0.52885 0.0016
Treehtm	0.49575 0.0033	0.39664 0.0223	0.50893 0.0025	1.00000	-0.01171 0.9484	0.55428 0.0008
NoBran	0.45948 0.0071	0.52509 0.0017	0.36488 0.0368	-0.01171 0.9484	1.00000	0.13804 0.4436
RCD	0.42864 0.0128	0.47958 0.0047	0.52885 0.0016	0.55428 0.0008	0.13804 0.4436	1.00000
Variables=	1) DM leafwt = DM leaf weight (g/tree); 2) Sum Bran = Sum of main branch diameters greater than 10 mm; 3) Treewidm = Tree width (m); 4) Treehtm = Tree height (m); 5) NoBran = Number of branches greater than 10 mm diameter; 6) RCD= Root Collar Diameter (mm).					

Table A.32: Pearson Correlation Coefficients, N = 33 Prob > |r| under H0: Rho=0 for calculated variables for 2000 compared to DM leaf weight (g/tree).

	DM leafwt	Area	Volcu	Volsqrt	Vol23	Volume
DM leafwt	1.00000	0.56430 0.0006	0.55622 0.0008	0.55135 0.0009	0.54333 0.0011	0.52085 0.0019
Area	0.56430 0.0006	1.00000	0.98412 <.0001	0.99186 <.0001	0.99462 <.0001	0.98847 <.0001
Volume	0.52085 0.0019	0.98847 <.0001	0.96488 <.0001	0.98131 <.0001	0.99217 <.0001	1.00000
Variables=	1) DM leafwt = DM leaf weight (g/tree); 2) Area (m^2), A=total height (H m) x tree canopy width (W m); 3) Volume (m^3), V=1/3 * p ((1/2 * tree width) ^2)*tree height).					

Table A.33: General linear model using sum of main branch diameters greater than 10 mm as the independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	854511.868	854511.868	117.96	<.0001
Error	31	224558.015	7243.807		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.791897	21.75314	85.11056	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	854511.8675	854511.8675	117.96	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	854511.8675	854511.8675	117.96	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	12.96723135	37.84982179	0.34	0.7342
Sum Bran	6.87143459	0.63266207	10.86	<.0001

Table A.34: General linear model using area (m^2) as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	343613.671	343613.671	14.48	0.0006
Error	31	735456.211	23724.394		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.318435	39.36735	154.0273	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Area	1	343613.6715	343613.6715	14.48	0.0006

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Area	1	343613.6715	343613.6715	14.48	0.0006

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	155.5350101	67.49303078	2.30	0.0281
Area	54.6991544	14.37285215	3.81	0.0006

Table A.35: General linear model using tree width (m) as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	292988.460	292988.460	11.55	0.0019
Error	31	786081.423	25357.465		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.271519	40.69973	159.2403	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treewidm	1	292988.4600	292988.4600	11.55	0.0019

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treewidm	1	292988.4600	292988.4600	11.55	0.0019

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	115.9131746	85.61495862	1.35	0.1856
Treewidm	188.0811396	55.33155932	3.40	0.0019

Table A.36: General linear model using volume (m^3) as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	292737.627	292737.627	11.54	0.0019
Error	31	786332.256	25365.557		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.271287	40.70622	159.2657	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Volume	1	292737.6266	292737.6266	11.54	0.0019

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Volume	1	292737.6266	292737.6266	11.54	0.0019

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	271.8807143	44.75997417	6.07	<.0001
Volume	63.2724512	18.62505589	3.40	0.0019

Table A.37: General linear model using tree height (m) as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	265205.244	265205.244	10.10	0.0033
Error	31	813864.639	26253.698		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.245772	41.41273	162.0299	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treehtm	1	265205.2438	265205.2438	10.10	0.0033

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treehtm	1	265205.2438	265205.2438	10.10	0.0033

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-374.4544514	242.5633420	-1.54	0.1328
Treehtm	265.4249674	83.5114380	3.18	0.0033

Table A.38: General linear model using number of branches greater than 10 mm diameter as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	227810.462	227810.462	8.30	0.0071
Error	31	851259.421	27459.981		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.211117	42.35344	165.7105	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	8.30	0.0071

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	8.30	0.0071

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	214.3864397	67.84489501	3.16	0.0035
NoBran	27.2743341	9.46929053	2.88	0.0071

Table A.39: General linear model using root collar diameter (mm) as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	198258.610	198258.610	6.98	0.0128
Error	31	880811.273	28413.267		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.183731	43.08233	168.5624	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
RCD	1	198258.6101	198258.6101	6.98	0.0128

Source	DF	Type III SS	Mean Square	F Value	Pr > F
RCD	1	198258.6101	198258.6101	6.98	0.0128

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	153.3645684	94.71808580	1.62	0.1155
RCD	37.1530016	14.06495876	2.64	0.0128

Table A.40: General linear model using root collar diameter sum (mm) as independent variable and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7051.098	7051.098	0.20	0.6547
Error	31	1072018.785	34581.251		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.006534	47.52903	185.9603	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
RCDS	1	7051.097847	7051.097847	0.20	0.6547

Source	DF	Type III SS	Mean Square	F Value	Pr > F
RCDS	1	7051.097847	7051.097847	0.20	0.6547

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	355.9387112	84.64825509	4.20	0.0002
RCDS	4.5173741	10.00410041	0.45	0.6547

Table A.41: General linear model using two variables: sum of main branch diameters greater than 10 mm and tree height (m) as the independent variables and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	880620.749	440310.375	66.56	<.0001
Error	30	198449.134	6614.971		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.816092	20.78751	81.33247	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SumBran	1	854511.8675	854511.8675	129.18	<.0001
Treehtm	1	26108.8816	26108.8816	3.95	0.0562

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SumBran	1	615415.5054	615415.5054	93.03	<.0001
Treehtm	1	26108.8816	26108.8816	3.95	0.0562

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-220.1820321	122.8030350	-1.79	0.0831
SumBran	6.3524588	0.6585998	9.65	<.0001
Treehtm	90.7223162	45.6650535	1.99	0.0562

Table A.42: General linear model using two variables: number of branches greater than 10 mm diameter and tree height (m) as the independent variables and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	498840.435	249420.217	12.90	<.0001
Error	30	580229.448	19340.982		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.462287	35.54494	139.0719	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	11.78	0.0018
Treehtm	1	271029.9727	271029.9727	14.01	0.0008

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	233635.1910	233635.1910	12.08	0.0016
Treehtm	1	271029.9727	271029.9727	14.01	0.0008

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-561.9996112	215.0736417	-2.61	0.0139
NoBran	27.6227064	7.9476065	3.48	0.0016
Treehtm	268.3423109	71.6835906	3.74	0.0008

Table A.43: General linear model using two variables: number of branches greater than 10 mm diameter and tree height (m) as the independent variables and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	451531.075	225765.537	10.79	0.0003
Error	30	627538.808	20917.960		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.418445	36.96564	144.6304	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	10.89	0.0025
Area	1	223720.6128	223720.6128	10.70	0.0027

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	107917.4034	107917.4034	5.16	0.0305
Area	1	223720.6128	223720.6128	10.70	0.0027

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	66.76062623	74.45820642	0.90	0.3771
NoBran	19.52788358	8.59743895	2.27	0.0305
Area	45.91351111	14.03935553	3.27	0.0027

Table A.44: General linear model using two variables: number of branches greater than 10 mm diameter and volume (m^3) as the independent variables and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	414523.818	207261.909	9.36	0.0007
Error	30	664546.064	22151.535		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.384149	38.04000	148.8339	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	10.28	0.0032
Volume	1	186713.3564	186713.3564	8.43	0.0069

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	121786.1919	121786.1919	5.50	0.0259
Volume	1	186713.3564	186713.3564	8.43	0.0069

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	158.6486431	63.88808943	2.48	0.0188
NoBran	20.6475772	8.80586437	2.34	0.0259
Volume	52.3197705	18.02104847	2.90	0.0069

Table A.45: General linear model using two variables: number of branches greater than 10 mm diameter and tree width (m) as the independent variables and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	383293.914	191646.957	8.26	0.0014
Error	30	695775.969	23192.532		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.355208	38.92357	152.2909	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	9.82	0.0038
Treewidm	1	155483.4517	155483.4517	6.70	0.0147

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	90305.4537	90305.4537	3.89	0.0577
Treewidm	1	155483.4517	155483.4517	6.70	0.0147

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	56.2161458	87.28898844	0.64	0.5245
NoBran	18.4437711	9.34688326	1.97	0.0577
Treewidm	147.1592232	56.83547386	2.59	0.0147

Table A.46: General linear model using two variables: number of branches greater than 10 mm diameter and root collar diameter (m) as the independent variables and DM leaf weight (g/tree) as the dependent variable for 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	374531.674	187265.837	7.97	0.0017
Error	30	704538.209	23484.607		
Corrected Total	32	1079069.883			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.347088	39.16790	153.2469	391.2564

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	227810.4621	227810.4621	9.70	0.0040
RCD	1	146721.2120	146721.2120	6.25	0.0181

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	176273.0640	176273.0640	7.51	0.0103
RCD	1	146721.2120	146721.2120	6.25	0.0181

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	27.54281859	97.59325368	0.28	0.7797
NoBran	24.22357311	8.84172240	2.74	0.0103
RCD	32.27024298	12.91062987	2.50	0.0181

Table A.47: Simple Statistics for Chapter 2 predication equation variables for combined 1999 and 2000 data.

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
DM leafwt	68	436.80260	237.05626	29703	92.50000	1226
Sum Bran	68	67.46662	34.97536	4588	18.90000	187.00000
Treewidm	68	1.83141	0.61136	124.53607	0.60800	3.30200
Treehtm	68	3.12294	0.44876	212.36000	2.23000	3.98000
NoBran	68	5.08824	2.83757	346.00000	1.00000	14.00000
Volume	68	3.19961	2.27323	217.57381	0.24582	11.36072
Area	68	5.87579	2.54254	399.55384	1.54432	13.14196
Variables=	1) DM leafwt = DM leaf weight (g/tree); 2) Sum Bran = Sum of main branch diameters greater than 10 mm; 3) Treewidm = Tree width (m); 4) Treehtm = Tree height (m); 5) NoBran = Number of branches greater than 10 mm diameter; 6) Volume (m^3), V=1/3 * p ((1/2 * tree width) ^2)*tree height); 7) Area (m^2), A=total height (H m) x tree canopy width (W m).					

Table A.48: Pearson Correlation Coefficients, N = 68 Prob > |r| under H0: Rho=0 for measured variables for combined 1999 and 2000 data compared to DM leaf weight (g/tree).

	DM leafwt	Sum Bran	Treewidm	Treehtm	NoBran
DM leafwt	1.00000	0.89365 <.0001	0.55382 <.0001	0.45974 <.0001	0.37948 0.0014
Sum Bran	0.89365 <.0001	1.00000	0.58755 <.0001	0.38618 0.0011	0.35551 0.0029
Treewidm	0.55382 <.0001	0.58755 <.0001	1.00000	0.57857 <.0001	-0.03124 0.8003
Treehtm	0.45974 <.0001	0.38618 0.0011	0.57857 <.0001	1.00000	-0.26944 0.0263
NoBran	0.37948 0.0014	0.35551 0.0029	-0.03124 0.8003	-0.26944 0.0263	1.00000
Variables =	1) DM leafwt = DM leaf weight (g/tree); 2) Sum Bran = Sum of main branch diameters greater than 10 mm; 3) Treewidm = Tree width (m); 4) Treehtm = Tree height (m); 5) NoBran = Number of branches greater than 10 mm diameter.				

Table A.49: Pearson Correlation Coefficients, N = 68 Prob > |r| under H0: Rho=0 for measured variables for combined 1999 and 2000 data compared to DM leaf weight (g/tree).

	DM leafwt	Volume	Area	Vol23	Volsqrt	Volcu
DM leafwt	1.00000	0.60314 <.0001	0.59577 <.0001	0.59230 <.0001	0.58373 <.0001	0.57278 <.0001
Volume	0.60314 <.0001	1.00000	0.98427 <.0001	0.99140 <.0001	0.98017 <.0001	0.96387 <.0001
Area	0.59577 <.0001	0.98427 <.0001	1.00000	0.99309 <.0001	0.99093 <.0001	0.98380 <.0001
Variables=	1) DM leafwt = DM leaf weight (g/tree); 2) Volume (m^3), V=1/3 * p ((1/2 * tree width) ^2)*tree height); 3) Area (m^2), A=total height (H m) x tree canopy width (W m).					

Table A.50: General linear model using sum of main branch diameters greater than 10 mm as independent variable and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3006852.480	3006852.480	261.72	<.0001
Error	66	758257.373	11488.748		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.798609	24.53868	107.1856	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	3006852.480	3006852.480	261.72	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	3006852.480	3006852.480	261.72	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	28.15829134	28.40770053	0.99	0.3252
Sum Bran	6.05698526	0.37440084	16.18	<.0001

Table A.51: General linear model using two variables: sum of main branch diameters greater than 10 mm and year as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3076660.736	1025553.579	95.34	<.0001
Error	64	688449.117	10757.017		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.817150	23.74437	103.7160	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Year	1	133002.499	133002.499	12.36	0.0008
Sum Bran	1	2937925.130	2937925.130	273.12	<.0001
Year*Sum Bran	1	5733.108	5733.108	0.53	0.4680

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	1936.317936	1936.317936	0.18	0.6728
Sum Bran	1	5676.946992	5676.946992	0.53	0.4702
Year*Sum Bran	1	5733.108389	5733.108389	0.53	0.4680

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-51488.83654	121354.1506	-0.42	0.6728
Year	25.75090	60.6946	0.42	0.6728
Sum Bran	-1293.88640	1781.0851	-0.73	0.4702
Year*Sum Bran	0.65038	0.8909	0.73	0.4680

Table A.52: General linear model using volume (m^3) as independent variable and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1369645.529	1369645.529	37.74	<.0001
Error	66	2395464.325	36294.914		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.363773	43.61518	190.5122	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Volume	1	1369645.529	1369645.529	37.74	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Volume	1	1369645.529	1369645.529	37.74	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	235.5594422	40.08673953	5.88	<.0001
Volume	62.8960580	10.23863877	6.14	<.0001

Table A.53: General linear model using two variables: volume (m^3) and year as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1516290.980	505430.327	14.38	<.0001
Error	64	2248818.873	35137.795		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.402722	42.91430	187.4508	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Year	1	133002.499	133002.499	3.79	0.0561
Volume	1	1365788.669	1365788.669	38.87	<.0001
Year*Volume	1	17499.813	17499.813	0.50	0.4829

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	105829.9745	105829.9745	3.01	0.0875
Volume	1	17571.4249	17571.4249	0.50	0.4820
Year*Volume	1	17499.8127	17499.8127	0.50	0.4829

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-310854.8989	179244.2784	-1.73	0.0877
Year	155.5634	89.6376	1.74	0.0875
Volume	37316.7960	52770.1234	0.71	0.4820
Year*Volume	-18.6268	26.3942	-0.71	0.4829

Table A.54: General linear model using area (m^2) as independent variable and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1336397.565	1336397.565	36.32	<.0001
Error	66	2428712.289	36798.671		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.354943	43.91682	191.8298	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Area	1	1336397.565	1336397.565	36.32	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Area	1	1336397.565	1336397.565	36.32	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	110.4184264	58.94441874	1.87	0.0655
Area	55.5472664	9.21745276	6.03	<.0001

Table A.55: General linear model using two variables: area (m^2) and year as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1559503.379	519834.460	15.08	<.0001
Error	64	2205606.474	34462.601		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.414199	42.49999	185.6411	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Year	1	133002.499	133002.499	3.86	0.0538
Area	1	1376472.793	1376472.793	39.94	<.0001
Year*Area	1	50028.088	50028.088	1.45	0.2327

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	139744.8977	139744.8977	4.05	0.0483
Area	1	50157.5665	50157.5665	1.46	0.2321
Year*Area	1	50028.0879	50028.0879	1.45	0.2327

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-557546.6726	276906.7128	-2.01	0.0483
Year	278.8511	138.4772	2.01	0.0483
Area	54695.5659	45337.5365	1.21	0.2321
Year*Area	-27.3204	22.6754	-1.20	0.2327

Table A.56: General linear model using the tree width (m) as independent variable and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1154817.870	1154817.870	29.20	<.0001
Error	66	2610291.983	39549.879		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.306716	45.52892	198.8715	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treewidm	1	1154817.870	1154817.870	29.20	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treewidm	1	1154817.870	1154817.870	29.20	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	43.5139541	76.67408115	0.57	0.5723
Treewidm	214.7460456	39.74120254	5.40	<.0001

Table A.57: General linear model using two variables: tree width (m) and year as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1357187.805	452395.935	12.02	<.0001
Error	64	2407922.048	37623.782		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.360464	44.40645	193.9685	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Year	1	133002.499	133002.499	3.54	0.0646
Treewidm	1	1130884.736	1130884.736	30.06	<.0001
Year*Treewidm	1	93300.571	93300.571	2.48	0.1202

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	155301.8777	155301.8777	4.13	0.0463
Treewidm	1	93463.0791	93463.0791	2.48	0.1199
Year*Treewidm	1	93300.5707	93300.5707	2.48	0.1202

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-748864.2913	368590.1350	-2.03	0.0463
Year	374.4901	184.3246	2.03	0.0463
Treewidm	302049.3262	191641.2759	1.58	0.1199
Year*Treewidm	-150.9306	95.8443	-1.57	0.1202

Table A.58: General linear model using the tree height (m) as independent variable and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	795790.581	795790.581	17.69	<.0001
Error	66	2969319.272	44989.686		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.211359	48.55917	212.1077	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treehtm	1	795790.5814	795790.5814	17.69	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treehtm	1	795790.5814	795790.5814	17.69	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-321.6156095	182.1543311	-1.77	0.0821
Treehtm	242.8538249	57.7433573	4.21	<.0001

Table A.59: General linear model using two variables: tree height (m) and year as the independent vari ables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	809090.926	269696.975	5.84	0.0014
Error	64	2956018.928	46187.796		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.214892	49.20151	214.9135	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Year	1	133002.4985	133002.4985	2.88	0.0946
Treehtm	1	676011.0971	676011.0971	14.64	0.0003
Treehtm*Year	1	77.3299	77.3299	0.00	0.9675

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	54.7192	54.7192	0.00	0.9726
Treehtm	1	641586.1813	641586.1813	13.89	0.0004
Treehtm*Year	1	77.3299	77.3299	0.00	0.9675

Table A.60: General linear model using number of branches greater than 10 mm diameter as independent variable and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	542185.389	542185.389	11.10	0.0014
Error	66	3222924.464	48832.189		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.144003	50.59037	220.9801	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
NoBran	1	542185.3892	542185.3892	11.10	0.0014

Source	DF	Type III SS	Mean Square	F Value	Pr > F
NoBran	1	542185.3892	542185.3892	11.10	0.0014

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	275.4941177	55.33229343	4.98	<.0001
NoBran	31.7022451	9.51413281	3.33	0.0014

Table A.61: General linear model using two variables: number of branches greater than 10 mm diameter and year as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1882129.561	627376.520	21.32	<.0001
Error	64	1882980.292	29421.567		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.499887	39.26881	171.5272	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Year	1	133002.499	133002.499	4.52	0.0374
NoBran	1	1082553.032	1082553.032	36.79	<.0001
Year*NoBran	1	666574.031	666574.031	22.66	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	100972.8626	100972.8626	3.43	0.0686
NoBran	1	666947.1813	666947.1813	22.67	<.0001
Year*NoBran	1	666574.0312	666574.0312	22.66	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-363584.7441	196327.5033	-1.85	0.0686
Year	181.8996	98.1889	1.85	0.0686
NoBran	182661.5801	38364.9282	4.76	<.0001
Year*NoBran	-91.3172	19.1850	-4.76	<.0001

Table A.62: General linear model using two variables: sum of main branch diameters greater than 10 mm and tree height (m) as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3064998.337	1532499.168	142.28	<.0001
Error	65	700111.517	10770.946		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.814053	23.75974	103.7832	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	3006852.480	3006852.480	279.16	<.0001
Treehtm	1	58145.857	58145.857	5.40	0.0233

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	2269207.755	2269207.755	210.68	<.0001
Treehtm	1	58145.857	58145.857	5.40	0.0233

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-170.2991636	89.73475929	-1.90	0.0622
Sum Bran	5.7043579	0.39300364	14.51	<.0001
Treehtm	71.1662558	30.62962381	2.32	0.0233

Table A.63: General linear model using three variables: sum of main branch diameters greater than 10 mm, tree width (m) and volume (m^3) and year as the independent variables and DM leaf weight (g/tree) as the dependent variable for combined 1999 and 2000 data.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	3077017.520	1025672.507	95.40	<.0001
Error	64	688092.333	10751.443		
Corrected Total	67	3765109.853			

R-Square	Coeff Var	Root MSE	DM leafwt Mean
0.817245	23.73822	103.6892	436.8026

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Sum Bran	1	3006852.480	3006852.480	279.67	<.0001
Treewidm	1	4753.708	4753.708	0.44	0.5085
Volume	1	65411.332	65411.332	6.08	0.0163

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Sum Bran	1	1692536.971	1692536.971	157.42	<.0001
Treewidm	1	44042.849	44042.849	4.10	0.0472
Volume	1	65411.332	65411.332	6.08	0.0163

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	156.8090943	72.16089402	2.17	0.0335
Sum Bran	5.6955485	0.45394145	12.55	<.0001
Treewidm	-135.3516405	66.87429121	-2.02	0.0472
Volume	44.8862889	18.19787073	2.47	0.0163

Table A.64: Chapter 3 Simple Statistics for Year 1999.

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
GDD	14	1134	423.26964	15870	683.00000	1702
RF(mm)	14	0.24857	0.22698	3.48000	0	0.53000
NDF	14	346.38714	83.58987	4849	236.72000	481.79000
ADF	14	226.29286	61.62827	3168	158.04000	336.35000
CELL	14	80.65429	11.13531	1129	64.93000	95.81000
ADL	14	145.67286	52.54915	2039	85.51000	242.29000
Nitrogen	14	48.13357	4.75741	673.87000	41.47000	57.31000
IVTDMD	14	643.48571	101.72999	9009	493.60000	805.50000
FR-phenol	14	6.55571	1.46090	91.78000	4.71000	9.02000
CT	14	7.29929	1.35163	102.19000	5.37000	9.59000
HT	14	7.19357	1.50967	100.71000	5.04000	9.95000
H	14	2.03464	0.59058	28.48500	1.35000	3.40000
HM	14	931.98925	590.25023	13048	218.11667	2074
Variables	GDD=Growing Degree Days (Min T (°F) + Max T (°F)/2 – 50 °F; RF (mm), Rainfall mm (averaged over two weeks prior to each sampling date); NDF= Neutral Detergent Fiber, ADF= Acid Detergent Fiber; CELL= Cellulose; ADL= Lignin; IVTDMD= *In vitro* true dry matter disappearance; FR -phenol= Folin-reactive phenolics; CT= Condensed Tannins; HT= Hydrolyzable Tannins; H= Height (m) and HM= Herbage mass (kg/ha)					

Table A.65: Chapter 3 Pearson Correlation Coefficients, N = 14Prob > |r| underH0: R ho=0 for year 1999.

	GDD	RF (mm)	NDF	ADF	CELL	ADL	Nitrogen	IVTDMD	FR-phenol	CT	HT	H	HM
GDD	1.00000	-0.93702 <.0001	0.96210 <.0001	0.92147 <.0001	0.95938 <.0001	0.87927 <.0001	-0.80403 0.0005	-0.90341 <.0001	-0.48164 0.0812	0.58075 0.0294	-0.50643 0.0646	0.76212 0.0015	0.87826 <.0001
RF (mm)	-0.93890 <.0001	1.00000	0.92123 <.0001	-0.88888 <.0001	-0.90862 <.0001	-0.85153 0.0001	0.82574 0.0003	0.88772 <.0001	0.47092 0.0892	0.54852 0.0422	0.54614 0.0433	-0.90044 <.0001	-0.83876 0.0002
NDF	0.96255 <.0001	-0.92123 <.0001	1.00000	0.98770 <.0001	0.91812 <.0001	0.96990 <.0001	-0.80325 0.0005	-0.94907 <.0001	-0.57647 0.0309	-0.65849 0.0104	-0.51910 0.0571	0.70659 0.0047	0.84890 0.0001
ADF	0.92219 <.0001	-0.88888 <.0001	0.98770 <.0001	1.00000	0.86414 <.0001	0.99352 <.0001	-0.77172 0.0012	-0.94787 <.0001	-0.61123 0.0202	-0.67985 0.0075	-0.52153 0.0558	0.67307 0.0083	0.82136 0.0003
CELL	0.95930 <.0001	-0.90862 <.0001	0.91812 <.0001	0.86414 <.0001	1.00000	0.80912 0.0005	-0.80607 0.0005	-0.86449 <.0001	-0.40819 0.1474	-0.54105 0.0457	-0.37990 0.1803	0.74745 0.0021	0.89708 <.0001
ADL	0.88013 <.0001	-0.85153 0.0001	0.96990 <.0001	0.99352 <.0001	0.80912 0.0005	1.00000	-0.74864 0.0021	-0.94044 <.0001	-0.61935 0.0182	-0.67441 0.0082	-0.50995 0.0625	0.63286 0.0151	0.78081 0.0010
Nitrogen	-0.80541 0.0005	0.82574 0.0003	0.80325 0.0005	-0.77172 0.0012	-0.80607 0.0005	-0.74864 0.0021	1.00000	0.84174 0.0002	0.14103 0.6306	0.19594 0.5020	0.24060 0.4073	-0.75319 0.0019	-0.72779 0.0032
IVTDMD	-0.90440 <.0001	0.88772 <.0001	0.94907 <.0001	-0.94787 <.0001	-0.86449 <.0001	-0.94044 <.0001	0.84174 0.0002	1.00000	0.43098 0.1239	0.49055 0.0749	0.37796 0.1827	-0.74695 0.0021	-0.83479 0.0002
FR-phenol	-0.48176 0.0811	0.47092 0.0892	0.57647 0.0309	-0.61123 0.0202	-0.40819 0.1474	-0.61935 0.0182	0.14103 0.6306	0.43098 0.1239	1.00000	0.96582 <.0001	0.65868 0.0104	-0.24335 0.4018	-0.42263 0.1322
CT	-0.58052 0.0295	0.54852 0.0422	0.65849 0.0104	-0.67985 0.0075	-0.54105 0.0457	-0.67441 0.0082	0.19594 0.5020	0.49055 0.0749	0.96582 <.0001	1.00000	0.66509 0.0094	-0.31099 0.2791	-0.53749 0.0475
HT	-0.50711 0.0642	0.54614 0.0433	0.51910 0.0571	-0.52153 0.0558	-0.37990 0.1803	-0.50995 0.0625	0.24060 0.4073	0.37796 0.1827	0.65868 0.0104	0.66509 0.0094	1.00000	-0.37722 0.1836	-0.51366 0.0603
H	0.76532 0.0014	-0.90044 <.0001	0.70659 0.0047	0.67307 0.0083	0.74745 0.0021	0.63286 0.0151	-0.75319 0.0019	-0.74695 0.0021	-0.24335 0.4018	-0.31099 0.2791	-0.37722 0.1836	1.00000	0.68670 0.0067
HM	0.87836 <.0001	-0.83876 0.0002	0.84890 0.0001	0.82136 0.0003	0.89708 <.0001	0.78081 0.0010	-0.72779 0.0032	-0.83479 0.0002	-0.42263 0.1322	-0.53749 0.0475	-0.51366 0.0603	0.68670 0.0067	1.00000
Variables	GDD=Growing Degree Days (Min T (°F) + Max T (°F)/2 – 50° F; RF (mm), Rainfall (mm) (averaged over two weeks prior to each sampling date); NDF= Neutral Detergent Fiber, ADF= Acid Detergent Fiber; CELL= Cellulose; ADL= Lignin; IVTDMD= *In vitro* true dry matter disappearance; FR-phenol= Folin-reactive phenolics; CT= Condensed Tannins; HT= Hydrolyzable Tannins; H= Height (m) and HM= Herbage mass (kg/ha)												

Table A.66: Chapter 3 Simple Statistics for Year 2000.						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
GDD	16	2165	473.28131	34640	1530	2711
RF (mm)	16	4.55500	2.73145	72.88000	1.83000	8.90000
NDF	16	385.23063	28.47436	6164	344.97000	458.99000
ADF	16	287.50125	33.20686	4600	243.25000	373.83000
CELL	16	87.06188	7.66403	1393	72.16000	99.08000
ADL	16	186.80750	29.92891	2989	153.71000	258.81000
Nitrogen	16	36.92938	2.86787	590.87000	33.02000	42.73000
IVTDMD	16	527.67500	38.51413	8443	456.60000	597.10000
FR-phenol	16	9.23875	2.35735	147.82000	6.02000	14.07000
CT	16	9.94125	2.67833	159.06000	6.22000	14.77000
HT	16	8.95875	2.95174	143.34000	5.22000	16.73000
H	16	2.96125	0.50464	47.38000	2.18500	4.20000
HM	16	2011	829.78750	32179	737.66667	3783
Variables	GDD= Growing Degree Days (Min T (°F) + Max T (°F)/2 – 50° F; RF (mm), Rainfall mm (averaged over two weeks prior to each sampling date); NDF= Neutral Detergent Fiber, ADF= Acid Detergent Fiber; CELL= Cellulose; ADL= Lignin; IVTDMD= *In vitro* true dry matter disappearance; FR-phenol= Folin-reactive phenolics; CT= Condensed Tannins; HT= Hydrolyzable Tannins; H= Height (m) and HM= Herbage mass (kg/ha)					

Table A. 67: Chapter 3 Pearson Correlation Coefficients, N = 16Prob > |r| under H0: Rho=0 for year 2000.

	GDD	RF (mm)	NDF	ADF	CELL	ADL	Nitrogen	IVTDMD	FR-phenol	CT	HT	H	HM
GDD	1.00000	0.74785 0.0009	-0.43631 0.0911	-0.56926 0.0214	-0.51390 0.0417	-0.43322 0.0937	-0.76209 0.0006	0.17739 0.5110	0.43975 0.0883	0.29930 0.2601	-0.26485 0.3215	-0.21905 0.4150	0.22910 0.3934
RF (mm)	0.74972 0.0008	1.00000	-0.30878 0.2446	-0.38500 0.1409	-0.70575 0.0023	-0.32031 0.2265	-0.42176 0.1037	-0.29894 0.2607	0.55612 0.0253	0.43788 0.0898	0.14876 0.5824	-0.15502 0.5665	-0.14025 0.6044
NDF	-0.43559 0.0917	-0.30878 0.2446	1.00000	0.96249 <.0001	0.32968 0.2124	0.93709 <.0001	0.10772 0.6913	-0.52423 0.0371	-0.71053 0.0020	-0.58674 0.0169	0.16440 0.5429	0.38540 0.1404	0.10461 0.6998
ADF	-0.56846 0.0216	-0.38500 0.1409	0.96249 <.0001	1.00000	0.33553 0.2039	0.96574 <.0001	0.23530 0.3803	-0.58888 0.0164	-0.69985 0.0025	-0.54660 0.0285	0.23373 0.3836	0.42586 0.1000	-0.01264 0.9629
CELL	-0.51517 0.0411	-0.70575 0.0023	0.32968 0.2124	0.33553 0.2039	1.00000	0.15160 0.5751	0.30387 0.2525	0.09554 0.7249	-0.38712 0.1385	-0.24685 0.3567	0.04787 0.8603	-0.29654 0.2647	-0.25149 0.3474
ADL	-0.43254 0.0943	-0.32031 0.2265	0.93709 <.0001	0.96574 <.0001	0.15160 0.5751	1.00000	0.08788 0.7462	-0.53377 0.0332	-0.70801 0.0021	-0.58088 0.0183	0.11806 0.6632	0.54342 0.0296	0.16274 0.5471
Nitrogen	-0.76162 0.0006	-0.42176 0.1037	0.10772 0.6913	0.23530 0.3803	0.30387 0.2525	0.08788 0.7462	1.00000	-0.21095 0.4329	-0.20733 0.4410	-0.16803 0.5339	0.27423 0.3040	0.08104 0.7654	-0.39359 0.1315
IVTDMD	0.17537 0.5159	-0.29894 0.2607	-0.52423 0.0371	-0.58888 0.0164	0.09554 0.7249	-0.53377 0.0332	-0.21095 0.4329	1.00000	0.05384 0.8430	-0.05111 0.8509	-0.51258 0.0423	-0.24662 0.3572	0.39687 0.1280
FR-phenol	0.43971 0.0883	0.55612 0.0253	-0.71053 0.0020	-0.69985 0.0025	-0.38712 0.1385	-0.70801 0.0021	-0.20733 0.4410	0.05384 0.8430	1.00000	0.95502 <.0001	0.35580 0.1762	-0.36546 0.1639	-0.27658 0.2997
CT	0.29896 0.2607	0.43788 0.0898	-0.58674 0.0169	-0.54660 0.0285	-0.24685 0.3567	-0.58088 0.0183	-0.16803 0.5339	-0.05111 0.8509	0.95502 <.0001	1.00000	0.41198 0.1128	-0.31671 0.2320	-0.30071 0.2578
HT	-0.26380 0.3235	0.14876 0.5824	0.16440 0.5429	0.23373 0.3836	0.04787 0.8603	0.11806 0.6632	0.27423 0.3040	-0.51258 0.0423	0.35580 0.1762	0.41198 0.1128	1.00000	-0.26135 0.3282	-0.58419 0.0175
H	-0.21841 0.4164	-0.15502 0.5665	0.38540 0.1404	0.42586 0.1000	-0.29654 0.2647	0.54342 0.0296	0.08104 0.7654	-0.24662 0.3572	-0.36546 0.1639	-0.31671 0.2320	-0.26135 0.3282	1.00000	0.61302 0.0116
HM	0.22791 0.3959	-0.14025 0.6044	0.10461 0.6998	-0.01264 0.9629	-0.25149 0.3474	0.16274 0.5471	-0.39359 0.1315	0.39687 0.1280	-0.27658 0.2997	-0.30071 0.2578	-0.58419 0.0175	0.61302 0.0116	1.00000
Variables	GDD=Growing Degree Days (Min T (°F) + Max T (°F)/2 – 50 °F; RF (mm), Rainfall (mm) (averaged over two weeks prior to each sampling date); NDF= Neutral Detergent Fiber, ADF= Acid Detergent Fiber; CELL= Cellulose; ADL= Lignin; IVTDMD= *In vitro* true dry matter disappearance; FR-phenol= Folin-reactive phenolics; CT= Condensed Tannins; HT= Hydrolyzable Tannins; H= Height (m) and HM= Herbage mass (kg/ha)												

Table A.68. Tannin data for indirect and direct cooling.

Date	Year	Method	FA-phenol	CT	HT	Sample date
6/23/1999	1999	DC intake	11.66336	6.929171	31.56935	23-Jun
6/23/99 fed on 6/26/1999	1999	indirect cooling	13.70428	11.13473	19.06786	23-Jun
6/25/1999	1999	DC intake	13.26885	11.26295	27.43815	25-Jun
6/25/1999 fed on 6/27/99	1999	indirect cooling	12.98643	12.7977	22.08074	25-Jun
6/25/1999 fed on 6/28/99	1999	indirect cooling	12.09799	12.70436	26.95027	25-Jun
06/28/99	1999	DC digestion	11.84026	10.07543	33.94786	28-Jun
6/28/1999 fed on 6/29/99	1999	indirect cooling	10.35057	6.345883	21.34358	28-Jun
06/29/99	1999	DC digestion	12.96063	10.07101	47.7629	29-Jun
6/29/99 fed on 6/30/1999	1999	indirect cooling	12.96942	11.6421	23.73159	29-Jun
6/30/2000	2000	DC intake	5.874614	4.984949	6.358428	30-Jun
6/30/00 fed on 7/01/00	2000	indirect cooling	6.585993	7.034011	12.85072	30-Jun
6/30/00 fed on 7/02/00	2000	indirect cooling	8.152174	8.735364	19.89201	30-Jun
7/7/2000	2000	DC intake	6.792522	6.404176	6.358428	7-Jul
7/7/00 fed on 7/8/00	2000	indirect cooling	4.402174	7.731827	15.21739	7-Jul
7/7/00 fed on 7/9/00	2000	indirect cooling	5.601064	6.048162	54.1903	7-Jul
7/7/00 fed on 7/10/00	2000	indirect cooling	12.18231	11.08418	68.47301	7-Jul
7/7/00 fed on 7/11/00	2000	indirect cooling	10.23018	11.61666	69.90622	7-Jul
7/10/2000	2000	DC digestion	7.176024	10.02797	10.27887	10-Jul
7/10/00 fed on 7/12/00	2000	indirect cooling	7.281383	7.547859	61.75173	10-Jul
7/13/2000	2000	DC digestion	9.801862	13.01197	11.04755	13-Jul
7/13/00 fed on 7/14/00	2000	indirect cooling	8.852716	8.458063	50.54616	13-Jul
06/30/99	1999	DC digestion	12.74381	12.04055	39.27886	6-Jun
7/3/2000	2000	DC intake	5.271928	5.999091	5.302227	3-Jul

Materials and Methods

During the intake and digestion trial we harvested daily BL foliage by hand and offered fresh to the goats. Harvested BL leaves were stored on large trays in a walk-in cooler (9°C) until feeding time. Leaves were either fed immediately (same day) or stored for a maximum of two days until fed. Sub-samples of BL foliage for chemical analysis were taken on a daily basis and immediately placed in liquid N. Because of limited freeze dryer facilities all samples were initially stored in a freezer (-20 °C) until they could be further processed.

The question of interest::

Do tannin concentrations change over time when BL foliage is stored in the cooler over two days? Data were collected to gather preliminary information and to understand if there would be any significant differences between immediately frozen (fed same day) compared to BL foliage collected two days ago and fed after sitting in the cooler.