ENERGY RESERVES AND AGRONOMIC CHARACTERISTICS OF FOUR LIMPOGRASSES (*Hemarthria altissima* (Poir) Stapf et C.E. Hubb) FOR FLORIDA'S FLATWOODS

BY

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Higher quality forages are needed for the four million hectares of flatwoods that are the base of the forage-livestock industry in Florida. The improved limpograss (*Hemarthria altissima* (Poir) Stapf et C.E. Hubb) cultivars 'Bigalta' and 'Redalta' were compared with PI 364888 and PI 349753 to find better yield, quality, and persistence, as well as to investigate morphological differences and total nonstructural carbohydrate (TNC) physiology as they relate to persistence.

Preliminary work in 1979 used regrowth-in-darkness studies that quantified the morphological differences in the four limpograsses. Larger stubble systems were positively correlated to TNC percentages in the stem bases. Frequent defoliation treatments in the field prior to the darkness study caused a shift in habit from upright to prostrate growth that permitted increases in axillary tiller formation with concomitant increases in TNC accumulation as measured by chemical means.

A field experiment was then initiated with nitrogen (N) fertilization added as a new variable at five levels which was combined with five defoliation frequencies using a response surface design. Dry matter (DM) yields, in vitro organic matter digestibility (IVOMD), crude protein (CP), and persistence were measured.

The best yield of all the genotypes was produced by PI 364888 (29 m ton/ha/yr) with two harvests on 27 July and 30 November, representing 18 week cutting frequencies. Nitrogen was applied at 240 kg/ha per harvest interval and yields were 18 and 11 m ton/ha for the first and second harvests, respectively.

Percentages of IVOMD and CP were very low, especially for CP, in the mid-summer period, but the quality increased substantially in the autumn when limpograss growth rates declined. Bigalta had superior quality among genotypes throughout the year.

Stored TNC decreased from a high in March to a low in July. The period of lowest percent TNC in stem bases corresponded to the duration of greatest limpograss losses in plots that were greatly stressed by frequent clipping and high N fertilization.

Limpograss PI 364888 represents an improvement in yield and persistence over Bigalta, an improvement in yield and quality over Redalta, and is scheduled for cultivar release in 1983.

INTRODUCTION

Limpograss (Hemarthria altissima (Poir) Stapf et C. E. Hubb) is a stoloniferous grass that has adaptability as an improved forage for flatwoods in Florida and the southeast. Of the cultivars now released, 'Bigalta' has high digestibility and low persistence while 'Redalta' has high persistence and low digestibility. The goal of this project was to evaluate two new limpograss genotypes, PI 364888 and PI 349753, based on their characteristics of yield, digestibility, and persistence, as well as to investigate the nonstructural carbohydrate physiology that releates to persistence.

A long term objective in Florida is to replace the low quality forages that commonly grow on flatwoods with higher quality forages possibly including limpograss. The flatwoods are the most important ecological site for the forage-livestock industry in the state.

Knowledge of the total nonstructural carbohydrate (TNC) content of a plant is necessary to understand how a plant grows. Studies of TNC, morphology, and observations of plant reactions to management can yield practical recommendations to be passed to agricultural producers.

This study was undertaken to thoroughly explore the flexibility of limpograss under a wide range of management practices to clearly identify its strengths and weaknesses as a forage plant for the flatwoods.

LITERATURE REVIEW

History, Potential, Limitations, and Development of Florida's Forage-Livestock Industry

In 1513 Ponce de Leon set out to find the land with the magic waters of Indian lore and discovered the mainland near St. Augustine. He named the place Florida.

One of the early victims of the reputed wealth of Florida was Hernando de Soto who had whetted his appetite for gold while accompanying Pizarro to Peru. He outfitted an expedition and landed in Florida in 1539. The next four years he spent marching through the southern states seeking gold, until in 1541 he came to a vast river, now known as the Mississippi. As the party returned from the present states of Arkansas and Louisiana, de Soto died. Although he dissipated his fortune in a vain quest for riches, he accomplished a far worthier result in opening a vast territory for those who followed (Collins, 1946).

The Indian inhabitants of Florida were hunters. Of the four major groups, the Calusa occupied the lower west coast, the Tesesta inhabited the lower east coast, the Timucua were located in the north, and the Apalache -- west of the Aucilla River. Fire was used to chase out game, and hence, these people were responsible for the creation of large expanses of grasslands (Hunter et al., 1979).

The Spaniards soon became pragmatic and recognized Florida's natural resources after the quest for gold fizzled. Large expanses of the landscape were grazed after Ponce de Leon landed cattle on the western coast of Florida in 1521.

The Spanish period lasted until the early 1700's, and during that time cattle herds utilized the natural prairies such as Paynes Prairie in Alachua County. The Spaniards left the landscape mostly undisturbed. Much of their effort went into establishing the port city of St. Augustine in 1565 which is the oldest permanent European settlement in the United States.

It was under British rule that St. Augustine became an important port for ship timbers. It has been said that almost all merchantable live oak within a few hauling miles of a navigable stream was cut by 1823. This harvesting of wood increased the potential area for grazing. Furthermore, in those days, 70 percent of the woodlands burned annually so the sight and smell of wood's fire smoke was as much a part of the Florida scene as pine trees and thunderstorms (Hunter et al., 1979).

Development of Grazing Land in Florida

The production practices in Florida for growing range animals remained essentially the same for over 200 years until the automobile replaced the horse and wagon. A problem arose with cattle confronting cars on the Florida highways, resulting in ever-increasing injury and loss. In 1948 the state legislature passed the "no fence law" that required livestock owners to fence in their animals.

This was an important departure from the roaming range practices of the past. Florida has serious mineral deficiencies, and fencing helped

ranchers to form a mental image of the mineral problems on their land. The cattlemen were aware of the nutrient rich areas on their open range; the areas were referred to as "hospital farms" where a nutrient deficient animal could graze and convalesce before being returned to the herd (Becker, personal communication).

In 1930, before mineral supplements were generally used, a study of 7,100 cattle on 44,516 hectares of range was conducted in central Florida. On flatwoods the calf crop averaged 34 percent, and 68 percent of the newborn calves died prior to 30 months of age. The market value was $10.33 per breeding cow per year or $2.54 per hectare (Becker, personal communication; Henderson, 1956).

Improved forages and better management have helped to alleviate many nutritional deficiencies. Supplementation with vitamins, minerals, protein, and energy has been an important aspect of management. McDowell et al. (1980) surveyed mineral status of beef herds on four soil types in Florida and concluded that mineral deficiencies were area specific. They indicated that phosphorus, selenium, and zinc deficiencies were present in all four of the regions studied. Protein, vitamin A, potassium, sodium, copper, and cobalt deficiencies were found in certain regions and were related to seasonality. Magnesium deficiency was most often associated with cows grazing winter pastures.

In general, the most satisfactory way of providing minerals to grazing animals is through the use of one complete mineral mixture offered free choice. The mix should contain a minimum of 6-8 percent phosphorus, and ideally, the calcium level should not exceed twice the level of phosphorus. Iron, zinc, manganese, copper, and cobalt should

provide 50-100 percent of the daily requirements in 2 ounces of the mixture (Ammerman, 1979).

History and Evolution of Florida's Grazing Research

The first forage crop specialist at the University of Florida began work in 1917, and the Agronomy Department was established in 1921. Since then research on improved pastures has expanded tremendously. The grazing experimentation was founded upon the observations made by centuries of cattlemen who noted that lack of sufficiently high quality forage during the winter months limited the carrying capacity of the range, retarded the development of immature animals, and affected the performance of mature animals. Average weight changes of mature cows on flatwoods rangeland from June 1933 to March 1938 were as follows: March to June, 34 kg gain; June to September, 10 kg gain; September to December, 11 kg loss; and December to March, 38 kg loss (Henderson, 1956).

The first grazing trials in Florida were conducted in 1929 for the purpose of evaluating four perennial summer grasses for use on well drained sands. The grasses used and the annual beef gains in kg/ha were as follows: carpetgrass (Axonopus affinis Chase), 195; common bermudagrass (Cynodon dactylon (L.) Pers.), 202; common bahiagrass (Paspalum notatum Flügge), 216; and centipedegrass (Eremochloa ophiuroides (Munro) Hack.), 248. The popularity of carpetgrass among cattlemen in the flatwoods area and discovery in 1937 of the requirements for successful production of clovers led to grazing trials during 1942-1945 comparing carpetgrass alone, unfertilized; carpetgrass alone, fertilized; carpetgrass-lespedeza (Lespedeza striata (Thunb.) H. & A.); and

carpetgrass-white clover (Trifolium repens L.). Average annual gains in kg/ha were as follows: carpetgrass, 84; fertilized carpetgrass, 168; carpetgrass-lespedeza, 246; and carpetgrass-white clover, 695. Meanwhile, superior grasses became available through plant introduction and breeding. In grazing trials conducted between 1943-1947, three of the new grasses--'Pangola' digitgrass (Digitaria decumbens Stent.), 'Pensacola' bahiagrass, and 'Coastal' bermudagrass--fertilized with 227 kg of 6-6-6 annually in the spring, produced yearly beef gains averaging 46 percent higher than gains produced by carpetgrass in earlier grazing trials (Henderson, 1956).

The importance of forages in Florida is obvious. From 1950 to 1973 the forage-livestock industry grew by 70 percent. One-third of the total range and pasture land in Florida is now planted to improved forages. In 1973, 270 million kg of beef were produced, totaling $223 million in agricultural income. By 1985 production is expected to reach 425 million kg of beef (Agriculture in an Urban Age (AGUA) Report, 1974).

Grazing Animal Improvement

Adams (1982) describes the rancher's attitude and general perception of his world "back in the old days."

> In the 40's we had a lot of trouble raising cattle in Florida. Cattle were cheap and wild. Pastures were large and the small boned cows could outrun some of the horses. Salt sickness was prevalent and we knew little about curing animal deficiencies. Tick fever had been eradicated but the screw worms were eating the cattle alive. Half the year cattle were bogged down trying to find a drink of water, and the other half they were standing in water up to their sides. Our main interest was survival; for the cattle and ourselves. There was no source of breeding stock available that had the heat tolerance and quality to meet the needs of a cowman. (Adams, 1982, p. 91)

In the past 30 years, the selection of animals has revolved around adaptation to the Florida forages and climate. Breeders have sought to combine the adaptability of Zebu, Brahman, or native animals with the performance capabilities of European stock.

According to Crockett (1982), it became popular in the 1960's to use sires representing breeds developed in Europe to increase beef production, and this led to diverse breeding combinations in the subtropical zone of the Atlantic coastal plain and the Gulf coast states. Crockett feels that breeding progress can be made in subtropical environments using Brahman-derivative breeds such as the Beefmaster, Braford, Brangus, or Santa Gertrudis. Fields and Hentges (1979) state that the genetic base of Florida cattle is primarily of Brahman extraction.

The characteristics needed in a commercial Florida herd are as follows: (1) high fertility, (2) tolerance to the temperature and humidity, (3) good foraging ability, (4) good maternal ability, (5) satisfactory feedlot performance, and (6) satisfactory carcass quality (Koger, 1982).

Florida's Role in the Forage-Livestock Industry

What must exasperate Florida producers is that there is an enormous appetite for beef in the state filled by producers from outside the state. Only 26 percent of the beef consumed annually by 10 million Florida residents and their visitors is produced in Florida. The remainder represents about 340 million kg of beef shipped in by other means. Approximately 300 million kg of the beef introduced to Florida is trucked from the high plains area of the United States (Baker, 1980).

A recent survey by Spreen and Shonkwiler (1982a) identifies Texas, Kansas, Nebraska, and Iowa as the largest suppliers of finished beef to Florida.

A most baffling feature of the Florida beef market is that cattlemen are selling calves to stockers 2250 to 3550 kilometers distant who then sell to feeders who sell to packinghouses who in turn spend close to $28 million annually to truck finished beef to Florida (Baker, 1980).

The question arises, "Why does Florida have a cow-calf industry, and what prevents producers from breaking into the stocker and finishing phases?" This is not an easily answered question, but one fact is that Florida does not produce the quantity and quality of feed required to keep calves in Florida until they reach the consumer. The nagging thought, however, is that people simply do not persist in doing things in a way that is less profitable than some logical alternative.

There are inherent cost advantages in keeping a geographically dispersed cattle production system. Baker (1980) reasoned that by virtue of escalating transportation expenses an economic incentive would slow the exodus of calves out of Florida. Stegelin and Simpson (1980), Spreen and Shonkwiler (1982b), and Ikerd (1981) all gave an economist's explanation to the contrary. In short, the index of transportation services in mid 1981 was about 300 percent of the 1974 level. But, during this same period, the total marketing cost index has increased by about 290 percent since 1968 and has increased 215 percent since 1974. Therefore, costs of transportation services have increased only 3-5 percent more than other marketing costs over the past 10-15 years in spite of rapidly rising fuel costs (Ikerd, 1981).

What are these locational advantages of geographical dispersion for different phases of beef production? The cow-calf phase is land extensive, utilizing the less productive land suited to pastures but little else, as in Florida. Stocker operations are located on areas with high quality pastures such as the wheat pasture areas of Oklahoma, Texas, and Kansas. Major cattle feeding areas are located near the major feed producing areas, and slaughter plants for cattle are located in the areas where cattle are fed (Ikerd, 1981). Ikerd's cost analysis gives a $32 per head advantage for shipping cattle to the plains states and trucking the meat back for consumption.

The economies of scale have allowed more cost efficiency for larger feedlots and packinghouses making establishment difficult for independent, small competitors. Indeed, it is more likely in the next 20 years for a continued trend toward fewer and larger specialized operations in all phases of the system. The areas where cow-calf and stocker operations are dominant are likely to remain dominant because of unique land and pasture requirements that preclude significant changes in the structure of these industries. The Florida cattle inventory reflects this trend. Between 1955 and 1980 there was a 62 percent increase in Florida cattle numbers compared to a 25 percent increase for the entire United States; however, in 1960 less than 20 percent of calves were outshipped, whereas in 1980 over 80 percent were outshipped (Spreen and Shonkwiler, 1982b).

Beef cattle production is capital intensive. An estimate of costs for land and livestock investment is currently $5,000 per cow in the United States (Ikerd, 1981). When tight money supply and rising interest rates squeeze marketing margins, packers and retailers buy less

cattle and carcasses. Demand is low, supply is high, and cattle prices decrease. The cattle feeders make up their losses by offering less money for stocker animals. The stocker operators, in turn, pass on the higher interest costs to the cow-calf man. There will be little expansion in the cattle business with high interest rates and low priced calves (Ikerd, 1981).

The Florida Opportunity for Finished Beef

The southeastern United States has the largest surplus of stocker-feeder cattle in the country; the seven states in this area provide about 5.5 million more calves each year than are needed for herd replacement or fed in southeastern feedlots (Baker, 1979).

If the calf crop is to be fed until slaughter in Florida, economics will necessitate fast, continuous, efficient growth from weaning until slaughter. The calves must be grown on forage (pasture and/or silage) with or without grain supplement with a finishing phase in the feedlot on a high energy ration.

Immediately, it is obvious to wonder, "Where will the grain come from?" The entire southeast is a grain deficient area, and corn will continue to cost $20/ton more in Florida than in major feeding states (Baker, 1979).

The unpredictability of the Florida climate makes corn production a risky business. Horton et al. (1981) and Horton and Mislevy (1981) grew corn and sorghum silages in south Florida, and their results showed that both corn and sorghum can be grown successfully and economically using multiple cropping. The viability of an intensive beef cattle industry

in Florida will depend on the ability to produce these locally grown, high energy feeds.

Provided the necessary feeding systems were developed, more cattle would remain on Florida ranches. The question of whether increased cattle numbers should precede the development of feedlot and packing industries is "putting the cart in front of the horse." Efficient feeding systems must evolve first, the cattle build up will follow, and count on the feedlot and packing people to fend for themselves. These businesses simply want a consistent, dependable supply of feed and animals (Kaplan, 1981).

As Erwin Bryan Jr. of the Central Packing Co. put it, "It is my feeling that we already have the know-how to feed cattle in Florida. The weakest point in the chain, and always has been, is grazing of the feeder calves until they are big enough to go into the feedlot" (Bryan, 1981, p. 46).

In conclusion, it would appear that 20 years from now a totally enclosed, self-reliant beef industry in Florida could be operational. The most likely stimuli will be (1) the development of new forage species and management systems that permit a shift from grain-fed to forage-fed livestock operations; (2) increased irrigation costs on the western high plains, decreasing grain availability; (3) solution of the agronomic problems for year round feed availability in Florida; and (4) lower interest rates to allow economic growth in the cattle business. These factors could shift the geographical advantage to the southeastern United States.

Barriers to Forage Production in Florida

There are three phases of the forage system--production, harvesting, and utilization. By identifying the present weaknesses in these phases, combinations of native range and improved pastures and/or supplemental pastures will be developed in order to provide a distribution of forage throughout the year. An even distribution of forages, more than any other factor, will ultimately be responsible for increased beef production in Florida (Mott, 1982b).

The biggest limitation to well distributed forage production is probably seasonality. Most forage production occurs from April to September, and two-thirds of the yearly growth of perennials occurs between July 1 and October 1 (Mott, 1982b).

Uneven distribution of forage production throughout the year makes management difficult, and supplemental feeding on pasture is often needed to efficiently utilize the forage produced. Sloan Baker summarizes Florida's current forage production problems. He states that cool season forages--rye (Secale cereale L.), ryegrass (Lolium multiflorum Lam.), and clovers (Trifolium spp.)--are excellent, yielding good gains of 0.5 to 0.9 kg/day for 90 to 160 days. Dry matter yields, however, are sometimes low due to weather. Warm season forages are more productive but give half the daily gains. Dry weather often limits production in spring and autumn; while in summer, rainfall and humidity are high enough to make hay curing difficult. Results with grass silage have been disappointing due to insufficient quality for good gains with young cattle (Baker, 1979).

The Climate in North Central Florida

The climate ranges from subtropical-oceanic in south Florida to a typical low-altitude, continental frontal pattern in the north (Hunter et al., 1979). The average year may be divided into two seasons: a warm, rainy season receiving about 60 percent of the annual rain and a cooler, dry season. The warm, rainy season runs from about the middle of June to the end of September. The cooler, dry season dominates the remainder of the year.

The summer rain occurs as afternoon thunderstorms, generated by strong surface heating and fed by a double sea breeze convergence. During the winter months, the differential cooling of land and sea, the occasional presence of stagnated high pressure cells, and the formation of low level inversions caused by nocturnal cooling act to maintain a high degree of atmospheric stability, suppressing frontal activity. A decrease in frequency of frontal movement across northern Florida is one cause of periodic drought on the average of once every 7 years (Dohrenwend, 1978).

The Bermuda high is common throughout the year, centered in the Caribbean with its strongest effect during winter. If it were not for large bodies of warm water on either side of the peninsula, Florida would be as arid as the great subtropical deserts at the same latitudes (Dohrenwend, 1978).

Flatwoods and Their Potential for Grassland Productivity

There are 14,236,908 hectares of land area in Florida. At present, there are approximately 1.21 million hectares of improved pasture, 1.62

million hectares of native range, and 2.02 million hectares of forestland that provide some grazing.

Seventeen major natural vegetation types are recognized in Florida. The most important ecotype from the standpoint of hectarage and potential for animal production from forages is the flatwood site. Flatwoods occupy 4.05 million hectares or almost 30 percent of Florida's land area.

Most of the state's timber production also occurs on flatwoods. Open woodland consists predominantly of one to three species of pines: longleaf (Pinus palustris Mill.), slash (P. elliottii Engelm.), and pond (P. serotina Michx.). Understory produces many grasses such as chalky bluestem (Andropogon capillipes Nash), broomsedge bluestem (Andropogon virginicus L.), paspalums (Paspalum spp.), wiregrass (Aristida stricta Michx.), indiangrass (Sorghastrum secundum (Ell.) Nash), and panicums (Panicum spp.). Associated forbs include grassleaf goldaster (Chrysopsis graminifolia (Michx.) Ell.), partridge pea (Cassia fasciculata Michx.), and beggerweed (Desmodium sp.). Shrubs are predominantly saw palmetto (Serinoa repens (Bartram) Small), wax myrtle (Myrica cerifera L.), blackberry (Rubus sp.), and gallberry (Ilex glabra (L.) Gray).

Amidst the flatwood areas are small hardwood forests, many cypress ponds, prairies, marshes, and bay tree swamps. The wet areas support very desirable forages such as maidencane (Panicum hemitomon Schult.) and little blue maidencane (Amphicarpum muhlenbergianum (Schult.) Hitchc.). The flatwoods are inhabited by deer and hogs, quail, gray squirrels, and turkey; hence, this is also the most important community type in Florida for hunting.

The soils are acid (4.0 - 5.5 pH), of the Spodosol Order, with a 4 cm surface horizon colored grey to grey-black by the presence of organic matter. The 10 to 45 cm stratum is dominated by white leached sands and from 45 to 55 cm a hard pan is found--fine particles cemented together by sesquioxides and other compounds. From 55 to 90 cm depth a brown, tannin-stained sand exists followed by white sand with further increases in the profile.

Due to the hardpan formed on Spodosols in Florida, a perched water table is created that is beneficial in reducing the rapid rate of percolation through the sands but is a problem when rainfall is so great as to cause standing water above the soil surface.

The acid soil condition of the flatwoods makes it unsuitable for crop production; however, acid and wet tolerant species like limpograss (Hemarthria altissima (Poir.) Stapf et C.E. Hubb) are well adapted to these lands.

The Need for Research on Limpograss

The days of quantum leaps in beef gains, such as those achieved on improved forages over native range, are over. An excellent inventory of improved forages is now available, and possibly the old problem of seasonal production can be partially solved by incorporating limpograss into the yearly forage system. Limpograss is a stoloniferous, C_4, perennial, summer-growing grass that has good cool-season production. Limpograss is adapted to the flatwoods habitat, can produce an abundance of biomass, and can be stockpiled, ensiled, or made into hay.

Research evaluating the first four limpograss introductions from 1964 culminated in the release of cultivars 'Redalta', 'Greenalta', and

'Bigalta' in 1978. Since that time many limpograsses were collected and evaluated in hopes of further exploiting the germplasm. Beneficial characteristics of quality, yield, persistence, morphology, winter-hardiness, and adaptability to flatwoods have given researchers evidence of the potential of limpograss as a forage-producing grass for the future.

Limpograss Literature to Present: An Overview

The literature on limpograss species to date will be grouped into five phases: (1) origin, distribution, and description; (2) characterization; (3) pest screening; (4) small plot trials and forage response to grazing; and (5) animal response and systems management. Limpograss research has just recently reached phase five.

Origin, Distribution, and Description

There are probably no more than 12 described species of limpograss (Kretschmer and Synder, 1979), and the one of most agrnonomic importance is Hemarthria altissima (Poir.) Stapf et C.E. Hubb. Agrostologists guess Hemarthrias' origin to be in tropical Africa, but it is also found in Madras, Burma, Malaysia, Malay, Siam, Turkey, Nigeria, Italy, Ethiopia, Tanzania, Ceylon, Northern India, Southeast Asia, and Argentina (Bor, 1960; Bogdan, 1977; Chippindall, 1955). The species are desirable, robust perennial fodder plants found in wet habitats such as river banks, seasonally flooded river valleys, seasonal swamps, and so forth.

Due to plant exploration and redistribution, Hemarthria introductions are now found in Australia, Brazil, Bolivia, Columbia, Ecuador, Hawaii, Malawi, Mexico, New Zealand, Paraguay, Uruquay, Venezuela, and the Virgin Islands (Quesenberry et al., 1978; Quesenberry et al., 1981; and Oakes, 1980).

Overwintering of H. altissima in the United States occurs in Alabama, Mississippi, Texas, Tennessee, and as far north as Beltsville, Maryland (Oakes, 1973; Oakes and Foy, 1980). Later, Oakes (1980) reported the survival of one limpograss accession from the 1964 collection (PI 299039 from Rhodesia) for three winters at Pullman, Washington. This diversity of winterhardiness may be used to extend the production range and grazing period for limpograss in the United States.

Some common names for grasses of the genus Hemarthria are limpograss, teagrass (Florida), Capim gamalote (Brazil), Pasto clavel, Gramilla canita (Argentina), Baksha, Panisharu (India), Swamp Couch, and Rooikweek (South Africa) (Bogdan, 1977; Chippendall, 1955).

> A description of the genus follows: Member of the Andropogoneae tribe. Perennials with short rhizomes and long spreading, decumbent, branched culms that root at the nodes; the upper part of the stems erect or suberect reaching 150 cm height but usually 30-80 cm. Leaves up to 20 cm long and 6 mm wide with membraneous ligules. The spike-like racemes are compressed, 6-10 cm long with spikelets appearing opposite--each pair composed of a bisexual sessile spikelet, 5-6 mm long, and a smaller, pedicelled male spikelet. Glumes nearly equal. First glume flat, 2-keeled, leathery; second glume coriaceous, fused to the hollowed-out face of the rachis internode (Bodgan, 1977; Hall, 1978).

Quesenberry et al. (1982) surveyed the USDA collection of Hemarthria (containing 76 species) and found 72 percent diploid, 27 percent tetraploid, and 1 percent hexaploid. Thirteen of 16 tetraploids

were originally found north of 24 S latitude in Africa. Diploids were more winterhardy than tetraploids.

The 1964, 1971, and 1976 USDA plant exploration missions to Africa provided the germplasm for vigorous limpograss experimentation in the United States for the past 15 years. Jack Oakes did most of the collecting. Three cultivars were cooperatively released on 19 April 1978 from the Institute of Food and Agricultural Sciences, University of Florida, and the Soil Conservation Service, USDA. The limpograsses are Redalta, Greenalta, and Bigalta, previously PI's 299993, 299994, and 299995, respectively (Quesenberry et al., 1979).

Redalta and Bigalta are two of the four limpograsses used in this dissertation. Oakes obtained these genotypes from the Rietondale Research Station near Pretoria, South Africa, in 1964 (Oakes, 1973). Redalta originated near the Pienaars River in west central Transvaal, and Bigalta is from an unspecified location near the Transvaal. Redalta is a diploid (2n = 18), and Bigalta is a tetraploid (2n = 36). Also included in this dissertation are PI 364888, collected in 1971 from a small island in the Luvuvhu River several kilometers above its confluence with the Limpopo River in Kruger National Park, and PI 349753, from Mt. Mbya, Kenya. These two limpograsses are tetraploids (Oakes, 1973).

There are many morphological differences among limpograsses. Leafiness, internode length, anthocyanin content, bunchiness, tillers per unit area, and stem thickness vary. These inherent differences aid in maintaining genotype identification.

Cytological investigations (Wilms et al., 1970) characterized the four limpograsses collected from Africa in 1964, and reported color differences for the anthers of Redalta (purple), Greenalta (brown), and

Bigalta (yellow). Schank (1972) characterized chromosome numbers, pollen stainability, and seed set for 11 more Hemarthrias in preparation for an intrageneric breeding program. Breeding efforts to date have not produced any limpograsses with better agronomic attributes than the vegetatively propagated plant introductions. The logistics of low seed set (Schank, 1972) may partially discourage plant breeders in this regard, as well as the difficulty in getting limpograss to flower in the greenhouse (Quesenberry, personal communication).

Schank et al. (1973) discovered that tetraploid limpograsses had higher in vitro organic matter digestibility (IVOMD) than diploid accessions. The mean decrease of IVOMD of the tetraploid, from 68.4 percent at 5 weeks, to 66 percent in mature plants, suggested a slower decline of quality than for most tropical grasses. Cross-sections of stems revealed significantly lower vascular bundle area in the tetraploid, as well as fewer sclerenchyma fiber cells.

Characterization

One of the tangential aspects of the incipient research on limpograss was that it was noted as having a tea-like odor and flavor. Killinger (1971) after collaboration with the USDA Northern Utilization Research Laboratory, filed for a beverage use patent on 25 January 1971. Thus, the name "teagrass" came into usage first promulgated, in-so-far as can be determined, by Eldridge D. Lee of the University of Florida Agronomy Farm. Killinger and Beckham obtained United States Patent 3,709,694 for Hemarthria beverage rights in 1973.

Oakes and Foy (1980) recommended limpograss for revegetating mine spoils due to an excellent tolerance to aluminum toxicity. Oakes (1973),

Oakes and Foy (1980), and Oakes (1980) also reported a wide diversity of cold tolerance, as mentioned earlier.

A recent finding was an alleopathic effect of some limpograsses (Ruelke and Quesenberry, 1981; Young and Bartholomew, 1981; Tang and Young, 1982). The alleopathic compound was suspected by Ruelke and Quesenberry (1981) in their limpograss mixtures with red clover and the action was attributed to the clover. The red clover isoflavinoids (Tamura et al., 1967; Chang et al., 1969) and the phenolic compounds from limpograss roots (Young and Bartholomew, 1981; Tang and Young, 1982) may have both been active. Bigalta was more growth depressive on 'Greenleaf' desmodium (Desmodium intortum (Mill.) Urb.) than was Greenalta limpograss. Bigalta root exudates also depressed the growth of Greenalta.

A series of four papers characterized 10 tropical forage grasses in Puerto Rico. Limpograss was included in these studies. The main intent was to survey: (1) fibrous carbohydrate fractions, (2) proximate nutrient composition, (3) mineral composition, and (4) the decline of in vitro true digestibility with advancing maturity of 10 tropical forage grasses. The 10 grasses were guineagrass (Panicum maximum Jacq.), Pangola digitgrass, congograss (Brachiaria ruziziensis Germain & Evrard.), African crabgrass (D. swazilandensis Stent.), Venezuelan elephantgrass (Pennisetum setosum (Swartz) L. Rich. in Pers.), giant Pangola digitgrass (D. valida Stent.), signalgrass (B. Brizantha (Hochst. ex A. Rich.) Stapf), buffelgrass (Cenchrus ciliaris L.), jaragua (Hyparrhenia rufa (Nees) Stapf), and limpograss. The identity of the Hemarthria was not reported, hence, care must be exercised in

extrapolating these results to other limpograsses (acknowledging variations among and within ploidy levels).

Paper No. 1 (Coward-Lord et al., 1974a) on carbohydrate fiber fractions discussed neutral-detergent fiber (NDF), acid-detergent fiber (ADF), acid-detergent lignin (ADL), hemicellulose, cellulose, and silica. The NDF fraction represents the total fiber fraction, its difference from 100 being the neutral-detergent solubles (NDS), or soluble nutrients. The ADF content is a measure of the ligno-cellulose fraction. The difference between NDF and ADF is an estimate of hemicellulose. Acid-detergent lignin (by the permanganate method) is an acid treatment of the ADF which leaves cellulose and silica. Ashing leaves silica.

Results showed that limpograss was one of three grasses with the highest levels of NDF, among the four lowest in ADF content, the highest in ADL, the highest in hemicellulose, among the lowest in cellulose, and the lowest in silica. In other words, among the tropical grasses studied, limpograss had a high percentage of total fiber--comprised of high quantities of lignin and hemicellulose relative to cellulose.

In Paper No. 2, Coward-Lord et al. (1974b) studied nutrient composition including crude protein (CP), dry matter, crude fiber (CF), ether extract, ash, and nitrogen-free extract (NFE). This methodology is slowly losing favor over the Goering and Van Soest (1970) methodology for fractionating feedstuffs for ruminant value. The definition of CF as a chemically uniform, non-nutritive substance cannot be reconciled because CF represents almost all the potentially digestible cellulose and also includes some lignin and hemicellulose. The imperfect CF methodology has allowed most of the liginin and hemicellulose to be

included in the NFE, which is supposed to represent available carbohydrate. In some cases the CF can be more digestible than the NFE, which is clearly incongruous with the aim of the fractionation. Nevertheless, results showed that limpograss had the highest mean NFE value of all 10 species at 53.3 percent over 180 days. The CP values dropped to 5.7 percent by the 90-day stage.

The third Puerto Rican Paper (Arroyo-Aquilú and Coward-Lord, 1974a) covered mineral composition. The ranges as percent of dry matter over 180 days for all 10 species and the averages for limpograss (in parentheses) were calcium, 0.11-0.43 (0.15); phosphorus, 0.08-0.39 (0.13); magnesium, 0.15-0.46 (0.20); and potassium, 0.68-7.33 (1.85). Limpograss had the lowest phosphorus percent of all grasses at 180 days (0.08)--clearly deficient for ruminants; magnesium and potassium were lowest at 30 days, 0.25 and 3.0 percent, respectively.

The final Puerto Rican paper (Arroyo-Aquilú and Coward-Lord, 1974b) discussed quality decline among the 10 tropical grasses. The mean rate of *in vitro* true digestibility decline was 24.1 units from 30-180 days. The largest decline (12.3 units) occurred between 30-60 days as compared to declines of 4.8, 3.9, 1.3, and 1.8 units between 30 day intervals from 60-180 days. All the grasses reacted similarly. This suggests that the tropical grasses studied may best be utilized between 30-60 days of growth.

Hodges and Martin (1975) included three limpograsses in a study of 23 perennial sub tropical grasses and reported that *Cynodons* and *Digitarias* were better cool season producers than the limpograsses at Ona, Florida. Seasonal yield distribution of numerous tropical grasses was studied by Taylor et al. (1976b) in New Zealand. In this study, a

tetraploid limpograss (identity not reported) yielded poorly during the low rainfall summer of the year. The total warm-season yield for limpograss nearly doubled under trickle irrigation, and the moist, cool-season yields were twice as great as the unirrigated warm-season yields.

The digestibility data for 22 C_4 grasses were analyzed in another Taylor et al. (1976a) publication to compare nutritive quality of the grasses grown at Kaitaia, New Zealand (35 S latitude), with the same grasses grown in more equatorial environments. The results for grasses grown at Kaitaia, which has a mean warm-season temperature of 18.4 C, were compared to the digestibility data for the same species grown at Lawes, Australia, with a mean temperature of 24.3 C. Slower rates of maturation in the cooler environment frequently resulted in higher tissue digestibilities.

The non-flowering limpograss used in the New Zealand studies produced tissue with moderate protein (15.7 percent in leaves, 6.1 percent in stems), moderate fermentable carbohydrate (7.2 percent in leaves, 6.0 percent in stems), and excellent digestibility (66.8 percent in leaves, 77.2 percent in stems). The stem digestibility was the highest of all the grasses studied.

Pest Screening

A limited number of references are available on limpograss susceptibility to nematodes and aphids. Boyd and Perry (1969) screened Redalta, Greenalta, and Bigalta resistance to sting nematodes (Belonolaimus longicaudatus Rau). This nematode is among the most persistent, serious pests in Florida's improved pasture--Pangola digitgrass, Pensacola bahiagrass, and Coastal bermudagrass are all

susceptible. The limpograsses had moderately low nematode counts but the authors attributed this to lack of roots rather than significant nematode resistance. Chlorosis was observed in Bigalta, and this genotype had higher counts than the two diploid limpograsses. In 1970, Boyd and Perry reported additional information on sting nematode damage to 17 pasture grasses. Redalta, Greenalta, and Bigalta were the three most favorable hosts.

Boyd et al. (1972) studied the interaction of soil temperature and sting nematodes and found that Greenalta grew best between 20 and 38 C in uninfested soils and best between 30 and 38 C in infested soils. Above 38 C, nematodes were reduced but the soil was too hot for good growth of the grass. Later, Quesenberry and Dunn (1977) received 54 more limpograsses which they screened for response to the sting nematode in the greenhouse. No available limpograss lines approached immunity, but a few introductions had greater tolerance. Of the 10 best lines, 50 percent were tetraploid or hexaploid, while all the least tolerant were diploid. The most tolerant introductions were collected from the islands of Mauritius in the Indian Ocean.

Pest problems in limpograss were reported by Oakes (1978) who studied resistance in Hemarthria to the yellow sugar-cane aphid Sipha flava (Forbes). Variable resistance was found for 54 H. altissima accessions. Two of the introductions included in this dissertation were evaluated in the Oakes study--PI 364888 was most susceptible and PI 349753 was moderately resistant, while all the limpograsses had better resistance to the sugar-cane aphid than found in Digitaria.

Small Plot Trials and Forage Response to Grazing

The second and third collections of limpograss were initiated in response to previous experimental results that recommended an extended search within Hemarthrias for favorable pasture grass attributes. Ruelke et al. (1976) evaluated 53 limpograsses in both greenhouse and small plot trials and this study satisfied their exploratory curiosity with respect to the identification of superior genotypes.

In 1976-77, Quesenberry and Ocumpaugh (1977) grew Redalta, Greenalta, and Bigalta as conserved forages. They presented their results at the American Society of Agronomy meetings at Los Angeles in 1977, and with more detail (Quesenberry and Ocumpaugh, 1980; Ocumpaugh and Quesenberry, 1980) following a second year of data. Stockpiling is an inexpensive way of filling the forage-deficient months of November-February in northern Florida. Stockpiled yields were greatest for Redalta in 1976-77 (over 10 m tons/ha). Yields were similar for the three cultivars in 1977-78 and lower, averaging 6 m tons/ha (Quesenberry and Ocumpaugh, 1980). Based on these results from the Green Acres Unit near Gainesville, Florida, stockpiling should begin by the beginning of August to allow 6-8 weeks of growth before frost.

The above study did not include data on animal acceptance; however, the authors observed satisfactory consumption of mature Bigalta. Other producers (Wendy J. Carpenter, personal communication) have reported animal rejection of similarly aged Bigalta.

Quesenberry and Ocumpaugh (1982) presented data on the tissue sampled from the stockpiling experiments. Potassium decreased from a high of 2.5 percent to below the National Research Council (NRC)

recommended level for ruminants (0.65-0.80) by early November. Phosphorus dropped below the NRC minimum for ruminants (0.16-0.24) by mid-October and in the second year was never above the minimum level. Magnesium was not considered a nutritional problem, while the mean calcium percent for both years (0.28) was adequate for mature pregnant beef cows (NRC = 0.16) but barely sufficient for lactating beef cows (NRC = 0.27). The authors' recommendation was to supplement with potassium, phosphorus, calcium, and protein after mid-October.

Quesenberry et al. (1978) coalesced the pertinent limpograss production data through 1978 for six sites in Florida. At Ft. Pierce, Bigalta had higher production in November and December than Redalta or Greenalta, but produced less in spring. At Gainesville, the late season production of limpograss was slightly less than that of the digitgrasses and one bermudagrass. Bigalta, however, had the highest total season production. Frequent clipping defoliation at Ona produced weed invasions and the limpograsses had intermediate yields compared to other tropical, perennial grasses. Results from Jay demonstrated slow establishment rates compared to Coastal bermudagrass, 'Transvala' digitgrass, and one bahiagrass. From work done at Quincy, it was concluded that limpograsses are not adapted to the dry, upland soils of the Florida panhandle. At Belle Glade, Coleman and Pate (Quesenberry et al., 1978) found good digestibility and acceptability of Bigalta by beef animals; however, St. Augustinegrass (Stenetaphrum secundatum Kuntz) was better adapted to the muckland soils at this south Florida site. Bigalta did not persist under heavy grazing. This led to the recommendation of rotational grazing for Bigalta.

Rotational grazing is too intensive for most Florida producers. It is fortunate that some of the new limpograss introductions will persist under continual use because this is an important criterion in a ranchers mind (Pate, personal communication).

Ruelke (1978) studied Redalta, Greenalta, and Bigalta and found significant yield responses to nitrogen up to 330 kg/ha/yr; however, severe losses occurred following frequent defoliation at high nitrogen rates, especially for Bigalta.

Bigalta's high digestibility combined with poor persistence was disconcerting. Quesenberry et al. (1981) found that PI 364888 was a digestible, persistent tetraploid. This accession was soon included in the limpograss studies with Bigalta and Redalta, while Greenalta was omitted from experimentation due to its similarity to Redalta.

Ruelke et al. (1978) included the promising accession in a limpograss establishment study. Denser stands, earlier production, and higher second year dry matter yields were obtained when planting material was sprigged, followed by disking to partially cover the stems, and cultipacked for firm contact between sprigs and soil. In the year after establishment, PI 364888 outyielded both Redalta and Bigalta.

Kretschmer and Synder (1979) compared growth of Redalta, Greenalta, and Bigalta to Transvala digitgrass, Pangola digitgrass, and 'Coastcross-1' bermudagrass and found that a 2 week cutting interval severely decreased the ability of limpograss to accumulate dry matter. Frequent clipping also diminished the efficiency of nitrogen usage. Delaying autumn fertilization at Ft. Pierce, Florida, from 17 September or 1 October to 29 October resulted in greatly reduced forage production when harvested on 17 December. The later nitrogen fertilization,

however, resulted in a better combination of yield and quality by raising the inherently low protein content in mature limpograss. Bigalta responded to cool-season nitrogen fertilization but gave way to weed encroachment during the summer which led to the recommendation that it should be rested sometime in the warm season to maintain plant populations.

Quesenberry and Ocumpaugh (1979) studied clipping and grazing defoliation methods for three years. The mob grazing method shortened the time necessary to advance the new limpograss germplasm through the early phases of agronomic evaluation. Quesenberry et al. (1981) summarized the events leading to the clip/graze experimentation. After preliminary testing of 53 clones in greenhouses and small plot clipping trials, 22 were selected for evaluation by clipping and 27 by grazing. Eight of the best genotypes were then evaluated at four frequencies of grazing (3, 5, 7, and 9 weeks). Ocumpaugh et al. (1981) identified PI 364888 as superior to Bigalta under grazing due to comparable digestibilities and higher persistence.

Meanwhile, Ruelke and Quesenberry (1982) obtained more data on seasonal productivity for PI 364888. They found nitrogen fertilization increased early spring growth; however, the responses they obtained were limited due to effects of spring drought and cold temperatures. In the autumn they studied deferred forage characteristics and suggested that after 10 weeks of age forage quality would decline to a maintenance level, and by 20 weeks maturity the forage would be rejected. This statement may reflect a change in attitude with respect to stockpiling.

The differences in persistence among limpograss genotypes led researchers to ask "why?" They reasoned that a knowledge of

nonstructural carbohydrate metabolism should contribute to an understanding of limpograss behavior. Christiansen et al. (1981) studied etiolated regrowth as an indicator of the stored energy in four limpograsses. Some of the results indicated that morphological differences were related to observations of agronomic performance. Frequent cutting treatments caused a drain of the energy reserves of Bigalta but not PI 364888. This suggested greater reserve energy storage for PI 364888.

Animal Response and Systems Management

Hodges and Pitman (1981) studied Bigalta limpograss, 'Callie' bermudagrass, 'Sarasota' stargrass (Cynodon nlemfuensis Vanderyst), and 'Ona' stargrass under year long grazing. During the cool season, Bigalta produced average daily gains of 0.20 kg as compared to 0.23, 0.21, and 0.13 kg for Ona, Sarasota, and Callie, respectively. Warm season average daily gains were similar for the four grasses and twice as high as the gains produced in the cool season. The stargrasses had much higher yearly production as reflected by animal grazing days per hectare: Sarasota, 1089; Ona, 1072; Callie, 911; and Bigalta, 783. Beef production over both seasons averaged 585, 558, 450, and 431 kg/ha for Ona, Sarasota, Bigalta, and Callie, respectively.

Ocumpaugh (1982) is presently conducting the second year of a three year animal production study comparing beef gains on Pensacola bahiagrass and PI 364888 limpograss. Preliminary results from 1981 indicate better average gain per yearling heifer on limpograss (78.6 vs. 57.7 kg/yr) and a 70 calendar day advantage in grazing days compared to

Pensacola bahiagrass. Ocumpaugh states that PI 364888 is being considered for cultivar release in June 1983.

Nonstructural Carbohydrates in Forages

Plants are the primary source of carbohydrates. Cellulose is the most prevalent organic compound on earth and is man's most important industrial carbohydrate. The staple grains are predominantly starch which is the chief carbohydrate in the human diet (Greenwood, 1970). This review will be confined to a summary of the carbohydrates important in forages. Structural carbohydrates are beta-linked molecules that are degraded into more utilizable forms by rumen microflora. These simpler substrates are then converted into energy and animal products useful to man. The nonstructural plant carbohydrates can be completely utilized in animal diets and also provide the energy necessary for bacterial preservation of silage.

In plants, nonstructural carbohydrates are used in growth and respiration and have been called "food reserves." The main objective of this review will be to synopsize the evolution of non-structural carbohydrate research in pasture plants during the past 60 years.

Quantities of nonstructural carbohydrates in plants fluctuate during each day as well as during the season. Variations in geography, environment, taxonomy, anatomy, and management (or experimental conditions) all add to the dynamics of carbohydrate flux.

Reactions to management vary from year to year depending on environment, species, and stress; hence, there are conflicts in the literature due to incongruities between imminent and long term results, between animal and pasture requirements, and between applied and

basic objectives. Three generations of researchers have often perpetuated a benign acceptance of conventional wisdoms.

The overwhelming quantity of carbohydrate studies created a need for review articles to summarize the research findings. Many theories were proposed to consolidate research on different species grown under different conditions for different purposes. The most important elucidations were (1) the definition of what organic substances should be considered reserves, (2) what methods were best to satisfactorily fractionate carbohydrate components, (3) what role carbohydrates play in regrowth mechanisms, (4) where plant foods accumulated, (5) how nonstructural carbohydrates varied by day and season, and (6) how carbohydrates in storage organs were affected by management. The reader is referred to the following publications as a chronological guide: Graber (1931), Weinmann (1952, 1955, 1961), Troughton (1957), May and Davidson (1958), Hunter et al. (1970), Sheard (1973), Smith (1973b), White (1973), and Noble and Lowe (1974). For work prior to the 1930's, the reader is referred to the literature citations of Graber et al. (1927) and Graber (1931).

The important findings of Cugnac (1931) are alluded to by nearly all carbohydrate reviewers. He separated the grasses into two groups--the fructose accumulating grasses native to temperature climates and grasses accumulating sucrose and starch that are mostly adapted to warm regions.

Graber (1931) assessed the condition of "low" or "high" organic reserves in pasture plants by means of dry matter yields, persistence, and weed encroachment. He saw that plant growth behavior was related to

available nutrients and that quantities of organic food were likewise correlated.

The Characterization of Carbohydrate Reserves

Leukel and Coleman (1930) in Florida measured carbohydrate fractions of bahiagrass and claimed that hemicellulose was transformed to lignin and cellulose under long cutting intervals and reduced to simpler sugars for use in tissue synthesis with a frequent defoliation regime.

McCarty (1935, 1938) studied seasonal carbohydrate fluctuations in several range grasses and concluded that sucrose and starch were the stored foods in California bromegrass (Bromus carinatus Hook. and Arn.). McCarty (1938) also conjectured that hemicellulose may have been converted into simpler components.

Sullivan and Sprague (1943) showed no hemicellulose utilization in the regrowth mechanism of ryegrass (Lolium perenne L.). Fructosan was the key reserve substance and these results were reinforced by Waite and Boyd (1953a, b) and Waite (1957, 1985). The concept that hemicellulose participated in respiration or tissue synthesis was essentially discarded by the late 1940's; however, fructosan storage characterization in important northern adapted grasses continued (Sprague and Sullivan, 1950; Waite and Gorrod, 1959; Okajima and Smith, 1964; Smith and Grotelueschen, 1966; Smith, 1967; Grotelueschen and Smith, 1968; Smith, 1975). Smith (1968) cataloged the carbohydrate storage tendencies of many North American grasses.

Tropical legume and grass carbohydrate characterization occurred later and remains an active area of research. Hunter et al. (1970) found no fructosan accumulation in the tropical plants he studied.

Noble and Lowe (1974) showed smaller seasonal variation of alcohol soluble carbohydrates in tropical grasses than in temperate grasses, and Wilson and Ford (1973) found that temperate grasses accumulated much higher concentrations of soluble carbohydrate than the tropical grasses.

A Progression of Nonstructural Carbohydrate Methods Used in Agriculture

A simple measure of energy reserves is obtained by the regrowth of a defoliated plant in darkness. The technique involves the removal of the sod, usually a 15 cm diameter plug, and allowing the defoliated plants to regrow in darkness with adequate moisture until the energy producing materials are exhausted. The weight of the clippings produced during the period give an index of the regrowth potential of the plant. Sheard (1973) reviewed etiolated regrowth studies, and his oldest reference is that of Burton and Jackson (1962); however, the regrowth-in-darkness technique goes much further back in time.

According to Smith (personal communication):

> The first use of growth in darkness that I know of is the early work of L. F. Graber (1927). I have always been intrigued with the technique, probably from being a student of Dr. Graber's. Where Graber got the idea I do not know, but he did his Ph.D. work with Dr. Kraus, a botanist at the University of Chicago, who worked a great deal with carbohydrate/nitrogen ratios in the growth of tomatoes and who may have used darkness studies. The first person to use growth in darkness on grasses was Vance Sprague (Sullivan and Sprague, 1943) at the USDA Pasture Lab in Pennsylvania. He picked up the technique from Graber when he was his Ph.D. student, and Blaser got the idea from Sprague (Ward and Blaser, 1961).

Pretreatment in darkness to vary carbohydrate concentration was used by Davidson and Milthorpe (1966b) and others that used the regrowth-in-darkness experimentation were Adegbola (1966), Adegbola and

McKell (1966b), Alberda (1966), Humphreys and Robinson (1966), Raese and Decker (1966), Matches (1969), Watson and Ward (1970), and Christiansen et al. (1981). Christiansen et al. (1981) found improved sensitivity in separating treatment differences by using stem base weights as a covariable to control variations in plant size.

Concerns over the unreliability of chemical fractions were due to the use of acids in early applications of carbohydrate methodology. Fructosans are water- and ethanol-soluble, as well as readily hydrolyzed to fructose monomers by acid treatment. Consequently, Sullivan and Sprague (1943) complained that acid analyses of starch were confounded by the contribution of fructose from fructosan in with glucose from starch in tests of reducing power.

In 1947, Weinmann published a method for total available carbohydrate (TAC) determination in plants. Total available carbohydrate was defined as "all those carbohydrates which can be used in the plant body as a source of energy or as a building material, either directly or indirectly after having been broken down by enzymes" (p. 279). In Weinmann's method, small samples of finely ground air-dry material are digested by takadiastase in water resulting in the breakdown of starch, dextrins, and maltose to glucose, while other sugars and fructosan are solubilized at the same time. The latter compounds are converted to hexose sugars by acid hydrolysis, following which the reducing power of the cleared, neutralized hydrolysate is determined (Weinmann, 1947).

Lindahl et al. (1949) made slight modifications in the Weinmann TAC method and hailed the efficacy of the procedure in agronomic applications. Their major modification was to change the enzyme from dialyzed takadiastase to "clarase"--a highly purified and concentrated

form of takadiastase containing invertase, maltase, and amylase. This procedure satisfied most agricultural needs for TAC determination throughout the 1950's; however, Smith continued to examine acid techniques from his lab in Wisconsin. The acid methods were faster, requiring 8 hours to complete analysis compared to 12 hours working time plus 44 hours incubation for the enzyme method.

Most of the hindrance of using an acid method was in finding the proper concentrations. Smith et al. (1964) varied sulfuric acid concentrations from 0.2 N - 0.8 N and compared the results to takadiastase as a standard. The 0.2 N H_2SO_4 method most nearly duplicated the takadiastase extraction while higher concentrations degraded hemicellulose. The results were supported by Burris et al. (1967) who added that the chances of obtaining erroneous data with acids were greatest when they analyzed bermudagrass during rapid growth phases and with tissue high in starch.

Groteluesch en and Smith (1967) qualified earlier work (Smith et al., 1964) after examining alfalfa (Medicago sativa L.), high in starch content. For fructosan accumulating tissue such as timothy (Phleum pratense L.), containing little starch, dilute acid (0.005 N H_2SO_4) procedures may be used for nonstructural carbohydrate extraction. On the other hand, with tissue high in starch such as legumes and tropical grasses, acid strengths beginning at 0.2 N H_2SO_4 degraded hemicellulose and incompletely hydrolyzed starch as well.

Greub and Wedin (1969) supported Groteluesch en and Smith's 1967 findings and also warned that above 0.2 N H_2SO_4 free fructose or fructose liberated from fructosan was being destroyed. The enzymatic

method was more accurate, demonstrated again at a later date, in Portugal by Chaves and Moreira (1977).

In 1969 Smith wrote his widely accepted version of the Weinmann/Lindahl procedure. It was and still is the most popular titrimetric nonstructural carbohydrate method. Smith suggested the term total nonstructural carbohydrate (TNC) as a more clearly definable term than TAC to both plant and animal investigators. The advantage of the Smith method was its organized, complete, "cookbook" presentation which included an information review, appendices, criteria for selecting a method, and sample preparation. Smith (1981b) revised the TNC method with minor modifications including an enzyme change. It was found that Mylase 100 has rapid saccharogenic activity and will completely digest the starch in tissues containing 30 percent or less of TNC in 20 hours.

Advances in carbohydrate methodology in the past 10 years have been centered around the shift toward colorimetry to increase speed, sample number, and efficiency (Haslemore and Roughan, 1976; Weier et al., 1977; daSalveira et al., 1978; Westhafer et al., 1982).

In colorimetric methods, specific color reactions for portions of carbohydrate molecules are due to formation of furfural or furfural homologues in strong acids, especially following heating. These furans or their reaction products are derived from oxidation, reduction, or condensation processes in strong acid and can form colored products upon reaction with sugars. There are treatmendous variabilities associated with the reactions depending on the sugar, reagent concentration, temperature, and time of heating (Dische, 1962). The search for specific, quantitative, and reproducible colorimetric assays for the determination of carbohydrates is an important area of research.

Colorimetric tests have advantages over titrimetric procedures due to their speed and equivalent precision. Two distinct steps involved in colorimetric reactions are (1) formation of a chromogen from the sugar, and (2) development of the color by a condensation of the chromogen with a specific reagent (Aminoff et al., 1970). It is important to recognize that most colorimetric methods are empirical. Since little is known about the reaction mechanism or the exact nature of the chromogen involved, absolute stochiometry is not often obtained. As long as the results obey Beer's Law within appropriate limits of concentration, the problem can be solved by using appropriate internal standards and blanks.

In quantitative analysis, two divergent objectives must be considered: (1) an overall analysis of all the sugars present by a very general reaction, and (2) the selective determination of one sugar in the presence of others. For example, Westhafer et al. (1982) found sucrose levels in turfgrass root tissue to have the most dramatic response to nitrogen treatment; thus, a TNC method would be less sensitive measure of carbohydrate concentrations changes than a test of sucrose.

The principal chemical methods for quantitative measurement of sugars use the action of sugar reduction on alkaline solutions of the salts of certain metals. The most extensively used metal in sugar analysis is copper. Most of the agricultural needs for quantitative sugar analysis have been satisfied with the titrimetric method of Somogyi (1945, 1952) and the following colorimetric techniques: phenol-sulfuric (Dubois et al., 1956), anthrone, and Nelson's (1944) test for

reducing sugars. An excellent discussion of reducing sugar techniques is given by Hodge and Hofreiter (1962).

Organic Substances Used in Regrowth

Perhaps the biggest controversy in the history of nonstructural carbohydrate research was conclusively proving that food reserves were used in the synthesis of new growth. May and Davidson (1958) stated that a general acceptance of the importance of carbohydrate reserves in regeneration seemed unjustifiable and argued that no causal role in shoot regrowth was suggested by decreases of carbohydrate in storage organs. May (1960) dismissed as conventional wisdom the Graber and Weinmann definitions of "reserves" on the basis that the term had become semi-technical and no longer subject to criticism. May (1960) also cited Archbold (1945) and Bernatwoicz (1958) who discarded the idea of stored sugars as a "purposive" reserve--"reserve" connotating provision for the future and as such signifying teleological thinking. A better definition was "accumulate" since it was non-committal concerning purpose or intent.

Carbon balance and labeling studies put an end to the controversy (Marshall and Sagar, 1965; Alberda, 1966; Davidson and Milthorpe, 1966a; Ehara et al., 1966; Wardlaw, 1968; Watson and Ward, 1970; Sheard, 1973). Davidson and Milthorpe (1966a) demonstrated that nitrogenous organic compounds were also mobilized under heavy stress to the plant; therefore, carbohydrates were only part of the labile pool used for respiration and new growth.

Location, Seasonal, and Daily Fluctuation of Reserve Foods

Nonstructural carbohydrates may be temporarily stored in all plant parts. Troughton (1957) concluded that the major storage regions were in the underground organs. Many other studies, however, have shown that major storage parts are stem bases, including stolons, corms, and rhizomes (Sullivan and Sprague, 1943; Baker and Garwood, 1961). Waite and Boyd (1953a, b) and Smith (1967) found percentages of fructosans and most sugars to be higher in the stems than in leaves in temperate grasses. For three tropical species, Hunter et al. (1970) found a higher concentration of reducing and total sugars in the stems than leaf blades plus sheath. Perry and Moser (1974) reported on the TNC content of eight range grasses and stressed the importance of locating the specific storage organ(s) of a grass before proceeding with TNC analysis.

The products of photosynthesis may be held in the leaf blades as sucrose or starch. Since sucrose is the main translocatable sugar and starch is stored in chloroplasts, it explains the frequent measurement of high amounts of these substances in leaves (Greenfield et al., 1974), especially with cool temperatures (Garrard and West, 1972; Carter and Garrard, 1976).

The environment governs seasonal assimilate distribution and carbohydrate metabolism is greatly affected by temperature. The accumulation of carbohydrates at low temperatures indicates that growth rates are more affected than are photosynthetic rates, and there is no conclusive evidence that reduced translocation is ever the primary cause of limiting growth under low temperatures (Wardlaw, 1968). Hence, for both temperate and tropical species, carbohydrate accumulation occurs in the autumn or cool season. However, in temperate areas autumn turns to

freezing winters and TNC reserves are necessary for survival until spring. Once perennial forages begin growth in the spring, TNC levels generally increase through vegetative stages to anthesis and later the carbohydrate reserves decline slowly through the summer when hot night temperatures cause high rates of respiration. Studies to support the above hypothesis for temperate grasses were published by Waite and Boyd (1953b), Baker and Garwood (1961), Trlica and Cook (1972), Smith (1975), and Mislevy et al. (1978).

For many tropical growing grasses carbohydrate accumulation occurs during the cool or dry season and carbohydrate drain is most intense during the summer due to high night temperatures and very active growth. It could be speculated that plant survival is dependent upon carbohydrate accumulations during the cool seasons. In northern Florida, for instance, frost will kill most above ground herbage but warm day temperatures and adequate day lengths permit basal leaf growth in species such as limpograss (Gaskins and Sleper, 1974). Studies showing the accumulation of carbohydrate in tropical grass species during the cool season were reported by Woods et al. (1959), Ferraris (1978), and Wilson and t'Mannetje (1978).

Daily carbohydrate fluctuations occur for all species but to different extents. Holt and Hilst (1969) showed that bromegrass (Bromus inermus Leyss.) utilized almost one-third of the TNC in the herbage during the night, but diurnal fluctuations were less for other grasses. Greenfield and Smith (1974) studied switchgrass (Panicum virgatum L.) and found that the diurnal trend was an increase of total sugars and starch from 6 am to 6 pm and then a decrease to midnight. Basal sheaths and internodes tended to increase in percent starch and TNC from 6 am

to midnight. Since these are storage parts, carbohydrates were presumably being translocated continuously from upper parts to these lower sinks for storage, especially after 6 pm.

Management Factors Affecting TNC: An Integrated Approach

Applied management of forage plants must optimize yield, quality, and plant persistence. Plant behavior can be modified by cultural practices but these procedures must integrate the plant physiology involved in maintaining vigor. Too often experiments disect plant response in order to study one variable at a time. A holistic approach that takes into account environment and plant growth stage will be necessary for management systems to be of any practical use.

While it is generally known that carbohydrate accumulation varies inversely with the growth rate of the plant (McCarty, 1935; Brown and Blaser, 1965; Colby et al., 1965; Blaser et al., 1966), few management systems are based upon these findings. Carbohydrate analyses alone cannot unambigously identify a superior management regime because of confounding variations in residual leaf area, crown structure, axillary bud number, leaf age, and altered root characteristics (Humphreys, 1966).

Much effort was expended in the understanding of residual leaf area, light interception, and carbohydrate reserves in explaining regrowth following defoliation (Ward and Blaser, 1961; Pearce et al., 1965; Davidson and Milthorpe, 1966b; Humphreys and Robinson, 1966). When moisture and nutrients are in adequate supply, residual leaf area is generally more important than food reserves; however, this concept

of explaining regrowth is dependent upon the intensity of defoliation, the presence or absence of buds, the age of leaf tissue, and species differences. Further, there is no clear relationship between rate of growth and leaf area index. Hence, this is an oversimplified model on which to base a management system (Milthorpe and Davidson, 1966).

The role of hormones in releasing apical dominance during frequent defoliation is often neglected. Certainly some plants have a better ability to adopt a prostrate growth habit with many small leaf blades that maintain assimilate supply to the plant. The classical study of bermudagrass by Weinmann and Goldsmith (1948) as reviewed by Weinmann (1961) comes to mind. Close cutting of a well fertilized green of Cynodon dactylon 91 times in a season did not result in TAC depletion due to high residual leaf area; however, complete defoliation by means of scissors, repeated at weekly intervals, nearly exhausted TAC reserves. Graber (1931) and Leukel and Coleman (1930) recognized the ability of plants to defend against frequent defoliation by altered morphology.

Nitrogen fertilization will stimulate herbage growth and, in general, will cause a reduction of carbohydrate reserves as they are used as carbon skeletons for protein synthesis (Waite, 1958; Alexander and McCloud, 1962; Colby et al., 1965; Adegbola and McKell, 1966a; Alberda, 1966; Auda et al., 1966; Gallaher and Brown, 1977; Wilson and t'Mannetje, 1978).

Nitrogen fertilization can cause increases or decreases in carbohydrate storage depending on the amount applied and time of sampling (Sprague and Sullivan, 1950). Carbohydrate reserves are only utilized for a short time following defoliation (2 days--Davidson and Milthorpe,

1965, 1966a, b; 6 days--Ehara et al., 1966; 7 days--Sullivan and Sprague, 1943). Moderate nitrogen fertilization promotes growth, photosynthesis, and TNC storage; therefore, sampling too late in non-stressed plants will not show a carbohydrate decline.

In frequently cut and highly fertilized swards, carbohydrate drain from storage organs can continue to the detriment of plant persistence. Alberda (1966) reported the death of tillers following severe defoliation stress and stated, "It may be supposed that a considerable part of . . . these tillers is broken down and translocated to the remaining tillers to be used for new leaf formation, but this has not been proven" (p. 147).

The rate of degradation of a stressed sward is accelerated once a state of tiller degradation occurs. As nitrogen fertilization forces herbage growth and frequent cutting disallows full leaf expansion, little assimilate is mobilized to roots and buds (Wardlaw, 1968). Energy reaching the roots is inadequate to meet the needs of respiration, root growth, and absorption have slowed or stopped, and root degradation occurs as other substances are scavenged as a last defense (Davidson and Milthorpe, 1966a).

Further complications in this theoretical example of high plant stress are imposed by aggressive weeds competing for nutrients, moisture, and light. Due to the combined effects of all the above factors, the botanical composition of the desired species declines and the sward degenerates. In conclusion, when TNC studies are used as a single tool complemented with other information, they greatly aid in understanding the total dynamics of plant behavior. However, emphasis must be placed on a balanced understanding of all factors involved in plant growth if

it is the objective of research to lead to sound management recommendations.

CHAPTER 1
REGROWTH IN DARKNESS AS INFLUENCED BY PREVIOUS CUTTING TREATMENT OF FOUR LIMPOGRASS GENOTYPES

Introduction

Limpograss (Hemarthria altissima (Poir.) Stapf et C.E. Hubb) is native to the humid subtropics of Africa. A brief resume of its introduction into American grassland agriculture has been reported by Oakes (1973). Agronomic evaluations (Kretschmer and Snyder, 1979; Quesenberry et al., 1978; Quesenberry and Ocumpaugh, 1980; Ruelke et al., 1978) have generated information supporting the view that limpograss has forage potential in subtropical regions and on soils that are intermittently flooded. This study of etiolated regrowth was performed as a means of characterizing the energy reserves and morphologies of four limpograsses.

The regrowth-in-darkness technique has been used by many investigators (Adegbola, 1966; Adegbola and McKell, 1966b; Burton and Jackson, 1962; Dovrat and Cohen, 1970; Matches, 1969; Raese and Decker, 1966; Ward and Blaser, 1961; Watson and Ward, 1970). The objective of this study was to determine whether frequent clipping in the field could reduce energy reserves below the critical amounts necessary to maintain stands through the dormant season. Although chemical tests were not conducted, much evidence exists substantiating a strong correlation between etiolated regrowth and nonstructural carbohydrates in storage organs (Adegbola, 1966; Adegbola and McKell, 1966b; Dovrat and Cohen, 1970; Raese and Decker, 1966).

No carbohydrate studies of limpograss could be found; hence, it was desired to learn the location of carbohydrate storage in these stoloniferous C_4 grasses before full scale chemical analyses were conducted in later phases of research.

Materials and Methods

A clipping study was conducted on four well-established plantings of limpograss on a Wachula sand, a poorly drained siliceous hyperthermic ultic haplaquod soil at the Beef Research Unit of the University of Florida near Gainesville. The grasses were PI 349753, PI 299995, PI 299993, and PI 364888. Grasses PI 299995 and PI 299993 have been released as 'Bigalta' and 'Redalta', respectively. Redalta is a diploid (2n = 18) and the other genotypes used here are tetraploids (2n = 36). The four blocks of grasses representing genotypes were unreplicated; hence, the design was a split plot without replication of main plots. The genotype*treatment (rep) term was used to test genotype, treatment, and interaction effects. Within main plots, clipping treatments were randomly assigned with three replications. Clipping treatments were initiated by mowing all plots to 5 cm on 27 July 1979 and harvesting at 2.5, 5, and 10 week intervals until 4 October 1979. Additional areas without replication were reserved to allow limpograss top growth to reach 15 and 25 weeks of age by 14 November 1979, when the regrowth-in-darkness Experiment 1 began.

The limpograsses were fertilized 28 May and 30 July 1979 with 280 kg/ha 17-5-10 (N-P_2O_5-K_2O) containing 1 percent of a microelement mix. Soil test results from samples taken on 3 December 1979 showed a pH of 6.6, 27 kg/ha phosphorus, and 22 kg/ha of potassium.

Experiment 1

On 14 November 1979, 15 cm diameter cores were removed from the 2.5, 5, 10, 15, and 25 week treatments in each of the four limpograss plantings. The cores were placed in black plastic pots with the plant material trimmed to 2.5 cm height. Two subsamples were taken from each of the 2.5, 5, and 10 week field plots, and six samples were taken from the areas assigned a 15 and 25 week cutting interval. Hence, there were six pots containing limpograss for each treatment, and they were arranged on shelves in a dark room using a randomized, complete block design. A small electric heater was used to maintain the air temperature at approximately 30 C. Plants were watered and sprayed with fungicide when necessary. The study was terminated after 3 weeks when growth had ceased. "Shoot" regrowth was removed before the remainder of the plant was washed free of soil and separated into "roots" and "stubble." All three components were then dried at 60 C and weighed. The dry matter (DM) yields were subjected to analysis of variance and regression analysis. The mean yields for treatments were subjected to the Waller-Duncan Multiple Comparison Test.

Data were analyzed using the Statistical Analysis System (SAS) on an Amdahl 470 V/6-11 with OS/MVS Release 3.8 and JES2/NJE Release 3. Computing was performed at the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

Experiment 2

A second etiolated regrowth experiment was begun on 6 February 1980 using new cores of PI 364888 from each of the five treatments described in Experiment 1. In this study plants were trimmed to ground level leaving short "stem bases" below ground. A completely randomized design was used with nine replications in the dark room. The regrowth period was 3 weeks in length, and plants were processed as in Experiment 1. In Experiment 1, various statistical models were employed to explain the etiolated regrowth as a function of the weight of "stubble" and/or "roots," and in Experiment 2, the reduced "stubble," i.e., "stem base" weight, was selected as a covariable in order to increase precision by accounting for variations in plant size.

Residual Effects

The limpograsses were clipped at a height of 5 cm on 30 April 1980. The forage was collected and dried at 60 C, weighed, and analyzed for yield differences created by the 1979 clipping treatments.

Results and Discussion

Experiment 1

The results obtained in Experiment 1 are summarized in Table 1. Clipping treatments caused etiolated regrowth yields to be different for PI 349753 and Bigalta ($P = 0.026$ and $P < 0.01$, respectively), whereas Redalta and PI 364888 were not affected by previous cutting treatment in the field ($P = 0.138$ and $P = 0.157$, respectively). For both PI 349753 and Bigalta, a maximum regrowth was observed at the 10 week cutting

Table 1. Average DM yields of etiolated "shoots" for four limpograsses following 3 weeks of growth in darkness

Clipping frequency (weeks)	Limpograss number or name			
	PI 349753	Bigalta	Redalta	PI 364888
	g/pot			
2.5	0.74 ab*	0.95 ab	0.43 a	0.36 a
5	0.76 ab	0.81 b	0.36 a	0.45 a
10	0.87 a	1.08 a	0.62 a	0.42 a
15	0.52 b	0.34 c	0.65 a	0.45 a
25	0.51 b	0.41 c	0.55 a	0.63 a

*Values within each column followed by the same letter are not significantly different ($P < 0.05$) based on the Waller-Duncan Multiple Comparison Test.

frequency with lower yields of regrowth for the 15 and 25 week treatments. Within the field plots representing the two longest cutting frequencies, new growth was noticed emerging from plant bases below a dense canopy. It is believed that this light-starved growth in PI 349753 and Bigalta contributed to a respiratory drain of the energy reserves. The same trend for lower DM yields in the 25 week treatment was observed for Redalta but not for PI 364888 which had more resistance to lodging allowing more light to reach new tillers beneath the canopy.

Weights of "shoot," "root," and "stubble" components were averaged for all cutting frequencies in order to compare plant form among limpograsses. As shown in Table 2, Bigalta and PI 349753 had significantly heavier "shoot" weights compared to Redalta and PI 364888. Bigalta and PI 349753 also had the lowest "stubble" weights. These data agree with field notes characterizing Bigalta and PI 349753 as having larger but fewer "shoots" per unit area than Redalta and PI 364888. Of the four grasses, PI 349753 had the lowest "root" weight. Redalta has a bunch-type growth habit and a large "root" mass, both of which may contribute to its excellent persistence.

These results suggest that the large amount of "stubble" in Redalta and PI 364888 might have provided a reservoir of energy reserves which buffered the cutting pressure on these two lines. Matches (1969) showed higher regrowth yields with increasing height of cutting, indicating an energy reserve sink in the stem bases of tall fescue (Festuca arundinacea Schreb.).

In this study of limpograss there was a possibility that a lower cutting height in darkness would reduce sink size and separate treatment effects in a significant way.

Table 2. Comparison of etiolated "shoot" DM yields and associated "root" and "stubble" components for four limpograsses averaged for all cutting treatments following 3 weeks of growth in darkness

Limpograss	Component		
	"Shoot"	"Root"	"Stubble"
	g/pot		
PI 349753	0.68 a*	9.66 c	2.46 c
Bigalta	0.72 a	14.47 b	2.33 c
Redalta	0.52 b	17.46 a	3.14 b
PI 364888	0.46 b	12.36 b	4.71 a

*Values within each column followed by the same letter are not significantly different ($P < 0.05$) based on the Waller-Duncan Multiple Comparison Test.

Experiment 2

The etiolated "shoot" weights from Experiment 1 were modeled as a function of the cutting treatments and "roots"; cutting treatments and "stubble"; and cutting treatments, "roots," and "stubble." The reduced models, which explained regrowth yields as a function of treatment and "stubble" (or treatment and "roots"), described etiolated regrowth as well as the full model for all four limpograsses. "Root" weights were affected ($P = 0.006$) by treatments in Experiment 1, whereas "stubble" weights were not ($P = 0.712$). Covariables are not supposed to be affected by treatments, and since the "stem bases" from Experiment 2 were actually that portion of the "stubble" below ground, the "stem base" weight was used as a covariable to explain some residual error in the analysis of Experiment 2 regrowth.

Analysis of variance of etiolated regrowth in Experiment 2 did not detect treatment differences ($P = 0.180$) but analysis of covariance did ($P = 0.026$). Table 3 shows the Waller-Duncan Multiple Comparison Test of average DM yields of "shoots" obtained for each clipping frequency in PI 364888. "Shoot" yields were lower and the range narrower than found in Experiment 1 for PI 364888, but the data followed the same trend. Lower yields can be attributed to the loss of some energy reserves that could be located in the above ground stem tissue removed prior to the start of Experiment 2.

Results agree with those of Matches (1969), who suggested that with shorter heights of cut the ranking order of treatments would remain nearly the same but the magnitude of difference of energy reserves might be less. Matches also stated that in etiolated regrowth experiments

Table 3. Average DM yields of etiolated regrowth from Experiment 2 following 3 weeks of growth in darkness

Clipping frequency (weeks)	PI 364888 "shoots" (g/pot)
2.5	0.28 c*
5	0.32 bc
10	0.34 abc
15	0.38 ab
25	0.42 a

*Values followed by the same letter are not significantly different ($P < 0.05$) based on the Waller-Duncan Multiple Comparison Test.

higher cutting heights permit more regrowth and allow greater differentiation of treatment effects. That statement is further substantiated by Watson and Ward (1970) who demonstrated a 25 percent reduction in food reserves when cutting height was reduced from 7.5 to 2.5 cm in dallisgrass (Paspalum dilatatum Poir.).

In this study the results of Experiment 2 show that trimming the plants to ground level required a covariable ("stem base" weight) to detect treatment differences. However, reducing rather than increasing residual plant height together with the small differences in regrowth yields between Experiment 1 and Experiment 2 inferred that the majority of food storage occurs in the very basal portions of the culm ("stem base").

Residual Effects

Yield data obtained from 30 April 1980 harvest of the limpograsses showed no evidence of a residual treatment effect on the sward. No sward damage was observed even in the limpograss plots harvested for four cycles of 2.5 weeks. Hence, the limpograsses were not sufficiently stressed to cause stand deterioration in 1980.

Conclusions

1. Experiment 1 showed that etiolated regrowth weights were maximized for the 10 week clipping treatment in Bigalta ($P < 0.01$) and PI 349753 ($P = 0.026$), but no differences were found due to previous cutting for Redalta ($P = 0.138$) or PI 364888 ($P = 0.157$).

2. Following the complete removal of above ground stem tissue at the start of Experiment 2, the production of considerable etiolated growth suggested energy reserve storage lower on the culm.

3. "Stem base" weights were used as a covariable in Experiment 2, and differences ($P = 0.026$) were found in yield of etiolated regrowth due to previous cutting pressures on limpograss PI 364888 with longer cutting intervals allowing greater yields of regrowth.

Summary

Limpograss (*Hemarthria altissima* (Poir.) Stapf et C.E. Hubb.) research has reached a stage where management recommendations are needed to fully implement limpograss' usefulness for the large hectarage of improved pastures on Spodosols like Florida's flatwood soils. A preliminary study of energy reserves in Redalta, Bigalta, and two other promising introductions was conducted using the regrowth-in-darkness technique.

Cutting frequencies of 2.5, 5, 10, 15, and 25 weeks on limpograss swards in the field were imposed to establish various levels of reserves. The reserve energy pool was then measured by regrowth yields of plant cores placed in a dark room.

Two regrowth-in-darkness experiments were conducted. Experiment 1 used a randomized, complete block design with six replications harvested at 2.5 cm "stubble" height. "Stubble" was classified as all the stem between the cutting height and "roots." The first experiment showed that etiolated regrowth weights were maximized for the 10 week clipping treatment in Bigalta and PI 349753 ($P < 0.01$ and $P = 0.026$, respectively), but Redalta and PI 364888, which had higher "stubble"

weights, showed no treatment effects on weight of etiolated "shoots." In Experiment 2, PI 364888, the line with the highest "stubble" weight from Experiment 1, was evaluated using a completely randomized design and nine replications with all plant material removed to ground level. Hence, only the "stem bases" remaining below the soil surface were responsible for regrowth in Experiment 2. No treatment effects were found ($P = 0.180$) until the data were analyzed with covariance techniques using "stem base" weight as a covariable. Statistical sensitivity improved, and increases ($P = 0.026$) in regrowth potential were detected with clipping treatments of a longer cutting interval. The covariable analysis represented an improvement in the regrowth-in-darkness technique.

Removing all plant material to the soil level prior to the beginning of the second experiment left 2-3 cm of "stem base" below ground. The close similarity of regrowth yields in Experiment 1, where plants were clipped to 2.5 cm above the soil surface, and those of Experiment 2 having no above ground tissue, suggested that the energy reserves were predominantly located in the bottom 2-3 cm of the stem.

CHAPTER 2
CUTTING FREQUENCY EFFECTS ON LIMPOGRASS MORPHOLOGY AND TOTAL NONSTRUCTURAL CARBOHYDRATE RESERVES

Introduction

Limpograss (Hemarthria altissima (Poir.) Stapf et C.E. Hubb) has promise as an adapted, warm-season grass for Florida's vast hectarage of acid flatwood soils. Unfortunately, 'Bigalta', most favored by ranchers because of its high forage quality, is also the least persistent of the cultivars which have been released (Quesenberry et al., 1978; Ruelke, 1978; Kretschmer and Snyder, 1979; Quesenberry et al., 1981). Other studies of limpograss have identified a few promising plant introductions that have comparable quality and better persistence than Bigalta (Ruelke, 1978; Quesenberry and Ocumpaugh, 1979; Ocumpaugh and Quesenberry, 1980; Ocumpaugh et al., 1981; Ocumpaugh, 1982).

In Chapter 1, Christiansen conducted regrowth-in-darkness experiments with limpograss that indicated the area of compressed, lower nodes on the stem base as the major site of energy reserve in these robust, stoloniferous, perennial fodder plants. The regrowth-in-darkness technique is a simple way to measure energy reserves by analyzing regrowth from defoliated plants placed in darkness (Burton and Jackson, 1962; Sheard, 1973). Perry and Moser (1974) stressed the importance of locating the specific carbohydrate storage organ of a grass before proceeding with total nonstructural carbohydrate (TNC) analyses. Studies of TNC content were deemed necessary to verify the regrowth-in-darkness results.

Many researchers have indicated a positive correlation between etiolated regrowth and TNC in the storage organs (Adegbola, 1966; Adegbola and McKell, 1966b; Dovrat and Cohen, 1970; Raese and Decker, 1966). Therefore, it was of interest to see if this positive correlation held for limpograss TNC versus etiolated regrowth.

Leukel and Coleman (1930) and Graber (1931) discussed the ability of various forage plants to transform an upright habit to a prostrate habit following frequent defoliation. Some limpograsses may be suspected to have greater flexibility than others in altering their growth habit. The objective was to study morphological changes induced by frequent cutting and elucidate subsequent differences in TNC accumulation for two promising and two released cultivars of limpograss.

Materials and Methods

The experiments to follow were conducted on four well established plantings of limpograss on a Wachula sand; a poorly drained siliceous hyperthermic ultic haplaquod soil, at the Beef Research Unit of the University of Florida. The grasses were PI 349753, PI 299995, PI 299993, and PI 364888. Both PI 299995 and PI 299993 have been released as Bigalta and 'Redalta', respectively. Redalta is a diploid (2n = 18) and the other genotypes used here are tetraploids (2n = 36).

The limpograsses were fertilized 28 May and 30 July 1979, with 280 kg/ha of 17-5-10 (N-P_2O_5-K_2O) containing 1 percent of a microelement mix. Soil test results from samples taken on 3 December 1979 showed a pH of 6.6, 27 kg/ha phosphorus, and 22 kg/ha of potassium.

Analysis of TNC in Plant Parts

Prior to the layout of the clipping experiment, whole plant samples of limpograss were taken on 3 July and 26 July 1979, representing 6 and 9 week old plant maturities, respectively. Five large plants of each genotype were randomly selected, dug, washed free of soil, and arranged in cotton sample bags. The cotton bags were then packed in a plastic bag and put on ice. Upon reaching the lab, the samples were dried in a Thelco forced-air oven at 100 C for 30-45 minutes after which the temperature was lowered to 70 C until the samples were removed 36-48 hours later (Smith, 1973a).

The plants were carefully fractionated into "shoots," "stubble," "crown," and "roots." In the plant part analysis, "crown" was designated as the bottom 2 cm of the stem base, and "stubble" was classified as the immediate 2 cm above the "crown." "Shoots" included all herbage above the "stubble," and "roots" were severed from the "crown" by knife. Each component was ground through a 1 mm screen in a small Wiley mill and then reground through a UDY Cyclone sample mill fitted with a 0.5 mm screen. The samples were stored in plastic, 20 ml Dilu-vials and then analyzed for TNC using enzymatic hydrolysis and spectrophotometric measurement of reducing sugars as described in Appendix A.

TNC as Related to Clipping, Season, and Genotype

A 10 week long clipping study was initiated by mowing all plots to 5 cm on 27 July 1979 and harvesting at 2.5 cm height with a Jari sickle bar mower at 2.5, 5, and 10 week intervals until 4 October 1979. The four blocks of grasses representing genotypes were unreplicated; hence,

the design was a split plot without replication of the main plots. The genotype * treatment (rep) term was used to test genotypes, clipping treatments, and interaction effects. Within main plots the clipping treatments were randomly assigned and replicated three times.

Samples for TNC analysis were dug prior to clipping on 13 August, 30 August, 17 September, 4 October, and 11 November and processed as before except that only "crown" (bottom 2 cm of stem base) samples were saved for TNC analysis. The "crowns" were scraped free of roots, leaf sheaths, and sand by using a wire buffing wheel attached to the extended shaft of a small electric motor (20 W, 1525 RPM). The "crowns" were then ground through a 0.5 mm screen in the UDY mill and stored until tested for TNC.

Data were analyzed using the Statistical Analysis System (SAS) on an Amdahl 470 V/6-11 with OS/MVS Release 3.8 and JES2/NJE Release 3. Computing was performed at the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville. The plant part analyses and the percent TNC data for all subsequent samplings were subjected to analysis of variance and means were compared using the Waller-Duncan Multiple Comparison Test.

Correlation of TNC versus Regrowth-in-Darkness

Prior to discussing the methods used in the correlation of TNC and etiolated regrowth, terminology will be reviewed for clarity. The regrowth-in-darkness experiments were completed before any chemical analysis took place. Hence, the first use of the term "stubble" was in Experiment 1 of Chapter 1 and represented the 2.5 cm of stem tissue

above the soil level as well as the stem base below ground. Recall that the stem material above ground level was removed at the start of the second regrowth-in-darkness study (Experment 2, Chapter 1), and the term "stem bases" was used to define the 2-3 cm long stem segments from below ground. In the plant part experimentation an effort was made to more specifically focus on the location of TNC accumulation. The "stem bases" from Experiment 2 in Chapter 1 were believed to contain most of the stored carbohydrate so in the plant part experiment the bottom 2 cm of the stem was called the "crown." The 2 cm of stem immediately above the "crown" was called "stubble" corresponding to common agronomic usage, i.e., "residual above ground tissue following defoliation."

Limpograss samples taken on 11 November 1979 were analyzed for percent TNC to quantitatively characterize the concentration of reserves present in the "crown" at the beginning of the regrowth-in-darkness study (Experiment 1, Chapter 1). The darkness study began on 14 November 1979 and lasted 3 weeks. The dry weights of etiolated "shoots," as well as weights of "stubble" (2.5 cm of stem above ground plus stem bases below ground) and "roots" per pot were recorded. The "crown" TNC data from 11 November 1979 were correlated against "stubble" and against "shoot" percentages of the "stubble" plus "roots" present in the pots (("Shoot"/("stubble" + "roots")) * 100).

Results and Discussion

Analysis of TNC in Plant Parts

Results for TNC analyses of plant parts are shown in Figure 1. The limpograsses were different ($P < 0.01$) in their percent TNC; however, significant two and three way interactions among parts, age, and

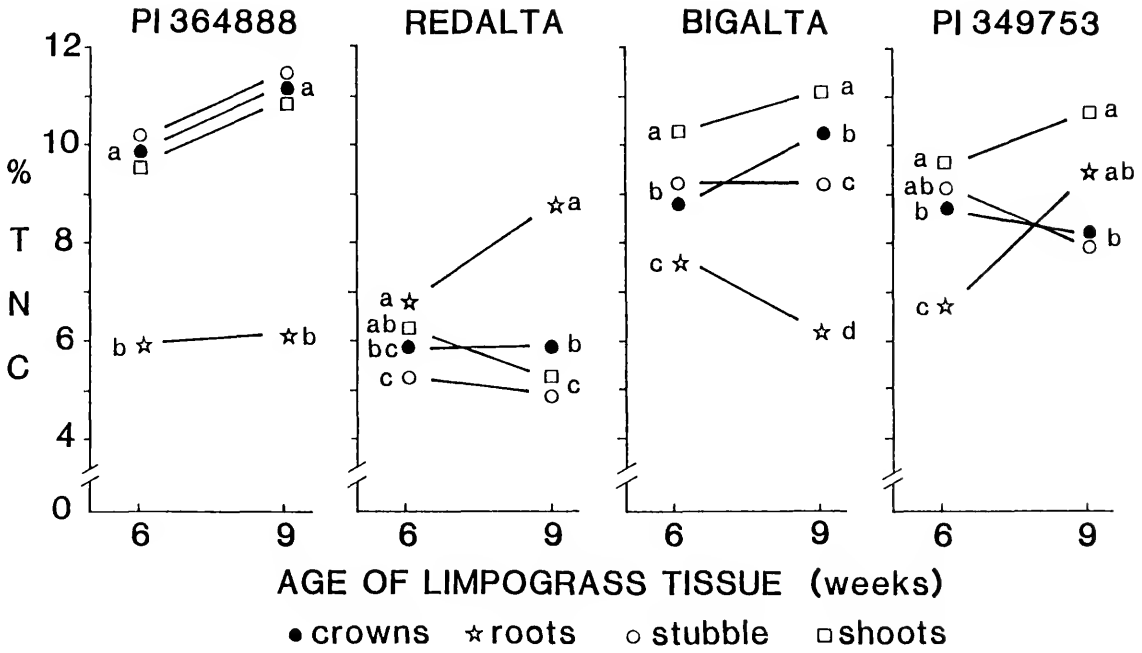

Figure 1. Total nonstructural carbohydrate (TNC) percent of dry matter of four plant parts in four limpograsses sampled at two maturities

(Different letters within an age of limpograss tissue indicate a significant difference ($P < 0.05$) of percent TNC in the plant parts.)

genotypes prohibited a comparison of overall means for the four limpograsses. When each limpograss was analyzed separately, all except PI 364888 showed different ($P < 0.01$) accumulation of carbohydrate to plant parts at 6 weeks as compared to 9 weeks. Between 6 and 9 weeks Redalta increased TNC to the "roots" at the expense of other parts, while Bigalta did just the opposite. In PI 349753 "roots" and "shoots" increased in TNC while "crown" and "stubble" declined between the two maturities. These results were interesting for Bigalta and Redalta because of the high or low contribution, respectively, of readily digested carbohydrates which would be measured in tests of *in vitro* organic matter digestibilities (IVOMD). Many studies have shown large IVOMD differences between these two grasses (Schank et al., 1973; Quesenberry et al., 1978).

Criteria necessary in selecting a plant part for TNC analysis were (1) disqualification of "shoots" because they were not perenniating parts, and (2) rejection of "roots" because of sand and dead tissue contamination. Figure 1 shows that the "crown" was statistically as high or higher in percent TNC than the "stubble" at all times. The bottom 2 cm of the stem base ("crown") was selected for further TNC analysis by chemical means.

TNC as Related to Clipping, Season, and Genotype

Table 4 shows the TNC results for four limpograsses subjected to three cutting frequencies in the 1979 growing season. In a combined analysis of sample dates, limpograsses, and cutting treatments, each factor influenced ($P < 0.01$) the TNC measured in "crowns." The two way interactions ($P < 0.01$) of limpograss and clipping frequency with

Table 4. The main effects of limpograss genotype and clipping treatment on the percent total nonstructural carbohydrates (TNC) in the bottom 2 cm of stem base ("crown")

Limpograss	1979 sampling date				
	8/13	8/30	9/17	10/4	11/11
	TNC %				
PI 364888	10.6b*	13.9a	12.4a	10.1a	13.6a
Redalta	6.6d	6.9d	6.4d	6.0d	10.8b
Bigalta	11.0a	11.8b	8.4b	9.9b	8.3c
PI 349753	9.1c	8.7c	8.0c	8.2c	8.1d
Clipping frequency (weeks)					
2.5	9.8a	10.5a	10.2a	8.7a	12.6a
5.0	9.9a	10.2a	8.8b	9.2a	10.6b
10.0	8.2a	10.2a	7.5c	7.7a	7.5c

*Different letters within a sampling date represent significant ($P < 0.05$) differences in percent TNC.

sampling date suggested that TNC concentration was independently altered by both cutting treatment and seasonal carbohydrate flux. There was no three way interaction ($P = 0.47$) or interaction between limpograss genotypes and clipping ($P = 0.76$); therefore, TNC concentrations were changing according to cutting frequency, but the reaction was similar among limpograsses.

Analysis of percentage TNC by sampling date revealed the rankings shown in Table 4. Bigalta was higher in TNC than other limpograsses on 13 August. From 30 August to 4 October PI 364888 was highest in TNC. In the last sampling on 11 November, the percent TNC in Redalta surged above Bigalta and PI 349753 but not above that of PI 364888. Perhaps the late autumn surge of TNC seen in PI 364888 and Redalta contributed to their higher persistence as opposed to the plateau or decline of TNC in PI 349753 and Bigalta.

Frequent cutting promoted lateral growth of stolons and increased the number of leaf blades per unit area. This change toward a turf-like morphology took some time to effect. In the bottom portion of Table 4 no differences in stored TNC were found for any frequency of cut in the limpograsses until 17 September. On 17 September and 11 November shorter cutting intervals enhanced TNC in the "crowns," suggesting an enhanced ability of the plants with a turf-like habit to accumulate TNC.

Correlation of TNC versus Regrowth-in-Darkness

In Figure 2 data from the regrowth study (Experiment 1, Chapter 1) was used to correlate against TNC in "crowns" of samples taken at the start of the regrowth experiment. The best correlations were found for

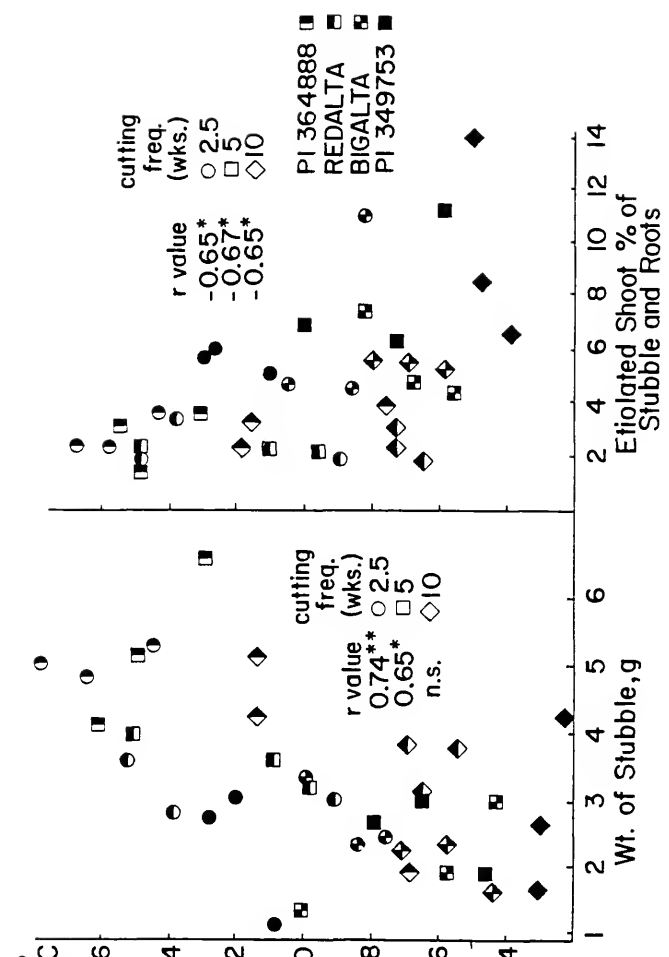

Figure 2. Total nonstructural carbohydrate (TNC) percentages in the "crown" correlated against "stubble" weights (left) and etiolated regrowth percent of whole plants (right) for four limpograss genotypes subjected to three clipping frequencies

*$P < 0.05$; **$P < 0.01$

TNC versus "stubble" and TNC versus percent plant regrowth in darkness (("shoots"/("stubble" + "roots")) * 100).

In Figure 2 the symbols represent the clipping frequencies used within each limpograss (genotypes are identified within a symbol by the coloration pattern). The important relationships are found within each cutting frequency.

Genotypes having greater "stubble" weight per pot had higher content of TNC in the "crowns." Frequent clipping induced a prostrate habit in all genotypes, but PI 364888 and Redalta provided more sites for leaf emergence--as reflected by the accumulation of TNC in "crowns" for the frequently cut treatments.

A negative correlation was observed in Figure 2 when the TNC in "crowns" were correlated against the "shoot" percentage of "stubble" plus "roots." Plants with more "stubble" (PI 364888) and "roots" (Redalta) (see Table 2, Chapter 1) produced small "shoots" that were greater in number and lower in weight; hence, were a lower percentage of the "stubble" plus "roots" (1 to 4 percent in Figure 2). Bigalta and PI 349753 had fewer but larger "shoots," as well as lower weights of "roots" and "stubble" (see Table 2, Chapter 1); hence, "shoots" were a larger percentage of the "stubble" plus "roots" in pots (4 to 14 percent in Figure 2).

When TNC was correlated directly against the dry weight of etiolated "shoots," a negative correlation was also found (-0.63) but not presented. Other experimenters (Adegbola, 1966; Adegbola and McKell, 1966b; Dovrat and Cohen, 1970; Raese and Decker, 1966) have found positive correlations of TNC versus etiolated regrowth, i.e., higher TNC in plants having longer rest intervals. These studies, however, used

upright instead of stoloniferous grasses, and used a single genotype of a species instead of four genotypes having variable morphologies.

Conclusions

1. Analysis of limpograss plant parts indicated that the bottom 2 cm of stem base ("crown") was a sight of TNC accumulation.

2. Cutting treatments and harvest date contributed independently to the significant ($P > 0.01$) variations seen in limpograss carbohydrate flux.

3. The more frequently clipped limpograsses effected a prostrate morphology which allowed more axillary tiller formation and TNC accumulation for all limpograsses.

4. Redalta and PI 364888 showed an autumn surge of TNC that might contribute to their better persistence as opposed to a decline or plateau of TNC for Bigalta and PI 349753.

5. Plants having greater weights of "stubble" also had higher concentrations of TNC in the bottom 2 cm of the stem base ("crown").

Summary

Limpograss (*Hemarthria altissima* (Poir.) Stapf et C.E. Hubb) could significantly contribute to the forage-livestock economy in the southeastern United States; however, cultivars must have good quality and high persistence before an impact will be made. Of the cultivars now available, 'Redalta' and 'Greenalta' have low digestibility and adequate persistence, while 'Bigalta' has excellent digestibility and poor persistence.

Current research efforts have identified PI 364888 limpograss as both persistent and digestible. The purpose of this study was to compare PI 364888 and another promising limpograss with Bigalta and Redalta to try to understand persistence relative to the total nonstructural carbohydrate (TNC) status of the plants.

The objective of this study was to locate the TNC storage site and characterize seasonal, genotypic, and clipping frequency effects on the TNC concentration in two released and two promising limpograsses. Secondly, it was desired to compare the chemical data with the regrowth-in-darkness results obtained in a previous study.

A sampling of 6 and 9 week old limpograss on 3 July and 26 July 1979, fractionated into four plant parts ("root," "shoot," "crown," and "stubble"), was chemically analyzed for percent TNC using an enzymatic sugar hydrolysis and spectrophotometric measurement of reducing sugars. The bottom 2 cm of stem base ("crown") was identified as the primary TNC storage site and, subsequently, served to compare the carbohydrate status in the four limpograsses.

A clipping study was conducted to variously deplete the reserves by cutting the limpograss field plots every 2.5, 5, or 10 weeks from 27 July to 4 October 1979. Results showed that frequent clipping induced a prostrate growth behavior and the turf-like condition allowed greater carbohydrate accumulation in the storage sites. Generally, PI 364888 was highest in stored TNC throughout the study and along with Redalta showed a surge of TNC in samples taken on 11 November 1979. Bigalta and PI 349753 did not react in this manner in late autumn, and they were also observed to be less persistent in previous studies. Limpograss

PI 364888 had the largest stolon system, stored the most TNC, and was very persistent.

The TNC results for 11 November 1979 were correlated against "shoot" and other plant weight data obtained from a 3 week regrowth-in-darkness study initiated on 14 November 1979. The results revealed a positive relationship ($r = 0.56$) across limpograsses for stubble weight versus TNC and a negative correlation ($r = -0.56$) for etiolated shoots expressed as a percent of the roots plus stubble. The significant ($P < 0.01$) regressions were caused by inherent differences in limpograss morphologies rather than cutting frequency effects within each limpograss.

CHAPTER 3
DRY MATTER YIELD, CRUDE PROTEIN, IN VITRO ORGANIC MATTER DIGESTIBILITY, TOTAL NONSTRUCTURAL CARBOHYDRATE, AND PERSISTENCE IN TWO PROMISING AND TWO RELEASED LIMPOGRASSES: EFFECTS DUE TO NITROGEN FERTILIZATION AND CUTTING FREQUENCY

Introduction

The southeastern United States is a subtropical zone that permits the growth of a wide variety of grasses; only a handful of which are agronomically important. Bahiagrass (*Paspalum notatum* Flügge) is the most widespread improved grass in Florida (Mott and Moore, 1977) because of its excellent persistence and broad adaptability. Bermudagrass (*Cynodon dactylon* (L.) Pers.) cultivars are economically important as hay and grazing crops due to successful plant breeding programs; but these cultivars generally grow better on upland sites. Digitgrasses (*Digitaria decumbens* Stent.) and stargrasses (*Cynodon nlemfuensis* Vanderyst) are grown more in south and central Florida due to their lower frost tolerance and winter hardiness. St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze and paragrass (*Brachiaria mutica* (Forsk) Stapf) have special adaptabilities for organic soils but are not widely used as pasture grasses elswhere in Florida.

Limpograss (*Hemarthria altissima* (Poir.) Stapf et C.E. Hubb) is a viable alternative to bahiagrass for flatwood sites. Limpograss can be equally persistent, but higher in quality than bahiagrass (Moore et al., 1981). Immature bahiagrass has potentially good quality; however,

quality rapidly declines in tissue greater than 6 weeks of age (Moore et al., 1970) and requires heavy utilization for optimum yield and quality (Beaty et al., 1980).

Ocumpaugh (1982) reported better yearly beef production and similar average daily gains from PI 364888 limpograss than from 'Pensacola' bahiagrass because of a 70 day grazing advantage. Gaskins and Sleper (1974) showed that daylength sensitivity was not a limiting factor for cool-season growth of limpograss but was for digitgrass and bermudagrass. Perhaps bahiagrass also falls into the latter category.

Bahiagrass is primarily used for grazing, not hay, and the major forage related problems in the southeastern United States are seasonal forage distribution and lack of preservation practices (Mott, 1982b). Limpograss is more seasonably flexible and can produce copious amounts of biomass. Killinger (1971) produced 15 m tons/ha of dry matter (DM) by 23 May and 23 m tons/ha by 14 August in north central Florida. This surpassed any of the yearly yields for five hybrid bermudagrasses, 'Pangola' digitgrass, and Pensacola bahiagrass reported in 1971 by Ruelke and Prine. Hodges and Martin (1975) studied the warm- and cool-season (1 November - 15 May) production of 23 subtropical grasses at Ona, Florida, and found the digitgrasses and Cynodons outyielded limpograsses; however, limpograsses were superior to Pensacola bahiagrass at all three fertility levels during the cool season. Kretschmer and Snyder (1979) obtained 21.8 m tons of dry matter (DM) with two harvests of 'Bigalta' limpograss using a 12 week cutting frequency and 168 kg/ha N per interval. In a management study, Ruelke obtained yields of 13.5-23.8 m tons for 'Redalta', 'Greenalta', and Bigalta limpograsses (Quesenberry et al., 1978).

The quality characteristics of tetraploid limpograsses are well known. Schank et al. (1973) identified high *in vitro* organic matter digestibilities (IVOMD) and a slower rate of IVOMD decline in the tetraploids. Taylor et al. (1976a), in New Zealand, also reported high *in vitro* digestibilities (leaves: 66.8 percent; stems: 77.2 percent). The tetraploid limpograss stem digestibility was the highest of all 25 summer grasses studied.

The above findings encouraged Quesenberry and Ocumpaugh (1977) to initiate stockpiling experimentation. They reported the beginning of August as the proper staging period to initiate regrowth of adequate yields of standing forage. Redalta produced 10.8-11.9 m tons DM but declined rapidly in quality, whereas Bigalta produced 6.2 m tons and maintained a 45 percent IVOMD when measured the following March. The rate of IVOMD decline was similar, but the intercept started 13 units higher in Bigalta than for Redalta and Greenalta (Quesenberry and Ocumpaugh, 1980).

Ruelke's management studies using limpograsses (Ruelke, 1978; Quesenberry et al., 1978) showed that close, frequent defoliation and high nitrogen fertilization led to the loss of Bigalta stands. Mob grazing and clipping trials by Quesenberry and Ocumpaugh (1979) also showed Bigalta's poor persistence. Quesenberry et al. (1981) identified PI 364888 as having superior persistence and comparable quality to Bigalta. Ocumpaugh et al. (1981) identified another promising limpograss (PI 349753) that persisted under mob grazing but that was of a slightly lower acceptability by animals than PI 364888 or Bigalta.

Total nonstructural carbohydrate (TNC) analysis has for decades been a useful technique in quantifying the fluctuation of organic

reserves in response to season (McCarty, 1935; Waite and Boyd, 1953a), defoliation (Sullivan and Sprague, 1943; May, 1960; Baker and Garwood, 1961; Marshall and Sagar, 1965), nitrogen fertilization (Adegbola and McKell, 1966a; Raese and Decker, 1966; Ford and Williams, 1973), and persistence (Graber, 1931; Alberda, 1966). The efforts of Dale Smith (1981a) and others in understanding the carbohydrate metabolism in alfalfa (Medicago sativa L.) is a prime example of the TNC studies yielding practical management recommendations. According to Graber (1931) "under field conditions, the limitations of root growth and the modifications of the internal environment resulting from low reserves may reduce the absorptive capacity of a plant so greatly and may so increase its susceptibility to drought, winter injury, weed encroachments, insect injury, and other hazards as to jeopardize its permanence" (p. 47).

Various reports have shown that high rates of nitrogen fertilizer have led to reduced persistence in digitgrass (Creel, 1957; Ruelke, 1960; Kien et al., 1975) and in orchardgrass (Dactylis glomerata L.) (Alexander and McCloud, 1962), but no carbohydrate analyses were presented. No chemical data relating TNC to persistence in limpograss could be located. Christiansen (Chapter 1) used regrowth-in-darkness studies as preliminary tests of reserve energy in limpograss. In Chapter 2 of this report the site of nonstructural carbohydrate accumulation was determined, as well as clipping effects on TNC percent of limpograss in 1979.

Redalta has poor quality but excellent persistence, while Bigalta has the reverse situation; neither combination being advantageous to the producer. The objective of this study was to thoroughly evaluate two

promising introductions against Bigalta and Redalta. The goal was to obtain a persistent, high quality, high yielding limpograss for flatwood sites in Florida and the southeast.

Materials and Methods

Two experimental areas were used in this study. The 1979 experimentation was conducted on four well established plantings of limpograss on a Wachula sand, a poorly drained siliceous hyperthermic ultic haploquod soil at the Beef Research Unit of the University of Florida. Another field was established in 1979 by vegetative propagation from material taken from the 1979 experimental area. The new planting was in an Adamsville sand. The limpograsses used were PI 349753, PI 364888, Redalta, and Bigalta. Redalta is diploid (2n = 18) and the other grasses used here are tetraploids (2n = 36).

1979 Establishment

The experimental area was 30 x 118 m and supported a mature stand of rye (Secale cereale L.) prior to cultivating with a Ground Hawg rototiller on 22 May 1979. The field was blocked into three sections and within each section four main plots (genotypes) were marked to measure 7 x 30 m with 3 m alleys between each main plot. The alleys were planted to 'Argentine' bahiagrass.

Waist high stands of each limpograss growing in the 1979 experimental area were mowed with a sickle bar set at 7.5 cm height, raked, and carried to an appropriately assigned, random location within each block. The herbage was evenly distributed over the soil, lightly disked to 15 cm, and the entire field was rolled using a cultipacker seeder to

assure good soil to stem contact. The field was irrigated when necessary to insure establishment and fertilized on 29 June and 23 August 1979 with 336 kg/ha of 17-5-10 ($N-P_2O_5-K_2O$) containing 3.4 kg/ha of a micronutrient mix. Soil test results from 7 June 1979 showed a pH of 6.2, 20.3 kg/ha phosphorus (P) and 40 kg/ha potassium (K). The field was flail chopped on 6 December 1979 to 7.5 cm height to remove frosted forage.

1979 Experimentation

Within each established limpograss sward in the 1979 experimental area plots were marked out for a clipping study. The blocks were unreplicated; hence, the design was a split plot design without replication of main plots. The clipping treatments within a limpograss block were randomly assigned and replicated three times. The genotype * treatment (rep) term was used to test genotype, clipping treatment, and interaction effects for the responses that were studied.

The clipping experiment was initiated by mowing all plots to 5 cm on 27 July 1979 and harvesting at 2.5, 5 and 10 week intervals until 4 October. The limpograsses were fertilized on 28 May and 30 July 1979 with 280 kg/ha of 17-5-10 ($N-P_2O_5-K_2O$) containing 1 percent of a micronutrient mix. Soil test results from samples taken on 3 December 1979 showed a pH of 6.6, 28.2 kg/ha P, and 22.5 kg/ha K.

The 2.5 week interval treatments were clipped at 5 cm height on 13 August, 30 August, 17 September, and 4 October 1979 using a Jari sickle bar mower. Yields were determined and "shoot" samples (tissue above cutting height) were taken for IVOMD and crude protein (CP). The 5 week interval treatments were harvested on 30 August and

4 October 1979. The 10 week treatment was harvested and sampled for IVOMD, yield, and CP only on 4 October 1979.

1980 Experimentation

Soil test results from the newly established field of limpograss revealed a pH of 6.1, 16.4 kg/ha P, and 53.1 kg/ha K. On 23 March 1980 the field was uniformly mowed to 7.5 cm height and treatments consisting of five levels of nitrogen (N) fertilization and five cutting frequencies (F) were imposed on the main plots. The two factors (N and F) were combined to represent $13/25^{ths}$ of a 5 x 5 complete factorial. Figure 3 shows a 3 x 3 factoral and the spatial arrangement of two factors, and Figure 4 shows a central composite design in three factors. The 13 treatment combinations used in this study are shown graphically in Figure 5 and the design points are plotted as grid coordinates in Figure 6. The arrangement of treatment combinations in Figure 6 shows the underlying difference between the classical central composite design (Figure 3) and the modified central composite design described by Littell and Mott (1975). Note in Figure 6 how information is concentrated in what was considered the "management realm" (0-120 kg/ha N) and 3 to 9 week defoliation frequencies. Also note the geometric scaling of N and the arithmetric progression for cutting frequency (with a skip at the 15 week level). As the dotted lines suggest, the design could be considered a superimposition of two complete factorials (2^2 and 3^2).

Consequently, the grasses were replicated as main plots in a split plot design, and the 13 treatments provided a response surface with all points replicated three times. Each main plot contained 13 plots

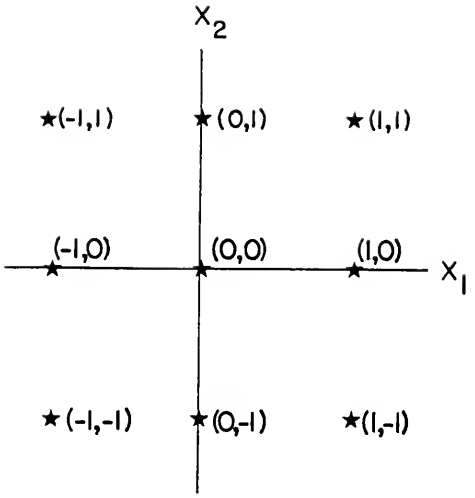

Figure 3. Spatial arrangement of a three level factorial design in two variables

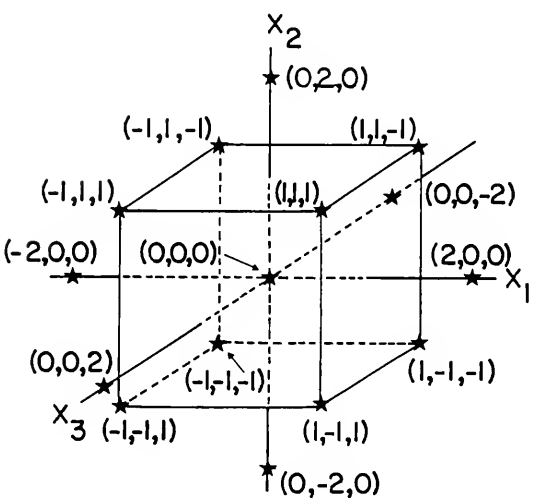

Figure 4. Spatial arrangement of a central composite design in three variables

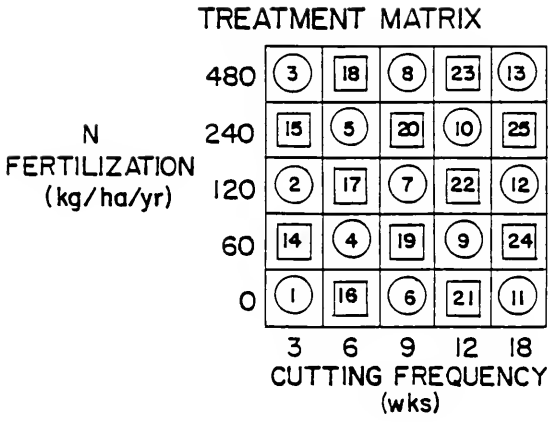

Figure 5. Treatment matrix showing treatment numbers inside each actual (circles) and absent (squares) treatment combination

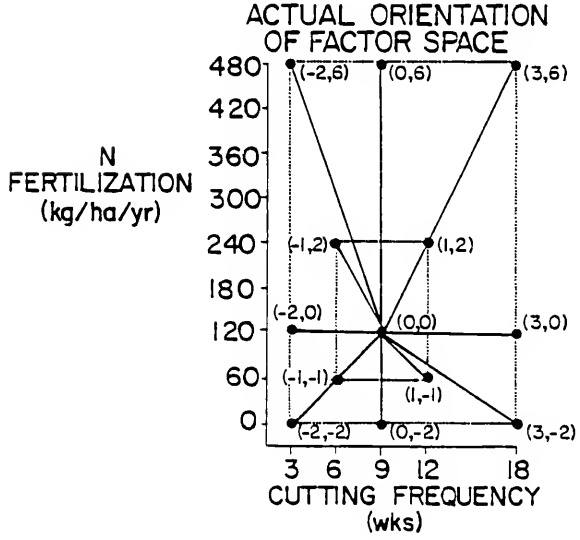

Figure 6. Treatment combinations of nitrogen (N) and cutting frequency plotted as grid coordinates with the origin at 120 kg/ha/yr N and 9 weeks cutting frequency

(2.3 x 7 m) for treatments. The entire field had 156 total plots (3 replications x 4 grasses x 13 treatments).

Figure 7 shows the treatment levels of N and F, the treatment number, and the harvest (H) number, date, and chronological stage of the 1980 experiment. Sampling for carbohydrate analyses followed visual estimations of percent limpograss but preceded sampling for herbage quality and clipping for yield. Fertilization followed clipping, and the N rates were applied by hand as ammonium nitrate dispersed in sand to ensure uniform application. The N application was split according to the number of defoliations; an equal rate applied at the start of the experiment and after every defoliation except the last. The entire field was fertilized with 300 kg/ha 0-10-20 (N-P_2O_5-K_2O) containing microelements prior to the start of the experiment and at the halfway point late in July.

The sampling schedule is shown by symbols in Figure 7 for IVOMD, CP (CP = 6.25 x N), DM yield determination, and TNC measurement. Forage cut for yield and quality was oven dried at 60 C. The dried forage was ground in a Wiley mill to pass a 1 mm screen and analyzed for percent N by the Kjeldahl procedure. The IVOMD determination used a modification of the Tilley and Terry technique (Moore et al., 1972). Total nonstructural carbohydrate (TNC) samples were randomly selected, dug, washed free of soil, and arranged in cotton bags. The cotton bags were then packed in a plastic bag and put on ice. Upon reaching the lab, the samples were dried in a Thelco forced-air oven at 100 C for 30-45 minutes after which the temperature was lowered to 70 C until the dried samples were removed 36-48 hours later (Smith, 1973a). The stem bases were scraped free of roots, leaf sheaths, and sand by using a wire

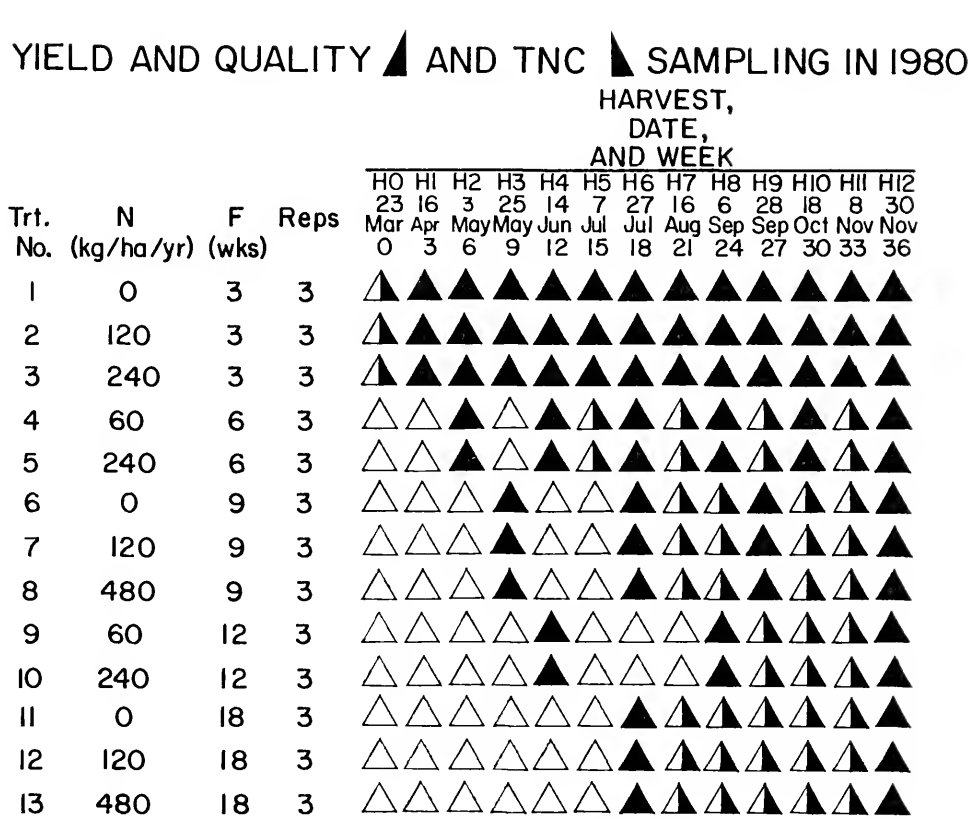

Figure 7. Yield, quality (IVOMD and CP), and total nonstructural carbohydrate (TNC) sampling schedule for 1980 (open symbols indicate no sample)

buffing wheel attached to the extended shaft of a small electric motor (20 W, 1525 RPM). The bottom 2 cm of the stem ("crowns") were severed and ground through a 0.5 mm screen in a UDY Cyclone mill and stored until tested for TNC. "Shoot" tissue was analyzed for TNC on the 27 July and 30 November dates. "Shoot" material was also ground through the UDY mill, and all samples were stored in plastic 20 ml Dilu-vials and then analyzed for TNC using an enzymatic hydrolysis and spectrophotometric measurement of reducing sugars as described in Appendix A.

1981 Experimentation

The 1980 experimental area was studied in 1981 for residual effects of cutting frequency in 1980 on 1981 DM yields. The limpograsses were harvested for three 9 week cutting intervals on 3 June, 3 August, and 7 October 1981. No fertilizer was applied. Soil test results from cores taken on 26 February 1981 indicated a pH of 6.2, 14.2 kg/ha P, and 46.5 kg/ha K.

Computing

Data were analyzed using the Statistical Analysis System (SAS) on an Amdahl 470 V/6-11 with OS/MVS Release 3.8 and JES2/NJE Release 3. Computing was performed at the Department of Agricultural Engineering and the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

Means were analyzed using the Waller/Duncan Multiple Comparison Test. Analysis of variance and regression were used to test response

surface models for adequate fit. A complete explanation of the SAS methodology for constructing surface plots is given in Chapter 4.

Results

Dry Matter Yields: 1979 Experimentation

Table 5 shows the DM yields obtained for limpograsses cut and weighed four times, twice, and once for a 10 week period beginning 27 July 1979 and ending 4 October 1979. Within limpograss genotypes each treatment had a significantly different DM production. Among grasses, no differences were found for combined yields from plots cut every 2.5 weeks, but if grasses were allowed to grow for two cycles of 5 weeks, Bigalta and PI 364888 had higher DM accumulation, as was seen again for the 10 week treatment yield.

Dry Matter Yields: 1980 Experimentation

The total limpograss DM yields in the 1980 experimentation were summed and modelled to construct response surface plots in Figure 8. For total DM yields in 1980 at an actual treatment combination, consult Table 6. Figure 8 and Table 6 both show DM yields for PI 364888 > PI 349753 > Bigalta > Redalta for the 480*18 (N*F) treatment. The surface plots show that Redalta and Bigalta plateaued in the surface region focused at the 480*18 treatment combination. These two limpograsses lodged at this combination of N and F, and it is believed that their decumbent habit caused some loss of DM.

Varying DM yield advantages were shown for limpograsses under different treatment regimes than 480*18. Bigalta, for instance, had a

Table 5. Total dry matter (DM) yields for four limpograsses clipped at three different frequencies for 10 weeks ending on 4 October 1979

Limpograss	Clipping frequency (wks)		
	2.5	5	10
	---------- DM (kg/ha) ----------		
PI 364888	1756 a*	3261 ab	8327 a
'Redalta'	1718 a	2328 c	4592 d
'Bigalta'	1903 a	3988 a	6616 b
PI 349753	1723 a	3133 b	5425 c

*Clipping treatments within a limpograss genotype are compared using the underlining technique and letters within a column compare limpograsses for a treatment. Any values sharing a commun underline or letter are not different ($P < 0.05$) using the Waller-Duncan Multiple Comparison Test.

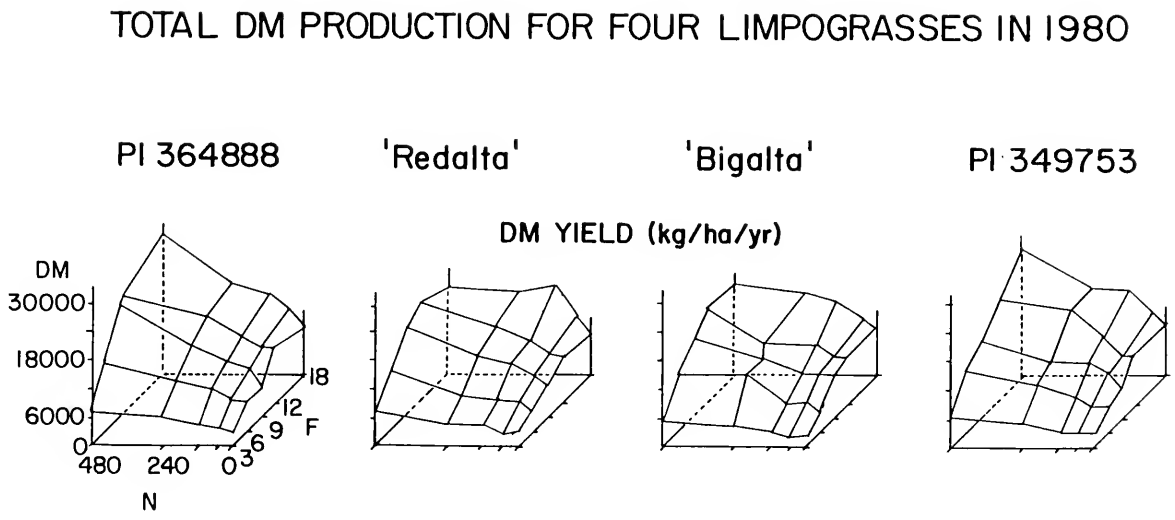

Figure 8. Total dry matter (DM) yield for four limpograsses subjected to five levels of nitrogen (N) fertilization and five frequencies (F) of defoliation throughout the 1980 growing season at the Beef Research Unit near Gainesville, Florida

Table 6. Effect of nitrogen (N) rates and cutting frequency (F) on dry matter (DM) yield, in vitro organic matter digestibility (IVOMD), crude protein (CP), harvest of protein, and fertilizer N efficiency

Fert. N (kg/ha)		Defoliation		Limpograss	Total DM yield (m ton/ha/yr)	DM yield* added by N (m ton/ha)	Harvest of protein (kg/ha/yr)	N harvested ÷ applied N (%)	27 July 1980			30 November 1980			Stand (%)
Per cut	Total	Freq F (wks)	Total no.						DM yield (m ton/ha)	IVOMD (%)	CP (%)	DM yield (m ton/ha)	IVOMD (%)	CP (%)	
0	0	3	12	PI 364888	2.7	---	171	---	0.5	49.9	7.5	0	51.9	6.6	92
				Redalta	3.3	---	221	---	0.7	41.5	7.4	0	40.0	5.9	92
				Bigalta	2.7	---	219	---	0.5	58.3	8.8	0	65.6	10.6	62
				PI 349753	3.1	---	215	---	0.3	47.0	9.1	0	52.6	7.5	92
10	120	3	12	PI 364888	4.4	1.7	361	26	0.6	50.7	9.0	0	56.8	9.8	82
				Redalta	4.7	1.4	357	18	1.0	39.8	7.6	0	42.1	8.2	90
				Bigalta	3.1	0.4	281	8	0.4	58.2	9.2	0	69.5	12.2	50
				PI 349753	2.9	0	225	2	0.4	45.0	7.3	0	57.5	10.3	83
40	480	3	12	PI 364888	6.8	4.1	708	18	0.5	51.8	9.7	0	61.8	12.7	78
				Redalta	7.6	4.3	744	18	0.8	44.3	9.8	0	47.9	10.4	88
				Bigalta	5.6	2.9	666	15	0.1	63.7	13.2	0	72.0	14.6	23
				PI 349753	6.1	3.1	643	14	0.4	45.2	9.7	0	61.5	12.5	68
10	60	6	6	PI 364888	7.1	0	384	47	3.5	49.8	4.4	0.2	56.0	7.6	95
				Redalta	7.4	1.8	413	37	2.6	40.8	5.1	0.2	44.1	8.3	97
				Bigalta	7.4	2.2	448	32	3.7	51.1	4.8	0.1	64.2	9.3	68
				PI 349753	6.4	0	371	27	2.6	43.3	5.3	0.2	54.6	8.3	97
60	240	6	6	PI 364888	11.7	4.5	731	35	4.6	48.3	4.7	0.4	60.0	10.3	100
				Redalta	8.2	2.6	557	19	3.4	39.4	6.0	0.1	69.4	10.3	82
				Bigalta	12.9	7.7	921	39	4.8	55.1	5.3	0.1	69.9	13.4	70
				PI 349753	10.7	4.3	704	29	4.1	49.1	5.4	0.2	60.7	10.6	95
0	0	9	4	PI 364888	5.9	---	262	---	3.8	45.8	2.9	0.1	53.0	6.6	98
				Redalta	6.5	---	329	---	2.5	41.6	4.8	0.2	45.9	7.7	98
				Bigalta	9.1	---	448	---	5.0	52.2	3.0	0.2	64.3	8.6	92
				PI 349753	6.8	---	324	---	4.0	45.8	3.7	0.1	49.9	6.3	98

Table 6. Continued

Fert. N (kg/ha)		Defoliation		Limpograss	Total DM yield (m ton/ha/yr)	DM yield* added by N (m ton/ha)	Harvest of protein (kg/ha/yr)	N harvested ÷ applied N (%)	27 July 1980			30 November 1980			Stand (%)
Per cut	Total	Freq F (wks)	Total no.						DM yield (m ton/ha)	IVOMD (%)	CP (%)	DM yield (m ton/ha)	IVOMD (%)	CP (%)	
30	120	9	4	PI 364888	12.1	6.2	544	38	7.2	43.3	2.6	0.3	58.5	8.4	98
				Redalta	11.6	5.2	592	35	4.4	38.3	3.9	0.4	45.9	9.0	98
				Bigalta	9.7	0.7	502	3	5.5	49.4	2.9	0.3	64.6	10.6	73
				PI 349753	12.4	5.6	506	38	6.3	41.0	3.5	0.3	56.9	9.6	100
120	480	9	4	PI 364888	24.4	17.5	1449	40	10.0	47.5	4.1	0.5	63.9	12.1	100
				Redalta	19.1	12.6	1200	29	6.6	41.2	5.3	0.2	51.0	10.6	100
				Bigalta	17.3	8.2	1229	25	7.6	50.0	5.1	0.5	67.8	13.3	93
				PI 349753	17.2	10.4	1100	26	7.1	44.0	4.7	0.4	63.9	12.1	100
20	60	12	3	PI 364888	12.3	0.4	429	47	11.6	44.0	1.4	0.8	51.0	4.9	98
				Redalta	11.0	0.7	384	24	4.0	38.0	3.2	1.0	40.4	4.6	97
				Bigalta	9.9	0	356	0	11.6	48.2	1.4	1.5	53.1	4.7	98
				PI 349753	10.0	0	375	20	6.7	42.0	2.4	0.8	47.5	5.8	93
80	240	12	3	PI 364888	19.1	7.2	756	33	14.4	43.4	1.2	3.1	53.4	6.0	100
				Redalta	16.2	5.8	707	28	9.7	36.2	2.9	1.8	42.0	6.8	100
				Bigalta	12.7	2.7	584	13	15.7	48.8	1.3	2.9	61.9	7.1	100
				PI 349753	20.0	8.9	830	35	10.5	41.0	2.0	3.0	52.6	6.1	100
0	0	18	2	PI 364888	10.4	---	252	---	7.2	43.1	2.4	3.2	40.8	2.3	98
				Redalta	8.8	---	253	---	5.4	33.7	3.3	3.4	32.7	2.7	96
				Bigalta	10.4	---	292	---	6.4	48.8	3.1	4.2	41.3	2.4	100
				PI 349753	11.0	---	284	---	7.8	37.2	2.6	3.2	37.3	2.5	98
60	120	18	2	PI 364888	17.4	7.0	366	16	9.0	41.0	2.0	8.4	37.0	2.1	98
				Redalta	19.0	10.1	405	21	11.1	31.5	2.1	7.9	29.2	2.2	100
				Bigalta	16.0	5.5	334	6	9.5	42.7	1.9	6.5	39.3	2.3	70
				PI 349753	18.6	7.5	387	14	11.4	33.3	2.1	7.2	33.9	2.0	98
240	480	18	2	PI 364888	29.3	18.9	992	25	18.3	42.0	2.4	11.0	42.3	4.3	98
				Redalta	18.3	9.5	611	12	12.0	33.0	2.7	6.3	33.0	4.2	93
				Bigalta	19.7	9.0	620	11	12.1	43.1	2.4	7.6	48.4	4.1	100
				PI 349753	26.8	15.7	760	16	18.0	34.6	2.3	8.8	38.9	3.3	98

*Total DM prediction equations gave DM values for 0 nitrogen at 6 and 12 week defoliations.

slight yield advantage in the treatment cut every 6 weeks in 1980, as was similarly shown in 1979 for Bigalta cut every 5 weeks (Table 5). What is clearly shown in Table 6, however, is the tremendous ability of all the limpograsses to respond to N and accumulate DM, especially at longer cutting intervals.

Table 6 also shows the increment of total DM yield caused by N fertilization. Brown (1978) termed productivity of biomass per unit of N applied as "nitrogen use efficiency." The DM production at zero N in each cutting frequency was subtracted from the production obtained with N applied in order to determine this statistic. It is shown that the maximum DM response to N was obtained with high N rates and long cutting frequencies (480*9, 240*12, 120*18, and 480*18). No clear cut advantage was observed for any one limpograss across all treatments.

The most protein harvested in herbage was obtained from the 480*9 treatment as shown in Table 6. The actual efficiency of nitrogen uptake was also calculated:

$$\left[\frac{\text{N in plants - N in plants receiving zero N}}{\text{N fertilizer applied}} \right] * 100$$

Limpograss PI 364888 was best for almost all treatments in this statistic, but it was not as effective at taking up N at zero N conditions (Table 6).

Dry matter (DM) yield distribution is as important as total production in Florida. The 36-week-long 1980 experiment was divided into three 12 week sections: the first interval termed spring and the last 12 week interval called autumn. Only the 3, 6, and 12 week treatments were included in spring and autumn because the 9 and 18 week cutting

frequencies did not match in harvest sequence. The graph in Figure 9 shows DM plotted against treatment combination. Spring production was greater than autumn production, especially in the treatments cut every 3 weeks. The decline in autumn yield for 3 week treatments with increasing N levels exemplified the effect of frequent defoliation and high N rates on limpograss. It is interesting to note that DM yield in the 480*3 treatment for Bigalta produced 3 m tons in spring but the subsequent stress permitted only 5.6 m tons total DM yield (Table 6).

Clearly, PI 349753 was the highest in spring production at the 240*12 treatment combination (Figure 9). Calculations using the 240*12 treatment data from Figure 9 and the total DM data presented in Table 6 allow a determination of summer yield for PI 349753 by difference. In this manner, PI 349753 was shown to produce 8 m tons of DM in spring, 6.9 m tons in summer, and 2.8 m tons in autumn.

Figure 10 has relevance to the use of limpograss as stockpiled forage. The PI 364888 limpograss was used in this analysis showing three staging dates that corresponded to the final intervals of the 9, 12, and 18 week cutting frequencies. A maximum of stockpiled forage was obtained for the 27 July > 6 September > 28 September staging dates. These dates agree with those of Quesenberry and Ocumpaugh (1980) who demonstrated the necessity of early August staging for adequate DM accumulation for stockpiling. At any staging date the second highest N rate produced nearly as much or more forage as the highest N rate.

In the 1979 experiment, PI 364888 yielded 8.3 m tons for the single 10 week cutting interval staged from 27 July 1979 to 4 October 1979 (Table 5). Nitrogen was applied on 30 July at a rate of 38 kg/ha. In the 1980 experiment, 60 kg/ha N was applied on 27 July 1980 for the

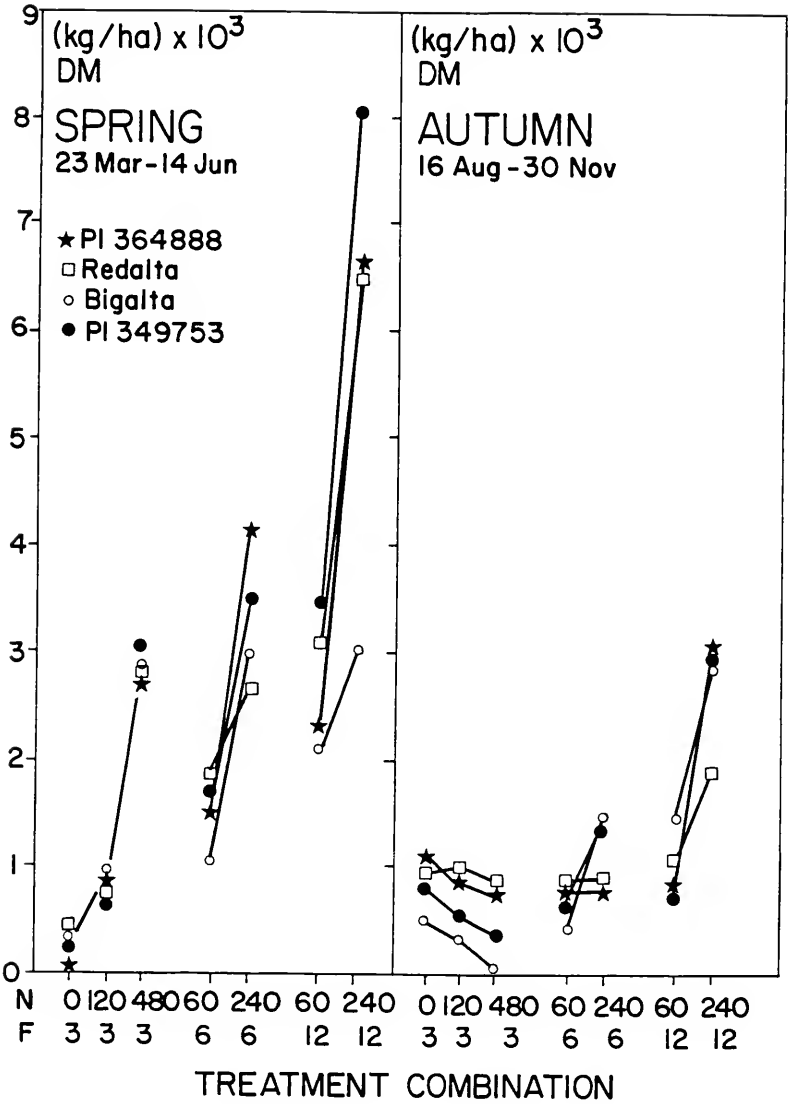

Figure 9. Spring and autumn seasonal distribution of dry matter (DM) yield for four limpograsses in 1980

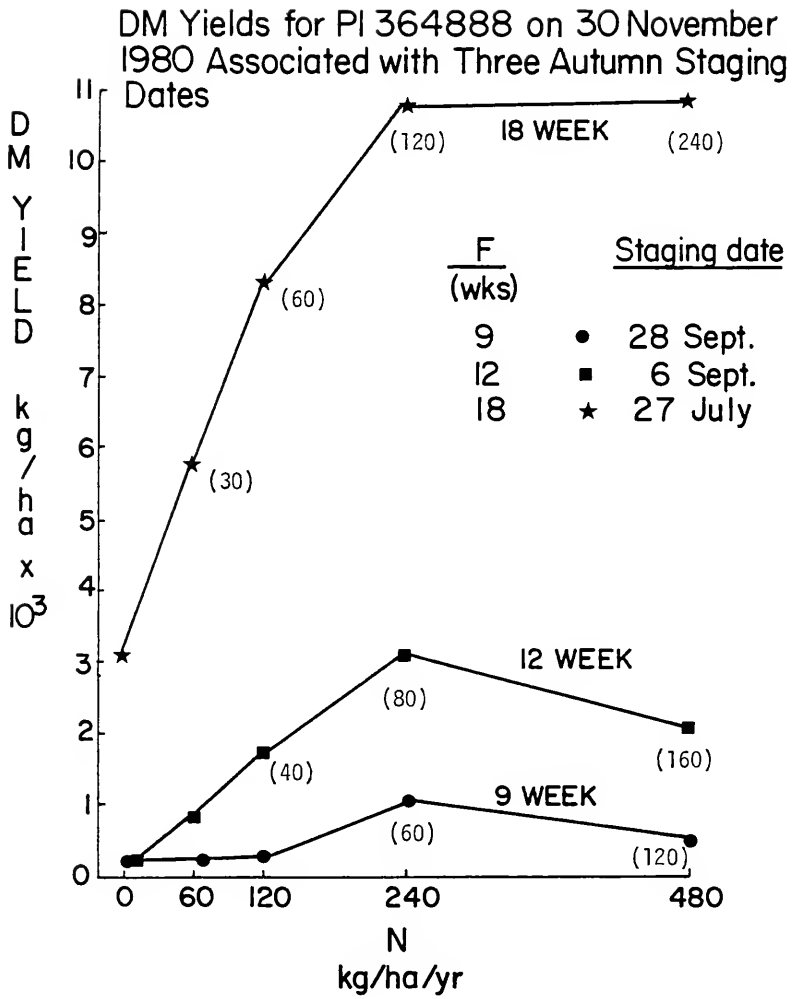

Figure 10. Dry matter (DM) yields for PI 364888 at three levels of nitrogen (N) fertility and staged at three different dates in the autumn of 1980

(Parentheses indicate the N(kg/ha) applied at the beginning of the interval to give the resultant yield.)

120*18 treatment, and it subsequently yielded 8.4 m tons (Figure 10). In 1981 Quesenberry and Ruelke (1982) applied 75 kg/ha N to PI 364888 on 4 August 1981 and measured 11.1 m tons stockpiled DM on 7 December 1981. This value is nearly identical to the 11 m tons DM measured in 1980 (Figure 10) for the 480*18 treatment (240 kg/ha N applied 27 July 1980).

Dry Matter Yields: 1981 Experimentation

In 1981, residual effects from the 1980 experiment were analyzed by measuring DM yields. The N applied later than 28 September must have remained as residual fertilizer in the soil at the beginning of the 1981 harvest season.

The surface plots shown in Figure 11 for PI 364888 indicate the highest yields at 480*9 and 480*3 for 3 June 1981. The 480*3 plots had lower percent limpograss at the close of the 1980 experiment following a year of frequent defoliation; however, by 3 August 1981 the plots had completely recovered in all the limpograsses due to the residual N fertilizer in the spring. Although not shown, the performance of Redalta during the first harvest interval of 1981 was superior to the other grasses. Redalta's 480*3 and 480*9 treatments averaged 4.2 m tons/ha DM, followed by PI 364888 (2.9 m tons/ha), PI 349753 (2.6 m tons/ha), and Bigalta (2.3 m tons/ha).

The 7 October harvest was not shown due to the similarity to the second harvest on 3 August. Yields in 1981 for all limpograsses showed similar response surface plots. The annual yields in all grasses were highest in the 480*3 and 480*9 treatments (10-12 m tons/ha) due to the yield advantage attained in the spring. The rest of the treatments were similar in annual DM production within a grass, but Bigalta yielded

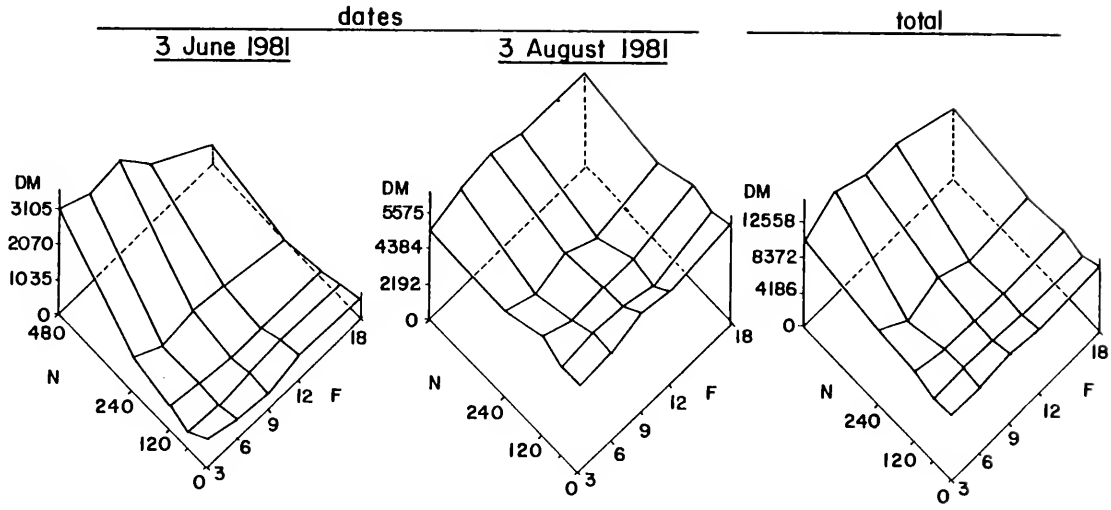

Figure 11. Residual clipping and fertilization effects on dry matter (DM) yield for PI 364888 harvested every 9 weeks in 1981 (3 June, 3 August, and 7 October (not shown)) and the total production for the year

Caution: DM yield units vary among graphs

higher DM (7.8-9.9 m tons/ha), followed by PI 349753 (5.9-9.3 m tons/ha), PI 364888 (6.1-8.2 m tons/ha), and Redalta (4.7-7.4 m tons/ha).

Crude Protein: 1979 Experiment

Table 7 reveals the most disappointing characteristic of limpograss: low CP content. In the 1979 experiment the plots were fertilized on 28 May and 30 July with 38 kg/ha N, but this was not sufficient to raise the CP levels in the 5 and 10 week treatments to the 7 percent value deemed necessary for maintenance in animals (Milford and Minson, 1965). Bigalta had the highest values for CP.

Crude Protein: 1980 Experiment

In Figure 12 the CP values are shown for the middle (27 July) and end (30 November) of the 1980 experiment. For 27 July note how quickly CP drops from the highest values at the left front edge of the plot (where N ranges from 0-480 kg/ha/yr in association with a 3 week cutting frequency). Not a single value from samples older than 3 weeks of age was above 7 percent CP (Table 6). The diploid (Redalta) and tetraploid limpograsses were alike in this regard. The peculiar aspect of Figure 12 was the prominent differences in CP percent of limpograss between the midsummer date and the autumn date.

Autumn temperatures allowed very little growth between the 8 November and 30 November sampling interval and, hence, in an effort to obtain an adequate sample older, residual forage was inadvertently included with the sample herbage in the 3 week treatments. Therefore, in the bottom portion of Figure 12, CP values for the 3 week frequency of cutting may have been unrealistically low. A ridge was formed at the

Table 7. Percent crude protein (CP) for four limpograsses clipped at three different frequencies for 10 weeks ending on 4 October 1979 (Samples taken on 4 October were analyzed for CP.)

Limpograss	Clipping frequency (wks)		
	2.5	5	10
	---------- Crude protein (%) ----------		
PI 364888	6.2 c*	4.9 a	3.1 b
'Redalta'	7.1 bc	4.4 a	4.6 a
'Bigalta'	9.4 a	5.2 a	3.2 b
PI 349753	7.3 b	5.0 a	3.5 b

*Clipping treatments within a limpograss genotype are compared by the underlining technique and letters within a column compare limpograss values for a treatment. Any values sharing a common underline or letter are not different ($P < 0.05$) using the Waller-Duncan Multiple Comparison Test.

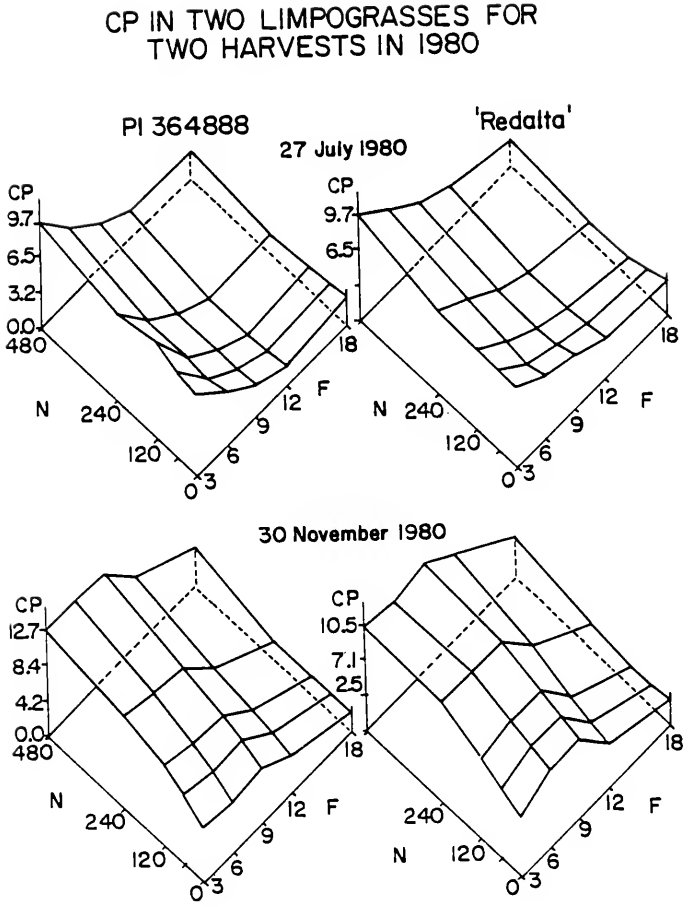

Figure 12. Comparison of crude protein (CP) percentages in the tissue of PI 364888 and 'Redalta' for two dates in 1980 in response to five levels of nitrogen (N) fertilization and five frequencies (F) of clipping

Caution: The CP scale is non-uniformly and non-linearly scaled on 30 November 1980

9 week level of cutting frequency, and beyond the ridge the maturity of the forage increased and CP values fell. Table 6 shows the substantial improvement in percent CP between 27 July and 30 November 1980 for all the grasses and all treatments except the 0*3 treatment and the 18 week old samples. On the 30 November sampling date, only four of 32 means for 3, 6, and 9 week old samples were below 7 percent CP (Table 6).

IVOMD: 1979 Experiment

Table 8 shows IVOMD data obtained from tissue collected on 4 October 1979 for the 10 week study conducted in 1979. The ranking of the limpograsses is similar to what will be presented later from the 1980 results. The main effect of clipping treatment is also shown in Table 8 and shows that quality was higher in the younger plant material.

IVOMD: 1980 Experiment

Figure 13 compared the IVOMD in all four limpograsses for 27 July and 30 November 1980. Treatments did not vary by a great degree as indicated by the topographical uniformity in the surface plots. One apparent feature of Figure 13 is the lower IVOMD found in Redalta. Other investigators (Schank et al., 1973; Quesenberry and Ocumpaugh 1980) have shown the low digestibility of diploids compared to tetraploid limpograsses. Redalta is a diploid while the remaining three grasses in this study are tetraploids. The 30 November plots showed a small but perceptively higher IVOMD and somewhat greater undulation; however, a tabular presentation was necessary for increased understanding. In Table 6, for all treatments, the ranking of limpograsses for percent IVOMD was Bigalta > PI 364888 > PI 349753 > Redalta. A glance at the

Table 8. The main effect of limpograss and clipping treatment on percent *in vitro* organic matter digestibility (IVOMD) of tissue sampled 4 October 1979

	IVOMD
	-- % --
Limpograss	
PI 364888	50.1 b*
'Redalta'	43.0 c
'Bigalta'	55.4 a
PI 349753	50.0 b
Clipping frequency (wks)	
2.5	51.6 a
5	50.4 b
10	46.9 b

*Values sharing a common letter within the column are not different ($P < 0.05$) using the Waller-Duncan Multiple Comparison Procedure.

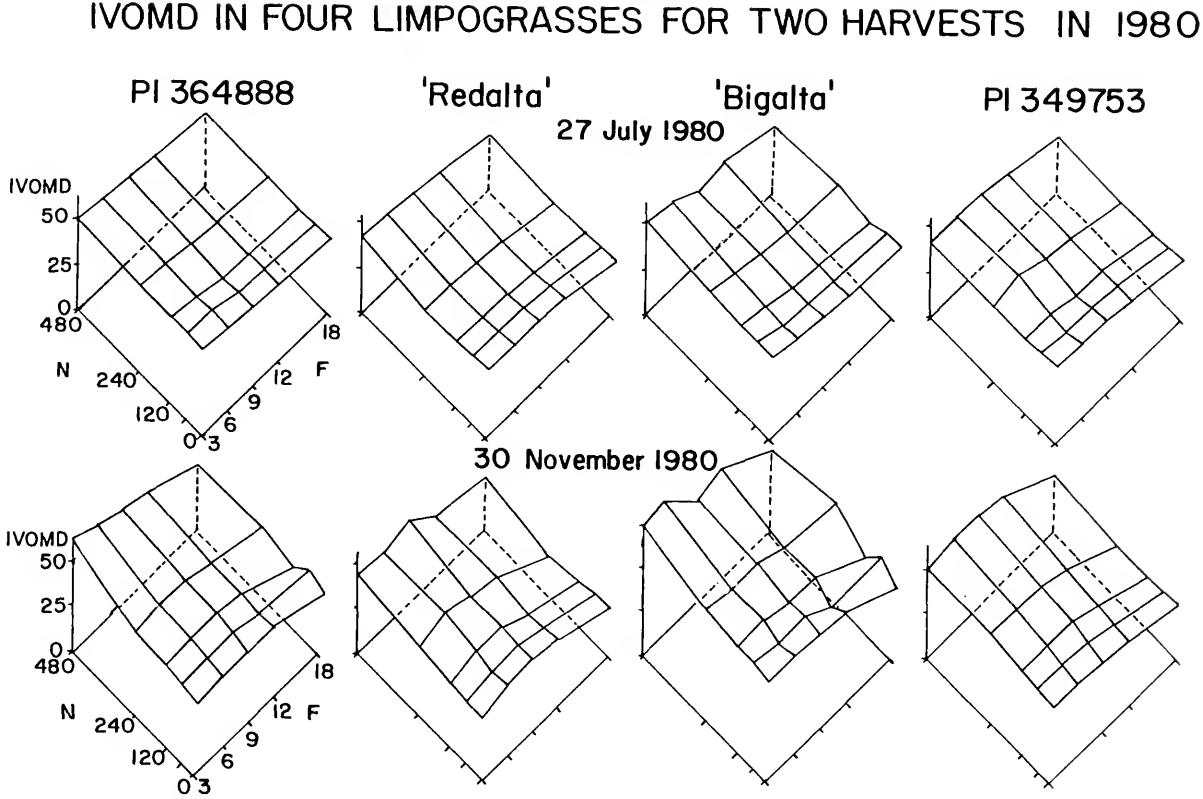

Figure 13. Comparison of in vitro organic matter digestibility (IVOMD) for four limpograsses at two 1980 harvest dates subjected to five levels of nitrogen (N) fertilization and five frequencies (F) of clipping

values across all treatments in the 27 July sampling explains the flatness of the surfaces in Figure 13. In the 30 November sampling there was a greater range of IVOMD values across treatments but the surface plots in Figure 13 were topographically too flat for easy detection of differences by the eye.

The increase in limpograss quality between 27 July and 30 November was reflected in the Table 6 IVOMD values. It was decided to examine the IVOMD values for Bigalta and PI 364888 more closely in Table 9 in order to compare the increases in digestibility from harvest 6 to harvest 12 (H6 to H12). For PI 364888 the quality increased in all but the 18 week treatments and nearly identical trends were shown in Bigalta. The largest differences between H6 and H12 were found in the 6 and 9 week old tissue in both grasses. Differences in IVOMD between Bigalta and PI 364888 were presented in Table 9 in order to demonstrate the consistently higher IVOMD of Bigalta. The advantage for Bigalta over PI 364888 was much more dramatic on the 30 November (H12) sampling date than on 27 July 1980 (H6).

Total Nonstructural Carbohydrate as Related to IVOMD

Heavily fertilized limpograss in the autumn appeared very lush compared to the stiffer stems from mid-season, and it was thought that accumulated starch might explain some of the autumn IVOMD increases in this study. Garrard and West (1972) and Carter and Garrard (1976) showed that starch accumulated in the leaves of digitgrass during cool nights. Response surfaces for "shoot" TNC were constructed for Bigalta, Redalta, and PI 364888 from data collected on 27 July and 30 November (Figures 14 and 15). In Figure 14 a rising plane is shown for PI 364888.

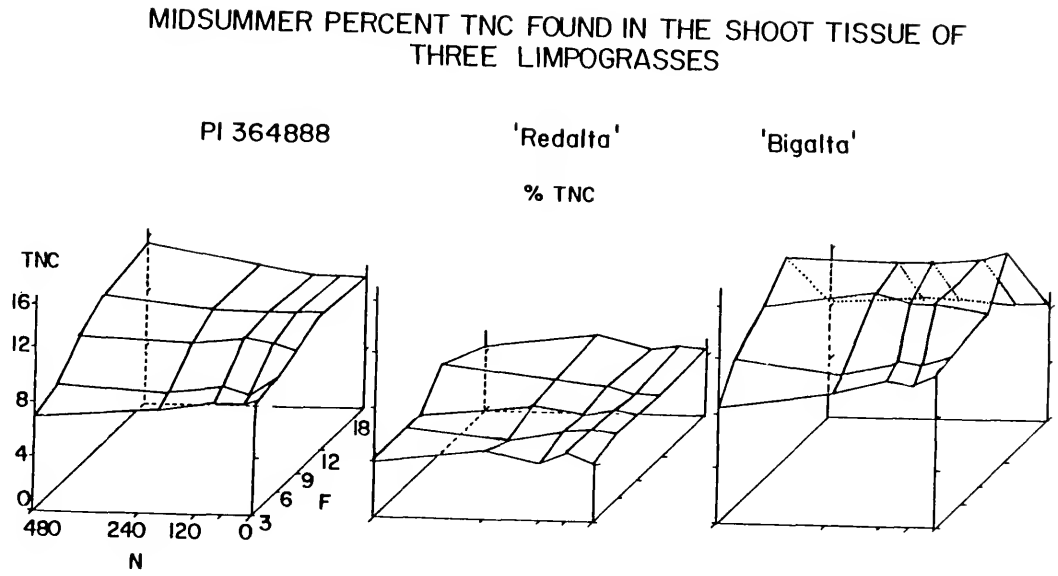

Figure 14. Percent total nonstructural carbohydrate (TNC) in the "shoot" tissue of three limpograsses on 27 July 1980 subjected to five levels of nitrogen (N) fertilization and five defoliation frequencies (F)

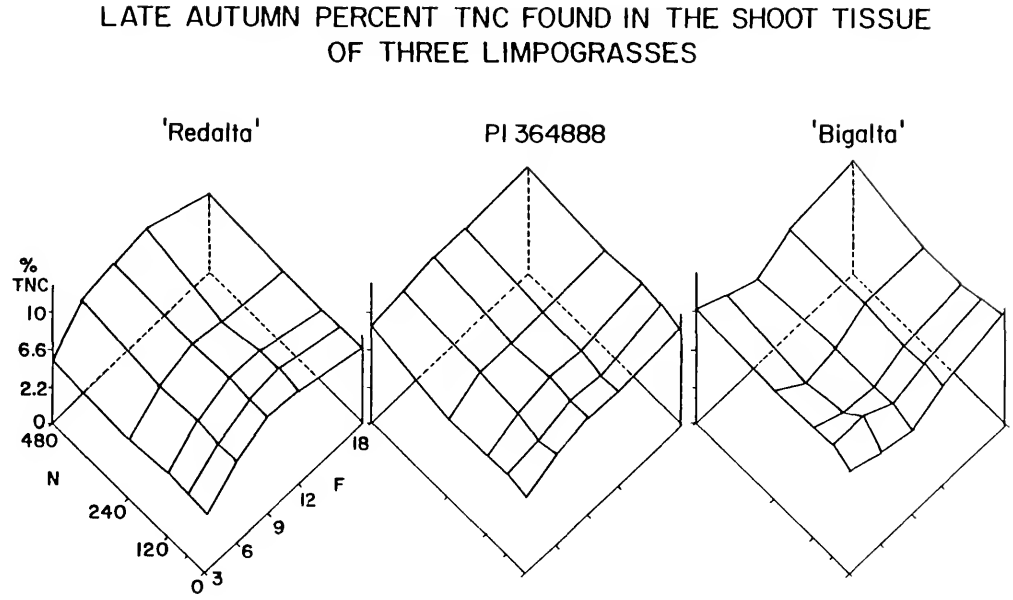

Figure 15. A comparison of total nonstructural carbohydrate (TNC) in the "shoots" of three limpograsses subjected to five levels of nitrogen (N) fertilization and five frequencies (F) of defoliation sampled on 30 November 1980

Redalta has a rising plane of a much shallower slope and of a lower magnitude than PI 364888. The Bigalta surface plot rose precipitously to a ridge at the 12 week cutting frequency and then the TNC in "shoots" fell back to a lower value at the rear edge of the cube (18 week clipping treatments). In Figure 15 Redalta and PI 364888 looked almost the same as for the July surfaces. Bigalta, on the other hand, increased carbohydrate content for 3 week treatments (front left of the plot); decreased TNC in 6, 9, and 12 week treatments greatly; and decreased the TNC percent in 18 week treatments very little. These effects inverted the response in Bigalta at 30 November from what was shown on 27 July 1980. These interpretations were verified by the actual "shoot" TNC percentages shown in Table 9. The occurrences described above were incongruous with the hypothesis that autumn increases in "shoot" TNC would increase percent IVOMD. What was observed was a decrease in "shoot" TNC and an increase in IVOMD especially in the 6, 9, and 12 week treatments.

The variation of TNC content in the "crowns" and "shoots" between 27 July and 30 November was studied in hopes of clarifying the relationship, if any, between TNC, IVOMD, and CP. In Table 9 the TNC accumulation patterns for PI 364888 between H6 and H12 showed an increase in the "crowns" and a decrease in the "shoots" for all except the 480*3 treatment combination. For PI 364888 it was logical to conclude that TNC produced in "shoots" in the summer was translocated to the "crowns" for storage in the autumn. In Bigalta a different trend emerged. The "shoot" TNC values decreased from H6 to H12; however, contrary to the situation in PI 364888, the Bigalta "crown" percent TNC also decreased

Table 9. Balance sheet of *in vitro* organic matter digestibility (IVOMD) and total nonstructural carbohydrates (TNC) in two limpograsses

Treatment				Harv no.	Limpograss						IVOMD difference (Bigalta minus PI 364888)
					PI 364888			Bigalta			
N/cut $kgha^{-1}$	Total N $kgha^{-1}yr^{-1}$	Cut freq	No. of harvests		TNC (%)		IVOMD (%)	TNC (%)		IVOMD (%)C	
					Shoot	Crown		Shoot	Crown		
0	0	3	12	H6	8.1	10.5	49.9	9.9	11.4	58.3	8.4
				H12	7.0	16.2	51.9	9.6	14.3	65.6	13.7
					-1.1	+5.7	+2.0	-0.3	+2.9	+7.3	
10	120	3	12	H6	7.6	10.9	50.7	9.7	11.3	58.2	7.5
				H12	7.4	17.1	56.8	10.0	13.9	69.5	12.7
					-0.2	+6.2	+6.1	+0.3	+0.6	+11.3	
40	480	3	12	H6	6.6	10.2	51.8	8.1	9.9	63.7	11.9
				H12	8.9	13.8	61.8	10.9	11.5	72.0	10.2
					+2.3	+3.6	+10.0	+2.8	+1.6	*8.3	
10	60	6	6	H6	11.1	13.0	49.8	11.6	13.6	51.1	1.3
				H12	7.8	14.6	56.0	10.1	13.4	64.2	8.2
					-3.3	+1.6	+6.2	-1.5	-0.2	+13.1	
60	240	6	6	H6	9.6	13.4	48.3	11.2	9.6	55.1	6.8
				H12	8.8	14.1	60.0	7.7	7.9	69.9	9.0
					-0.8	+0.7	+11.7	-3.5	-1.7	+14.8	

Table 9. Continued

Treatment				Harv no.	Limpograss						IVOMD difference (Bigalta minus PI 364888)
					PI 364888			Bigalta			
N/cut kgha^{-1}	Total N kgha^{-1}yr^{-1}	Cut freq	No. of harvests		TNC (%)		IVOMD (%)	TNC (%)		IVOMD (%)	
					Shoot	Crown		Shoot	Crown		
0	0	9	4	H6	11.8	12.7	45.8	16.1	11.3	52.2	6.4
				H12	8.9	13.9	53.0	7.6	6.0	64.3	11.0
					-2.9	+1.2	+7.2	-8.5	-5.3	+12.1	
30	120	9	4	H6	12.4	10.4	43.3	16.2	10.6	49.4	6.1
				H12	9.4	11.8	58.5	8.1	7.2	64.6	6.1
					-3.0	+1.4	+15.2	-8.1	-3.4	+15.2	
120	480	9	4	H6	12.4	8.3	47.5	16.3	10.4	50.0	2.5
				H12	9.9	11.7	63.9	7.8	9.3	67.8	3.9
					-2.5	+3.4	+16.4	-8.5	-1.1	+17.8	
20	60	12	3	H6	13.0	*	44.0	18.9	*	48.2	4.2
				H12	8.3	16.3	51.0	9.3	12.3	53.1	2.1
					-4.5	*	+7.0	-9.6	*	+4.9	
80	240	12	3	H6	13.2	*	43.4	18.2	*	48.8	5.4
				H12	8.9	15.7	53.4	9.7	11.2	61.9	8.5
					-4.3	*	+10.0	-8.5	*	+13.1	

Table 9. Continued

Treatment				Harv no.	Limpograss						IVOMD difference (Bigalta minus PI 364888)
					PI 364888			Bigalta			
N/cut $kgha^{-1}$	Total N $kgha^{-1}yr^{-1}$	Cut freq	No. of harvests		TNC (%)		IVOMD (%)	TNC (%)		IVOMD (%)	
					Shoot	Crown		Shoot	Crown		
0	0	18	2	H6	12.4	10.2	43.1	11.1	13.9	48.8	5.7
				H12	8.9	16.8	40.8	10.1	11.2	41.3	0.5
					-3.5	+6.6	-2.3	-1.0	-2.7	-7.5	
60	120	18	2	H6	12.3	11.9	41.0	11.2	12.2	42.7	1.7
				H12	9.7	17.9	37.0	9.5	11.4	39.3	2.3
					-2.6	+6.0	-4.0	-1.7	-0.8	-3.4	
240	480	18	2	H6	15.0	10.7	42.0	10.7	11.1	43.1	1.1
				H12	9.8	14.7	42.3	10.5	10.2	48.4	6.1
					-5.2	+4.0	-0.3	-0.2	-0.9	+5.3	

(or remained the same) for all treatments except those cut every three weeks.

The question was, "What was responsible for the increase in quality seen in all limpograsses between midsummer and late autumn?" From the above data for limpograss it was postulated that the high levels of TNC in summer "shoot" tissue along with very low CP contents may have allowed complete digestion of the nonstructural carbohydrates but restricted the rumen bacteria from degrading the cell wall materials. In the summer all the absorbed N would be sequestered by rapid growth and tissue synthesis, as opposed to autumn when growth rates decline. Hence, in autumn it was thought that TNC was partially bound up in compounds such as protein in the stems or leaves that was not measured in TNC analyses but was readily digested by rumen microorganisms in tests of IVOMD. The hypothesis was especially attractive for Bigalta because of the large decreases of TNC in shoots for 6, 9, and 12 week treatments along with concomitantly large increases in IVOMD and CP for the same treatments between the two dates. The IVOMD and CP changes between H6 and H12 for all four limpograsses can be studied in Table 6. The same situation was observed to a lesser degree in PI 364888; however, this plant appeared to partition more TNC into the storage organs than Bigalta. This explanation was appealing because of the excellent persistence reported for PI 364888 and the poor persistence reported for Bigalta (Quesenberry et al., 1978).

Seasonal TNC Trends

The primary objective of using the TNC analyses was in studying storage of carbohydrates in limpograss. The means for all limpograsses

for any given harvest date and treatment are shown in Appendix B. The overall seasonal trends can be constructed given this information. The trends were (1) highest TNC in the spring, (2) lowest TNC in midsummer, (3) an increase in TNC from August to mid-September, (4) a decrease in TNC to November, and (5) a sharp increase again from December until spring. The TNC increases in the autumn were most predominant for PI 364888.

The decrease of stored TNC in "crowns" in summer may be associated with rapid growth rates and maximum day and night temperatures causing a severe demand on TNC in the storage organs. In summer TNC was also highest in the "shoots" because of active growth and photosynthesis (McCarty, 1935; Brown and Blazer, 1965; Blaser et al., 1966). As night temperatures cooled off in early autumn, the respiratory demand may have decreased and active photosynthesis in the day allowed a net storage of TNC. Wardlaw (1968) stated that, "Growth of established shoots has priority over root and bud growth under conditions of assimilate deficiency" (p. 86). After reserves began to increase again in early autumn, the tillers and roots began active growth; however, this new growth would require substrate which may explain the small decrease of TNC in "crowns" between September and November. In this study TNC may have been translocated to sites of root and tiller synthesis instead of becoming localized in the shoots as observed by Garrard and West (1972) and Carter and Garrard (1976). Chatterton et al. (1972) studied tillering as a variable on the effect of cool night temperatures on Pangola digitgrass. They showed that actively tillering plants accumulated no starch, while non-tillering plants accumulated starch during the day that localized in the chloroplasts during cool nights.

Wardlaw (1968) stated that temperature affects growth (i.e., development) greater than photosynthesis and translocation. The final surge of carbohydrate shown in limpograss "crowns" during December and beyond was possibly caused by the photosynthesizing basal tillers during warm days in the cool season.

Total Nonstructural Carbohydrates and Persistence

Another intention of using TNC analysis was to study differences in persistence observed in the limpograsses. In Figure 7 the sampling schedule for TNC shows that complete sampling for all treatments was begun on 6 September (H8). Until that point the treatments were allowed to take their effect. In Figure 16 data obtained from "crowns" harvested on 28 September and 30 November from PI 364888 and Bigalta are plotted. A predominant ridge along the front left edge of all plots was explained by the change in morphology effected by clipping every 3 weeks. Plants were prostrate, had more tillers per unit area, and hence, high photosynthetic activity was able to provide assimilates for storage in the stolons and stem bases. A trough or basin shown for both PI 364888 and Bigalta in the 28 September plots was caused by the 6 week treatments in this portion of the response surfaces. These treatment combinations were not short enough to promote a prostrate habit and not long enough to allow complete stem elongation. Elongation requires carbohydrate compounds, and before the shoots were able to resupply the storage organs with photosynthate, it was defoliated and the cycle began again. Beyond the 9 week level of cutting frequency the TNC concentration in "crowns" increased gradually for 28 September samples, whereas

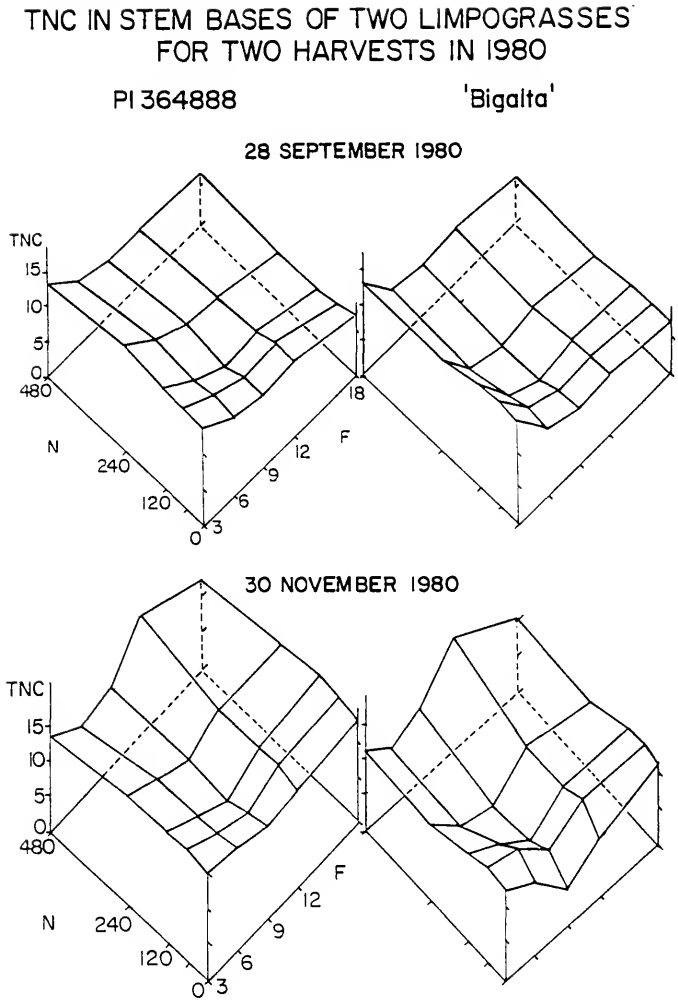

Figure 16. Percent total nonstructural carbohydrate (TNC) in the stem base ("crowns") of two limpograsses on two dates as affected by nitrogen (N) fertilization and defoliation frequency (F)

the increase was more dramatic for 30 November, ending with a plateau along the rear edge of the surface plots.

In Figure 17 the TNC percent of the 3 week treatments is plotted across the 1980 season for PI 364888 and Bigalta. The effect of N caused an increase in TNC in the "crowns" early in the year when stored carbohydrate compounds were able to provide the carbon skeletons for protein and tissue synthesis. The rapidly growing tissue photosynthesized enough carbohydrate in Bigalta in the 480*3 treatment to maintain more stored substrate than the other levels of N until the fourth harvest. At this time the TNC values dropped in both limpograsses as respiration and growth exerted a heavy demand on stored TNC.

From midsummer onward in Figure 17 the 480 N level caused a depression in stored TNC for the 3 week treatments. The sharp rise of TNC at H8 indicated that environmental stress had diminished in comparison to midsummer. New root and tissue synthesis was most likely the cause of the H8-H11 TNC decline. The TNC increased again in the November samplings of PI 364888 but Bigalta did not respond in the same manner, as was shown in November samples from the 1979 experiment (Chapter 2, Table 4).

Sampling logistics prevented an analysis of actual quantities of TNC per unit area of plot. Only percent TNC was measured and, as shown in Figure 17, there were no large differences between the persistent (PI 364888) and nonpersistent (Bigalta) limpograsses in percent TNC. Visual estimates of percent limpograss, however, did permit an analysis of persistence (Figure 18).

Figure 18 shows how the 480 N level caused rapid growth early in the season, as indicated by the high percent of limpograss in both stands until H4. Midsummer stress caused by frequent defoliation, high

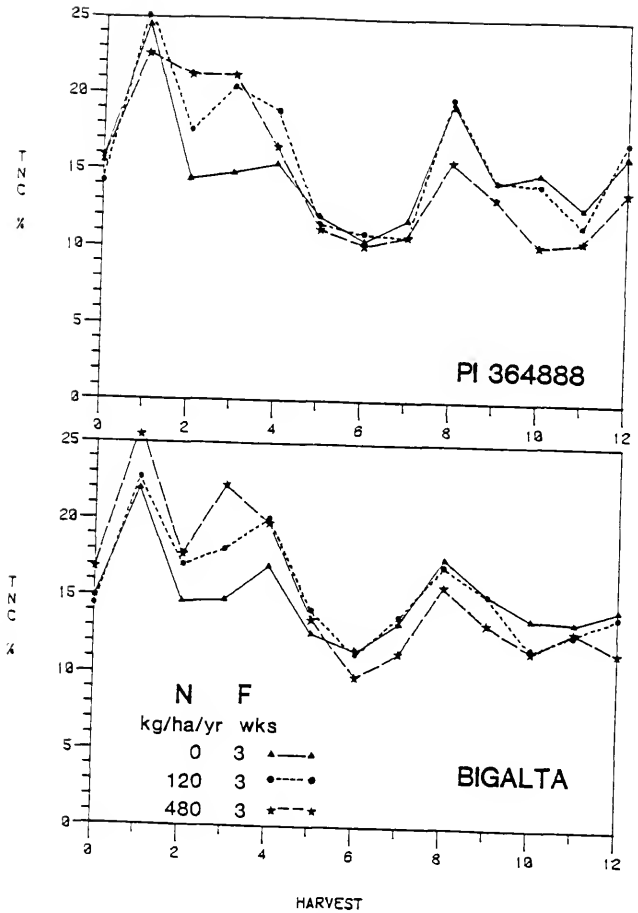

Figure 17. A comparison of total nonstructural carbohydrate (TNC) percent in the "crowns" of two limpograsses subjected to 3 week defoliation frequencies (F) and fertilized at three levels of nitrogen (N) during the 1980 growing season

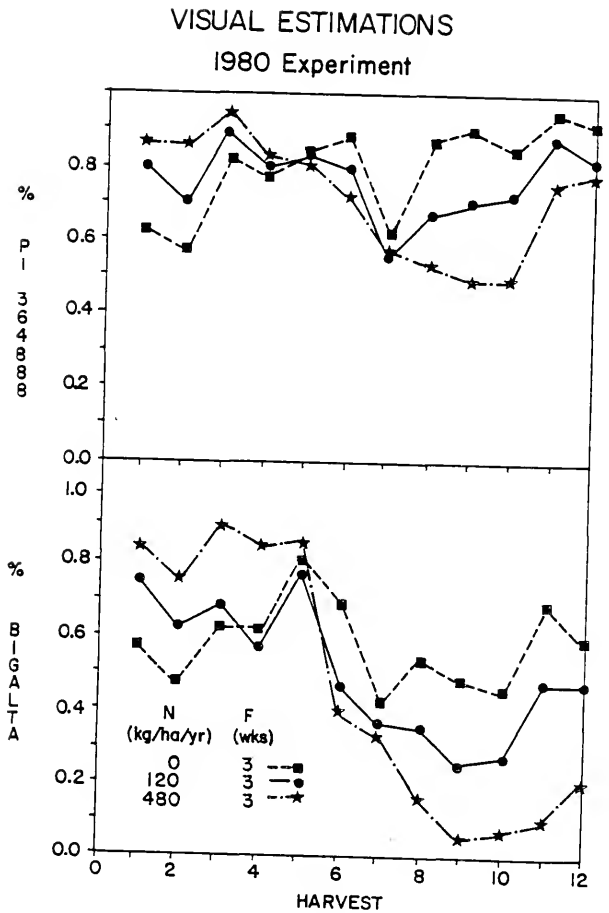

Figure 18. Visual estimations of percent 'Bigalta' and PI 364888 for treatments having 3 week defoliation frequencies (F) and three levels of nitrogen (N) in the 1980 growing season

fertilization, as well as TNC demands from growth and respiration caused a tremendous loss of limpograss in plots. The loss of Bigalta was much greater because this grass had fewer stolons to serve as a carbohydrate reservoirs when N was applied. Tissue degradation and competition from summer weeds further depressed percent Bigalta and PI 364888 until H10 when the summer weeds died and the limpograss continued growing; therefore, becoming a greater percent of the vegetation in the plots. This effect was present for all three treatments.

In Figure 19 the 1980 temperature and rainfall data are presented. The weather data was averaged for the 3 week periods prior to each harvest in order to represent the temperature and rainfall regimes under which the samples were taken. It was hoped that weather data could help explain the TNC peak centered about H8 in Figure 17. The maximum and minimum average daily temperatures for the 3 week period prior to H8 were almost as high as in midsummer; however, these data did not reflect the duration of heat for a summer day as opposed to an early autumn day. Hence, even though the minimum and maximum average daily temperatures did not appreciably decline until the beginning of October (H9-H10), the actual temperature stresses on the limpograsses probably diminished prior to October.

Water deficits have been reported to cause increases in stored TNC but the 3 week period before H8 had more rain (14 cm) than most of the 3 week periods in the 1980 harvest season (Figure 19). Hence, lack of water cannot be used to explain the TNC increase at H8.

In Figure 18 the percent limpograss began to rise again between H7-H8 (16 August-6 September) for the 0*3 and 120*3 treatments in PI 364888 and in the 0*3 Bigalta treatment. This increase in limpograss percent

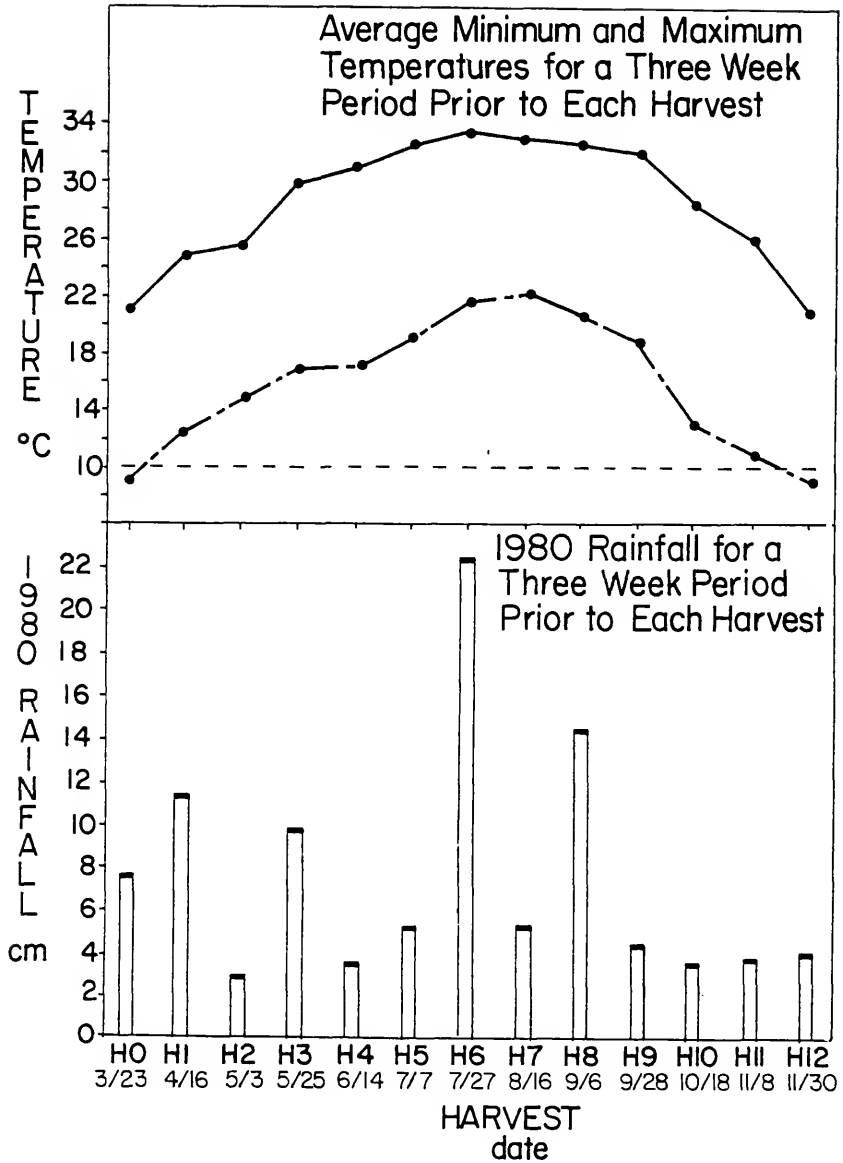

Figure 19. Rainfall and temperature data for the 1980 growing season taken at the Beef Research Unit near Gainesville, Florida

of stands matches the autumn TNC peak at H8 in Figure 17. Further increases were made in percent limpograss in stands once the annual summer weeds died out at H10. Between H10 and H11 the average minimum daily temperature dropped below 10 C (Figure 19) and the limpograsses grew very slowly. Green tissue was present throughout the winter period, however, even after freezing temperatures.

In Figure 20 the visual estimations for all limpograsses between 14 June and 6 September (H4, H6, H8) were plotted as response surfaces. Notice how limpograss dominated sward composition on 14 June but by 27 July had diminished appreciably for the 3 week cutting frequency treatments. By 6 September the non-persistent limpograsses (Bigalta and PI 349753) were identified and their persistence declined further at 28 September. Values for percent limpograss at the end of the 1980 experiment on 30 November may be found in Table 6. It was once again believed that the persistence of both Redalta and PI 364888 was due to a larger reservoir of carbohydrate imparted by morphological differences amongst the grasses.

Discussion

The top DM yields obtained for the limpograsses in this study were excellent. Limpograss PI 364888 produced a total of 29.3 m tons in 1980 and this was not matched in the literature. If Killinger (1971) had continued his measurement of yield beyond August, his Bigalta yields might have increased beyond the 27 m tons he reported. By August the limpograsses in this study produced 12-18 m tons (Bigalta and Redalta produced 12 m tons).

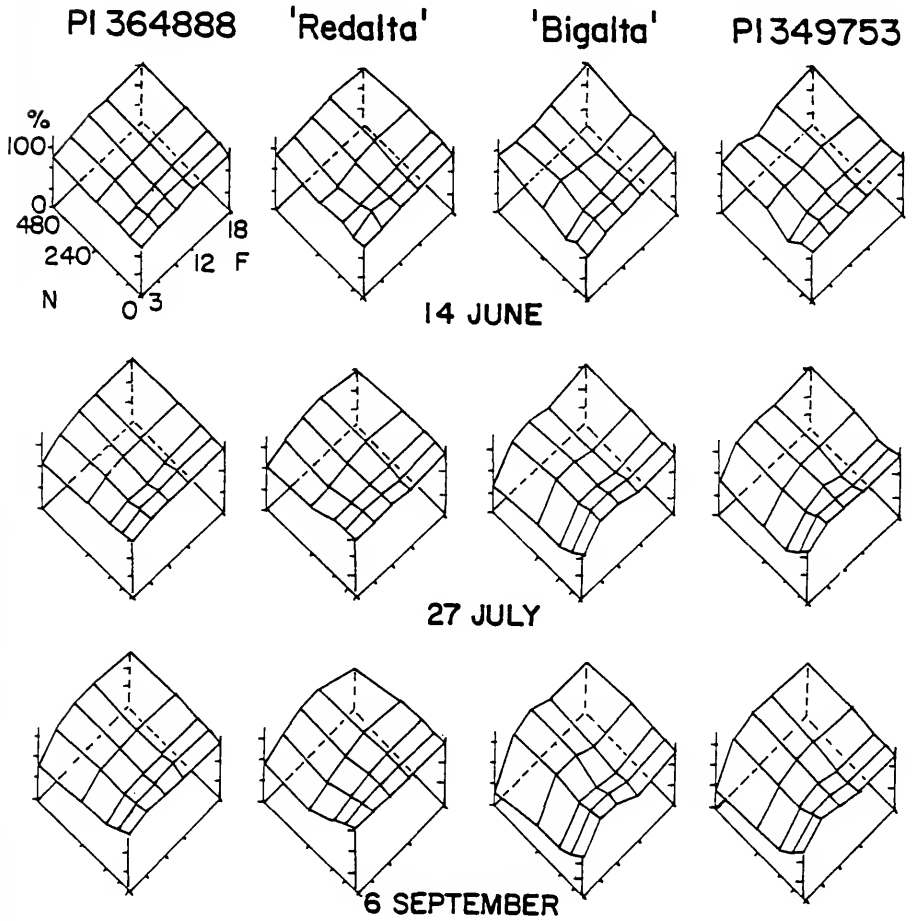

Figure 20. Visual estimates of four limpograsses subjected to five levels of nitrogen (N) fertilization and five frequencies (F) of clipping across three summer harvests

The yield potential of limpograss would be unimpressive if it were not for its autumn and spring production. There is no lack of poor quality biomass in Florida in the summer. Cool-season production is a greater problem in north Florida than further south. Colder temperatures in north Florida restrict the growth of some improved forages that do well in south Florida. Limpograss is well adapted (Oakes, 1980). Stockpiled limpograss may not be the answer for limpograss utilization even though it is an inexpensive technique. Limpograss is supposedly difficult to cure as hay but there is nothing in the literature to suggest anyone has tried. The increasing acceptance of round bales as well as hay ammonification procedures could improve the utilization of limpograss to take advantage of the tremendous potential for DM accumulation. Ruelke and Quesenberry (1982) are now studying the early application of N to produce large quantities of limpograss forage in the spring. Big bales could preserve this material before summer rains in June.

Ammonification sounds intuitively more appealing than late N fertilization (Blue et al., 1961; Kretschmer, 1965) as a way of increasing the N content of mature limpograss. Another method to increase N in limpograss forage is to mix legumes in the stand. With no N applied the limpograss canopy is very open and might be conducive to the association of a legume such as joint vetch (Aeschynomene americana L.). Work is presently initiated in this regard. The limpograsses have recently been attributed an alleopathic effect (Ruelke and Quesenberry, 1981; Young and Bartholomew, 1981; Tang and Young, 1982) which may inhibit weeds and legumes as well.

Limpograss PI 364888 has the potential to add flexibility to the flatwoods forage system. If producers have bahiagrass pastures they can graze PI 364888 in spring and autumn (Ocumpaugh, 1982). Bahiagrass could be grazed in the summer and limpograss could accumulate DM until the summer rains end, at which time it could be baled (if ammonification works) or ensiled and then staged for stockpiling until October when the bahiagrass stops its growth. When winter ensues the limpograss silage or hay could be fed.

Table 10 is presented at this point to show the treatment combinations representing several combinations of yield and quality in the present study. It exemplifies the high quality attained in PI 364888 without the production of DM, or the high yield without adequate quality. Clearly a protein supplement must be injected into a limpograss feeding program that utilizes some of the combinations shown in Table 10.

Florida has both a summer and winter slump (Mott and Moore, 1977) in which cattle perform poorly. The summer slump could be considered both an animal and plant problem. Midsummer CP and IVOMD values reported for limpograss on 27 July of this study were as poor as any that were found for limpograss in the literature. The samples for quality evaluation were clipped at 5 cm and as such would not represent what an animal would selectively consume; however, the objective was not to inject sampling bias by plucking rather than uniformly sampling the plants. The IVOMD analyses were conducted confounding replications with laboratory runs and included internal plant standards whose results did not vary from their accustomed values. In the Moore et al. (1972) modification to the Tilley and Terry two stage test for IVOMD, soybean meal is fed to the fistulated steer a short time prior to collecting the

Table 10. Treatment combinations in PI 364888 representing the best compromise between yield and quality in the 1980 experiment

FERT N		Defoliation		Var*	Date of harvest											DM Total
Per cut	Total	FREQ of	No. of		3 May	25 May	14 Jun	7 Jul	27 Jul	16 Aug	6 Sep	28 Sep	18 Oct	11 Nov	30 Nov	
- (kg/ha) -																(kg/ha)
60	240	6	6	DM	0.6		2.7		4.6		2.1		0.4		0.3	10.7
				IVOMD	57.3		46.5		48.4		48.7		52.0		59.7	
				CP	6.0		3.5		4.7		5.5		7.1		10.2	
120	480	9	4	DM		6.1			10.1			6.8			0.4	23.4
				IVOMD		49.7			47.6			45.8			63.9	
				CP		4.5			4.1			4.0			12.1	
80	240	12	3	DM			6.7				9.3				3.1	19.1
				IVOMD			42.9				39.6				53.5	
				CP			2.8				3.1				6.0	
240	480	18	2	DM					18.3						11.0	29.3
				IVOMD					42.0						42.3	
				CP					2.4						4.3	

*DM (m ton/ha); IVOMD (%); CP (%).

rumen fluid for the test. It is possible that the soybean meal did not supply sufficient N to the rumen bacteria *in vitro*, therefore causing a limitation on the normal degree of cell wall degradation.

Jolliff et al. (1979) showed that forage of the same chronological age can vary widely in nutritive value when sampled at different times of the year. The study, conducted in Texas, had a similar seasonal temperature regime as present in Gainesville, Florida, and it was found that percent changes in CP and digestibility in 8 week old forage varied by as high as 4.6 and 12.7 percentage units within a 30 day period. These findings support the seasonal changes of percent CP and IVOMD found between 27 July and 30 November in this study. The higher quality in the autumn could be due to slower maturation in lower temperature regimes (Wilson and t'Mannetje, 1978).

The seasonal flux of TNC in this study was similar to that shown by wiregrass (*Aristida stricta* Michx.) in the western sand hills of Florida (Woods et al., 1959). Carbohydrate reserves in wiregrass roots were in least supply during mid-July, and were highest around the first of February. In Australia Wilson and t'Mannetje (1978) showed a TNC decrease in summer and higher levels in the spring for leaves of buffelgrass (Cenchrus ciliaris cv.'Biloela') and green panic (*Panicum maximum* var. *trichoglume* cv. 'Petrie'); however, the magnitude of seasonal TNC fluctuations were far less (8-12 percent) than observed here for limpograss (4.2 percent in PI 349753 (480*9), 18 October to 26.0 percent in Bigalta (480*3), 16 April).

The TNC studies did not entirely explain the loss of persistence in Bigalta and PI 349793. The literature warned of the pitfalls of percent data in TNC studies (Humphreys, 1966; May, 1960), but the logistical

problems were too immense to consider the extra work involved in producing TNC data on actual amounts of carbohydrate per unit area or per shoot. Visual estimations of percent limpograss were taken on all plots prior to each harvest by the same experimenters. This method was reliable enough to characterize the treatment effects on the limpograss persistence.

The results of this study showed that Bigalta was superior in quality and is an excellent grass when properly managed. This contention is borne out by the fact that it recovered so quickly in 1981 from below 20 percent of stands after stressful treatments in 1980. Redalta had the worst quality and has a limited future. Careful management is also necessary in order for PI 349753 to persist. Of all limpograsses, PI 346888 had higher yield and persistence than Bigalta and PI 349753; higher yield and quality than Redalta and PI 349753; and has been recommended for cultivar release in 1983.

Conclusions

1. The four limpograsses included in this study had different advantages in yield potential at various levels of N and F. Bigalta produced well at intermediate cutting frequencies, while PI 364888 produced more DM with high nitrogen and long cutting frequencies.

2. The four limpograsses differed in their seasonal distribution of DM yield. Bigalta was lowest in spring production and PI 349753 was highest. Stockpiled forage from 27 July 1980 to 30 November was greatest for PI 364888.

3. Values for IVOMD were lower than shown in other published reports, especially for samples taken during midsummer. The IVOMD

percents increased in all grasses in the autumn and throughout the year Bigalta was superior in IVOMD percent than the other limpograss genotypes.

4. Crude protein was uniformly low in all genotypes, especially during midsummer. As with the IVOMD values, CP increased in the samples collected in the autumn. Bigalta was highest in CP content.

5. Studies of TNC revealed a maximum percent of organic food reserves in stem bases in March and a low in July. As temperature stressed decreased in September and October, TNC increased and then decreased again until November when new roots and tillers may have been using the newly accumulated TNC. After November a slow accumulation of TNC occurred through the winter.

6. The 3 week cutting frequency and 480 kg/ha/yr N rate caused limpograss deterioration in the plots. Bigalta and PI 349753 were most susceptible to the treatment stresses while Redalta and PI 364888 were most persistent.

7. The period of low TNC reserves in midsummer coincided with the period of most severe limpograss deterioration in the plots. Perhaps the high amounts of absorbed N in the plant demanded more carbohydrate for tissue and protein synthesis than the plants were able to supply. Weed encroachment and competition for nutrients, light, and water were contributory factors in the decline of limpograss stands.

Summary

Research on limpograss (Hemarthria altissima (Poir) Stapf et C.E. Hubb) has identified the potential of this grass for flatwood forage-livestock systems due to better quality and dry matter yield potential

than bahiagrass (Paspalum notatum Flügge). A current problem is that the limpograss cultivars 'Bigalta' and 'Redalta' have attributes that make them less than ideal forages. Recent evaluations of PI 364888 suggest that this grass adequately combines the desirable characteristics of yield, persistence, quality, and animal acceptability. The present study was designed to thoroughly understand why, how, and in what categories the new limpograss was superior to the already released cultivars.

Results from an extensive field study and thorough laboratory analyses showed that PI 364888 did, indeed, possess better attributes for dry matter (DM) yield and persistence than Bigalta; better digestibility and yield than Redalta; however, it fell short of Bigalta in crude protein (CP) and in vitro organic matter digestibility (IVOMD).

Seasonal DM distribution and total nonstructural carbohydrate (TNC) flux were also studied as influenced by a complete array of nitrogen fertilization (0, 60, 120, 240, and 480 kg/ha/yr) and a wide range of defoliation frequencies (3, 6, 9, 12, and 18 weeks) in a modified central composite, response surface design.

Spring DM production was highest for another new limpograss (PI 349753) that yielded 8 m tons in the 12 week period prior to 14 June 1980. Bigalta had the lowest spring yields and was the most frost susceptible in the autumn. Limpograss PI 364888 produced 11 m tons of DM when staged to grow as a stockpiled forage from 27 July to 30 November 1980, and also produced the most annual DM (29 m tons).

Total nonstructural carbohydrate (TNC) in the storage organs decreased from a high in March to a low in July. In vitro organic matter digestibility (IVOMD) and CP values in tissue of equivalent

chronological age varied seasonally with regard to quality. Crude protein in samples taken during the summer were extremely low in all the limpograsses--well below the 7 percent necessary to maintain body weight in ruminant animals. The midsummer period was marked by lowest quality, highest stress on the reserves, and most marked decreases in percent limpograss in the stands.

CHAPTER 4
STATISTICAL ANALYSIS SYSTEM (SAS) METHODOLOGY FOR CONSTRUCTING RESPONSE SURFACE GRAPHICS

Introduction

The characterization and quantification of relationships between "independent" and "dependent" variables was traditionally approached by testing one factor at a time while holding other factors constant. It was recognized that information of much wider generality could be produced if several factors were investigated simultaneously (Hader et al., 1957).

Complete factorals are one statistical approach to understanding complex relationships among experimental factors but these designs consume profligate amounts of time, resources, and money because of the exponential explosion of treatment combinations with greater than three factors. Fractional factorials represent some improvement in reducing the size of the experiment, but they require extreme care in avoiding confounded effects because of pratial internal replication (Cochran and Cox, 1957).

Box and Wilson (1951) were the first to develop and describe the response surface design. Response surface methodology decreases the large number of treatment combinations necessary in factorial experiments. The first applications were in the chemistry and engineering fields. By 1966 the literature was full of diverse applications in a variety of fields (Hill and Hunter, 1966).

A factorial arrangement of design points for a two factor experiment are shown in Figure 3, and Box and Wilson's (1951) composite design in three factors is graphically represented in Figure 4. The latter has the property of symmetry when viewed from any axis. According to Hader et al. (1957), Box and Hunter thoroughly explored designs for fitting second order surfaces and eventually produced a new class called rotatable designs. Rotatable designs are defined as those whose estimations are equally reliable at any equal distance from the design origin. Hence, the standard error of the estimated response is dependent on distance rather than direction.

Rotatability is a reasonable property for exploratory work when the experimenter does not know in advance how the response surface will look. Consequently, he/she has no rational basis for specifying that the standard error of the estimate should be smaller in some directions than in others (Cochran and Cox, 1957).

In some industrial applications of response surfaces a series of short experiments could be planned so as to use the results of the first to plan the treatments for the second. A perpendicular course up or down the contours would head the experimenter towards the desired "optimum." This path of steepest ascent or descent could be used to "fix" the origin in the second and subsequent experiments (Cochran and Cox, 1957; Myers, 1971; Box et al., 1978). Canonical analysis is another method used in sequential testing that consists of shifting the origin to a new point and rotating the axes so they correspond to the axes of the contours. When the response surface is oriented to the new set of axes, the second order equations are greatly simplified and their

topographical nature becomes more obvious (Box et al., 1978; Myers, 1971).

In lines of work where an experiment must extend over a long period of time in order that the treatments produce their effects, the natural strategy is to try to discover the optimum combinations at the end of a single experiment (Cochran and Cox, 1957). Some experimenters have modified response surface designs in order to have treatment combinations that better fit the needs of their situation. The modifications are usually in the form of adding design points outside the experimental region of the formal design, or to shift points around within the experimental region to concentrate information in certain areas of interest (Littell and Mott, 1975). Mott (1982a) has referred to designs of this sort as modified central composite designs.

Littell and Mott (1975) described the use of contour diagrams using SAS; Henderson and Robinson (1982) presented but did not describe the formation of SAS surface plots. Schoney et al. (1981) described contour and response surfaces from computer graphics, but their University of Wisconsin-Madison computing package is not as widely available as SAS.

The objective of this paper is to show a "cookbook" procedure using SAS (Council and Helwig, 1979) and SAS/GRAPH (Council and Helwig, 1981) in plotting various surface plots for agronomic variables in a modified central composite design. Tests for lack of fit (LOF), precautions, and some pitfalls are discussed.

Materials and Methods

Vegetative sprigging was used to establish four limpograss genotypes on an Adamsville sand; a poorly drained siliceous hyperthermic

ultic haplaquod soil at the Beef Research Unit of the University of Florida. The grasses were PI 364888, PI 349753, 'Redalta', and 'Bigalta'. Florida Experimental Station accession numbers were 297, 886, 553, and 554, respectively.

Establishment Year: 1979

The experimental area was 30 x 118 m and supported a mature stand of rye (Secale cereale L.) prior to cultivating with a Ground Hawg rototiller on 22 May 1979. The field was blocked into three sections, and within each third, four main plots were marked to measure 7 x 30 m with 3 m alleys between each main plot. The alleys were planted to 'Argentine' bahiagrass (Paspalum notatum Flügge).

Waist-high stands of each limpograss were cut at 7.5 cm height, raked, and carried to an appropriately assigned, random location within each block. The herbage was evenly distributed over the soil, lightly disked to 15 cm, and the whole field was rolled using a cultipacker seeder to assure good soil to stem contact. The field was fertilized on 29 June and 23 August 1979 with 336 kg/ha 17-5-10 (N-P_2O_5-K_2O) containing 1 percent of a microelement mix. Soil test results from 7 June showed a pH of 6.2, 20.3 kg/ha phosphorus (P), and 40 kg/ha potassium (K). The field was flail chopped on 6 December 1979 to 7.5 cm height in order to remove frosted forage.

Experimental Year: 1980

Soil test results from samples taken on 6 March 1980 revealed a pH of 6.1, 16.4 kg/ha P, and 53.1 kg/ha K. On 23 March 1980, the field was uniformly mowed to 7.5 cm height and treatments consisting of five

levels of nitrogen (N) fertilization and five cutting frequencies (F) were imposed on the main plots. The treatments represented $13/25^{th}$ of a 5 x 5 factorial as depicted in Figure 5. Grasses were blocked (or replicated) as main plots in a split plot design, and the treatments provided a response surface with all points replicated three times. Each main plot contained 13 plots (2.3 x 7 m) for treatments. The entire field had 156 total plots (three blocks x four grasses x 13 treatments).

The treatment combinations represented a modified central composite response surface design that maximized information in what was considered the "management realm" (0-120 kg/ha/yr N and 3-9 week defoliation frequencies). Note the geometric scaling of N and the arithmetic progression for cutting frequency (with a skip at the 15 week level) in Figure 6. As the dotted lines in Figure 6 suggest, the design could be considered a superimposition of two complete factorials (2^2 and 3^2).

Figure 7 shows the treatment levels of N and F, the treatment number, and the harvest number, date, and chronological stage of the 1980 experiment. Sampling for carbohydrate analyses followed visual estimations of percent limpograss but preceded sampling for herbage quality and yield. Fertilization followed clipping, and the N was applied by hand as ammonium nitrate mixed with sand. The N application as split according to the number of defoliations; an equal portion applied at the start of the experiment and after every defoliation except the last.

The sampling schedule is shown by symbols for <u>in vitro</u> organic matter digestibility (IVOMD), N analysis, dry matter (DM) yield determination, and total nonstructural carbohydrate (TNC) measurement.

Full information on all treatments is necessary for the formation of surface plots and stem base ("crown") data obtained from TNC samples taken on 28 September 1980 satisfied this requirement in the example to follow. The TNC analysis is fully described in Appendix A.

Data were analyzed using SAS on an Amdahl 470 V/6-11 with OS/MVS Release 3.8 and JES2/NJE Release 3. Computing was performed at the Northeast Regional Data Center and the Agricultural Engineering Department of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

An analysis of LOF preceded surface plotting. The following SAS commands were used to generate the models necessary to test for LOF in this example. (A cubic model described the TNC response after a quadratic model failed.)

Complete model:

```
(LINE = limpograss genotype)
PROC ANOVA; CLASS LINE TRT REP;
MODEL TNC9=REP LINE LINE*REP TRT LINE*TRT;
```

Fractionated model:

```
PROC GLM; CLASS LINE REP;
MODEL TNC9=REP LINE REP*LINE
           N N*N N*N*N
           F F*F F*F*F
           N*F N*N*F N*F*F
           LINE*N  LINE*N*N  LINE*N*N*N
           LINE*F  LINE*F*F  LINE*F*F*F
           LINE*N*F  LINE*N*N*F  LINE*N*F*F/SOLUTION;
```

The test of LOF was accommodated by an F-test of the two previously described models: (Sums of Squares (SS); Degrees of Freedom (DF); Mean Square Error (MSE)).

$$F(LOF) = \frac{\left[\frac{\text{SS error (fractionated)}}{\text{DF (fractionated)}}\right] - \left[\frac{\text{SS error (complete)}}{\text{DF (complete)}}\right]}{\text{MSE (complete)}}$$

Often an elimination procedure for nonsignificant terms is useful for adding SS and DF back into the error term for the fractionated model. When a model was finally deemed acceptable, the computer provided estimates that were solved for the individual limpograss intercepts and parameters (N and F).

The equation for one limpograss line (PI 364888 or Florida number 297) was used to demonstrate the program for surface plotting (Table 11). The program has the following steps: (1) load the raw data from the TNC analyses and create means; (2) load the previously described surface equations; (3) assign treatment numbers (Figure 5) for the predicted treatment combinations; (4) use the surface equation to solve for TNC at the "missing" levels (Figure 5) of N and F; (5) merge actual plus predicted data; (6) assign N and F levels for the actual data; (7) "trick" the computer to start at zero on the TNC axis; and (8) plot the surface using PROC G3D (Figure 21).

Results and Discussion

It will be assumed the reader has a minimum knowledge of SAS because a detailed explanation of the program would be both laborious and out of place. Tables 12 and 13 are shown in order to allow the reader to trace the values from the computer output to the test for LOF in Table 14. In this example, LOF is determined with an F-test of models. The complete model contains the minimum possible sums of squares because it represents the best explanation of treatment

Table 11. A SAS program using a previously fitted regression to obtain values for the dependent variable to fill the holes in the treatment matrix in order to merge with actual data and use PROC G3D

```
1   //TNC9 JOB (1001,1401,29,9,0,),'CHRISTIANSEN;CLASS=2
2   /*PASSWORD     08,SCOTT
3   /*ROUTE PRINT  REMOTE13
4   //  EXEC SAS,REGION=372K,PLOT=
5   GOPTIONS DEVICE=GOULD;
6   DATA CR80;
7   INPUT NO 1-4 M 6-7 D 9-10 Y 12-13 LINE 15-17 TRT 19-20 REP 22
8   INC 24-25 DEV 27-28 WT 30-34 P1 36-37 DILN 39-40 P2 42-43 OD1 45-47
9   OD2 49-51 OD3 53-55 OD4 57-59;
10  DATE=MDY(M,D,Y); JULIAN=JULDATE(DATE); DROP DATE;
11  IF JULIAN=80272 THEN HARV=9;
12  ODA=(OD1+OD2+OD3+OD4)/4;
13  CARDS;
14
15  DATA CR80; SET CR80;
16  IF DEV=52 THEN MICROGM=0.1163113*ODA+4.3782886;
17  IF DEV=53 THEN MICROGM=0.1088506*ODA+3.2966179;
18  IF DEV=54 THEN MICROGM=0.1232033*ODA+4.5434298;
19  MGCHO=(MICROGM*0.011*DILN)/(P1*P2);
20  CHO=MGCHO*100/WT;
21  IF DEV=52 THEN DO; ADJCHO=CHO/1.0835; IPS=9.46; ADJIPS=8.73; END;
22  IF DEV=53 THEN DO; ADJCHO=CHO/0.9751; IPS=8.64; ADJIPS=8.86; END;
23  IF DEV=54 THEN DO; ADJCHO=CHO/1.0177; IPS=8.66; ADJIPS=8.51; END;
24
25  DATA TNC9; SET CR80;
26  IF LINE=297
27  PROC SORT; BY TRT;
28  PROC MEANS NOPRINT; BY TRT
29  VAR ADJCHO; OUTPUT OUT=NEW MEAN=TNC;
30
31  DATA DUMB13;
32  DO LINE=297;
33  DO N=0, 60, 120, 240, 480, 480.1;
34  DO F=3, 6, 9, 12, 18, 18.1
35  MACRO AA IF LINE=%
36  MACRO BB THEN TNC=%
37  MACRO GG IF N=%
38  MACRO HH AND F=%
39  MACRO II THEN TRT=%
40  MACRO JJ; ELSE TRT=.; %
41  GG     480.1     HH     18.1     II     26 JJ
42  GG         0     HH        6     II     16;
43  GG         0     HH       12     II     21;
44  GG        60     HH        3     II     14;
```

Table 11. Continued

```
45   GG        60     HH        9     II     19;
46   GG        60     HH       18     II     24;
47   GG       120     HH        6     II     17;
48   GG       120     HH       12     II     22;
49   GG       240     HH        3     II     15;
50   GG       240     HH        9     II     20;
51   GG       240     HH       18     II     25;
52   GG       480     HH        6     II     18;
53   GG       480     HH       12     II     23;
54   COMMENT TNC9 CROWN 80 297;
55   AA 297 BB 20.61946875 + 0.01089278*N + 1.5022467E-05*N*N
56   - 7.2858312E-08*N*N*N - 2.78981175*F + 0.25778999*F*F
57   - 7.7240594E-03*F*F*F - 0.00295115*N*F + 3.3195286E-06*N*N*F
58   + 6.2476724E-05*N*F*F;
59   OUTPUT; END; END; END;
60
61   DATA DUMMY; SET DUMB13;
62   IF TRT=16 OR TRT=21 OR TRT=14 OR TRT=19 OR TRT=24 OR TRT=17 OR
63   TRT=22 OR TRT=15 OR TRT=20 OR TRT=25 OR TRT=18 OR TRT=23 OR TRT=26;
64
65   PROC SORT; BY TRT;
66   PROC PRINT;
67
```

68 OB	LINE	N	F	TRT	TNC	OB	LINE	N	F	TRT	TNC
69											
70 1	297	0.0	3.0	.	14.36	19	297	240.0	3.0	15	15.42
71 2	297	0.0	6.0	16	11.49	20	297	240.0	6.0	.	11.40
72 3	297	0.0	9.0	.	10.76	21	297	240.0	9.0	20	9.79
73 4	297	0.0	12.0	21	10.92	22	297	240.0	12.0	.	9.34
74 5	297	0.0	18.0	.	8.88	23	297	240.0	18.0	25	6.90
75 6	297	0.0	18.1	.	8.78	24	297	240.0	18.1	.	6.80
76 7	297	60.0	3.0	14	14.59	25	297	480.0	3.0	.	13.31
77 8	297	60.0	6.0	.	11.33	26	297	480.0	6.0	18	9.29
78 9	297	60.0	9.0	19	10.27	27	297	480.0	9.0	.	7.96
79 10	297	60.0	12.0	.	10.17	28	297	480.0	12.0	23	8.05
80 11	297	60.0	18.0	24	7.81	29	297	480.0	18.0	.	7.50
81 12	297	60.0	18.1	.	7.71	30	297	480.0	18.1	.	7.44
82 13	297	120.0	3.0	.	14.91	31	297	480.1	3.0	.	13.31
83 14	297	120.0	6.0	17	11.32	32	297	480.1	6.0	.	9.29
84 15	297	120.0	9.0	.	10.01	33	297	480.1	9.0	.	7.96
85 16	297	120.0	12.0	22	9.72	34	297	480.1	12.0	.	8.04
86 17	297	120.0	18.0	.	7.19	35	297	480.1	18.0	.	7.50
87 18	297	120.0	18.1	.	7.08	36	297	480.1	18.1	26	7.44

```
88
89   DATA SURFACE; MERGE NEW DUMMY;
90   BY TRT;
91   DATA RESPONSE; SET SURFACE;
92   MACRO CC IF TRT=%
```

Table 11. Continued

```
 93   MACRO DD THEN DO; N=%
 94   MACRO EE F= %
 95   MACRO FF; END; %
 96   CC  1      DD   0      EE  3      FF
 97   CC  2      DD 120      EE  3      FF
 98   CC  3      DD 480      EE  3      FF
 99   CC  4      DD  60      EE  6      FF
100   CC  5      DD 240      EE  6      FF
101   CC  6      DD   0      EE  9      FF
102   CC  7      DD 120      EE  9      FF
103   CC  8      DD 480      EE  9      FF
104   CC  9      DD  60      EE 12      FF
105   CC 10      DD 240      EE 12      FF
106   CC 11      DD   0      EE 18      FF
107   CC 12      DD 120      EE 18      FF
108   CC 13      DD 480      EE 18      FF
109   IF TRT=26 THEN TNC=0;
110   IF N=480.1 AND F=18.1 THEN TNC=0;
111   TITLE 1 ACTUAL PLUS PREDICTED TNC;
112   TITLE 2 % TNC FOR 28 SEPTEMBER 1980 IN PI 364888 CROWNS;
113
114   PROC SORT; PROC PRINT
115                    ACTUAL                          PREDICTED
116   TRT      TNC        N        F        TRT      TNC        N        F
117     1    14.42       0.0      3.0       14    14.59      60.0      3.0
118     2    14.56     120.0      3.0       15    15.42     240.0      3.0
119     3    13.28     480.0      3.0       16    11.49       0.0      6.0
120     4    12.05      60.0      6.0       17    11.32     120.0      6.0
121     5    11.85     240.0      6.0       18     9.29     480.0      6.0
122     6    10.25       0.0      9.0       19    10.27      60.0      9.0
123     7     8.96     120.0      9.0       20     9.79     240.0      9.0
124     8     7.95     480.0      9.0       21    10.92       0.0     12.0
125     9    11.05      60.0     12.0       22     9.72     120.0     12.0
126    10     9.24     240.0     12.0       23     8.05     480.0     12.0
127    11     8.80       0.0     18.0       24     7.81      60.0     18.0
128    12     7.18     120.0     18.0       25     6.90     240.0     18.0
129    13     7.51     480.0     18.0       26     0.00     480.1     18.1
130
131   PROC G3D; PLOT N*F=TNC;
132
133   //EXEC PXPLOT
134   /*EOF
```

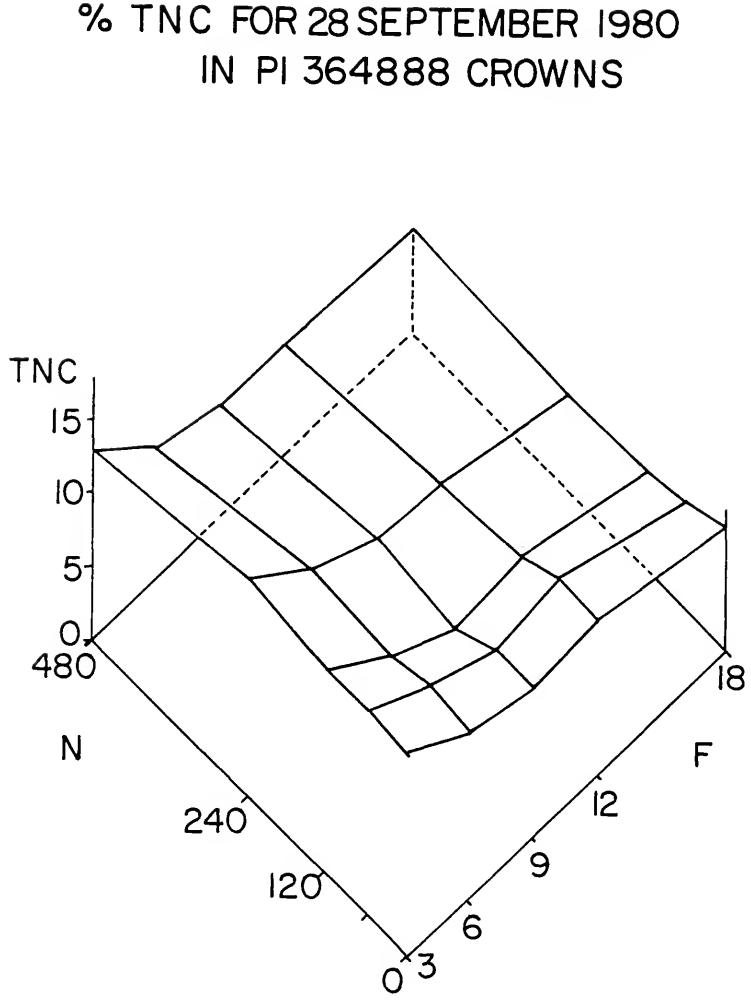

Figure 21. An example of the response surface plotted using SAS/GRAPH computer assistance

Table 12. A SAS analysis of variance procedure for a model that quantifies the maximum treatment (TRT) sum of squares (SS) for total nonstructural carbohydrate (TNC) in harvest 9 of the 1980 experiment

INDEPENDENT VARIABLE: TNC9

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	P > F	R-SQUARE	C.V.
MODEL	59	1234.35	20.92	21.37	0.0001	.93	11.16
ERROR	96	93.98	0.98				
CORRECTED TOTAL	155	1328.33		STD DEV	TNC9 MEAN		
				0.99	8.86		

SOURCE	DF	ANOVA SS	F VALUE	PR > F
REP	2	27.67	14.13	0.0001
LINE	3	240.60	81.92	0.0001
LINE*REP	6	17.19	2.93	0.0115
TRT	12	854.82	72.76	0.0001
LINE*TRT	36	94.06	2.67	0.0001

TEST OF HYPOTHESES USING THE ANOVA MS FOR LINE*REP AS AN ERROR TERM

SOURCE	DF	ANOVA SS	F VALUE	PR > F
LINE	3	240.60	27.99	0.0006

Table 13. A cubic SAS general linear model that fractionates the treatment sum of squares (SS) into SS explainable by nitrogen (N) fertilization and frequency (F) of defoliation for total non-structural carbohydrate (TNC) in harvest 9 of the 1980 experiment

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	P > F	R-SQUARE	C.V.
MODEL	47	1213.99	25.83	24.40	0.0001	0.92	11.61
ERROR	108	114.34	1.06				
CORRECTED TOTAL	155	1328.33					

STD DEV	TNC 9 MEAN
1.03	8.86

	SOURCE	DF	TYPE I SS	F VALUE	PR > F	TYPE IV SS	F VALUE	PR > F
	REP	2	27.67	13.07	0.0001	27.67	13.07	0.0001
	LINE	3	240.60	75.75	0.0001	18.76	5.91	0.0010
	LINE*REP	6	17.19	2.71	0.0173	17.19	2.71	0.0173
TRT	N	1	90.74	85.71	0.0001	3.88	3.67	0.0581
	N*N	1	29.48	27.84	0.0001	0.38	0.36	0.5502
	N*N*N	1	0.01	0.00	0.9634	0.83	0.78	0.3790
	F	1	501.73	473.91	0.0001	114.63	108.27	0.0001
	F*F	1	164.28	155.17	0.0001	69.92	66.05	0.0001
	F*F*F	1	55.37	52.30	0.0001	53.55	50.58	0.0001
	N*F	1	2.57	2.43	0.1221	0.01	0.01	0.9237
	N*N*F	1	0.62	0.58	0.4466	0.88	0.83	0.3630
	N*F*F	1	0.12	0.11	0.7392	0.13	0.12	0.7245

Table 13. Continued

	SOURCE	DF	TYPE I SS	F VALUE	PR > F	TYPE IV SS	F VALUE	PR > F
LINE*TRT	N*LINE	3	8.52	2.68	0.0495	9.19	2.89	0.0380
	N*N*LINE	3	10.68	3.36	0.0212	4.89	1.54	0.2069
	N*N*N*LINE	3	6.97	2.20	0.0913	5.23	1.65	0.1816
	F*LINE	3	20.01	6.30	0.0006	12.14	3.82	0.0120
	F*F*LINE	3	15.35	4.83	0.0035	8.11	2.55	0.0583
	F*F*F*LINE	3	8.04	2.53	0.0598	6.30	1.98	0.1190
	N*F*LINE	3	2.84	0.90	0.4479	10.04	3.16	0.0272
	N*N*F*LINE	3	6.34	2.00	0.1170	5.85	1.84	0.1422
	N*F*F*LINE	3	4.83	1.52	0.2121	4.83	1.52	0.2121

Table 14. An F-test of models for determining a statistically significant response lack of fit (LOF) and an elimination method for nonsignificant terms

TEST FOR LOF

$$F(LOF) = \frac{\left(\frac{\text{SS complete model - SS fractionated model}}{\text{DF complete model - DF fractionated model}}\right)}{\left(\frac{\text{SS complete model}}{\text{DF complete model}}\right)}$$

$$F(LOF) = \frac{114.34 - 93.98/108 - 96}{93.98/96}$$

$$= 1.73$$

Ftab (0.05,12,80) = 1.85
(0.05,12,100) = 2.36

Ftab (0.01,12,80) = 1.83
(0.01,12,100) = 2.33

CONCLUDE: 1.73 n.s. LOF

ELIMINATION METHODOLOGY:

SOURCE	DF	TYPE I SS	P > F
ERROR	108	114.34	----
N*N*F*LINE	3	6.34	0.1170
N*F*F*LINE	3	4.83	0.2121
	114	125.51	

$$F(LOF) = \frac{125.51 - 93.98/114 - 96}{93.98/96}$$

$$= 1.76$$

Ftab (0.05,18,80) = 1.73
(0.05,18,100) = 2.18

Ftab (0.01,18,80) = 1.72
(0.01,18,100) = 2.14

CONCLUDE: *LOF(P < 0.05)

variability. The cubic model with N and F parameters explained enough of the treatment variability to give a nonstatistical LOF.

The elimination procedure depicted in Table 14 must be used with adherence to parameter hierarchy, i.e., if the N*N term, for example, is nonsignificant but the LINE*N*N term is significant, then the former cannot be removed from the model due to the dependence of the latter. Evaluation of P > F term is begun from the bottom parameter (TYPE I SS) upwards until a significant term is reached and at that time P > F decision is based upon TYPE IV SS. The reason for the above is because TYPE I P > F is calculated for each term prior to the addition of subsequent terms. TYPE IV P > F gives an analysis of each term given that all other terms are present.

A final general rule in the attempt for a more parsimonious model in the process of eliminating nonsignificant terms: try to pick the parameters with small SS and large DF because this will further reduce the fractionated model MSE (SS/DF) when the SS and DF are added back into this portion of the numerator shown in Table 11. A reduced numerator will decrease the F value in the test for LOF.

Tables 15 and 16 explain how the estimates are obtained for each individual line and model parameter. In this example, the cubic 297 limpograss equation has coefficients for parameters that can be easily traced back to their origin for illustrative purposes. The other limpograsses will not be considered beyond this point to avoid redundancy.

The major objective of the program in Table 11 was to fill "holes" in the response surface treatment matrix in order to make use of SAS/GRAPH. The resultant surface shown in Figure 21 represents a combination of 13 actual data points and 12 predicted points. The inclusion of actual

Table 15. The SAS general linear models procedure solution for estimates of intercept adjusted for the limpograss (line) and rep parameters

PARAMETER			ESTIMATE				INTERCEPT FOR LIMPOGRASS LINE
INTERCEPT			18.95925501 +	0.32606522	= 19.28532023		
REP	1		0.48188699				
	2		0.49630868	$\bar{x}$	= 0.32606522		
	3		0.00000000				
LINE	297		0.88462663 +	0.44952189	= 1.33414852		20.61946875
	553		1.75620304 +	(-0.04744913)	= 1.70875363	+ 19.28532023 =	20.99407386
	554		9.72148784 +	(-0.30779278)	= 9.41369506		28.69901529
	886		0.00000000 +	0.00000000	= 0.00000000		19.28532023
LINE*REP	297	1	-0.20409839				
		2	1.55266406	$\bar{x}$ = 0.44952189			
		3	0.00000000				
	553	1	-0.61035359				
		2	0.46800535	$\bar{x}$ = -0.04744913			
		3	0.00000000				
	554	1	-0.68583224				
		2	-0.23754609	$\bar{x}$ = -0.30779278			
		3	0.00000000				
	886	1	0.00000000				
		2	0.00000000	$\bar{x}$ = 0.00000000			
		3	0.00000000				

Table 16. The SAS general linear models procedure solutions for estimates of nitrogen (N) and cutting frequency (F) treatment parameters adjusted for each limpograss (line)

TREATMENT PARAMETER	ESTIMATE	
N	-0.02030927	(A)
N*N	6.3981011E-05	(B)
N*N*N	-9.5484194E-08	(C)
F	-3.19230044	(D)
F*F	0.27822672	(E)
F*F*F	-0.00771670	(F)
N*F	0.00054597	(G)
N*N*F	9.9283955E-07	(H)
N*F*F	-3.5145052E-05	(I)

TREATMENT PARAMETERS ADJUSTED FOR LINE ESTIMATES

N*LINE	297	0.03120205				0.01089278	
	553	-0.01659688	+	(A)	=	-0.03690615	*N
	554	0.01919086				0.00111841	
	886	0.00000000				-0.02030927	
N*N*LINE	297	-4.8958544E-05				1.5022467E-05	
	553	3.6548348E-05	+	(B)	=	1.0052936E-04	*N*N
	554	-0.00014040				-0.00007642	
	886	0.00000000				6.3981011E-05	

Table 16. Continued

TREATMENT PARAMETERS ADJUSTED FOR LINE ESTIMATES

N*N*N*LINE	297	2.2625882E-08				-7.2858312E-08	
	553	8.0519150E-09	+	(C)	=	-8.7432279E-08	*N*N*N
	554	2.3065701E-07				1.3517282E-07	
	886	0.00000000				-9.5484194E-08	
F*LINE	297	0.40248869				-2.78981175	
	553	0.13348428	+	(D)	=	-3.05881616	*F
	554	-2.58616658				-5.77846702	
	886	0.00000000				-3.19230044	
F*F*LINE	297	-0.02043673				0.25778999	
	553	-0.02705211	+	(E)	=	0.25117461	*F*F
	554	0.23902603				0.51725275	
	886	0.00000000				0.27822672	
F*F*F*LINE	297	-7.3594410E-06				-7.7240594E-03	
	553	0.00109042	+	(F)	=	-0.00662628	*F*F*F
	554	-0.00678204				-0.01449874	
	886	0.00000000				-0.00771670	
N*F*LINE	297	-0.00349712				-0.00295115	
	553	0.00082619	+	(G)	=	0.00137216	*N*F
	554	0.00027520				0.00082117	
	886	0.00000000				0.00054597	
N*N*F*LINE	297	2.3266890E-06				3.3195286E-06	
	553	-2.7248721E-06	+	(H)	=	-1.7320326E-06	*N*N*F
	554	-1.1295575E-06				-1.3671795E-07	
	886	0.00000000				9.9283955E-07	
N*F*F*LINE	297	9.7621776E-05				6.2476724E-05	
	553	9.5997150E-06	+	(I)	=	-2.5545337E-05	*N*F*F
	554	8.4502848E-06				-2.6694797E-05	
	886	0.00000000				-3.5145052E-05	

THE FINAL EQUATIONS FOR TNC9 IN 297 LIMPOGRASS:

297 TNC9 = 20.61946875 + 0.01089278*N + 1.5022467E-05*N*N - 7.2858312E-08*N*N*N -2.78981175*F + 0.25778999*F*F - 7.7240594E-03*F*F*F - 0.00295115*N*F + 3.3195286E-06*N*N*F + 6.2476724E-05*N*F*F

data provides a verification of the model equation if the actual and predicted points blend evenly. This was considered important because some models that were plotted were statistically adequate but none the less failed to fit in some regions of the surface plot.

The Table 11 program will be discussed from top to bottom using the program line number for easy reference. On line 7 the information associated with the raw data is inputed. For a more thorough investigation into the mechanics used in processing the TNC data, consult Appendix A. On lines 25-29 the TNC means are created. The DATA DUMB13 (line 31) is the data set containing the previously determined surface equation and instructions to code for treatment, N, and F. Only the missing treatments shown in Figure 5 are given treatment numbers as well as one more (TRT 26), which will be used later to trick the computer into starting the TNC axis at zero. The surface equation is used to solve for TNC at the specified levels of N and F (lines 54-59), and on line 89 the actual and predicted data sets are merged. The actual data is assigned N and F levels in lines 92-108 and lines 109-110 set the base line for TNC at zero. The remainder of the procedure is standard plotting in SAS/GRAPH.

The computer output is often not "publication perfect" because certain additions are generally added more easily by a graphics person. As yet some details of SAS/GRAPH are yet to be worked out. One problem is that no uniform scaling routine is yet available; hence, graphical comparisons of limpograsses must be uniformly scaled by hand. Also, sometimes the computer uses a non-linear scale for the "Z" axis that is clearly undesirable. In general, however, by using photoreduction a graphics person can easily trace the surface plot in the proper size for use in dissertations, etc., with a minimum of manipulation.

The smoothing techniques employed by Schoney et al. (1981) are clearly more advanced than the methodology described here; however, SAS/GRAPH is not yet so sophisticated. The major precaution this paper delivers is that before the smooth, fitted function is used in SAS surface plots (and their corresponding contour plots), the predicted points should be plotted amidst the actual data for a visual test of LOF. Also, beware of the interpretation of a surface based upon a non-linear scaling of the "Z" axis.

Computer generated graphics have been promoted by Cady and Fuller (1970), and it is believed that these graphics will be more widely used in the future. "Through graphics, the distribution, physical response, and interaction characteristics of a large volume of data can be readily analyzed" (Schoney et al., 1981, p. 437).

Conclusions

1. Response surface designs are more efficient in space, time, and cost, and the three dimensional representation of the surface gives an overview of the entire response while the contour plot gives an easily perceivable value of "Z" for any level of "X" and "Y."

2. The F test of models gives a test of LOF SS by the difference method: The MSE for a fractionated prediction model minus MSE for a model explaining a maximum amount of treatment variability yields MSE LOF.

3. Intercept and parameter estimations provided with the /SOLUTION option gave the intercept and coefficients needed to obtain a prediction equation.

4. A merger of predicted and actual data provided a verification of the predicted equation for a visual test of LOF.

5. Comparisons of surfaces must be made with extreme caution because of the non-uniform scaling produced from one graph to the next and the non-linearity of the scaling on the "Z" axis.

Summary

Computer assisted graphics look great but too often experimenters do not fully document the routine necessary to construct these graphs. Response surface methodology was used in a field experiment conducted between 1979-1981 that gave an opportunity to use three dimensional plots in studying the effect of nitrogen (N) fertilization and defoliation frequency (F) on four limpograss (Hemarthria altissima (Poir.) Stapf et C.E. Hubb) genotypes. Total nonstructural carbohydrates (TNC), forage quality, persistence, and yield were measured, and from those responses a cubic model describing TNC in the "crowns" (lowest 2 cm of stem) of PI 364888 limpograss harvested 28 September 1980 was selected to exemplify the computer procedures necessary for constructing surface plots.

The plotting procedures in SAS/GRAPH cannot accept "holes" in the treatment combination matrix. A procedure was presented showing how to fill the "holes" with predicted numbers derived from a regression equation fitting the surface response.

Comparison of surfaces is difficult because no uniform plotting program is yet available in SAS/GRAPH. Due to the limitation of non-uniform scaling and sometimes non-linear scaling of the "Z" axis, extreme caution is advised in the interpretation of surface plots.

SUMMARY AND CONCLUSIONS

The introduction of limpograss into the United States occurred in 1964 and since that time it has grown popular in the southeast as an improved forage for the flatwood site. Research prior to 1978 culminated in the identification, characterization, and cultivar release of 'Bigalta', 'Redalta', and 'Greenalta'. More limpograss germplasm became available for evaluation after plant explorations in 1971 and 1976, and several of the plant introductions were suspected to have agronomic characteristics that were superior or equal to the released cultivars.

In this study Bigalta and Redalta were compared to PI 364888 and PI 349753 in a series of experiments during 1979-1981. Bigalta is a highly digestible tetraploid limpograss that has not persisted under management practices involving frequent defoliation and/or heavy applications of nitrogen fertilizer. Redalta, a diploid, has excellent persistence but its overall nondigestibility is undesirable. The promising limpograsses PI 364888 and PI 349753 were suspected to have higher persistence than Bigalta along with higher quality than Redalta.

Working with established stands of limpograss in 1979, the limpograsses were fractionated and analyzed to determine the site of total nonstructural carbohydrate (TNC) storage. At the conclusion of the plant part sampling, five intervals of cutting were imposed in order to differentially drain the storage organs of their carbohydrate reserves.

Carbohydrate levels were tested chemically and by the use of regrowth-in-darkness techniques. Weight data on etiolated shoots, stubble, and roots provided the basis for a morphological comparison of genotypes. Positive correlations between weight of stubble and TNC stored in the bottom 2 cm of stems suggested that large stubble systems (Redalta and PI 364888) may aid in plant persistence.

In 1980 an extensive field experiment was conducted with five levels of N fertilization (0, 60, 120, 240, 480 kg/ha/yr) and five frequencies of defoliation (3, 6, 9, 12, and 18 weeks). Dry matter (DM) yields, in vitro organic matter digestibility (IVOMD), crude protein (CP), persistence, and TNC in shoots and storage organs were measured.

Bigalta produced adequate DM at intermediate cutting frequencies, while PI 364888 produced more DM with high N and long cutting frequencies. Bigalta was lowest in spring forage production and PI 349753 was highest. Limpograss PI 364888 was the best genotype for use as a stockpiled forage.

Bigalta's IVOMD and CP percentages were clearly superior to the other limpograsses. The spring and autumn forage was of a very high quality, but the midsummer values for IVOMD and CP were quite low.

Studies of TNC showed a maximum percent of organic food reserves in stem bases in March and a low in July. As temperature stress decreased in September and October, the TNC values increased. The period of low TNC accumulation in midsummer coincided with the period of most severe limpograss deterioration in the plots. The 3 week cutting frequency in combination with the 480 kg/ha/yr N rate was the most stress provoking treatment. Bigalta and PI 349753 were most sensitive to the treatment stresses while Redalta and PI 364888 were most persistent.

Results from this study show that the DM yield production in spring could be exploited in management systems. Limpograsses may be utilized for grazing in the spring of the year until bahiagrass begins its growth.

The summer period was marked by active limpograss growth, low percentages of stored TNC, and very low values for CP and IVOMD. The summer period also seemed to be the time when limpograss was most susceptible to stress and loss of stand. The recommendation to rest the limpograss during the summer has been suggested; however, the accumulated forage must be removed, burned, ensiled, or made into hay so that the field may be clean at the beginning of August for the onset of stockpiling.

Heavy summer rains may prohibit limpograss utilization as hay; however, silage making may be feasible. From data presented in this study it would appear that protein may be limiting in summer-harvested limpograss. A promising area of research may be the ammonification of the ensiled limpograss or even big bales of hay. The TNC values from shoots (studied in Chapter 3) showed that carbohydrate levels in the tissue may be adequate for silage making. Even if they were not, the technology exists for the addition of carbohydrate to silage.

Limpograss studied as stockpiled forage did not effectively utilize fertilizer N applied at rates above 120 kg/ha. The 1 August staging date was critical for sufficient DM accumulation; however, the standing forage should be utilized before frost in order to take maximum advantage of the relatively good forage quality present in autumn limpograss herbage.

Finally, if a preservation technique were studied and elucidated, the limpograss could be fed with supplemental protein and minerals during the winter. In conclusion, it would appear that limpograss can add much flexibility to the flatwood forage system and continued efforts in this direction seem justified.

APPENDICES

APPENDIX A
TOTAL NONSTRUCTURAL CARBOHYDRATE (TNC) PROCEDURE

TOTAL NONSTRUCTURAL CARBOHYDRATE (TNC) PROCEDURE

UNIVERSITY OF FLORIDA
AGRONOMY DEPARTMENT
GAINESVILLE, FL 32611

AUGUST, 1982

FOURTH REVISION

Scott Christiansen, K. J. Boote, and E. B. Blazey

Preliminary Steps

Sample Acquisition

After thorough consideration of experimental design, the sampling frequency and plant part to be analyzed may be determined. Total nonstructural carbohydrate (TNC) samples must be hand plucked if shoots are to be studied or dug if roots or stem bases are of interest. In the case of stem bases, they must be dug, selected, and removed from the basal body of the plant as single shoots to avoid dead residue in the sample. The samples are then washed and enclosed in a labeled diaper and placed on ice to avoid excess respiratory losses. Samples should be kept on ice to minimize respiration but kept relatively dry to facilitate drying without formation of Maillard products or fermentation which both result in loss of carbohydrates.

P.O.Box 1400
Sun City, AZ 85372
7 February 1983

To Whom It Concerns:

This note is in regard to the availability of Mylase 100 enzyme suggested for use in Wisconsin Agric. Exp. Sta. Res. Report R2107 (1981) entitled "Removing & analyzing total nonstructural carbohydrates from plant tissue". In the first 3 lines of column 1 on page 5, it states that the chemical company producing Mylase 100 is in Des Plaines, IL. The company has moved from Illinois to Charlotte, NC, and they can be contacted as outlined below:

G.B. Fermentation Industries Inc.
P.O. Box 241068
Charlotte, NC 28244

Tel. 704-527-9000
800-438-1361

Sincerely,

Dale S.

Dale Smith
Emeritus Professor of Agronomy
Univ. of Wisconsin-Madison

P.S. Send me your copy of Dissertation of Scott Christiansen

D.A.R.

Drying

The drying procedure is best effected using a small forced-air drier such as those made by Thelco. The drier may be preheated to 120 C so that by the time samples are loaded the temperature will have dropped to 100 C. Keep samples at this temperature for 30-45 minutes and then lower to 70 C. Remove when dry, approximately 36-48 hours later.

Storage

As frequently occurs, samples must be stored for some time until grinding. Keep samples in air-tight plastic bags. This serves a dual purpose in stablizing humidity and preventing sample damage by insects. Store in a cool, dry place or freeze if possible. Freeze drying is the best preservation technique; however, this is often impractical for large numbers of samples.

Grinding

Grind samples as soon as possible as it reduces sample bulk and it simplifies the logistics of sample preservation. Samples taken by hand will usually be of a size that can be ground entirely so as to maintain total plant representation. Grinding in a large Wiley mill may be used to pulverize the sample. With mills that are frequently used, blades are dull or misfitted resulting in a differential grind, i.e., leaves pass the 1 mm screen easily but lignin and other structural components remain behind. Hence, it is important to collect all the tissue in the grinding chamber as well as tissue having passed through the screen. This material can then be placed in a Whirlpac bag and pressed close to

exclude any remaining air space. This coarsely ground tissue should subsequently be reground through a small Wiley mill, a ball mill, or a UDY Cyclone mill to pass 0.5 mm or a 40 mesh screen. The second grinding has the advantage of thoroughly mixing the tissue of each sample. Remember to reseal the Whirlpac bags without excess internal air space. Dilu-vials also work well to hold samples.

Carbohydrate Analysis

Procedure

1. Open Whirlpac bags or Dilu-vials containing sample and place in a 50 C forced-air drier for 6-8 hours. After removal of the sample from the bags, eliminate air space while reclosing.

2. With an analytical balance, weigh out 0.1000 to 0.1099 g of sample and place it into a 25 ml erlenmeyer flask. For samples weighed out ahead of time for analysis, cork the flasks to prevent dust contamination. Record the weight. (Often it is better to use 80 column computer forms such as IFAS Form 2625 to log your data instead of a lab book. This eliminates a step between the analysis and the computer by putting your numbers in a form that can be punched directly onto computer cards or a terminal. See Table A-1 for an example of a job submitted using SAS at NERDC.)

3. Prepare the enzyme mix. (Have 0.2 M acetate buffer made prior to this operation.) The enzymes necessary for TNC analysis and supplier information may be found in another section of this procedure.

4. Add 5 ml of distilled water to erlenmeyer flasks holding samples, internal plant standards, glucose for determination of glucose

Table A-1. SAS job as submitted on cards to the computer

```
//TNC JOB (4001,1490,5,5,0,),'USEIFASFORM2625',CLASS=A
/*PASSWORD
// EXEC SAS
DATA PARTS;
COMMENT THE CHALLENGE OF LEARNING SAS IS YOUR PROBLEM;
INPUT M 2-3 D 5-6 Y 8-9 LINE 11-13 AGE $ 18-24 PT $ 21-22 RP 24 INC 26-
27 DV 29-30 WT 32-36 P1 38-40 DN 41-42 P2 44-45 OD1 48-50 OD2 52-54 OD3
56-58 OD4 60-62; DROP OD1-OD4; DATE=MDY(M,D,Y); JULAN=JULDATE(DATE);
DROP DATE;
IF PART='DO' THEN P='R';
IF PART='CR' THEN P='C';
IF PART='SH' THEN P='T';
IF PART='ST' THEN P='S';
ODA=(OD1+OD2+OD3+OD4)/4;
CARDS;
 07  03  79  297  6WKRO  2  24  19  104.3  .5  10  01  250  260  265  255
 07  03  79  297  6WKCR  2  24  19  107.7  .5  10  01  428  383  381  448
 07  03  79  297  6WKST  2  24  19  104.3  .5  10  01  420  419  421  441
 07  03  79  297  6WKSH  2  24  19  104.5  .5  10  01  320  311  395  395
 07  03  79  553  6WKRO  2  24  19  106.1  .5  10  01  320  325  317  342
 07  03  79  553  6WKCR  2  24  19  106.3  .5  10  01  190  195  192  190
 07  03  79  553  6WKST  2  24  19  105.4  .5  10  01  175  171  174  178
 07  03  79  553  6WKSH  2  24  19  108.0  .5  10  01  190  199  240  241
 07  03  79  554  6WKRO  2  24  19  106.2  .5  10  01  305  309  328  325
 07  03  79  554  6WKCR  2  24  19  102.3  .5  10  01  282  280  318  321
 07  03  79  554  6WKST  2  24  19  104.8  .5  10  01  408  370  362  390
 07  03  79  554  6WKSH  2  24  19  106.1  .5  10  01  478  489  465  463
 07  03  79  886  6WKRO  2  24  19  104.1  .5  10  01  345  331  348  362
 07  03  79  886  6WKCR  2  24  19  102.8  .5  10  01  411  431  475  440
 07  03  79  886  6WKST  2  24  19  104.7  .5  10  01  412  452  469  475
 07  03  79  886  6WKSH  2  24  19  106.1  .5  10  01  459  440  498  500
DATA PARTS; SET PARTS;
IF DEV=19 THEN MICGM=0.106235*ODA+6.5827748;*
MGCHO=(MICGM*0.011*DN)/(P1*P2);
CHO=MGCHO*100/WT;
IF DEV=19 THEN ADCHO=CHO/.9703;
PROC PRINT;
/*EOF
```

*If the enzyme has a sugar content subtract it here, e.g., MICROGM= (0.106235*ODA+6.5827748)-(MICROGM for the enzyme blank). Careful! Depends on the same dilution as the unknown sample.

NOTE: See Table A-2 for example of returned job.

Table A-2. Statistical analysis system

OB	M	D	Y	LINE	AGE	PT	RP	INC	DV	WT	P1	DN	P2	JULAN	P	ODA	MICGM	MGCHO	CHO	ADCHO
1	7	3	79	297	6WK	RO	2	24	19	104.3	0.5	10	1	79184	R	257.50	33.94	7.47	7.16	7.38
2	7	3	79	297	WK	CR	2	24	19	107.7	0.5	10	1	79184	C	410.00	50.14	11.03	10.24	10.56
3	7	3	79	297	WK	ST	2	24	19	104.3	0.5	10	1	79184	S	425.25	51.76	11.39	10.92	11.25
4	7	3	79	297	WK	SH	2	24	19	104.5	0.5	10	1	79184	T	355.25	44.32	9.75	9.33	9.62
5	7	3	79	553	WK	RO	2	24	19	106.1	0.5	10	1	79184	R	326.00	41.22	9.07	8.55	8.01
6	7	3	79	553	WK	CR	2	24	19	106.3	0.5	10	1	79184	C	191.75	26.95	5.93	5.58	5.75
7	7	3	79	553	WK	ST	2	24	19	105.4	0.5	10	1	79184	S	174.50	25.12	5.53	5.24	5.40
8	7	3	79	553	WK	SH	2	24	19	108.0	0.5	10	1	79184	T	217.50	39.69	6.53	6.05	6.23
9	7	3	79	554	WK	RO	2	24	19	106.2	0.5	10	1	79184	R	316.75	40.23	8.85	8.33	8.59
10	7	3	79	554	WK	CR	2	24	19	102.3	0.5	10	1	79184	C	300.25	38.48	8.47	8.28	8.53
11	7	3	79	554	WK	ST	2	24	19	104.8	0.5	10	1	79184	S	382.50	47.22	10.39	9.91	10.22
12	7	3	79	554	WK	SH	2	24	19	106.1	0.5	10	1	79184	T	473.75	56.91	12.52	11.80	12.16
13	7	3	79	886	WK	RO	2	24	19	104.1	0.5	10	1	79184	R	346.50	43.39	9.55	9.17	9.45
14	7	3	79	886	WK	CR	2	24	19	102.8	0.5	10	1	79184	C	429.25	53.25	11.71	11.40	11.74
15	7	3	79	886	WK	ST	2	24	19	104.7	0.5	10	1	79184	S	452.00	54.60	12.01	11.47	11.82
16	7	3	79	886	WK	SH	2	24	19	106.1	0.5	10	1	79184	T	474.25	56.96	12.53	11.81	12.17

recovery, and the empty flasks used to determine the TNC content of the enzyme. A Cornwall pipet works well for this addition. Clamp the flasks into the sample bed and top with the stopper/policemen.

5. Adjust the water level in the boiling bath higher than the level of the liquid in the flasks. Immerse the sample bed into the boiling (100 C) water for 10 minutes. Use a timing clock.

6. Remove the sample bed from the boiling water bath and add 5 ml Of 0.2 M acetate buffer to each flask when they have cooled. The Cornwall pipet is useful for this operation also.

7. Add 1 ml of enzyme mix to the flasks for samples, glucose recovery, plant standards, and enzyme blanks using the Oxford triple range micropipet sampler. Add 1 ml of water instead of enzyme to the glucose flasks. (The enzyme slurry should be vigorously stirred on a stirring plate during this step to insure a homogenous mix.) Replace stopper/policemen to minimize evaporation.

8. Return the sample bed with the samples to the shaker unit. The water in the bath should be at 48 C and set at a reasonable shaking oscillation. Allow a digestion time of whatever has been determined necessary in preliminary experiments. Make sure the distilled water level in the shaking water bath remains above the level of liquid in the flasks.

9. Perch funnel-folded filter paper disks (11 cm diameter) on the rim of 100 ml beakers. Arrange the 100 ml beakers on a tray and empty the contents of the erlenmeyer flasks into the filter paper funnels when the sample slurry is cool. Maintain sample identity.

10. The test tube racks will hold 40 lipless culture tubes (16 x 150 mm) arranged in a 10 x 4 configuration. In the first dilution each

rack holds the 20 test tubes needed for 10 samples. Remove two aliquots from the filtrate in the 100 ml beakers and place the liquid in test tubes with the corresponding numbers (Figure A-1).

NOTE: The aliquot volume is dependent upon the dilutions needed in samples and sugar standards to result in an optical density that can be read on the absorbance scale of the spectrophotometer. The dilutions must be determined experimentally for the particular tissue being analyzed. For example, with limpograss shoot tissue, 0.5 ml of liquor is transferred using an Oxford triple range pipet. Then, 9.5 ml of distilled water is added to make 10.0 ml. Therefore, 10.0 ml is the volume for the first dilution. The Cornwall pipet speeds the addition of the distilled water.

11. Once all the samples have been transferred and the appropriate volume of distilled water is added, vortex the tubes to assure an even concentration of the liquid. Now continue to the second dilution.

12. Sort the racks for the first dilution so that the sample numbers run sequentially. Taking one rack at a time, pair the racks for dilution 1, with the racks for dilution 2. Dilution 2 racks hold 40 tubes instead of 20. Remove two aliquots from each of the two originally diluted tubes and dispense into the four tubes associated with the dilution 2 rack (Figure A-2). For example, with limpograss again, a 0.5 ml aliquot is taken from the tubes in the dilution 1 rack and placed in the tubes for dilution 2.

13. Add 1 ml of alkaline reagent to each tube. Vortex each rack as a unit rather than by individual tubes within a rack. Load the rack for dilution 2 onto the tray designed for dipping the samples into the boiling water bath. When the water has reached a boil (100 C) lower the

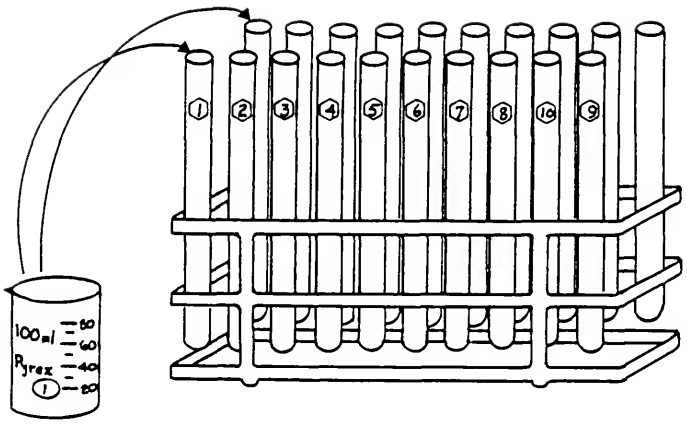

Figure A-1. Dilution one

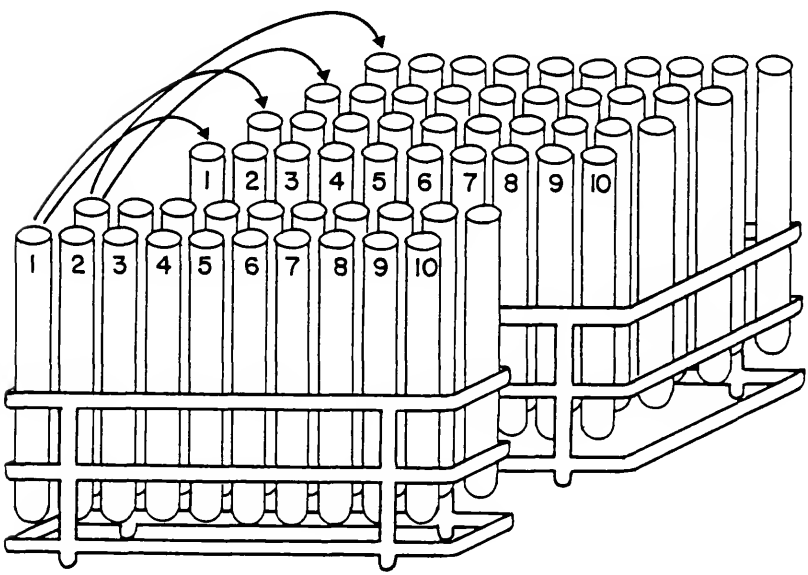

Figure A-2. Dilution two

dipping tray into the bath for 20 minutes. Have the water adjusted to be above the level of samples in the tubes. Moderate the gas for the burner so that splashes from the boil are kept from entering the tubes.

14. After 20 minutes, raise the dipping tray and transfer it to the cooling bath.

15. Remove the racks out of the dipping tray when the samples are cooled and arrange on the lab bench as before. Add 1 ml of arsenomolybdate reagent (the color forming reagent) with a manostat pipet containing no metal parts. Vortex the entire rack as a unit rather than one tube at a time.

NOTE: Do not try to use a Cornwell pipet for this operation because the arsenomolybdate reagent will be contaminated by contact with the metal in the Cornwall pipet.

16. At this point, the samples may be diluted to their final volume of 10 ml. For example, if you used a 0.5 ml volume of sample, 1 ml of alkaline reagent, 1 ml of arsenomolybdate, and 7.5 ml of distilled water is added. Using the Cornwall pipet with the proper technique, you can direct the stream of water directly at the wall at the surface of the sample in the tube to get a good mixture. This is important because the water must get thoroughly mixed with the ingredients in the tube.

NOTE: The sugar standards use an aliquot volume of 1 ml for the determination of the Beer's law relationship--plus 1 ml of alkaline reagent and 1 ml of arsenomolybdate giving a total of 3 ml in the tube. Therefore, 7.0 ml of distilled water is added to obtain the final volume of 10 ml in these tubes.

17. Warm up the Coleman Jr. II spectrophotometer for 5 minutes and set at 540 nm. Make sure the display is pushed to the right (since it

is loose). The cuvette should be scrupulously clean. Pour a small amount of distilled water into the funnel of the vacuvette cell. Fill the vacuvette cell with the reacted water blank (described in the section on glucose standards) and adjust the instrument to zero absorbance. Vortex each tube before placing in the cuvette. Read the absorbance of the samples and standards. Clean the cuvette after using by flushing with a mild detergent solution and rinsing in distilled water several times. To avoid aspiration of liquid waste into the pump, the pump reservoir should be removed and emptied whenever it gets two-thirds full. Disconnect the pump from the AC line, then unscrew the reservoir from the pump; be careful not to tip or agitate the assembly in such a way as to allow liquid to splash or flow into the pump intake.

18. Multiply the absorbance by 1000 for convenience and transfer the information to the 80 column data sheet. The 1000-fold multiplication makes the data easier to punch onto computer cards and does not change the relationship with the standards.

Glucose Standards

Carefully weigh out 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 g anhydrous D-glucose into 1 L volumetric flasks and fill to the mark with the distilled water. The glucose should be kept in a desiccator prior to weighing. Pour the amount of standard needed for the duration of the experimentation into two or more sets of plastic bottles and freeze them. Before using the standards, thaw and allow the solution to reach room temperature.

Sugar standards enter the procedure at Step 13 after all sample tubes have been brought through the second dilution. Sugar standards

and water blanks enter the procedure at Step 13 after all sample, plant standard, enzyme blank, and glucose recovery tubes have been brought through the second dilution.

Using the Oxford pipet add 1 ml of standard to each of the four tubes in the sample rows for each standard. One ml of alkaline reagent is added to these tubes and the 1 ml water blank tubes exactly as is done for the samples. Vortex. The standards are then treated exactly the same as samples in the remainder of the procedure. Remember to include at least four water blank tubes to be combined and used to zero the spectrophotometer.

Enzymes

A. Amyloglucosidase from Rhizopus

Stock #A7255 500 g $50.00 (6/24/81)

Supplier: Sigma Chemical Co.
P. O. Box 14508
St. Louis, MO 63178

Toll free: (800) 325-3010

Customer Service Phone: (314) 771-5765 (call collect)

B. Invertase concentrate from yeast (in glycerol)

Stock #39020 4E 250 ml $30.00 (6/24/81)

Supplier: Gallard Schlessinger Chem. Mfg. Corp.
584 Mineola Avenue
Carle Place, NY 11514

Phone: (516) 333-5600

NOTE: The enzymes should be kept in the freezer. The amyloglucosidase should be kept desiccated while in the freezer.

Enzyme mix.

45 ml distilled H_2O

5.0 ml 0.1 M Acetate buffer

2.5 ml Invertase concentrate

1.25 g amyloglucosidase

0.1 g thymol

Make enzyme mix fresh daily and stir on a magnetic stirrer when removing a 1 ml aliquot for addition to the sample. The buffer and water take the reducing sugars into solution. The invertase hydrolyzes the bond between glucose and fructose in sucrose and the amyloglucosidase hydrolyzes the starch molecules into monomers. The resulting monomers are measured by the Nelson's reducing sugar test of which this procedure is an adaptation.

Enzyme bank. By using buffer, invertase, and amyloglucosidase in this procedure, a small carbohydrate content is added by the enzyme which should be determined and subtracted out.

One ml of enzyme is added to an erlenmeyer flask having 5 ml of 0.2 M acetate buffer at Step 7. The enzyme blank is subsequently treated like the samples.

Glucose Recovery

In order to check whether there is any loss or gain of liquid during incubation, filtering, etc., a known quantity of glucose can be tested with the assay to check for concentrating or diluting effects. This may be done in two ways.

1. Weigh out 10 mg of glucose and treat it as you would a dry sample except add 1 ml of water instead of enzyme solution. A 0.5 ml aliquot diluted up to 10 ml (add 9.5 ml of distilled water) in the first dilution will result in a mid-range optical density using a 0.5 ml aliquot in the second dilution.

2. Weigh out 10 g glucose and dissolve in 1 L distilled water. At the beginning of each run put 1 ml of stock (10 mg/ml) into the erylenmeyer flasks. Add only 4 ml of water to give 5 ml in the initial boil so that volumes between glucose recovery flasks are comparable to volume of sample flasks. Use the same dilutions as described above.

After subtracting the quantity of carbohydrate added by the enzyme from plant standards and samples, an adjustment is made to 100 percent glucose recovery (see calculations).

Beer's Law

A regression equation must be used to obtain the relationship between sugar content of the samples and the optical density read at 540 nm. The standards have a known quantity of carbohydrate and serve as the reference and must be included in every set due to a multitude of factors that can change the color development from run to run.

It is faster to determine the regression equation by hand calculator so that the computer may be fed the remainder of the information in SAS input to complete the computations.

A few words on theory are necessary. We assume the glucose standards to be accurate, thus it will be the independent variable (X). The optical density values will, therefore, be dependent values (Y). Since we are using a least squares method of fitting the regression line and

the standards are assumed correct, then our analysis reveals variability in the technique (Figure A-3).

We are not interested in predicting optical densities; therefore, the equation is rearranged to get the inverse relationship. Consider the following set of data for standards. Punch into the TI-55 calculator:

[µg gluc (µG)]		[optical density (OD)]		display
10	x ⇄ y	93	Σ	1
20	"	150	"	2
30	"	222	"	3
40	"	312	"	4
50	"	432	"	5
60	"	469	"	6
70	"	558	"	7
80	"	700	"	8

Press 2nd SLOPE 2nd INTCP, for the equation $OD = m(\mu G) + b$.

$OD = 8.5119048 * \mu g + (-16.035714)$

$$\mu g = \frac{OD + 16.035714}{8.5119048}$$

$$\mu g = \frac{1}{8.5119048} * OD + \frac{16.035714}{8.5119048}$$

$$\mu g = 0.1174825 * OD + 1.8839$$

Calculations

1. Standards were made by placing 0.01-0.08 g of D-glucose in 1 L volumetric flasks.

$$\frac{0.01g}{1\ L} * \frac{1000\ mg}{g} * \frac{1\ L}{1000\ ml} = 0.01\ mg/ml$$

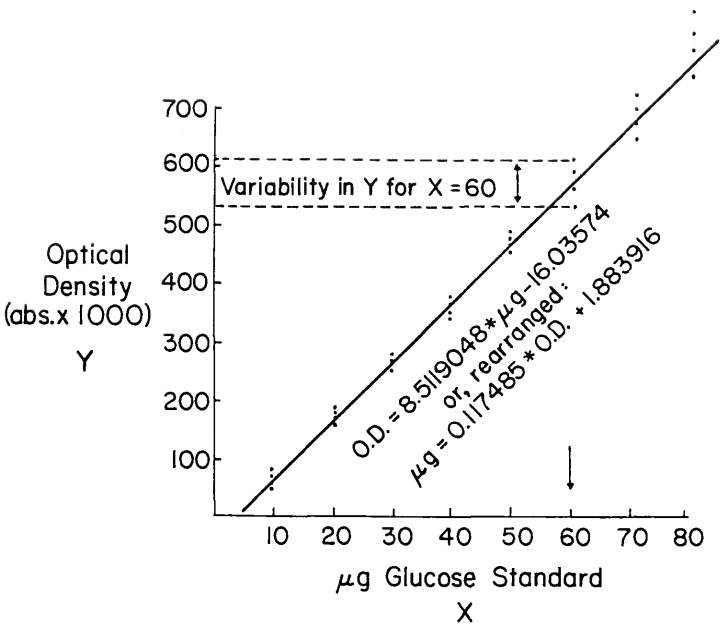

Figure A-3. The regression of optical density (OD) versus glucose

2. The regression equation determinated in the last section was based on µg.

$$0.01 \text{ mg/ml or } 0.01 \text{ mg/tube} * \frac{1000 \text{ µg}}{\text{mg}} = 10 \text{ µg glucose}$$

3. Enzyme blanks, glucose recovery, and internal plant standard values are determined as a function of the Beer's law regression for the sugar standards. The enzyme blank can be determined first if it has an identical dilution series as the unknown sample (e.g., OD = 45).

$$\begin{aligned} \text{µg (for enzyme blank)} &= 0.1174825 * \text{OD} + 1.8839 \\ &= 0.1174825 * 45 + 1.8839 \\ &= 7.1706 \end{aligned}$$

4. Sample carbohydrate content (subtracting the enzyme blank) (e.g., OD = 175).

$$\begin{aligned} \text{µg (for sample or plant standard)} &= 0.1174825 * \text{OD} + 1.8839 \\ &= 0.1174825 * 175 + 1.8839 \\ &= 22.4433 \end{aligned}$$

$$\begin{aligned} \text{Subtraction of enzyme CHO} &= \text{µg for sample} - \text{µg for enzyme blank} \\ &= 22.4433 - 7.1706 = 15.2727 \end{aligned}$$

5. Convert to mg carbohydrate. (NOTE: If a different dilution was used for the enzyme blank bring both sample and enzyme blank to the level of mg CHO and then subtract out the mg CHO for the enzyme blank, i.e., mg CHO = mg CHO (sample + enzyme) - mg CHO (enzyme blank).)

$$\text{mg CHO} = 15.2727 \text{ µg} * \frac{1.0 \text{ mg}}{1000 \text{ µg}} * (\text{1st dilution}) * (\text{2nd dilution}).$$

$$= 15.2727 * \frac{1.0}{1000} * \frac{11.0 \text{ ml}}{0.5 \text{ ml}} * \frac{10.0 \text{ ml}}{0.5 \text{ ml}}$$

$$= 6.72$$

6. Convert to % CHO (assume sample weight = 105.5 mg)

$$\% \text{ CHO} = \frac{6.72}{\text{wt}} * 100$$

$$= 6.37\%$$

7. Glucose Recovery--Method I (assume wt = 10.5 mg D glucose; OD = 180).

$$\mu g = 0.1174825 * \text{OD} + 1.8839$$

$$= 0.1174825 * 180 + 1.8839$$

$$= 23.0308$$

$$\text{mg CHO} = 23.0308 * \frac{1.0 \text{ mg}}{1000 \text{ g}} * \frac{11.0 \text{ ml}}{0.5 \text{ ml}} * \frac{10.0 \text{ ml}}{0.5 \text{ ml}}$$

$$= 10.13$$

(assume weight of glucose = 10.5)

$$\% \text{ of glucose recovery} = \frac{10.13}{10.5} * 100 = 96.51\%$$

8. Adjust sample values to 100 percent glucose recovery.

$$\text{ADJ CHO\%} = \frac{\text{sample value (enzyme blank subtracted out)}}{\text{glucose recovery}}$$

$$= \frac{6.72}{0.9651}$$

$$= 6.96\%$$

Reagents

Alkaline reagent. Dissolve 25.0 g anhydrous sodium carbonate (Na_2CO_3), 25.0 g potassium sodium tartrate ($KNaC_4H_4 \cdot 4H_2O$), 20.0 g

sodium bicarbonate ($NaHCO_3$), and 200.0 g anhydrous sodium sulfate (Na_2SO_4) in 700 ml distilled water and then dilute to 1000 ml in a volumetric flask. Dissolve 6.0 g cupric sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in 40 ml distilled water followed by one drop of concentrated sulfuric acid (H_2SO_4). Combine and mix the two solutions. Follow these directions!

Arsenomolybdate reagent. Dissolve 25.0 g ammonium molybdate tetrahydrate [$(NH_4)(6MO_7O_{24}4H_2O)$] in 450 ml distilled water, then add 21 ml concentrated sulfuric acid while stirring. Dissolve 3.0 g sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) in 25 ml distilled water. Combine and mix the two solutions and store in a brown bottle for 24 hours at 37 C. The reagent should be yellow with no green tint and should be remade after 10 days.

NOTE: Arsenomolybdate reagent is easily contaminated by metal surfaces so avoid metal bottle caps, etc. Follow directions!

Acetate buffer, 0.2 M, pH 4.5. Prepare the following stock solutions:

0.2 M Acetic acid--To approximately 500 ml distilled water in a 1 liter volumetric flask add slowly and carefully 11.6 ml glacial acetic acid. Cool and make to 1 liter with distilled water.

0.2 M Sodium Acetate--Dissolve 16.4 g $NaC_2H_3O_2$ or 27.2 g of $NaC_2H_3O_2 \cdot 3H_2O$ per liter of distilled water.

For 0.2 M buffer, combine 300 ml 0.2 M acetic acid with 200 ml sodium acetate. Titrate the final buffer solution to pH 4.5 by addition of either stock solution.

Refrigerate.

0.1 M Acetate Buffer. Dilute the 0.2 M buffer accordingly.

APPENDIX B
TOTAL NONSTRUCTURAL CARBOHYDRATE (TNC) RESULTS

Table A-3. Percent total nonstructural carbohydrate (TNC) means for all treatment, dates, and limpograsses in 1980

OB	N*	F**	H0	H1	H2	H3	H4	H5	H6	H7	H8	H8	H10	H11	H12
			23 Mar	16 Apr	3 May	25 May	14 Jun	7 Jul	27 Jul	16 Aug	6 Sep	28 Sep	18 Oct	8 Nov	30 Nov
PI 364888															
1	0	3	15.7	24.5	14.4	14.9	15.5	12.0	10.5	12.0	19.4	14.4	15.0	12.8	16.2
2	120	3	14.1	25.5	17.6	20.4	18.9	11.5	10.9	10.8	19.7	14.6	14.3	11.6	17.1
3	480	3	15.6	22.6	21.2	21.1	16.5	11.2	10.2	10.8	15.7	13.3	10.3	10.6	13.8
4	60	6	.	.	19.1	.	18.0	14.7	13.0	9.6	13.3	12.0	11.4	10.2	14.6
5	240	6	.	.	19.7	.	18.1	14.4	13.4	9.0	14.2	11.8	9.9	9.0	14.1
6	0	9	.	.	.	16.5	.	.	12.7	10.5	16.0	10.2	11.9	10.2	13.9
7	120	9	.	.	.	16.0	.	.	10.4	8.9	13.9	9.0	6.9	9.2	11.8
8	480	9	.	.	.	18.3	.	.	8.3	8.3	12.8	7.9	6.2	9.9	11.7
9	60	12	.	.	.	.	13.8	.	.	.	13.6	11.0	9.5	10.6	16.3
10	240	12	.	.	.	.	13.4	.	.	.	11.0	9.2	10.1	10.6	15.7
11	0	18	.	.	.	.	.	.	10.3	7.8	15.4	8.8	8.4	9.4	16.7
12	120	18	.	.	.	.	.	.	11.9	6.7	12.7	7.2	7.4	8.1	17.9
13	480	18	.	.	.	.	.	.	10.7	7.2	13.1	7.5	6.0	7.7	14.7

Table A-3. Continued

OB	N*	F**	H0	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
			23 Mar	16 Apr	3 May	25 May	14 Jun	7 Jul	27 Jul	16 Aug	6 Sep	28 Sep	18 Oct	8 Nov	30 Nov
Redalta															
14	0	3	15.4	18.5	12.0	13.2	12.8	9.8	8.6	10.5	11.8	13.5	11.2	13.4	15.6
15	120	3	16.2	19.8	13.0	13.5	13.6	8.1	8.0	8.2	14.0	11.5	9.3	11.9	12.6
16	480	3	16.5	20.3	14.5	11.1	12.3	6.9	6.4	6.8	11.4	10.3	8.4	11.6	8.7
17	60	6	.	.	14.9	.	15.7	8.3	8.4	6.3	9.4	9.1	9.1	9.2	9.0
18	240	6	.	.	13.2	.	9.9	6.4	6.3	4.8	8.3	6.7	7.1	9.1	6.4
19	0	9	.	.	.	12.2	.	.	8.5	6.4	10.1	9.4	7.5	10.4	9.4
20	120	9	.	.	.	9.0	.	.	5.7	4.7	8.5	6.5	4.9	10.0	7.4
21	480	9	.	.	.	8.1	.	.	6.0	5.8	6.7	6.2	4.4	9.7	7.0
22	60	12	.	.	.	.	11.4	.	.	.	9.6	7.4	6.7	10.2	9.9
23	240	12	.	.	.	.	9.2	.	.	.	6.9	7.0	5.9	8.9	10.7
24	0	18	.	.	.	.	.	.	9.9	6.2	9.8	8.7	9.2	12.2	14.5
25	120	18	.	.	.	.	.	.	9.3	4.4	7.3	7.1	7.0	11.0	11.5
26	480	18	.	.	.	.	.	.	6.3	4.5	6.8	5.1	5.7	9.0	10.3
Bigalta															
27	0	3	14.9	22.0	14.6	14.8	17.0	12.6	11.4	13.4	17.5	15.2	13.7	13.5	14.4
28	120	3	14.4	22.7	17.0	18.2	20.1	14.1	11.3	13.8	17.1	15.2	11.7	12.8	13.9
29	480	3	16.8	22.6	17.7	26.0	19.8	13.6	9.9	11.4	15.7	13.4	11.6	13.0	11.5
30	60	6	.	.	17.4	.	20.2	12.8	13.6	11.5	14.4	10.0	12.4	10.8	13.4
31	240	6	.	.	16.8	.	19.0	11.8	9.6	9.5	10.4	7.4	9.1	7.3	7.9
32	0	9	.	.	.	15.0	.	.	11.3	10.5	11.3	8.3	6.6	8.6	6.0
33	120	9	.	.	.	14.0	.	.	10.6	8.6	9.5	6.8	6.2	7.4	7.2

Table A-3. Continued

OB	N*	F**	H0 23 Mar	H1 16 Apr	H2 3 May	H3 25 May	H4 14 Jun	H5 7 Jul	H6 27 Jul	H7 16 Aug	H8 6 Sep	H9 28 Sep	H10 18 Oct	H11 8 Nov	H12 30 Nov
34	480	9	.	.	.	12.7	.	.	10.4	9.7	11.1	7.1	6.5	8.8	9.3
35	60	12	.	.	.	.	14.4	.	.	.	8.9	8.7	8.2	8.4	12.3
36	240	12	.	.	.	.	13.3	.	.	.	8.3	7.6	8.4	8.6	11.2
37	0	18	.	.	.	.	.	.	13.9	8.8	10.6	7.6	8.4	7.6	11.2
38	120	18	.	.	.	.	.	.	12.2	8.1	10.4	7.5	7.0	7.2	11.4
39	240	18	.	.	.	.	.	.	11.1	7.3	10.4	6.8	7.0	7.5	10.2
PI 349753															
40	0	3	12.8	20.8	15.7	18.0	17.3	13.2	9.2	10.1	13.5	12.0	9.6	9.8	8.4
41	120	3	16.7	23.5	16.5	17.3	17.0	11.8	11.5	11.4	12.7	10.2	9.6	8.9	8.7
42	480	3	17.2	25.1	18.9	20.7	16.3	12.8	10.7	10.4	9.8	7.8	7.7	8.8	6.6
43	60	6	.	.	17.3	.	19.5	10.6	10.6	8.9	9.5	8.3	7.7	7.4	8.4
44	240	6	.	.	16.5	.	14.1	9.2	10.6	7.7	7.6	6.7	5.3	6.2	6.9
45	0	9	.	.	.	17.2	.	.	11.6	7.9	9.9	7.0	7.5	7.1	10.5
46	120	9	.	.	.	15.2	.	.	8.8	5.6	8.8	5.9	4.7	6.9	8.5
47	480	9	.	.	.	13.5	.	.	8.8	6.9	8.8	4.8	4.2	7.3	8.4
48	60	12	.	.	.	.	15.4	.	.	.	8.2	7.0	6.6	7.6	7.9
49	240	12	.	.	.	.	10.6	.	.	.	6.8	6.5	5.0	7.5	11.4
50	0	18	.	.	.	.	.	.	12.9	8.2	11.0	7.1	5.9	8.4	11.4
51	120	18	.	.	.	.	.	.	9.8	6.1	8.6	5.1	4.9	6.7	9.4
52	480	18	.	.	.	.	.	.	10.7	5.4	8.2	4.8	4.4	6.1	8.3

*Nitrogen (kg/ha/yr).

**Frequency (weeks).

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BIOGRAPHICAL SKETCH

Scott Christiansen was born the son of Carl August Christiansen, Jr. and Mary Jean (Boller) Christiansen on 21 June 1954 at 10:53 p.m. in Buffalo, New York (43 N 79 W). He led an idealistic youth surrounded by the love of sibs and parents, and had an all American boy adolescence.

He moved to Madison, Wisconsin, on 9 June 1974, after two semesters of liberal arts courses at the State University of New York at Buffalo. In Madison he obtained the B.S. degree in August, 1977, and continued for a M.S. degree that was conferred in May, 1979. On 5 January 1979, he moved to Gainesville, Florida, and after four years expects to receive the Doctor of Philosophy degree in December, 1982. In July, 1982, Scott accepted a research agronomist position with the USDA-ARS Southwest Livestock and Forage Research Unit in El Reno, Oklahoma.

Scott became engaged to be married on 1 July 1982 to Caroline Mirabel Kitts and wed 29 August 1982. When the couple moves to Oklahoma in September, 1982, Carrie will continue her education in music, studying oboe, piano, and the Suzuki method of music pedagogy.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

O. Charles Ruelke

O. Charles Ruelke, Chairman
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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