GROWTH ANALYSIS OF FLORIGRAZE RHIZOMA PEANUT (*Arachis glabrata* Benth.)

BY

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TO MY PARENTS:

OLGA AND ABELARDO

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	ix
ABSTRACT	x
INTRODUCTION	1
CHAPTER I - LITERATURE REVIEW	3
Arachis glabrata Benth.	3
Agronomic Characteristics of Florigraze Rhizoma Peanut	4
Potential Use	5
Forage Management and Utilization	6
Forage Regrowth	7
Forage Quality	15
CHAPTER II - GROWTH ANALYSIS OF AERIAL AND UNDERGROUND PLANT PARTS OF FLORIGRAZE RHIZOMA PEANUT	25
Introduction	25
Materials and Methods	27
Results and Discussion	32
Shoot Growth	32
Rhizome Growth	39
Total Growth	43
Summary	46
CHAPTER III - DIFFERENTIAL ACCUMULATION OF TOTAL NONSTRUCTURAL CARBOHYDRATES AND NITROGEN IN PLANT PARTS OF FLORIGRAZE RHIZOMA PEANUT	55
Introduction	55
Materials and Methods	57
Results and Discussion	59
Ice vs Dry Ice	59
Total Nonstructural Carbohydrates	61
Nitrogen	67
Summary	73

Page

CHAPTER IV - EFFECT OF DEFOLIATION ON GROWTH, TOTAL NONSTRUCTURAL CARBOHYDRATES AND NITROGEN IN FLORIGRAZE RHIZOMA PEANUT RHIZOMES. 76

Introduction 76
Materials and Methods. 77
Results and Discussion 80
Rhizome Growth 80
Total Nonstructural Carbohydrates. 86
Percent Nitrogen 91
Summary. 100

CHAPTER V - EFFECT OF GRINDING METHOD ON in vitro ORGANIC DIGESTIBILITY OF FORAGES. 102

Introduction 102
Materials and Methods. 103
Results and Discussion 108
Grasses. 111
Legume 118
Summary. 125

CHAPTER VI - GENERAL DISCUSSION 128

LITERATURE CITED. 132

BIOGRAPHICAL SKETCH 146

LIST OF TABLES

Table		Page
1	Sampling schedule for three data sets to record the growth and regrowth of Florigraze rhizoma peanut.	30
2	Mathematical equations to describe the growth of Florigraze rhizoma peanut components in three data sets	33
3	Regression analyses of complete data and individual comparisons between data sets for growth components in Florigraze rhizoma peanut	34
4	TNC percentage in cooled (ice) and frozen (dry ice) samples in 1981 regrowth (II) and 1981 (III) of Florigraze rhizoma peanut	60
5	Regression analyses of complete data and individual comparisons between data sets for TNC percent in plant parts of Florigraze rhizoma peanut	63
6	Regression analyses of complete data and individual comparisons between data sets for N percent in plant parts of Florigraze rhizoma peanut	69
7	Soil mineral analysis in samples from field A and field B in February 1981.	72
8	Sampling and defoliation schedule for 1981 treatments imposed on Florigraze rhizoma peanut.	79
9	Regression analyses of complete data and individual comparisons between defoliation treatments for growth components of Florigraze rhizoma peanut	82
10	Mathematical equations to describe the effects of frequency of defoliation on the growth of rhizomes in Florigraze rhizoma peanut.	84
11	Regression analyses of complete data and individual comparisons between defoliation treatments for TNC percent in plant parts of Florigraze rhizoma peanut . .	88

Table		Page
12	Regression analyses of complete data and individual comparisons between defoliation treatments for N percent in plant parts of Florigraze rhizoma peanut	93
13	Weight of shoots per plant for defoliation treatments at sampling dates in Florigraze rhizoma peanut. . .	97
14	List of grasses and sampling dates for Florigraze rhizoma peanut.	104
15	Differential IVNDFD in relation to grinding treatment	105
16	Diagram of the laboratory experiment for grasses and legume.	107
17	Analyses of variance for all forages, grasses, and legume samples	110
18	Analyses of variance for grasses separated by genus, and for legume samples separated into leaf and stem.	114
19	Effect of grinding treatment and incubation time on IVOMD by grass, and IVOMD means by grass by treatment	116
20	Regression analyses per year and plant fraction for treatments and incubation effects upon IVOMD of Florigraze rhizoma peanut, and treatment means by year and plant fraction.	121
21	Regression and correlation coefficients for prediction of Organic Matter Digestibility (OMD) and Organic Matter Intake (OMI) from IVOMD of nine grasses with four grinding treatments and with three incubation times.	123

LIST OF FIGURES

Figure		Page
1	Total growth and components of growth in Florigraze rhizoma peanut for three data sets. . .	49
2	Growth rates of plant components in Florigraze rhizoma peanut for three data sets	50
3	Relative growth rates of plant components in Florigraze rhizoma peanut for three data sets .	51
4	Production of shoots in Florigraze rhizoma peanut for three data sets	52
5	Temperature and rainfall recorded in Gainesville in 1980 and 1981	53
6	Primary and secondary rhizomes	54
7	Percent TNC (organic matter basis) in plant parts of Florigraze rhizoma peanut for three data sets.	62
8	Percent Nitrogen (organic matter basis) in plant parts of Florigraze rhizoma peanut for three data sets.	68
9	Effect of defoliation treatments on growth of rhizomes in Florigraze rhizoma peanut.	81
10	Effect of defoliation treatments on TNC percent in plant parts of Florigraze rhizoma peanut. . . .	87
11	Effect of defoliation treatments on N percent in plant parts of Florigraze rhizoma peanut. . . .	91
12	Effect of defoliation treatments on the number of shoots in Florigraze rhizoma peanut	97
13	Effect of grinding treatment and incubation on IVOMD of nine grasses.	113
14	Effect of grinding treatment and incubation on IVOMD of seven samples of Florigraze rhizoma peanut	119

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GROWTH ANALYSIS OF FLORIGRAZE
RHIZOMA PEANUT (*Arachis glabrata* Benth.)

By

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A series of studies was conducted to determine growth characteristics and responses to defoliation under field conditions; and quality characteristics of Florigraze rhizoma peanut. Growth of shoots and rhizomes was monitored in undefoliated plants for two consecutive years (1980 growth and 1981 regrowth). The first year growth was replicated (1981 growth) in another field. During the second year (1981 regrowth), growth of rhizomes was recorded in plants defoliated every 2, 6, and 8 weeks. Total nonstructural carbohydrates (TNC) and nitrogen (N) were determined in plant parts. Rhizoma peanut forage samples (leaf and stem) were compared to nine tropical grass samples, as to the effect of four grinding treatments (Udy vs Wiley mills, 1.0- and 0.5-mm screen) and three incubation times on *in vitro* organic matter digestibility (IVOMD).

In undefoliated plants relative growth rates were higher in 1980 and 1981 growth than in 1981 regrowth, indicating a reduction in relative growth potential. The cause of this appears to be N deficiency in early

regrowth. In both years rhizome growth paralleled shoot growth until September. From then on, shoot growth declined and rhizomes continued to grow, at least until the first frost. Defoliation reduced plant biomass, especially rhizome growth. Regardless of defoliation frequency, little growth was observed in rhizomes after September. In the rhizomes of undefoliated plants, TNC accumulation was higher in 1981 regrowth than in 1980 and 1981 growth. In November 1981 the rhizomes of undefoliated plants (1981 regrowth) contained about 70% TNC compared to 45 to 50% TNC for defoliated plants; rhizomes of 8-week frequency had higher TNC than 2-week and 6-week frequencies ($P = 0.0141$ and $P = 0.0001$, respectively). The TNC accumulation in rhizomes started with the reduction of growth rates of shoots and rhizomes in September and continued to increase until the end of the growing season regardless of defoliation treatments.

All grasses and legume samples responded to grinding treatment. Grinding treatment had a greater effect on low quality forages than on high quality forages. In grasses, 0.5-mm grinding increased IVOMD. In the legume samples 0.5-mm grinding increased IVOMD also, but 1.0 mm Udy gave higher IVOMD than 0.5-mm Wiley. The IVOMD of legume samples was affected very little by incubation time. Fine-grinding in grasses, increased accuracy of prediction of organic matter digestibility (*in vivo*) from IVOMD.

INTRODUCTION

Forage legumes may be the most economical way of introducing nitrogen (N) in pastures to enhance animal production. This might be particularly important in those production systems where economic returns are not sufficient to justify the use of N fertilizer. As opposed to temperate areas, legumes in the tropics constitute a relatively new area of research. In the last 20 years, researchers in tropical areas have devoted considerable effort to understand the dynamics of legume-grass associations. However, the results are controversial. The failure in a lot of instances is associated with lack of complete understanding of the physiological capabilities of the legumes. The purpose of the association is to provide N to the system while increasing the nutritive level of the animal diet. Therefore, to achieve this goal it is necessary to study the management or alternate managements in the associates to determine their compatibility.

Intensity and frequency of defoliation are the two most important factors in forage management. Forage plants respond differently, physiologically and morphologically, to these two factors. These responses determine the survival of the plants. Morphology and regrowth mechanisms in grasses allow them to survive a relatively wide range of defoliation managements, whereas legumes are more restricted. In North Florida, most perennial tropical legumes do not survive the winter and the annuals do not start growing until late June. Florigraze rhizoma peanut

has a great potential for North Florida because it survives the winter and starts growing very early in the spring. Some observations on this legume suggest that it might be associated with bahiagrass (*Paspalum notatum* Flügge.) under grazing conditions. The persistence of this legume to grazing is related to its rhizomatous nature. The rhizomes are also used for vegetative propagation since it does not produce seed. This fact represents an inconvenience because up to 2 years are required for establishment of pastures.

The objectives of this research were to study the growth characteristics of Florigraze rhizoma peanut, the role of carbohydrate reserves and N, the responses to defoliation, and the comparison of its forage quality to tropical grasses of known *in vivo* quality data.

CHAPTER I

LITERATURE REVIEW

Arachis glabrata Benth.

Peanuts (*Arachis* spp), also known as groundnuts, are the most widespread and potentially the most important food legume species of the world; they are grown in some 82 countries (Varnell and McCloud, 1975).

The genus *Arachis* originated in South America where it is widely distributed from the Amazon River through Brazil, Bolivia, Paraguay, Uruguay and northern Argentina to about 35° south latitude (Higgins, 1951). The genus *Arachis* comprises several species. Herman (1954) described eight species of wild peanuts from which seven are perennial. Gregory et al. (1973) stated that on the basis of collected material the genus could be grouped into 30 to 50 different species. The three perennial *Arachis* species most widely distributed in South America are *glabrata*, *marginata*, and *villosa*. Their limit of distribution is about 35° south latitude which in the northern hemisphere would almost correspond to the northern boundary of the state of Georgia in the U.S.A. This feature suggested that some ecotypes among those perennial species could be adapted to North Florida (Prine, 1973).

Arachis glabrata Benth. was introduced into the United States in 1936, by W. Archer, as PI 118457 and released to farmers with the variety name 'Arb' (Prine, 1964). Since then other introductions of rhizoma

peanut have been named and distributed to farmers for testing (Prine et al., 1981). In 1962, a rapidly spreading plant was observed in an Arb rhizoma peanut plot, by G. M. Prine, who isolated it and tested it as Gainesville Selection No. 1 (GS-1) (Prine, 1973). It is suspected that this selection is a seedling from Arb. This selection was released with the cultivar named 'Florigraze' in 1979 (Prine et al., 1981).

Agronomic Characteristics of Florigraze Rhizoma Peanut

Florigraze rhizoma peanut is a rhizomatous legume. It produces very little seed; therefore, its establishment is accomplished by vegetative propagation (sprigging) or by planting rhizomes. The latter is the most recommended method of propagation. A potato digger was one of the first mechanical devices used for harvesting the rhizomes from a well-established rhizoma peanut field (Adjei, 1975). One square foot (930 cm^2) mats were planted in hills 7 to 10 cm deep separated 1.8 m, but better coverage is obtained if the mats are broken up into four pieces and planted 0.9 m apart in every direction (Adjei, 1975; Adjei and Prine, 1976). Recently, the rhizome material is being dug with a bermudagrass sprig digger. Using this machine the rhizomes come out cut in small pieces, and according to Prine et al. (1981), this may reduce the efficiency of propagation. Hence, they suggested that a modification to the machine may help to obtain larger pieces of rhizomes. However, at the present time there is no experimental evidence to conclude such a loss in efficiency (Prine, 1982. Personal communication).

Florigraze is adapted to well-drained soils. Prine et al. (1981) emphatically stated that it is not adapted to flatwoods soils or to

any soil subjected to high water tables. Like any other legume, phosphorus (P) and potassium (K) should be provided for good growth, but special attention should be focused on calcium (Ca) and magnesium (Mg) (Prine et al., 1981). To ensure good nodulation, it is recommended to inoculate rhizomes at planting with Rhizobium japonicum (Adjei, 1975).

Florigraze is a slow establishing crop with fastest growth during the summer months. It takes from 2 to 3 years to get a solid sward (Prine, 1979). It is drought resistant, and if drought is severe, top growth may die and then regenerate from rhizomes following rainfall (Prine et al., 1981). Some reports indicate that well established plots resisted frequent defoliation (Breman, 1980; Beltranena, 1980).

The rhizomatous habit of A. glabrata makes it suitable for grazing. Some studies have shown that it produces approximately the same amount of underground dry matter as it does above ground (Breman, 1980). Even though other reports indicate that it tolerates grazing and trampling by cattle, alone and in association with grasses (Blickensderfer et al., 1964; Otero, 1946), there is no information regarding its persistence in relation to its physiological or morphological responses to defoliation.

Potential Use

Prine (1964) planted Arb rhizoma peanut in association with 'Pensacola' bahiagrass, 'Coastal' bermudagrass (Cynodon dactylon L.) and 'Pangola' digitgrass (Digitaria decumbens Benth.); he found that the highest dry matter yield was produced when associated with bermudagrass (5,040 kg/ha) or in pure stand (4,360 kg/ha). In another study, in pure stands, Florigraze produced more dry matter yield (10,300 kg/ha)

than Arb (8,740 kg/ha) (Prine, 1973). Quality determinations of Florigraze rhizoma peanut in vivo (Prine et al., 1981) indicate no palatability problems; voluntary intake was well above the maintenance requirement in two hays with different maturity. In a preliminary study, Saldivar et al. (1981) reported that suckling calves creep grazing Florigraze rhizoma peanut gained 160 g/day more than calves without creep grazing.

The persistence, high quality, and yield of Florigraze rhizoma peanut make it a forage with a great potential. Its slow establishment may be overcome by its perenniality and persistence under diverse production systems. Currently, research is being conducted at the University of Florida to determine the feasibility, from both practical and economical standpoints, of intercropping Florigraze rhizoma peanut with cash crops to reduce establishment costs. Preliminary results on these investigations indicate that some cultural practices may enhance the establishment rate during the second year (Franca-Dantas, 1982).

Forage Management and Utilization

Forage utilization is determined by type, intensity, and economics of livestock production operations. In any production system, forage utilization involves the removal of foliage; this is technically defined as defoliation, which means removal of leaves, but it more commonly refers to removal of shoots (leaves and stems) in forage species. The frequency and degree of defoliation determine the intensity of forage utilization (Humphreys, 1981). Yet, time of defoliation, in relation to plant development, may be as important (Humphreys, 1966). Degree of defoliation for mechanized harvesting can be defined

as cutting height in cm because the homogeneity of defoliation, with respect to plant structure, promotes a high correlation with leaf area. In grazing situations, defoliation is not homogeneous; therefore, two parameters have been used. The first one defines degree of defoliation as residual leaf area index (LAI) after grazing or cutting (Humphreys and Robinson, 1966; Brown et al., 1966a; Brown and Blaser, 1968) whereas the second one takes into account age effect in photosynthetic activity of leaves (Brown et al., 1966b) as well as photosynthesis of stems (Ludlow and Wilson, 1971) and defines degree of defoliation as residual dry matter/ha (FDM/ha) after grazing (Mott, 1973). On the other hand, frequency of defoliation is defined as the period of time between defoliations. Time of defoliation deals with the physiological changes occurring in the plants that may affect growth.

The response of forage plants to the interaction of frequency, degree and time of defoliation may be of physiological and/or morphological importance, and it has been a primary concern to pasture scientists because the concurrent manipulation of all three factors determines yield and persistence of forage. Forage utilization involves the animal or the transformer of forages into products for human consumption (Blaser et al., 1966); therefore, a compromise between plant and animal yield may be pertinent (Humphreys, 1966).

Forage Regrowth

Plant growth depends on photosynthesis as a source of energy, and nutrients from the soil. Any factor affecting photosynthesis will also affect growth. Complete defoliation temporarily eliminates the photosynthetic apparatus (shoots), stopping growth until new shoots are

developed. This replacement of shoots after defoliation is termed regrowth. The regrowth is associated with meristematic tissue, which is responsible for cell division, and is found in growing centers known as buds (Jewiss, 1972a; Humphreys, 1981). Buds remain dormant until stimulation is produced. Development of buds into shoots is hormone regulated (Phillips, 1969). When a shoot develops, the apical meristem produces new leaves and new buds are formed which will remain dormant until stimulation occurs. Every bud has potential to produce a new shoot, or branch, depending on the growth habit of the plant. However, apical dominance restrains the development of these buds by an inhibitory mechanism controlled by hormones (Thimann, 1937) and metabolic sinks (Phillips, 1969). When the apical meristem is removed, regrowth is stimulated and axillary buds develop into shoots. The number of buds that grow and the rate of growth of these newly developed shoots will depend on nutritional factors (Sachs and Thimann, 1964). In the decapitated shoot, leaf and bud formation is stopped and further leaf and bud formation will depend on the new shoots. In grasses, this process is less understood, especially in sod-forming species where apical dominance is difficult to track (Humphreys, 1981); however, it is better understood in dicotyledonous species (Phillips, 1969). Defoliation of grasses above the apical meristem does not stop growth since mutilated leaves continue to elongate (Jewiss, 1972a) unless a leaf has completed expansion (Langer, 1954). In grasses with determinate growth, the apical meristem undergoes a series of changes and inflorescence formation initiates at the onset of reproductive phase. This process stops leaf formation in the shoot and inhibits the

development of axillary buds. In legumes the shoot apex remains vegetative whereas axillary buds develop inflorescences. The inhibition of axillary buds development receeds shortly after stem elongation is almost complete and inflorescence emerges (Jewiss, 1972a). When shoot development is initiated in the buds, energy is required for cell division and elongation. The source of energy could be nutrient reserves in the plant, which are translocated from the storage organs (Smith, 1981) to the growing sink, or from photosynthesis in the tissue remaining after defoliation (Blaser et al., 1966). The degree of contribution of the two sources may be quite variable, and they will be discussed later in more detail.

Forage yield and persistence are often correlated with a vigorous regrowth, and in turn three factors are recognized to affect forage regrowth: 1) Total non-structural carbohydrates (Graber, 1931; Weinmann,1961; Smith, 1981); 2) Number of growing points (Booysen et al., 1963; Jewiss, 1972b; Kretschmer, 1976; Knight, 1970); 3) Leaf area left after defoliation (Brown and Blaser, 1968; Valle, 1977; Andrade, 1979). The first two are forage species dependent whereas the third one is an external factor that modifies the other two.

Non-structural carbohydrates. Non-structural carbohydrates, commonly referred as total non-structural carbohydrates (TNC) (Smith, 1981) or total available carbohydrates (TAC) (Weinmann,1961) are the primary source of energy to initiate regrowth in perennial and biennial forage plants (Smith, 1981); particularly after a period of dormancy where no photosynthesis occurs. The accumulation of TNC in undisturbed plants follows a particular seasonal trend in every

species (Weinmann, 1948, 1961; Smith, 1975, 1981). It usually increases in summer and decreases during periods of active growth (Humphreys, 1981). The levels of concentration of TNC in storage organs (roots and/or crowns) can be reduced by liberal applications of N fertilizer (Brown et al., 1966a) because more assimilate is utilized in shoot growth and less is mobilized to roots and stem bases (Hojjati et al., 1968). Moderate shading also decreases the concentration of TNC (Burton et al., 1959; Blaser et al., 1966) without decreasing growth, unless liberal N is supplied (Burton et al., 1959; Colby and Drake, 1966). Conversely, water stress and suboptimal temperatures favor TNC accumulation (Eaton and Ergle, 1948; Blaser et al., 1966). This indicates that when photosynthesis exceeds carbohydrate utilization, TNC accumulates. The opposite occurs when growth exceeds photosynthesis (Wardlaw, 1968). Blaser et al. (1966) stated that low temperature affects growth more than it affects photosynthesis. Some tropical grasses are especially sensitive to low temperatures (West, 1969; Carter et al., 1972; Chatterton et al., 1972) and carbohydrate produced in leaves is not translocated to stems and roots, resulting in reduction of photosynthesis. Another example of this relationship can be seen in some legumes where N fixation does not meet the N demand for growth, thus carbohydrate may accumulate in roots but low applications of N maintain low levels of root TNC (Barta, 1979). In some circumstances, concentration of TNC may not be a good parameter because a decrease in concentration might be produced by increased plant mass (Weinmann and Goldsmith, 1948; Humphreys and Robinson, 1966). A drop in TNC has been observed after defoliation (Graber, 1931; Weinmann, 1947, 1952) and it has been positively correlated with

plant regrowth (Wolf, 1978). For these reasons the accumulation of high levels of TNC in roots and basal stems of forages has been the objective in grazing and cutting practices (Humphreys, 1966; Smith, 1981). However, other studies have failed to correlate regrowth with levels of TNC (Davidson and Milthrope, 1965; Weinmann, 1947; Humphreys and Robinson, 1966). There is a general agreement in that a minimum level of TNC is required to initiate regrowth in the spring. The controversy arises in regard to regrowth after defoliation because some grasses do not show a marked reduction in concentration (Weinmann, 1947) unless frequently defoliated (Weinmann and Goldsmith, 1948). It seems that the interaction between growth habit and carbohydrate levels might be the answer to this matter because complete defoliation is seldom achieved in decumbent plants, whereas plants with erect growth are more easily defoliated (Rossiter and Collins, 1980),

Growing points. As it was discussed previously, regrowth mechanisms are essentially the same in all plants but their morphology is highly variable. The morphology of grasses varies from bunch-type to rhizomatous types, and intermediate types that produce stolons and types that modify their morphology from upright growth to horizontal growth (Humphreys, 1981). Legumes, on the other hand, present very simple forms with one main stem that branches as it grows. These simple types might be of erect growth or climbing type of growth. They also present forms that have crowns from where several shoots are produced (Oakes, 1968; Brewbaker, 1976). Little is known about actual numbers of buds, but more research has been done on the position

of growing points and the hierarchical distribution. When shoots elongate during the reproductive phase, lateral buds arise also; therefore, if shoots are cut or grazed, most lateral buds may be removed; hence regrowth will initiate from basal buds (Jewiss, 1972b) which means that fewer shoots may be formed. This type of relation varies between species (Branson, 1953) due to the proportion of vegetative to reproductive shoots. This proportion changes also with the season (Knight, 1970). In some grasses, early removal of vegetative shoots increases the proportion of reproductive shoots, resulting in a considerable increase in seed production. In some grasses, internodal elongation occurs during the vegetative stage resulting in the elevation of vegetative buds above the soil surface and susceptibility to removal (Booysen et al., 1963).

In legumes, the hierarchical distribution of buds is better understood (Phillips, 1969). However, growing points move away from the soil along with the shoot apex, and the growth suppression on lateral buds depends on nutritional status and light. Defoliation of legumes close to the ground eliminates most growing points; therefore, regrowth must start at the crown of the plants. Variation has been found in the numbers of shoots that legumes produce after defoliation (Kretschmer, 1976; Imrie, 1971). Poor regrowth has been related to deficiency of basal bud sites in some erect types of Stylosanthes guianensis (Grof et al., 1970). These results indicate that some legumes may show little resistance to defoliation. This may be particularly important in legumes with climbing habit unless they are able to form rooting stolons where new growing points are generated (Hutton and Beall, 1977).

Some woody legumes have an excellent regrowth capacity from buds near the crown (Brewbaker, 1976; Gildersleeve, 1982). Grasses and legumes with rhizomatous habits maintain growing points below cutting height; therefore, more vigorous growth after defoliation is often observed. The pattern of shoot formation is typical of every plant; therefore, defoliation at a particular time may reduce shoot production.

Degree of defoliation. As discussed previously, defoliation means the removal of shoots, but from the standpoint of forage regrowth, what is left of the plant is important. Single plants may not reflect the response of a plant community (Alexander and McCloud, 1962) because light interception (Brougham, 1955), plant morphology (Ludlow and Wilson, 1970), and plant age (Brown and Blaser, 1968) interact in the rate of regrowth. The plant material that remains after defoliation contributes to the process of regrowth through photosynthesis and for nutrient reserves. The extent of photosynthesis will depend primarily on leaf material. It has been shown that increasing leaf area increases the growth rate until 95% of the light was intercepted (Brougham, 1955, 1956). The point where growth rate reaches its maximum was termed optimum LAI (Davidson and Donald, 1958), and at this point the leaves under the canopy are at their compensation point. However, different growth rates can be observed with similar LAI's when the canopies differ in age (Begg and Wright, 1964; Brown et al., 1966b). This means that a lenient defoliation does not necessarily result in a higher regrowth (Bryant and Blaser, 1961). It has been observed that higher productions are obtained with less frequent but complete defoliations than with several partial defoliations (Ridgman, 1960;

Reid, 1966; Vickery, 1981). Blaser et al. (1966) suggested that high degree of defoliation is recommended, especially after a long period of growth. Vickery (1981) concluded that lenient defoliations are not favorable to plant regrowth since animal and mechanical defoliations remove most of the newer leaves which are in the top of the canopy. These leaves are more photosynthetically active than the ones in the lower canopy. Plant morphology also affects the degree of defoliation and consequently the rate of regrowth. Plants with prostrate habits are more difficult to defoliate (Rossiter and Collins, 1980; Humphreys, 1981) but are less affected by changes in photosynthetic efficiency since most of their leaves are equally exposed to light. Therefore, lenient defoliations can maintain a higher growth rate (Ludlow and Charles-Edwards, 1980).

Interaction of factors. From the previous examination of factors affecting regrowth, it is clear that no one by itself can be considered as the key to forage management. In this discussion, very little was said about environmental factors which undoubtedly play an interactive role. With the understanding of the physiological mechanisms of regrowth in plants, it can be easier to understand their potential adaptability to a given environment which will encourage changes in the plant to ensure its survival. From an agronomist's point of view, survival of plants is not enough; they must produce under defoliation, but management of defoliation may be the key to plant survival. Therefore, knowing when and how much can be defoliated constitute the parameters in forage production. But with the animal as utilizer, forage production per se may not be very meaningful; thus animal production becomes the determinant in forage utilization.

Forage Quality

Barnes (1981) defined the term forage as "the vegetative portions of plants that are consumed by animals" (p. 2). From a nutritional point of view, Van Soest (1973) defines it as "coarse livestock food that is composed of leaves, stems, and sometimes grain" (p. 53). Then, forage quality refers to the efficiency of edible plant materials to provide nutrients for growth, reproduction, etc. Mott (1959) suggested that forage quality must be expressed ultimately in terms of output per animal. The use of output per animal as a measure of forage quality takes into consideration the interactions, known and unknown, between animal, plant, and environment that determine animal production. According to Mott (1973), forage quality comprises the nutritive value and the consumption or "intake" of forages. Nutritive value is a function of the chemical composition of a forage, its digestibility and the nature of digested products. On the other hand, intake is a function of the acceptability, rate of passage, and availability. With these concepts in mind, Moore and Mott (1973) pointed out that forage quality can be expressed in terms of output per animal provided that forage availability is not limiting and animal potential does not vary between treatments. In a strict sense, forage quality cannot be defined in terms of a single component; however, the quantification and/or qualification of one or more of those components may lead to an approximate estimation of forage quality.

The qualitative evaluation of forages calls for comparison methods that allow the selection of the most productive forages. Those methods must be simple, inexpensive, and easy to reproduce. Over the years it

has been widely accepted that the best measure of forage quality is animal performance; however, in very many instances it is not possible to compare several forages in animal trials; this is particularly critical in plant breeding programs where a large number of varieties and small samples are evaluated (Marten, 1981). Minson (1981a) stated that two other reasons for using alternate methods of forage evaluation are the formulation of rations to feed animals, and identification of problems when animals fail to achieve the expected performance. For these reasons, several approaches have been taken to elucidate the relative influence of the known components of forage quality. Digestibility and intake of forages seem to be the most widely accepted parameters of forage quality. Raymond (1969) combined digestibility and intake to develop the parameter termed nutrient intake, which is defined as the intake of feed x the digestibility of feed x efficiency of utilization of digested feed. Although this particular index requires animal trials to determine the consumption of the forages under evaluation, it can be very useful if its components were estimated from laboratory analyses. Since both intake and nutritive value are in turn affected by various factors, they will be discussed separately.

Forage intake. Different approaches must be taken to define the components of forage intake, i.e., in the pasture situation intake is a function of the acceptability, rate of passage, and availability (Mott, 1973, whereas, in confinement, availability may not be limiting. It has been postulated that intake of forage has a greater effect on forage quality than does nutritive value (Ventura et al., 1975) and it is attributed to the "distension" mechanism by which certain level of

fill in animals causes the cessation of consumption (Campling, 1970). According to Van Soest (1973), the attempts to establish measurements of intake as a feed evaluation characteristic have been difficult because the urge to eat varies among animals depending upon their physiological requirements. For comparison purposes, this can be overcome by utilizing several animals per treatment (Heaney, 1970) and animals of the same age and condition. Abrams (1980) summarized six factors responsible for the control of forage intake, when the control mechanism is distension. The six factors are 1)rumen fill, 2) potentially digestible and indigestible pool sizes, 3) initial particle size distribution, 4) rate of particle size reduction, 5) rate of passage, and 6) rate of digestion. In the discussion of these factors Abrams pointed out that they do not operate independently and their interrelationships will vary from forage to forage. This type of fractionation has been utilized in modeling for prediction of intake and digestibility. The models tested have been promising although the results so far are not satisfactory (See Waldo et al., 1972; Martens and Ely, 1979; Golding, 1976; Abrams, 1980).

The prediction of intake from laboratory analyses has not been very successful, and this, in the opinion of Donefer (1970), has been due to lack of complete understanding as to what physiochemical mechanisms, plant and animal, influence the voluntary intake by ruminants.

It is a general agreement that if intake is controlled by distension, the intake must be regulated by the unload rate of forage from the reticulo-rumen. Grovum and Williams (1973) provided experimental

evidence to confirm that the reticulo-rumen is the limiting compartment in the passage of dry matter. Furthermore, the physical form of the material found in the subsequent compartments indicates that most physical breakdown of forage occurs in the same compartment (Bailey et al., 1976). This suggests that the rate of forage unload depends upon mechanical and chemical degradation (Laredo and Minson, 1973). So far, it has not been possible to delineate the relative influence of these two mechanisms. In an attempt to characterize the physical degradation in the rumen, Chenost (1966) proposed a "fibrousness" index, obtained by measuring the energy required to grind forage samples through a 1-mm screen. He found a 0.90 correlation coefficient with six temperate grasses between the fibrousness index and dry matter intake. Laredo and Minson (1973) utilized the same method on five tropical grasses, separated in leaf and stem. They reported that more energy was required to grind stems than leaves. Their overall correlation coefficient between grinding energy and dry matter intake was 0.70 for leaves and stems together. Worrell (1982) found negative correlations between energy required for regrinding and organic matter digestibility and neutral detergent fiber digestibility.

Nutritive value. The second major component of forage quality is the nutritive value, and it refers to the relative supply of available nutrients contained in a forage. Mott (1973) summarized the constituents of nutritive value into three: chemical composition of the forage, its digestibility, and the nature of digested products. Due to the nature of forages, digestibility is the most widespread parameter of forage quality. It can be applied not only to dry matter, but also

to the various chemical fractions of the forage; e.g., organic matter, cell wall (Neutral Detergent Fiber), lignin and energy (Hacker and Minson, 1981).

Digestibility. Digestibility refers to the disappearance of forage material due to the truly chemical breakdown of the chemical constituents of forages into compounds absorbed by the animal. Estimation of digestibility with animals, along with forage intake, is the best parameter to estimate forage quality. In most cases, *in vivo* digestibility refers to apparent digestibility, since true digestibility is seldom estimated; the difference between true and apparent digestibility of dry matter is approximately 12.9% units (Van Soest and Moore, 1965). Therefore, in most cases, the digestiblity values reported for forages are underestimations of true digestibility. It is known that digestibility varies among species of plants (Moore and Mott, 1973) and among genotypes of both temperate and tropical grasses (McLeod and Minson, 1969; Burton et al., 1967) as well as tropical and temperate legumes (Moore and Mott, 1973).

It is unknown what causes digestibility to change, but the changes associated with maturity of a forage have supported the conclusion that digestibility is more a function of plant factors than animal factors, though it is recognized that efficiency of digestion is related to many animal factors such as species, level of intake, etc. (Minson, 1976). The relationship between digestibility and chemical composition of forages has been approached through two general methods—the first one involves the fractionation of feed and feces into chemical compounds (Gaillard, 1962; Jarrige, 1965), the second one fractionates the forage into cell wall and cell contents (Van Soest, 1967). Both

methods demonstrated that the availability of the cell contents is not limited by the cell wall. According to Van Soest (1976), these findings support the conclusion that cell wall digestibility is the limiting factor in forage quality. Chemical changes occur when a plant matures, the cell walls become relatively more important, and the fibrous constituents increase and become more lignified. These changes result in progressively lower dry matter digestibilities (Sullivan, 1969). Regressions have been used to relate digesbitility of forages to lignin, acid detergent fiber (Van Soest and Moore, 1965; Van Soest, 1965; McLeod and Minson, 1971), cellulose and hemicellulose (Russo, 1981), cell wall (Abrams, 1980) but none of them explains the variability found among and within species. Van Soest (1976) stated that the error with the use of lignin is particularly increased through the interaction of legumes and grasses, since grasses tend to have a higher cell wall content and lower lignin content than legumes at the same digestibility.

Unlike forage intake, the estimation of in vivo digestibility through laboratory analysis has been very successful. This has been accomplished by several methods; however, two of them are the most widely accepted by the scientific community. The one developed by Tilley and Terry (1963) is known as two-stage in vitro technique. It is based on the simulation of the digestive processes. The first stage involves the incubation of the forage sample with rumen fluid-buffer solution; and the second stage, a treatment with acid-pepsin. This method has been considered the most accurate laboratory method to predict dry matter digestibility in vivo of temperate and tropical

forages (Barnes, 1973; Minson, 1981a); and it has been used in plant breeding and selection programs in many parts of the world (McLeod and Minson, 1978). Another method to estimate in vivo digestibility was proposed by Donefer et al. (1963) and it is based on the utilization of purified enzyme extracts containing cellulase. Jones and Hayward (1975) described a two-stage technique in which a sample is incubated first with acid-pepsin to remove cell contents, followed by the incubation of the residue with the cellulase. According to Minson (1981a), the application of this method became a successful routine when an inexpensive "broad spectrum" cellulase became commercially available; this enzyme had cellulase, hemicellulase and pectase activity.

The accuracy of estimation is measured in terms of correlation coefficients between the in vivo digestibility and the in vitro values of digestibility. Minson (1981a) stated that the most useful criterion for comparing the laboratory methods is the residual standard deviation (RSD) of the regressions. He further stated that RSD is the minimum error that must always be applied to any in vivo digestibility value predicted from the regression.

Relationship between forage intake and digestibility. The digestion and passage of forage through the animal depends upon its chemical and physical breakdown. From the factors listed by Abrams (1980), the rate of particle reduction is the only factor that may exert the most influence over the other factors. It is also the one factor where most overlapping occurs between plant and animal effects. Much attention has been focused on the chemical aspects of forage degradation and very little to the mechanical counterpart. The latter involves chewing,

rumen motility, and rumination which may directly affect the rate of passage and digestion. This is illustrated with the results of Blaxter et al. (1956) who reported that rate of passage increased as particle size decreased, regardless of feeding level. In the same study, they found that digestibility of dry matter also decreased as particle size decreased, attributing it to lower retention time. This is an example of the continuous process occurring in the reticulo-rumen, where particles from a piece of forage may have different digestibilities due to reduced or increased fermentation times. Rodrigue and Allen (1960) observed reduced digestibility of cellulose and/or crude fiber, in relation to fineness of grinding. They concluded that the finer the hay was ground, the greater was the depression in digestibility of the total ration and the faster the rate of excretion. Numerous examples show that for both temperate and tropical forages, digestibility and intake show a variable degree of correlation (Minson, 1972; Moore and Mott, 1973; Ventura et al., 1975; Chaves, 1979; Abrams, 1980). This means that forages with low digestibility may have higher intake than forages with high digestibility.

A number of studies have demonstrated that for a given forage species, the leaf fraction is more digestible than the stem fraction (Clements et al., 1970; Hacker, 1971; Laredo and Minson, 1975; Hacker and Minson, 1981). Therefore, if digestibility affects intake, it would be expected to find higher intake of leaf than stems. Some _in vivo_ trials with sheep have proved this hypothesis right; however, the digestibility of the stem fraction has been slightly higher or equal to the leaf (Laredo and Minson, 1973; 1975). The same results were obtained with sheep and cattle (Poppi et al., 1981a, b). These studies explained the differences in intake in terms of retention time and rate of

excretion. If digestibility is the same but retention time is not, then the rate of particle size reduction must be increased. In vitro observations indicate that longer fermentation times result in higher digestibilities; also, the rate of digestion can be increased by reducing the particle size (Troelsen and Hanel, 1966; McLeod and Minson, 1969; Laredo and Minson, 1975). The study by Poppi et al. (1981a) showed that the digestibility of the fiber (NDF) was lower in the leaf than in the stem fraction. All these observations indicate that the rate of particle-size reduction is a more important factor than digestibility in the control of forage intake. Furthermore, they suggest that the rate of particle-size reduction will determine the extent of digestion and hence digestibility. Histological observations (Akin et al., 1977; Hanna et al., 1976; Akin and Burdick, 1975) of different forages indicate that there are marked differences in the extent of digestion in the different tissues. It is possible that the disappearance of the most digestible cells, in combination with mechanical action of the animal, increase the fragility of the tissue and stimulate the particle reduction.

It appears that more research is needed to gain understanding in the animal mechanisms that regulate forage intake. In the vegetal counterpart, it is necessary to understand the changes that occur in the plants which alter digestibility. It should also be pointed out that the in vitro digestibility must be used cautiously and should not be used alone as the only parameter of forage quality. Therefore, with the level of understanding of the factors of forage quality, it would be safer to base forage evaluation on in vitro digestibility,

mineral balance, and leafiness plus all the desirable agronomic characteristics (Minson, 1981b).

CHAPTER II

GROWTH ANALYSIS OF AERIAL AND UNDERGROUND PLANT PARTS OF FLORIGRAZE RHIZOMA PEANUT

Introduction

Growth analysis is a tool to assist in the interpretation of morpho-physiological changes occurring in plants throughout their life cycles. It is based on the mathematical description of the growth of a plant, either as a whole or as its components. Several concepts of growth analysis have been proposed and were summarized by Radford (1967) and Evans (1972). In general, all concepts are analyzed as a function of time with the purpose of studying the seasonal growth which in turn is a function of the environment. The selection of the form of analysis depends on the objectives of the study. For plant breeding purposes, Snyder and Carlson (1978) used the ratio of taproot to leaf weight in sugarbeet (*Beta vulgaris* L.) to select for higher taproot yield. Duncan and Hesketh (1968) found genetic differences in net photosynthetic rates of different races of maize (*Zea mays* L.) with respect to temperature regimes. Comparing several peanut (*Arachis hypogaea* L.) cultivars, Duncan et al. (1978) suggested that the yield of cultivar 'Early Bunch' could be increased by improving crop growth rate whereas that of 'Florunner' could be increased by improving partitioning. McCollum (1978) studied the effect of P-fertilizer regimes on the growth and yield of potato (*Solanum tuberosum* L.)

Milthorpe and Moorby (1979) suggested that fitting growth data into curves, for comparison purposes, gives a greater overall understanding of the effect of the experimental variables. They also pointed out that the experimenter must keep in mind that drastic changes in growth caused by changes in the environment may not show up in the fitted curves. Radford (1967) indicated that fitting curves with regression has the advantage of pooling errors inherent in sampling plants grown in the field.

Rhizoma peanut, one of several perennial species in the genus Arachis, has shown considerable potential for improvement of livestock production in North and Central Florida. The most outstanding selection was released by the University of Florida in 1979 with the cultivar named 'Florigraze' (Prine et al., 1981). The rhizomatous habit of the plant makes it able to withstand grazing. A serious disadvantage of Florigraze rhizoma peanut is the lack of seed production; therefore, its establishment must be achieved by vegetative propagation. The most rapid method is by planting rhizome pieces late in the winter (Prine et al., 1981). There is little information regarding the seasonal growth and development of the aerial parts in relation to the underground rhizome system.

In North and Central Florida, the aerial part dies in the winter whereas the rhizome system remains dormant and initiates growth early in the spring. Since both perennation and persistence depend on the rhizome part of the plant, it is necessary to understand the dynamics of growth in the plant. With this in mind, the objectives of the present study were to describe the growth pattern of Florigraze rhizoma

peanut and the proportion of plant parts relative to the recovered plant biomass under field conditions.

Materials and Methods

The experiments were carried out in 1980 and 1981 in two areas of land (A and B) of approximately 0.2 ha each, at the University of Florida Agronomy Department's "Green Acres" farm located about 18 km west of Gainesville, FL. The experiment in field A was conducted for 2 years, starting in 1980, whereas the one in field B was conducted only during 1981. The soil type in both areas was Arredondo loamy sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult). In 1980, preparation of the land area A included harrowing and disking; treatment with 2.3 kg active ingredient (ai) ha^{-1} of vernolate (S-propyl dipropylthiocarbamate) and 3.0 kg ai ha^{-1} of benfluralin (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine) for preemergence control of nutsedges (*Cyperus* spp) and annual grasses (*Digitaria sanguinalis* L. and *Cenchrus pauciflorus*, Benth.) respectively. The soil was fertilized in January 1980 with approximately 52 kg of P, 200 kg of K, and 2 kg of minor elements (FTE 503 mix) per ha. Rhizome material was planted on 27 Feb. 1980 in hills every 2 m in rows 3 m apart. Each hill was composed of three to five rhizome pieces, to make up a weight of 30±2 g (fresh weight). The rhizome material used for the planting was dug with a potato digger (Prine et al., 1981). Each rhizome piece was of approximately the same length (25 to 30 cm) and diameter (3 to 4 mm) in an attempt to maintain morphological uniformity. Rhizomes were planted at about 7-cm depth and were inoculated with a commercial granular peanut

inoculum by sprinkling a few mg of granules over the rhizomes prior to covering them with soil. Weed control during 1980 included spraying with 0.96 kg ai ha^{-1} of bentazon (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) for control of broadleaf weeds 1 month after emergence (April 1980). After the April spraying, hand weeding was done in and around the rhizoma peanut plants to allow the use of a rotary mower between rows without physical damage to the plants. The spaces between plants were mowed in June and August 1980. Field A was uniformly mowed in January 1981 to eliminate dead material. In 1981, field A was sprayed with 1.15 kg ai ha^{-1} of alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide) and 0.34 kg ai ha^{-1} of 2,4-DB (4-(2,4-dichlorophenoxy) butyric acid) shortly after emergence of rhizoma peanut (late March) to control broadleaf weeds. Mowing between the plants was the only weed control practiced in field A during the growing season in 1981.

Field B was planted in 1981 as a duplication of the 1980 planting in field A. In field B the land area was rototilled on 15 Dec. 1980. On 20 Feb. 1981 the area was sprayed with 2.0 kg ai ha^{-1} of vernolate and 0.75 kg ai ha^{-1} of trifluralin (α,α,α-Trifluoro-2,6-dinitro-N, N-dipropyl-p-toluidine). The herbicides were incorporated with a harrow immediately after application. On 17 Feb. 1981 the soil was fertilized with 18 kg of P and 67 kg of K per ha. Rhizomes were planted in hills on 4 March 1981 with distribution of hills, selection, planting and inoculation of rhizomes similar to the 1980 planting on field A, except that digging of rhizomes was performed with a bermudagrass sprig digger (Prine et al., 1981). In field B, in 1981, weed control consisted of

postemergence application of 0.34 kg ai ha^{-1} of 2,4-DB to control broadleaf weeds and spot applications of ghyphosate (isopropyl-amine salt of N-(phosphonomethyl) glycine) to eliminate perennial and annual grasses.

In both 1980 and 1981, plant spacing was designed to ensure that all plants had enough land to grow without competition from the adjacent plants and to be able to differentiate one plant from another. The center of the plants was marked with a wooden stake at planting. A sampling schedule (Table 1) was arranged for both years. In field A the 1980 sampling was termed 1980 growth (or data set I) and the 1981 sampling was termed 1981 regrowth (or data set II). The 1981 sampling in field B was termed 1981 growth (or data set III). During 1980 in field A, 10 randomly selected hills were dug at each sampling period. The randomization was restricted to one half of the plants, digging in alternate rows and columns such that at the end of 1980 one half of the initial number of plants were left in the field with a spacing of 4 x 3 m between plants. In 1981, in both fields, the number of hills per sampling was reduced to seven. The plants were dug by loosening the soil with a garden fork, around and within the hill; then from the center of the hill outward the rhizomes were lifted carefully by hand following its direction. One radial section was dug at a time to avoid breaking the hill into pieces. After digging the hills (which remained intact), the sand was shaken off and all foreign plant material was removed. When digging was completed, the plants were carried to a nearby water faucet and carefully, but thoroughly, washed on perforated aluminum trays to remove any soil adhering to the plants. After washing, each plant sample was separated into shoots and rhizomes, and the number of shoots recorded. The two

Table 1. Sampling schedule for three data sets to record the growth and regrowth of Florigraze rhizoma peanut.

Data sets		
Field A		Field B
1980 Growth (I)	1981 Regrowth (II)	1981 Growth (III)
Sampling Dates		
28 Feb (59)†	3 Mar (62)	
4 Apr (94)		27 Mar (86)
2 May (122)		
23 May (143)	20 May (140)	20 May (140)
6 Jun (157)	8 Jun (159)	9 Jun (160)
20 Jun (171)	26 Jun (177)	
4 Jul (185)		
19 Jul (200)	15 Jul (196)	9 Jul (190)
1 Aug (213)	3 Aug (215)	7 Aug (219)
15 Aug (227)		
4 Sept(247)	1 Sept(244)	5 Sept(248)
26 Sept(269)	3 Oct (276)	2 Oct (275)
17 Oct (290)		
7 Nov (311)	1 Nov (305)	31 Oct (304)
11 Dec (345)	27 Nov (331)	26 Nov (330)

†Julian days.

sections were dried at 70 C until weight equilibration (about 36 hours). When dried, the sections were weighed and each one further separated into two parts—shoots into leaf and stem and rhizomes into primary and secondary rhizomes—and their weight recorded. Secondary rhizomes were considered those cylindrical in form, rather thin (less than 3 mm in diameter), whereas primary rhizomes are conical and show a thickening from where branches are produced. After the third sampling period of the 1981 regrowth in field A, a plant subsample was taken from the hill according to the following procedure: the initial rhizome pieces, planted the year before, had developed into taproot-like structures from 10 to 15 mm in diameter. Therefore, by untangling one of these rhizomes from the remainder of the hill, it was possible to recover all its branches and acceptably maintain the proportion of shoots to rhizomes. This subsample was then processed as previously described; the remainder of the plant sample was freed from other plant materials, washed, dried, and weighed to record total growth and to estimate the total growth of the different parts. The 1981 growth in field B was processed like 1980 growth in field A.

Total growth and growth of the components were fitted to an exponential function of the form

$$W(t) = W\, e^{at + bt^2 + ct^3} \qquad (1)$$

where:

$W(t)$ = Predicted dry weight (g)

W = observed dry weight (g)

t = days

e = 2.78

a,b,c = linear, quadratic, and cubic coefficients.

Comparisons between growth and regrowth curves were performed with General Linear Model procedure of SAS (SAS Institute Inc., 1982) using the logarithmic form of equation (1)

$$\text{Ln } W(t) = \text{Ln } W + at + bt^2 + ct^3 \qquad (2)$$

and data set as class variable to test differences in linear, quadratic, and cubic coefficients among curves. The first partial derivative of equation (1) was used to estimate growth rates (g hill^{-1} day^{-1}) in total growth and growth of components (Radford, 1967). The first partial derivative of equation (2) was used to estimate relative growth rates (g g of hill^{-1} day^{-1}) also for total growth and growth of components (Radford, 1967).

Results and Discussion

The 1980 growth (data set I), 1981 regrowth (data set II) and 1981 growth (data set III) were plotted against time (Fig. 1). Prediction equations for the data-set curves are shown in Table 2. The regression analyses to compare data sets of all plant fractions is presented in Table 3. To facilitate understanding, the results will be discussed by individual plant fractions and total growth.

Shoot Growth

The shoot growth presented a growth curve characterized by a rapid increase in growth from June to September (Figs. 1A, 1B, 1C) followed by a plateau which maintained until late October. The comparisons among shoot growth curves (Table 3) indicated that all curves were different from each other especially with respect to their linear coefficients.

Table 2. Mathematical equations to describe the growth of Florigraze rhizoma peanut components in three data sets.

Component		Intercept	Coefficients†		
			Time‡	Time^2	Time^3
Total growth					
1980 growth	(I)	3.8166	-0.0413*	3.29 E-4§	-5.576 E-7*
1981 regrowth	(II)	6.6244	-0.0168*	1.31 E-4	-2.202 E-7
1981 growth	(III)	3.4934	-0.0252*	1.76 E-4	-2.476 E-7
Shoot growth					
1980 growth	(I)	-6.2341	0.0822*	-1.74 E-4*	8.360 E-8
1981 regrowth	(II)	8.7398	-0.0707*	4.16 E-4*	-6.780 E-7*
1981 growth	(III)	-7.5550	0.0890*	-2.21 E-4*	1.910 E-7
Rhizome growth					
1980 growth	(I)	5.0050	-0.0644*	4.07 E-4*	-6.231 E-7*
1981 regrowth	(II)	7.2453	-0.0235*	1.20 E-4	-1.564 E-7
1981 growth	(III)	8.0344	-0.0799*	3.48 E-4	-4.039 E-7
Number of shoots					
1980 growth	(I)	8.4953	-0.0794*	4.13 E-4	-6.191 E-7*
1981 regrowth	(II)	8.0961	-0.0172	6.04 E-5	-7.126 E-8
1981 growth	(III)	-4.9340	0.1101*	-4.74 E-4	6.804 E-7
Leaf growth					
1980 growth	(I)	-.2781	2.47 E-3*	1.47 E-4*	-3.378 E-7*
1981 regrowth	(II)	7.4579	-0.0552*	3.39 E-4*	-5.678 E-7*
1981 growth	(III)	-7.9557	0.0904*	-2.29 E-4*	1.982 E-7
Stem growth					
1980 growth	(I)	-6.6587	0.0731*	-1.37 E-4*	3.951 E-7
1981 regrowth	(II)	9.3843	-0.1024*	5.75 E-4*	-9.110 E-7
1981 growth	(III)	-8.4561	0.0821*	-1.84 E-4*	1.407 E-7
Primary rhizomes					
1980 growth	(I)	4.0790	-0.0610*	3.71 E-4*	-5.649 E-7*
1981 regrowth	(II)	5.9507	-0.0412*	2.41 E-4	-3.500 E-7
1981 growth	(III)	5.1432	-0.0502*	2.09 E-4	-2.011 E-7
Secondary rhizomes					
1980 growth	(I)	4.2245	-0.0616	4.01 E-4*	-6.176 E-7*
1981 regrowth	(II)	6.7326	-0.0159	0.75 E-4	-0.909 E-7
1981 growth	(III)	8.2322	-0.0912	4.01 E-4	-4.821 E-7

†Linear, quadratic and cubic ‡Days §10^{-x}
*Significant ($P > 0.05$).

Table 3. Regression analyses of complete data and individual comparisons between data sets for growth components of Florigraze rhizoma peanut.

Plant part	Data set comparisons			
	Data sets I, II, III	Data set I vs Data set II	Data set I vs Data set III	Data set II vs Data set III
	PR = F§			
Total Growth				
Data set†	0.0001	0.0001	0.0001	0.0001
Data set x time‡	0.0001	0.0001	0.0439	0.0001
Data set x time^2	0.0461	0.6063	0.0036	0.4362
Data set x time^3	0.2261	0.0980	0.3959	0.9583
Shoot Growth				
Data set	0.0001	0.0001	0.0001	0.0001
Data set x time	0.0001	0.0001	0.0528	0.0001
Data set x time^2	0.1954	0.0768	0.2836	0.5973
Data set x time^3	0.3656	0.1385	0.8092	0.2909
Rhizome Growth				
Data set	0.0001	0.0001	0.0001	0.0001
Data set x time	0.0001	0.0001	0.0001	0.0002
Data set x time^2	0.0008	0.9691	0.0001	0.1042
Data set x time^3	0.0823	0.0218	0.5457	0.6449
Number of Shoots				
Data set	0.0001	0.0001	0.0001	0.0001
Data set x time	0.0001	0.0001	0.0022	0.0043
Data set x time^2	0.4984	0.1712	0.3703	0.9384
Data set x time^3	0.0032	0.2211	0.0002	0.2979
Leaf				
Data set	0.0001	0.0001	0.0001	0.0001
Data set x time	0.0001	0.0001	0.0496	0.0001
Data set x time^2	0.6652	0.3934	0.9195	0.5287
Data set x time^3	0.4995	0.6955	0.3099	0.3447
Stem				
Data set	0.0001	0.0001	0.0001	0.0001
Data set x time	0.0001	0.0027	0.0090	0.0002
Data set x time^2	0.5813	0.3053	0.4101	0.7254
Data set x time^3	0.2712	0.1043	0.8469	0.2183

Table 3 - continued.

Plant part	Data set comparisons			
	Data sets I II III	Data set I vs Data set II	Data set I vs Data set III	Data set II vs Data set III
	PR = F			
Primary Rhizome				
Data set	0.0001	0.0001	0.0005	0.0001
Data set x time	0.0003	0.0001	0.0065	0.5459
Data set x time^2	0.0002	0.9122	0.0001	0.0268
Data set x time^3	0.3501	0.2528	0.2769	0.7503
Secondary Rhizome				
Data set	0.0001	0.0001	0.0001	0.0001
Data set x time	0.0001	0.0001	0.0001	0.0001
Data set x time^2	0.0030	0.8546	0.0001	0.1448
Data set x time^3	0.0831	0.0196	0.7404	0.5126

†Intercept

‡Time in days

§Significance level

Since a logarithmic model was employed to compare the curves, a significant difference among quadratic coefficients would suggest differences in relative growth rates (Figs. 3A, 3B, 3C). This is so, because to obtain the first partial derivative of the growth curve, the linear coefficient becomes the intercept of relative growth rate curve; also, two times the quadratic coefficient becomes the linear coefficient in the relative growth rate curve, and so on. In 1980 growth (I) and 1981 growth (III) the quadratic coefficients were negative (Table 2) while in 1981 regrowth (II) it was positive. Although among quadratic coefficient there was a difference only between 1980 growth (I) and 1981 regrowth (II), the relative growth rates can be ranked in the following order: 1980 growth, 1981 growth, and 1981 regrowth. At the time of the last sampling, the shoot growth of 1981 growth (III) (Fig. 1C) was still on the plateau; in the same year but in separate fields, this was clearly not the case of the 1981 regrowth (II). The plant canopies in 1980 growth (I) and 1981 regrowth (II) were very dense and began to show signs of senescence in October, particularly at the base of old shoots where light was probably limiting. In other temperate and tropical species, it has been shown that photosynthesis is lower at the base of a canopy (Brown and Blaser, 1968) because less direct sunlight penetrates to the base of closed canopies (Ludlow and Wilson, 1970). The 1981 growth (III) plants did not close the canopy; in fact, they remained rather prostrate. No leaf senescence or leaf loss was observed in spite of low minimum temperatures (Fig. 5B) recorded in November 1981. In contrast, frost damage was observed in the plants of 1981 regrowth (II); there was no explanation to this, since the fields were about 600 m apart.

The shoot growth was closely associated with the number of shoots (r^2 = 0.98, 0.63, and 0.88 for 1980 growth (I), 1981 regrowth (II), and 1981 growth (III), respectively). These are plotted against time in Fig. 4A. The weight of individual shoots also increased with time. The shoot growth rates increased until September in all three data sets (Figs. 2A, 2B, 2C). The shoot production rates (Fig. 4B) also increased for the 1980 growth (I) and 1981 regrowth (II) until September whereas the 1981 growth (III) reached its minimum shoot production rate in September and started to increase from then on. These results suggest that after September, plants started to reduce number of shoots and the individual shoot weight. In the case of 1981 growth (III), the increase in total shoot weight was due to the increasing number of shoots and not to an increase in the weight of individual shoots. Nineteen eighty-one was a dry year, and a severe drought occurred until the end of June (Fig. 5C). In the 1981 growth (III), newly formed shoots died during the drought, leaving only the old ones. These old shoots branched abundantly after the drought and formed a thick stem. This branching occurred from lateral buds on the stem, as opposed to the new shoots which originate from buds on the rhizomes. This helps to explain the decreasing rates of shoot production in the 1981 growth (Fig. 4B) where the canopy was formed as a result of shoot branching. During the drought it was observed that rhizomes produced numerous roots and no shoots. A response like this was not observed in 1981 regrowth (II), probably because old primary rhizomes were very well rooted; although shoot production was affected (Fig. 4A). This was reinitiated at an increasing rate after the dry period.

When looking at the growth of leaves and stems (Figs. 1D, 1E, 1F), it can be noticed that the leaf fraction is the shoot component that declines earlier in the year, while the stem fractions remain high for a longer period. The statistical comparisons between coefficients (Table 3) gave the same results as shoots. Curves depicted in Figs. 2D, 2E, and 2F showed sharper decline in growth rates (September) in leaf fractions than in stem fractions. The declining rates of growth in the leaf fraction were related to the size and structure of the plant canopies. The highest rate of decline was observed in 1981 regrowth (II) followed by 1980 growth (I) and 1981 growth (III). Even though leaf area was not measured, it was observed that plant canopies were fuller in 1980 growth (I) or 1981 regrowth (II) than in 1981 growth (III). Probably, a full canopy was reached, causing a steep decline in leaf growth rates, as opposed to the 1981 growth (III) where the rate of decline was very small. The rate of increase of shoot numbers in the 1981 growth (III) partially accounts for the slow decline in leaf and stem growth rates, although the size of shoots did not increase after September. Early in the year, when shoots started to emerge, stems were heavier than leaves, but in 2 to 3 weeks, the weight of the leaves accounted for up to 75% of the weight of the shoots. As the plants grew older, the leaf fraction decreased steadily due to leaf loss and to stem development. Furthermore, the shoots produced later in the year had very small leaves, and even though they were counted as shoots, the leaf mass was not greatly increased. The fact that stem growth rates did not decline at the same time that the leaf growth rates, may have been due to a slower rate of decomposition in the stems, which means that after a shoot looses its leaves, the stem

remains longer. In this experiment, dead shoots were not counted, but shoots which remained green with at least one leaf were counted. In corn, Williams et al. (1965) reported that crop growth rates increased with increasing plant populations with concurrent reduction in the size of individual plants. Similar results were reported by Knight (1970) for orchardgrass (Dactylis glomerata L.), where he found that removal of tillers enhanced the development of others, making up for the space left. In this context, with respect to the ranking of relative rates of shoot production (Fig. 4C) and relative growth rates of shoots (Figs. 3A, 3B, and 3C), it appears that low shoot growth in 1981 regrowth (II) was due to a low rate of shoot production. The 1981 regrowth (II) showed an increasing relative growth rate of shoots reaching a maximum in August, and declining thereafter. The reason for this was that the emergence of shoots did not occur at the same time; therefore, growth was not a result of development of the initial shoots, but the result of shoot growth and shoot emergence.

Rhizome Growth

As opposed to shoot growth which reached a plateau in September, rhizome growth continued at least until the last samples were taken at the end of the year (Figs. 1A, 1B, and 1C for 1980 growth (I), 1981 regrowth (II), and 1981 growth (III), respectively). The regression analyses (Table 3) showed differences between rhizome growth curves, particularly between linear coefficients (Table 2), suggesting differences in growth rates. There were no differences among quadratic coefficients, except between 1980 growth (II) and 1981 growth (III), but the linear coefficients determined the differences in relative growth rates

(Figs. 3A, 3B, and 3C). The ranking in relative growth rates can be determined with the quadratic coefficients and it follows the same trend as shoot relative growth rate. Even though the linear coefficient of 1981 regrowth (II) was the smallest, it ranked last because the quadratic coefficient (Table 2) was also the smallest and did not counteract the effect of the linear coefficient. The relative growth rates of rhizomes in 1980 growth (I) (Fig. 3A) almost doubled those of 1980 regrowth (II) (Fig. 3B) with the ones of 1981 growth (III) (Fig. 3C) in the middle. Rhizomes grew almost parallel to shoots from the beginning of the year until September. It was previously believed that rhizomes grew only in the fall (G. M. Prine, 1980. Personal communication). The results of these studies indicate that shoots stop growing early in the fall and rhizomes continue to grow, indicating an increase in partitioning of assimilates towards rhizome growth. This phenomenon is clearly illustrated with the growth rate curves (Figs. 2A, 2B, and 2C) where the growth rates of shoots are declining in September while rhizome growth rates either continue to increase (1980 growth and 1981 growth) or are reaching a plateau (1981 regrowth). Throughout the year rhizomes were continuously produced, but in September of both years it was more evident; probably because fewer shoots were produced and it changed the proportion of aerial and underground parts. Figures 2A, 2B and 2C also show that growth rates of rhizomes in 1980 growth (I) and 1981 growth (III) were comparatively higher than shoots. This did not occur in 1981 regrowth (II). Various degrees of assimilate partitioning have been reported for plants with determinate growth. The variation can be genetic (Duncan et al., 1978; Snyder and Carlson, 1978) or can be caused by nutrient imbalances that,

in turn, cause alteration of 'sinks' (McCollum, 1978). Rhizoma peanut is perennial and day neutral plant. In this experiment it flowered all year and very few pods were found. Most pods were empty, although some were found in different stages of pod filling, indicating that pod fill was completed early in October. With the observations of these experiments it is not possible to determine whether there was a physiological change that made rhizomes a stronger sink for assimilates, or if it was only induced by reduction in growth in the aerial part.

From the rhizomes, two fractions were separated, primary and secondary rhizomes. In Figs. 1D, 1E, and 1F it can be seen that secondary rhizomes constituted the bulk of the rhizome network and the largest component of the entire plant. The secondary rhizomes (cylindrical) seemed to play a role of spreading the plant and maintaining it as one unit (Fig. 6A). Most nodules were found on secondary rhizomes and small roots associated with them (Fig. 6B). On the other hand, primary rhizomes (conical) developed from secondary rhizomes at points where shoots surface out. These conical structures were the point of origin of new rhizomes and shoots (Fig. 6C). Rhizomes grew radially, from the center, occasionally some were observed growing perpendicularly to the center, but almost never towards the center. In fact, in the 1981 regrowth (II) some plants started to overlap and it was easy to determine the origin of a rhizome by determining the direction of its growth. Any rhizome produced will grow several centimeters and its tip will emerge as a shoot (Fig. 6A). As the shoot grows, a primary rhizome is produced, but only if the shoot develops. Otherwise, the rhizome will branch under the ground in one or more directions. These branchings do not produce primary

rhizomes. Primary rhizomes thicken according to the number of branches produced from this point. Roots also develop from these points. Most roots were found associated with primary rhizomes but not all primary rhizomes produced deep roots. In the 1980 growth (I) the ratio of secondary to primary rhizomes increased as the growing season progressed; the same trend was observed, to a lesser extent, in the 1981 growth (III). In the 1981 regrowth (II) the ratio was maintained rather constant throughout the year. The growth rates (Figs. 2D, 2E, and 2F) for both rhizome fractions presented the same trends as the growth curves; which means that in 1981 growth (I) and 1981 growth (II) the growth rates of secondary rhizomes increased faster and were much higher than in primary rhizomes. In the 1981 regrowth (II), growth rates of both fractions were very similar, indicating that more primary rhizomes were produced during the regrowth phase than during growth. This was further confirmed by the relative growth rates, which were higher for the primary rhizomes. Data in Table 3 show, for primary rhizomes, that the quadratic coefficient of 1981 growth (III) was different from both 1980 growth (I) and 1981 regrowth (II) ($P = 0.0001$ and $P = 0.0268$, respectively). These results indicated that relative growth rates for primary rhizomes in 1980 growth (I) and 1981 regrowth (II) were very similar and at the same time higher than in 1981 growth (III). Conversely, for secondary rhizomes, Table 3 shows differences in linear and quadratic coefficients between 1980 growth (I) and 1981 growth (II), (Table 2) and differences in linear and cubic coefficients between 1980 growth (I) and 1981 regrowth (II). These results indicated differences in relative growth rates, with the highest for 1980 growth (I) followed by 1981 growth (III) and 1981

regrowth (II). These results can be visualized in Figs. 3D, 3E and 3F; in 1980 growth (I) and 1981 growth (III) relative growth rates were higher for secondary rhizomes than for primary. The reverse effect was observed in the 1981 regrowth (II).

The reduction in relative growth rates of secondary rhizomes, along with the reduction in relative rates of shoot production, in the 1981 regrowth (II), strongly suggested that efficiency of both plant propagation and production was lost in the second year. On the other hand, the increase in primary rhizomes may indicate that an equilibrium is reached although rhizome growth was reduced.

Total Growth

Total growth comprised shoot and rhizome fractions (Figs. 1A, 1B, and 1C) and it was characterized by a rapid increase from June until October, when top growth reduced its growth rates. A regression analysis including all three data sets (Table 3) showed differences among intercepts and among time (linear) and time^2 (quadratic) coefficients of the curves. However, the individual comparisons showed only differences in quadratic coefficients between 1980 growth (I) and 1981 growth (III) ($P = 0.0036$); indicating that sets reached their plateau at different times. The cubic coefficients were not different ($P = 0.2261$) among total growth curves, indicating that a decline in total growth occurred approximately at the same time, although in the 1981 growth (II) the inflexion point did not occur within the observed range of time. Nineteen eighty-one was very dry, and a drought was experienced until the end of June. Consequently, 1981 growth (III) (Fig. 1C) was delayed until July, and thereafter rhizoma peanut grew at a nearly linear rate until

at least 27 November when the last samples were dug. The drought effect is seen in both 1981 regrowth (II) (Fig. 1B) and 1981 growth (III) (Fig. 1C), in the solid line at the beginning of the growing season. It occurred earlier in the 1981 regrowth (II) because the shoots emerged about 3 weeks before the 1981 growth (III). In addition, the plants were much larger and occupied more space; therefore, water in the upper profile was probably depleted and the plants showed the effect. Conversely, the plants of the 1981 growth (III) had almost no competition and they were very small. Drought stress was observed in some plants after it started raining at the end of June. The amount of rainfall was not enough for the incipient root system of young plants in 1981 growth (III), but it was enough for the well-rooted plants in the 1981 regrowth (II). The number of shoots (Fig. 4A) also followed the same trend as that of total weight, indicating that the early season decrease in both 1981 sets were associated with losses in shoot numbers. This confirmed the observations in the field of shoots dying during and after the drought.

Maximum growth rates for total growth occurred between mid-September and mid-October (Figs. 2A, 2B, and 2C), suggesting a decline in photosynthetic activity. The decline in growth rates coincided with the seasonal decline in temperature (Figs. 5A and 5B); however, other effects must have been associated with it because high growth rates in 1981 growth (III) (Fig. 2C) were maintained over a longer period of time. It was indicated that the structure of the canopy could have affected photosynthesis. An illustration of this can be found in growth rates of shoots (Figs. 2A, 2B, and 2C). In 1980 growth (I) and 1981 growth (III), the decline in total growth rates was closely associated with the decline

in rhizome growth rates. In the 1981 regrowth (II) the decline was associated with the declining rates of shoot growth, indicating that net assimilation was reduced. It has been reported that Pangola digitgrass reduces photosynthesis with night temperatures around 10 C (West, 1969) particularly if low temperature occurs for 3 consecutive days (Karbassi et al., 1972. In Siratro (Macroptilum atropurpureum [D.C.] Urb.) very little growth occurs below 21 C and 14 C, maximum and minimum, respectively (Jones, 1967; Whiteman and Lutham, 1970). It seems that rhizome peanut is sensitive to the decline in temperature that occurs in September and October, and reduces shoot growth. Rhizome growth is totally dependent on the supply of sugars from the shoots; and soil temperature does not decline as fast as air temperature. Therefore, rhizome growth will continue for as long as the shoots remain photosynthetically active. In these experiments, the foliage was not disturbed, so it was thought that the age of the shoots could decrease photosynthesis regardless of the structure of the canopies, resulting in a reduction of shoot growth rates due to constant respiration. Prine et al. (1981) provided data that refutes this hypothesis. In one of their experiments, Florigraze rhizoma peanut was harvested three times in 2 consecutive years. In both years, the third harvest which represented growth from mid-August to mid-October produced 50% less than the previous two harvests, indicating that indeed the plant reduces its shoot growth in September. To understand further, the differences among growth curves, it was necessary to examine the relative growth rates because the obvious differences in plant size. Data in Table 3 show that for total growth, the quadratic coefficients of 1980 growth (I) and 1981 growth (III) were different

(P = 0.0036) indicating differences in relative growth rates. The quadratic coefficient for 1980 growth (I) was almost double the coefficients of either 1981 regrowth (II) or 1981 growth (III), and despite a low intercept, it was translated into higher relative growth rates. Data in Fig. 3A, 3B and 3C show that highest relative growth rates were achieved in the 1980 growth, followed by 1981 growth and 1981 regrowth. These results suggest that Florigraze rhizoma peanut grows more efficiently during the first year. The observations in these studies indicate that for a given mass of rhizome material, more rhizomes are produced in the first year of growth than in the second. Also, more shoots, relatively speaking, are produced during the first year. What causes these changes in the proportion of plant components cannot be explained on the basis of experimental evidence. Visual observations of plants in September 1982, in their third growing season, indicated that the rate of spreading decreased every year. Nevertheless, it takes less time to fill in the canopy when the growing season starts. This makes the plant competitive with weeds since it starts growing early in April unless it has just been planted; in newly planted fields, growth will be started a month or so later.

Summary

Growth analysis is a valuable tool for interpretation of morphological changes in plants as a result of environment or treatment effects. Two field experiments were conducted in 1980 and 1981 to describe the growth pattern of Florigraze rhizoma peanut and the proportion of plant parts relative to recovered plant biomass. The experiments were

conducted at the University of Florida Agronomy Department's "Green Acres" farm, 18 km west of Gainesville. Experiment A was conducted for 2 years (1980 growth and 1981 regrowth). Experiment B was conducted only in 1981 (1981 growth) as a replication of 1980 growth of experiment A. Ten and seven plants (1980 and 1981, respectively) were dug at all samplings. Sampling schedule covered the entire growing season in both experiments. Plants were dug, washed, separated into shoots and rhizomes, and dried. The dry weights were recorded and plant fractions further separated—shoots into leaves and stems, and rhizomes into primary and secondary rhizomes. Data were fitted into logarithmic form of exponential function and analyzed with regression analyses.

Statistical differences were associated with environmental effects. The 1981 regrowth had lower relative growth rates than the 1980 and 1981 growth. Shoots and rhizomes grew parallel to each other until September. From then on, shoot growth declined and rhizome growth continued to increase. The decreasing rates of shoot growth as opposed to the increasing rates of rhizome growth suggested that photosynthates were being translocated to the rhizomes. The cessation of growth in shoots with the concurrent growth in rhizomes suggested that differences in gradient temperature were affecting shoot formation but not rhizome formation, indicating that photosynthesis had not been stopped.

Secondary rhizomes constitute the bulk of rhizoma peanut biomass. Like total growth, the relative growth rate of secondary rhizomes was lower in 1981 regrowth than in 1980 or 1981 growth. The same occurred with the relative rates of shoot production (number of shoots), indicating a strong relationship between shoots and secondary rhizomes,

which confirmed field observations that secondary rhizomes emerged as shoots. Roots were found associated with primary rhizomes and nodules with secondary rhizomes. Higher relative growth rates in rhizomes of 1981 growth than in 1981 regrowth indicated that efficiency of propagation was lost in the second year of growth.

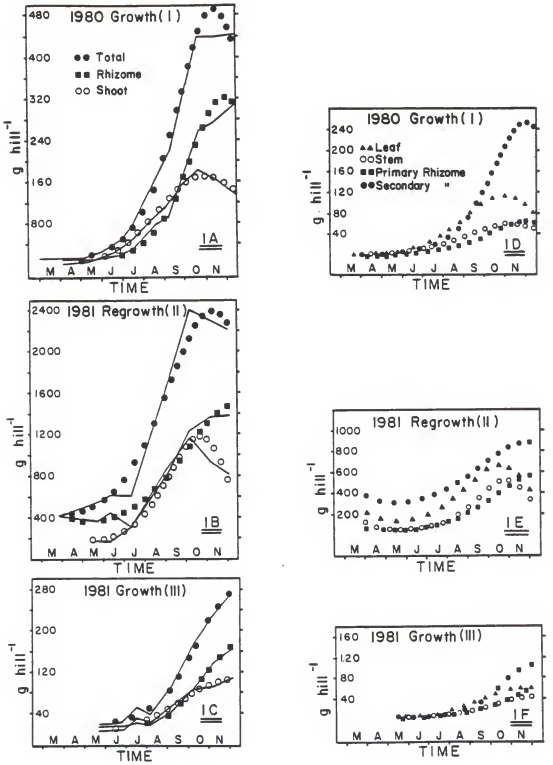

Fig. 1. Total growth and components of growth in Florigraze rhizoma peanut for three data sets.
1A, 1B, 1C = Total growth, rhizome, and shoot growth.
1D,1E, 1F = Leaf, stem, primary rhizome, and secondary rhizome growth.

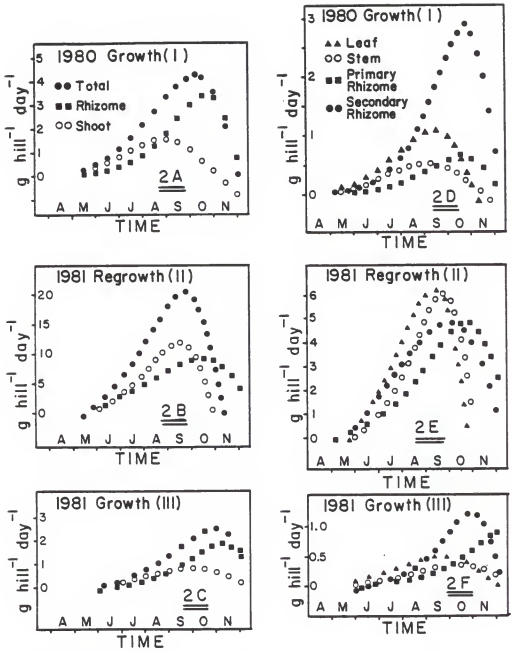

Fig. 2. Growth rates of plant components in Florigraze rhizoma peanut for three data sets.
2A, 2B, and 2C = Total, shoot and rhizome growth rates.
2D, 2E, and 2F = Leaf, stem, primary rhizome, and secondary rhizome growth rates.

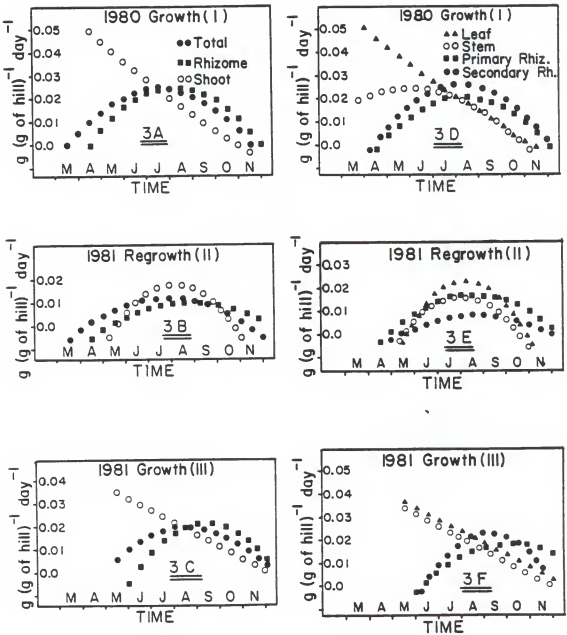

Fig. 3. Relative growth rates of plant components in Florigraze rhizoma peanut for three data sets.
3A, 3B, and 3C = Total, rhizome and shoot relative growth rates.
3D, 3E, and 3F = Leaf, stem, primary rhizome, and secondary rhizome relative growth rates.

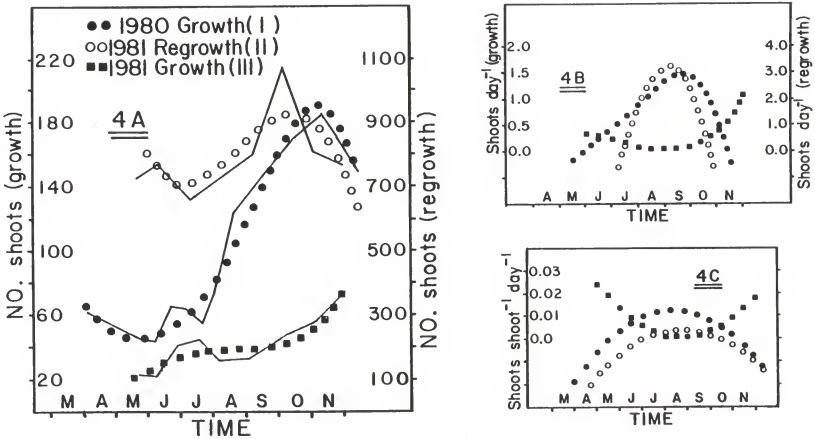

Fig. 4. Production of shoots in Florigraze rhizoma peanut for three data sets.
4A = Production of shoots.
4B = Rates of shoot production.
4C = Relative rates of shoot production.

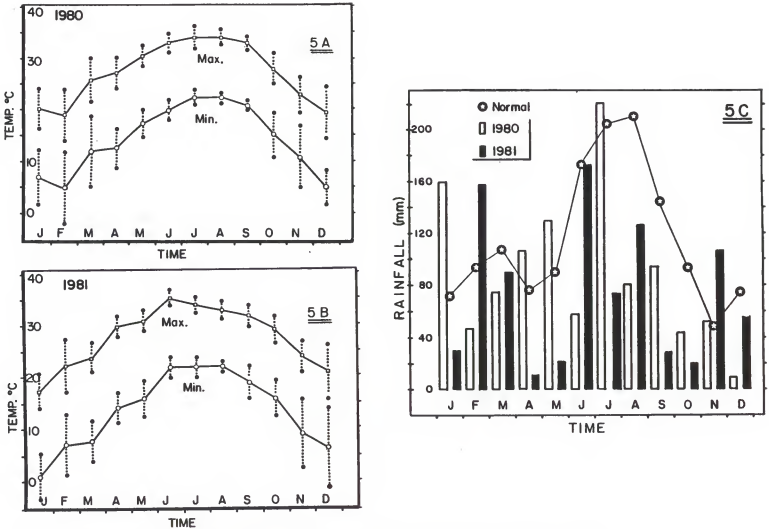

Fig. 5. Temperature and rainfall recorded in Gainesville in 1980 and 1981.

6 A

6 B

6 C

Fig. 6. Primary and secondary rhizomes.
6A - branching in a secondary rhizome;
6B - nodules in secondary rhizome
6C - primary rhizome with shoots and secondary rhizomes.

CHAPTER III

DIFFERENTIAL ACCUMULATION OF TOTAL NONSTRUCTURAL CARBOHYDRATES AND NITROGEN IN PLANT PARTS OF FLORIGRAZE RHIZOMA PEANUT

Introduction

It has been shown that forage species have a typical pattern of total nonstructural carbohydrate (TNC) accumulation and mobilization (Weinmann, 1948; Smith, 1975, 1981). This pattern can be modified by the environment and by management practices (Eaton and Ergle, 1948; Brown et al., 1966). A typical pattern shown a decrease in TNC accumulation in periods of rapid growth (Brown and Blaser, 1965) and an increase in periods when growth is reduced, indicating that photosynthesis is less affected by temperature than is growth (Brown and Blaser, 1968). The nonstructural carbohydrate fraction stored in a particular plant varies with the forage species (Smith, 1968); therefore, it is imperative to know what type of carbohydrate is accumulated in a particular species to choose the proper laboratory analysis. Also, it is necessary to determine the plant part which accumulates and stores carbohydrates in order to properly sample a plant.

The persistence of perennial forage species that remain dormant during the winter depends, in part, on the availability of TNC for regrowth in the spring (Humphreys, 1966). Nitrogenous compounds are also stored, and utilized for regrowth (Davidson and Milthorpe, 1966). The regrowth mechanism is a complex one and energy is required to

activate it until photosynthetically active tissues are developed. The absence of a minimum level of TNC may result in poor yields and persistence because the initial regrowth can be retarded, thus making the plant less competitive for light, water, and nutrients.

Florigraze rhizoma peanut is a rhizomatous plant introduced into the United States by W. Archer in 1936 (Prine, 1964) from the state of Mato Grosso, Brazil (Prine et al., 1981). It is drought tolerant and is very well adapted to Florida's well-drained soils (Prine et al., 1981). It is sensitive to low temperatures and above ground plant tissue is frost-killed in the winter, but the rhizomes remain dormant until the spring. The main disadvantage of Florigraze rhizoma peanut is that it does not produce seed, hence, it has to be vegetatively propagated. Due to the slow rate of establishment, maximum yield and utilization may take from 2 to 3 years (Adjei and Prine, 1976). Franca-Dantas (1982) suggested that propagation methods should be improved or rhizoma peanut should be intercropped during the first and second years of establishment in order to maintain a profitable production system.

In order to examine the possibility of improving the propagation methods in Florigraze rhizoma peanut and to design management systems to increase the efficiency of utilization, a series of studies was conducted to gain some understanding in the growth characteristics of this cultivar. In the previous chapter, results were reported pertaining to morphological changes in Florigraze rhizoma peanut during the first 2 years of establishment.

The objectives of the study reported herein were to determine the TNC and N accumulation patterns of undisturbed plants. A second objective was to determine the plant part where carbohydrates accumulate. Since plants keep respiring after being cut or dug, and sampling in the field usually takes several hours, a third objective in this study was to determine whether there was an effect of preservation method, during sample processing, on the level of TNC recovered.

Materials and Methods

Two field experiments in two locations were carried out in 1980 and 1981 at the University of Florida Agronomy Department's "Green Acres" farm, 18 km west of Gainesville, FL. In one of the locations, termed field A, an experiment was conducted for 2 years. In this experiment 300 rhizoma peanut hills were planted with rhizome pieces in February 1980. Approximately 150 hills were dug in 1980 and about 70 in 1981. These samples will be referred to as 1980 growth or data set (I) and 1981 regrowth or data set (II). In the other location, or field B, 300 rhizoma peanut hills were planted in March 1981. Approximately 70 hills were dug in 1981. These samples will be referred to as 1981 growth or data set (III). The details of field design, soil type, and general practices were described in Chapter II. In 1980 and 1981, 10 and 7 samples, respectively, were taken at all sampling periods (Table 1, Chapter II) for laboratory plant tissue analyses. The samples were taken immediately after digging the rhizoma peanut hills. The procedure for sampling was similar to that previously described (Chapter II) to determine the relative percentage, in large

hills, of the four plant fractions characterizing the growth. The procedure consisted of selecting one well rooted primary rhizome, then two branches of the selected rhizome were untangled from the rhizome mat. All other branches of that primary rhizome were pruned off at the base. The sample was quickly separated into shoots and rhizomes. In 1980, the plant fractions were put in paper bags and cooled by placing them on crushed ice in a polyurethane ice chest. The ice chest was constantly drained to prevent samples from soaking up water. In both the 1981 experiments the samples were separated into two similar subsamples. One subsample was cooled on ice as above, and the other was frozen _in situ_ by placing it in a plastic bag with crushed dry ice. These plastic bags were stored in another ice chest. In both years, when digging was completed in the field, the samples were taken to a laboratory and transferred to dry paper bags. The paper bags with the samples were immediately placed (standing open) in an oven at 110 C for 20 to 40 minutes, depending on the load. The bags were then transferred to a forced-air oven set at 60 C where the samples remained for 36 hours. Once dried, the samples were separated into four plant fractions, as indicated in Chapter IV and weighed. Leaf and stem fractions were put back together, leaving three plant fractions (shoots, primary rhizomes, and secondary rhizomes). Rhizoma peanut hills were categorized into small, medium, and large according to their weight. For laboratory analyses, samples were somposited according to the size of the hill they came from. Samples from small hills were combined into one, as were samples from medium and large hills. Hence, three composite samples (small, medium, large) represented every plant fraction at

every sampling period. The composite samples were ground in a Udy cyclone mill to pass a 0.5 mm screen and stored in plastic containers until analyzed. The samples were analyzed for total nonstructural carbohydrate (TNC) following the procedure outlined by Smith (1981) with the modifications reported by Valle-Melendez (1981) and Christiansen (1982). Nitrogen analyses were performed with a micro-Kjeldahl, aluminum block digestor and a Technicon Autoanalyzer™ (Gallaher et al., 1975) at the Agronomy Research Support Laboratory of the University of Florida. Organic matter was determined by placing a 0.5 g sample in a muffle furnace for 5 hours. Nitrogen and TNC concentrations were expressed on organic matter basis. The data were analyzed by fitting second degree regression equations, with time as a continuous variable. Seasonal variation among TNC curves and among N curves was tested by comparing linear and quadratic regression coefficients. The statistical tests were performed using General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 1982) with data set as class variable.

Results and Discussion

Ice vs Dry Ice

Data in Table 4 show the results of the comparison between cooled and frozen samples. Total nonstructural carbohydrates, as percentage of organic matter, were slightly higher in frozen samples than in cooled samples; however, there were no statistical differences between TNC values ($P = 0.3940$) and no significant time x ice and time^2 x ice interactions ($P = 0.6662$ and $P = 0.6089$, respectively). These small differences might have been caused by broken cell membranes in the frozen samples, which

Table 4. TNC percentage in cooled (ice) and frozen (dry ice) samples of 1981 regrowth (II) and 1981 growth (III) of Florigraze rhizoma peanut.

Sampling date	Plant parts					
	Shoots		Primary rhizomes		Secondary rhizomes	
	Ice	Dry ice	Ice	Dry ice	Ice	Dry ice
1981 Regrowth (II)	TNC %					
27 March	--†	--	50.2	56.9	47.6	54.1
20 May	15.1	16.7	28.3	34.6	28.7	33.0
9 June	11.9	14.0	43.4	44.1	44.0	42.7
9 July	18.6	19.5	22.5	26.4	21.4	22.4
7 Aug.	14.3	16.8	37.4	36.2	33.9	32.6
5 Sept.	11.4	10.7	36.3	38.6	48.0	45.5
2 Oct.	13.0	16.0	44.2	47.5	52.1	50.8
31 Oct.	15.8	15.1	59.0	61.2	66.1	70.3
26 Nov.	16.5	15.7	66.6	65.8	72.1	72.8
1981 Growth (III)						
20 May	--‡	9.8	--	16.0	--	18.2
8 June	--	11.0	--	16.6	--	16.8
26 June	--	13.3	--	11.0	--	10.6
15 July	6.4	8.6	8.6	10.4	9.9	8.1
3 Aug.	15.6	16.9	16.5	17.1	12.2	17.6
1 Sept.	9.8	8.4	16.7	16.1	17.4	16.9
3 Oct.	9.6	15.1	24.2	29.0	26.6	33.7
1 Nov.	12.1	13.3	37.5	38.1	39.2	40.9
27 Nov.	12.6	15.0	39.8	38.4	42.2	40.1

†No shoot growth.
‡Not enough sample.

increased the solubility of nonstructural carbohydrates; or it may have been an actual reduction in respiration. Nevertheless, these results suggest that very little carbohydrate was lost when the samples were cooled in crushed ice. Haslemore et al. (1980) used a range of temperatures from -15 C to 40 C in postharvest storage and found very little effect on N-soluble sugars, and starch analyses. Nevertheless, they suggested that plant samples should be cooled until they can be dried. Since the 1980 samples for TNC analysis were cooled, the results from here on will only include the TNC values obtained with samples cooled in ice.

Total Nonstructural Carbohydrates

The TNC values as percentage of organic matter were plotted against time and are shown in Fig. 7 for shoots, primary and secondary rhizomes. In shoots from all three data sets, TNC varied within a relatively narrow range from 8 to 19% (Fig. 7A). A regression analysis showed nonsignificant Data set x time and Data set x $time^2$ interaction effects (Table 5) or TNC percent in shoots. The curves, then, were very similar in shape, and although there were peaks and valleys in all of them (Fig. 7A), TNC remained relatively constant throughout the year in every experiment. However, the intercepts of the curves were different ($P = 0.0077$). The intercept of 1981 regrowth (II) was significantly higher than the intercepts of 1980 growth (I) and 1981 growth (III). This difference was translated into a higher overall TNC percentage in the shoots of 1981 regrowth (II) than in the other two data sets. The trends of TNC in the shoots were not linear, but the increases and decreases cannot be explained on the basis of diurnal variations. Holt

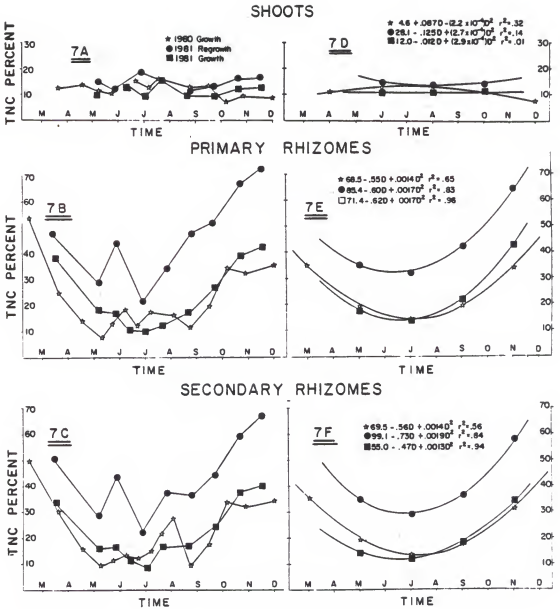

Fig. 7. Percent TNC (organic matter basis) in plant parts of Florigraze rhizoma peanut for three data sets.
7A, 7B, 7C = observed data
7D, 7E, 7F = estimated data

Table 5. Regression analyses of compplete data and individual comparisons between data sets for TNC percent in plant parts of Florigraze rhizoma peanut.

Plant part	Data set comparisons			
	Data sets I II III	Data set I vs Data set II	Data set I vs Data set III	Data Set II vs Data set III
	PR = F†			
Shoots				
Time‡	0.4939	0.5286	0.2729	0.7308
$Time^2$	0.2699	0.1469	0.1606	0.1125
Data set§	0.0077	0.0109	0.5497	0.0001
Data set x time	0.3884	0.3551	0.2115	0.9510
Data set x $time^2$	0.2157	0.1424	0.3797	0.3474
Primary rhizome				
Time	0.0001	0.0001	0.0001	0.0001
$Time^2$	0.0001	0.0001	0.0001	0.0-01
Data set	0.0001	0.0001	0.3062	0.0001
Data set x time	0.0001	0.0001	0.0113	0.0037
Data set x $time^2$	0.2914	0.1847	0.4356	0.7919
Secondary rhizome				
$Time_2$	0.0001	0.0001	0.0002	0.0001
$Time^2$	0.0001	0.0001	0.0001	0.0001
Data set	0.0001	0.0001	0.5021	0.0001
Data set x time	0.0650	0.0091	0.0466	0.1783
Data set x $time^2$	0.0007	0.0833	0.8571	0.0734

†Significance level.
‡Time in days (Julian).
§Intercept.

and Hilst (1969) showed diurnal fluctuations in carbohydrate percentage, but minimum levels were found early in the morning. In this study the samples were taken at relatively wide intervals, and all samples were taken on sunny days between 9 and 12 A.M. Therefore, those cyclic changes in shoot TNC must be related to morpho-physiological changes occurring in the plant.

In the trends corresponding to TNC in the two rhizome fractions (Figs 7B and 7C), there was an increase in mid-June, while shoots showed a decrease (Fig. 7A); once more in early July, TNC in rhizomes decreased and shoot TNC increased. These cycles corresponded to cycles of shoot emergence observed in the field, when rhizome development preceded shoot formation and emergence. Wardlaw (1967) reported high levels of carbohydrate assimilates in leaves of wheat (Triticum aestivum L.) with drought stress. A similar trend was found with wheat and oats (Avena sativa L.) by McCaig and Clarke (1982). These reports may partially explain the high TNC percentage in the shoots of 1981 regrowth, since 1981 was a rather dry year and drought stress was observed at the onset of the growing season and at the end. In the middle of July, when rapid growth started, the trends of TNC in shoots and rhizomes seemed to follow a similar pattern; however, the magnitude of the changes in rhizomes were by far bigger than in shoots. This was expected since shoots are not storage organs and even though some physiological changes may increase the demand for carbohydrates, these results indicated that rhizoma peanut shoots maintained a low level of TNC; this suggested that, if photosynthesis remained constant, the excess of assimilates was translocated to the rhizomes.

Some reports in the literature state that carbohydrates accumulate during the cool temperatures of the fall, when growth rates have decreased (Brown and Blaser, 1965; Wardlaw, 1969; Christiansen, 1982); the TNC levels in rhizomes of rhizoma peanut also began to increase at the end of September when rates of growth were decreasing. The TNC accumulation patterns in both rhizome fractions were very similar; however, primary rhizomes had higher TNC than secondary rhizomes ($P = 0.050$). For practical purposes, either rhizome can be sampled for TNC since the overall mean differed by less than 2.0 percentage units. Furthermore, considering the size of the fractions (see Chapter II), more TNC was found in secondary rhizomes than in primary ones. The fractions seemed to play different roles; secondary rhizomes spread the plant while primary rhizomes were centers of growth and development of the root system. Nevertheless, secondary rhizomes are capable of branching and producing more shoots and rhizomes, and can eventually become primary rhizomes. Therefore, either one of them would give an indication of the status of the plant.

The regression analysis of TNC in primary rhizomes (Table 5) showed significant Data set x time interaction in all three comparisons between data sets. The fitted curves (Fig. 7E) show that less TNC was mobilized in the 1981 regrowth (II) than either the 1980 growth (I) or 1981 growth (III). Also, more TNC was accumulated in the 1981 regrowth (II) than in the other two data sets, which were very similar. Therefore, the rates of TNC accumulation were highest for the 1981 regrowth (II) followed by 1981 growth (III) and 1980 growth (I). The

same trends with the same ranking or data sets were observed for TNC percentage in secondary rhizomes (Fig. 7F), although statistical results of the comparisons between curves of data sets were not the same (Table 5). In the 1980 growth (I) and 1981 growth (III) curves, the linear coefficients were not different (P = 0.0466) indicating higher TNC accumulation in 1981 growth (III) than in 1980 growth (I) (Fig. 7F). Between 1981 regrowth (II) and 1981 growth (III) there was no difference in the rates of accumulation (linear P = 0.1783 and quadratic P = 0.0734). This result suggested that differences among TNC patterns in the secondary rhizomes of those two data sets were due to initial TNC percentage (intercepts); however, the observed curves for primary and secondary rhizomes (Figs. 7B and 7C) show that initial TNC percentages, in all three data sets, were very similar. Furthermore, the percent TNC curves of secondary rhizomes (Table 5) for the 1980 growth (I) had significantly different intercept and linear coefficient from 1981 regrowth (II). Therefore, TNC accumulation rates in secondary rhizomes also ranked highest for 1981 regrowth (II) followed by 1981 growth (III) and 1980 growth (I). The nature of these slight discrepancies between TNC trends in primary and secondary rhizomes seemed to originate between July and September (Figs. 7B and 7C) when cyclic changes in percent TNC were more drastic in secondary rhizomes. During early and later parts of the year both fractions followed similar patterns. These results suggested that an equilibrium existed between the two rhizomes. Since most growth occurred in secondary rhizomes, the percent TNC was higher in secondary than in primary rhizomes in periods of rapid growth. However, an equilibrium was reached later

between the two fractions. To illustrate this point, the period between July and September (Fig. 7C) shows a sharp TNC increase and decline in the 1980 growth (I); in the same period, 1981 regrowth (II) and 1981 growth (III) show an increase followed by a constant period (Fig. 7C). The same trend is observed, to a lesser degree, in primary rhizomes (Fig. 7B). This period corresponds to the period of fast growth in rhizomes reported in Chapter II, indicating that photosynthetic rates were exceeded by rhizome growth rates in 1980; whereas in 1981 rhizome growth rates may have been smaller than photosynthetic rates, because TNC increased in quantity, although it is not reflected on a percent basis. These observations clearly demonstrated that, at this point in time, the rhizomes became a strong sink for assimilates in undisturbed plants.

Nitrogen

The N percentage in shoots was plotted against time; regression equations were also generated and plotted. The observed fitted curves for N percentage (organic matter basis), for shoots, primary and secondary rhizomes are shown in Fig. 8. The regression analyses for shoots (Table 6) showed that the intercept, linear coefficient (time), and quadratic coefficient ($time^2$) of 1981 regrowth (II) were different from those of 1980 growth (I) and 1981 growth (III). On the other hand, between 1980 growth (I) and 1981 growth (III) there were only significant differences between linear coefficients and intercepts ($P = 0.0008$ and $P = 0.0086$, respectively). Therefore, 1981 growth (III) had the highest N percent followed by 1980 growth (I) and 1981 regrowth (II). Nitrogen concentration in shoots (Fig. 8A) declined until September and then

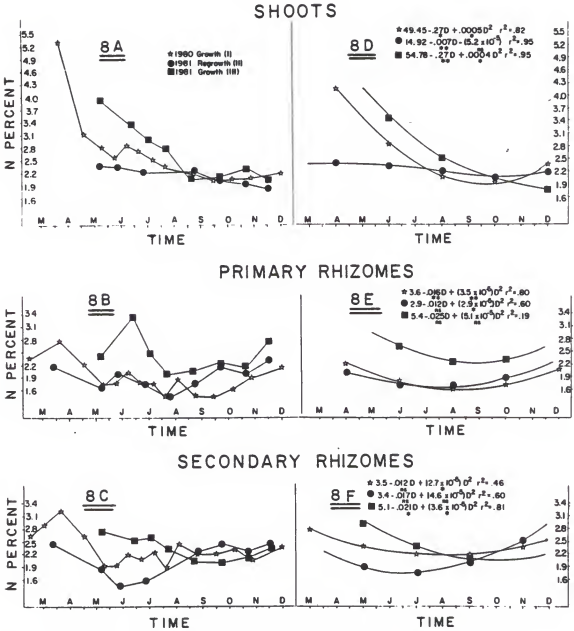

Fig. 8. Percent Nitrogen (organic matter basis) in plant parts of Florigraze rhizoma peanut for three data sets.
8A, 8B, 8C = observed data.
8D, 8E, 8F = estimated data.

Table 6. Regression analyses of complete data and individual comparisons between data sets for N percent in plant parts of Florigraze rhizoma peanut.

Plant part	Data sets I II III	Data set comparisons: Data set I vs Data set II	Data set I vs Data set III	Data set II vs Data set III
	PR = F†			
Shoots				
Time‡	0.0001	0.0001	0.0001	0.0001
$Time^2$	0.0001	0.0001	0.0001	0.0345
Data set§	0.0001	0.0097	0.0008	0.0001
Data set x time	0.0002	0.0315	0.0086	0.0001
Data set x $time^2$	0.0081	0.0048	0.6889	0.0001
Primary rhizome				
Time	0.1769	0.0001	0.0130	0.6288
$Time^2$	0.0001	0.0001	0.0001	0.4192
Data set	0.0001	0.1257	0.0001	0.0003
Data set x time	0.0016	0.0005	0.3212	0.0598
Data set x $time^2$	0.3769	0.6676	0.4824	0.5368
Secondary rhizome				
Time	0.0519	0.0748	0.0001	0.5814
$Time^2$	0.0001	0.0001	0.0001	0.0266
Data set	0.0001	0.0019	0.0134	0.0001
Data set x time	0.0001	0.0001	0.0019	0.0001
Data set x $time^2$	0.8502	0.0908	0.5023	0.6179

†Significance level.
‡Time in days (Julian).
§Intercept.

remained constant until the end of the experimental periods. The 1981 regrowth (II) showed a rather constant pattern from start to end, probably because the first sampling was taken when shoots were over a month old, although the subsequent samples were also lower than those of the 1981 growth (III). The N percent in plants declines with age, as cell walls thicken and lignin deposits. The N levels found for shoots are within the range reported by Prine et al. (1981) for Florigraze rhizoma peanut during several growing seasons and several locations in Florida. But the 1981 regrowth (II) N percent was maintained at the lowest extreme of the reported ranges. Only the 1980 growth (I) and 1981 growth (III) followed the decreasing pattern reported by Breman (1980) and Beltranena (1981).

In both rhizome fractions, N percentage (Figs. 8B and 8C) also declined until about August and then it slowly increased until the end of the experiments. The fluctuations that occurred throughout the growing season did not correlate with any of the morphological changes observed in the plant. The fitted regressions (Figs. 8E and 8F) showed that N percentage followed a declining trend at the beginning of the growing seasons, followed by a relatively constant stage and ended with a slight increase. The regression analyses for primary rhizomes (Table 6) showed that 1981 growth (III) had similar linear and quadratic coefficients to 1980 growth (I) and 1981 regrowth (II), although the intercepts were different. Between 1980 growth (I) and 1981 regrowth (II) only the linear coefficients were different. In secondary rhizomes the intercepts and linear coefficients were different among all three curves (Table 6). These results indicated, on one side, that more N was found in secondary rhizomes than in primary rhizomes. On the other side, the results also

indicated that the concentration of N in rhizomes decreased during periods of rapid growth and increased at the end of the year. The decline of N at the beginning of the growing season, without concurrent increase in rhizome mass, indicated that N was mobilized to shoots. Davidson and Milthorpe (1966) reported mobilization of nitrogenous compounds from the roots of Dactylis glomerata L., whereas Smith and Silva (1969) found the same in alfalfa roots.

The plants in this study were grown on Arredondo loamy sand soil which is characterized by low organic matter and poor N retention (USDA, 1976). Soil pH was 5.9 and 6.0 in field A and field B, respectively (Table 7). No N fertilizer was applied during the experimental periods; therefore, the N sources for plant growth were residual N and N fixation from the symbiosis of rhizoma peanut with Rhizobium japonicum (Adjei and Prine, 1976). Maximum N fixation occurs when nodules have enough available carbohydrates (Bergersen, 1975). Data in Figs. 7D, 7E, and 7F show that consistently more TNC was accumulated in the plant parts of the 1981 regrowth (II) than either the 1980 growth (I) or 1981 growth (III). On the other hand, the N results (Figs. 8D, 8E, and 8F) show that N percentage was also consistently lowest in the 1981 regrowth (II). McIntyre (1967) found that low N levels favored rhizome formation in couchgrass (Agropyron repens L. Beauv.). Also, low temperatures had a similar effect. In potato (Solanum tuberosum L.), increasing N supply increased leaf area, but there was a strong interaction with P (Watson, 1963). In these experiments the low levels of N observed in the 1981 regrowth (II) suggest that N deficiency, particularly at the onset of the growing season, did not allow maximum growth. Soil analysis (Table 7) indicated that P was

Table 7. Soil mineral analysis in samples from Field A and Field B in February 1981.

Analysis	Field A†			Field B‡		
	ppm	s.d.	c.v.	ppm	s.d.	c.v.
P	48.8	10.2	20.9	50.7	11.8	23.2
K	40.3	10.5	26.2	36.4	11.2	30.6
Ca	138.0	42.0	30.4	140.6	58.8	41.8
Mg	20.3	9.9	48.5	10.7	6.1	57.7
Fe	14.9	4.8	32.2	15.0	-3.5	23.1
Mn	3.9	0.6	16.1	3.1	0.6	20.7
Cu	0.5	0.1	31.3	0.2	0.03	11.5
Zn	0.6	0.2	29.6	0.8	0.3	33.0
pH (H_2O)	5.87	0.142	2.42	5.98	0.176	2.95
pH (KCl)	4.53	0.137	3.02	4.61	0.197	4.27

†Means of 12 composite samples.

‡Means of 20 composite samples.

within acceptable range for this type of soil, although K, Ca, and Mg were low with respect to recommended standards to grow peanuts (Arachis hypogaea L.) (Rhue and Sartain, 1979). The same levels were present in both fields. These observations suggested that N limited growth. It has been reported that N fixation does not fulfill the demand for growth in some legumes (Allos and Bartholemew, 1959). This might be the explanation for low relative growth rates found in 1981 regrowth (Chapter II). This was probably not the case in the 1980 growth (I) and 1981 growth (III) because some residual N from previous crops was available to the plants. The N deficiency seemed to have occurred early in the growing season of 1981. Immediately after emergence of the 1981 regrowth plants, a drought was experienced and very few nodules were found. In the 1981 growth (III), no nodules were found either, but the plants developed a very branched root system and very few shoots (Chapter II). Field observations suggest that rhizome growth of rhizoma peanut is reduced in heavy soils. Some coiling of rhizomes has occurred in this type of soils (G. M. Prine, personal communication). In these experiments, at the end of 2 years, plants remained together in spite of multiple ramifications and rooting; therefore, it might be possible that rhizome growth was reaching a plateau. In fact, the primary rhizomes developed more than secondary rhizomes in the 1981 regrowth as compared to 1980 growth or 1981 growth (Chapter II). Also, observations during 1982 seemed to indicate that the rate of spreading was reduced even more than in 1981.

Summary

All plants show typical patterns of TNC accumulation. Usually there is a decrease of TNC in periods of slow growth. The objectives

of the present study were to determine TNC and N accumulation patterns of undisturbed Florigraze rhizoma peanut plants; to determine where TNC accumulates; and to determine the effect of cooling and freezing forage samples in the field on TNC recovery. Two field experiments (A and B) were carried out in 1980 and 1981, 18 km west of Gainesville, FL. Experiment A was conducted during 2 years (1980 growth and 1981 regrowth), experiment B only 1 year (1981 growth). Samples were taken for TNC and N analyses according to a sampling schedule that covered the entire growing season. Ten and seven samples (1980 and 1981, respectively) were taken at all samplings. The sample was split in two, one sample was cooled (ice) and the other frozen (dry ice). Samples were separated into shoots, primary rhizomes and secondary rhizomes. Samples were composited later into three samples using plant size as compositing criterion. Samples were analyzed for TNC and N. The results were fitted into a quadratic model and statistically analyzed with regression analyses.

There was no statistical difference between cooling and freezing samples, although there was a tendency of frozen samples to show higher TNC recoveries. The TNC percentage was consistently higher in 1981 regrowth in all plant fractions than 1980 and 1981 growth, whereas the opposite occurred with N, suggesting that low relative growth rates of 1981 regrowth were due to N deficiency. More TNC was found in secondary rhizomes in periods of rapid growth than in primary rhizomes. With the same trend for N, these results suggested that secondary rhizomes are a more active metabolic center; therefore, in periods of rapid growth they will not give an indication of the status of the plant.

The TNC accumulation increased at the end of the growing season indicating that photosynthesis exceeded growth, and it continued to increase at least until the last samples were taken. Initial decline of TNC was not related to increase in rhizome weight, indicating that TNC was respired and/or used in shoot growth. This kind of response was observed in cycles but the extent of reduction was very small indicating that enough photosynthates were produced at all times. This maintained increasing levels of TNC at the end of the growing season, in spite of the growth of rhizomes.

CHAPTER IV

EFFECT OF DEFOLIATION ON GROWTH, TOTAL NONSTRUCTURAL CARBOHYDRATES, AND NITROGEN IN FLORIGRAZE RHIZOMA PEANUT

Introduction

Regrowth in perennial plants occurs after defoliation or after the top growth is killed by low temperatures in the winter. Plants need active meristematic tissue and nutrient reserves to initiate regrowth. Some researchers have stressed the importance of total nonstructural carbohydrates (TNC) to initiate regrowth (Weinmann, 1961; Smith, 1981) while others have questioned it (May and Davidson, 1958; Blaser et al., 1966). However, this controversy only arises with respect to the initiation of regrowth after defoliation because it is generally accepted that TNC is needed in regrowth after winter (Humphreys, 1981). It has been demonstrated that stored N also plays an important role in regrowth (Davidson and Milthorpe, 1966) although its relative contribution in relation to TNC may be low (Smith and Silva, 1969). The number of shoots is species dependent; however, the removal of apical meristems triggers shoot formation from lateral meristems (Thimann, 1937), increasing the number of shoots. In legumes during the reproductive stage, lateral meristems develop inflorescences (Phillips, 1969); therefore, if apical meristems are removed, new shoots must develop from basal meristems which is a long and inefficient process.

Florigraze rhizoma peanut, a legume native to South America, is an important forage crop in Florida. However, a serious disadvantage is the lack of seed production; therefore, it has to be vegetatively propagated (Prine et al., 1981). This process is costly and slow, because rhizomes must be dug for its propagation. The establishment usually takes from 2 to 3 years (Adjei and Prine, 1976). Due to its rhizomatous habit, numerous lateral buds remain underground; therefore, shoot formation after defoliation should only be limited by nutrient reserves. Reports by Beltranena et al. (1981) and Breman (1980) indicate that maximum yields of Florigraze rhizoma peanut are obtained when it is defoliated every 8 weeks. In Chapters II and III, results were reported on the total growth, morphological changes throughout the year, and storage and translocation of TNC and N in undisturbed plants. With those patterns as reference, an objective of this study was to quantify the effects of frequency of defoliation upon the growth and development of rhizoma peanut rhizomes and the translocation and accumulation of TNC and N. Another objective was to determine the effect of defoliation upon shoot formation.

Materials and Methods

A field experiment was conducted in 1980 and 1981 at the University of Florida Agronomy Department's "Green Acres" farm, 18 km west of Gainesville, FL. The details on field design, soil type and general practices were described in Chapter II. The plants used in this study were planted in February 1980 (Field A, see Chapter II) and they were allowed to grow throughout 1980 without disturbance or plant competition.

The foliage was frost-killed in December 1980, and all dead material was removed by mowing in January 1981. Growth data for these plants during 1980 and 1981 were reported in Chapter II. The 1981 regrowth data, which comprised the growth of undefoliated hills during the second season of establishment were used in this study as an undefoliated control treatment. In addition to this, in the same field, three defoliation frequencies were randomly assigned to 91 hills in 1981. The frequencies were every 2, 6, and 8 weeks. The 2-week frequency was assigned to 42 hills, the 6-week to 28 hills, and the 8-week to 21 hills. Defoliation was done by mowing at 3.5 cm height with a rotary lawn mower. All 91 hills were defoliated on 10 June 1981 to initiate the experiment. Within every treatment a sampling schedule was randomly assigned. Seven hills per treatment were dug at all sampling periods. The sampling and defoliation schedules are presented in Table 8. The sampling schedule was designed to match, as much as possible, that of the undefoliated control. At all sampling periods all hills were dug; a sample was taken from every hill, as described in Chapter II, to determine the relative weight of shoots, primary rhizomes, and secondary rhizomes. The sample and the remainder of the hill were freed of extraneous plant materials, washed to eliminate sand, and dried in cloth bags at 60 C for 48 hrs. Immediately after digging, samples were also taken of all three plant fractions and placed on ice. These samples were taken to a laboratory, dried at 110 C for 30 minutes and transferred to a forced-air oven set at 60 C, where they remained for 36 hours. All samples were ground in a Udy cyclone mill with a 0.5 mm screen, and stored in plastic containers.

Table 8. Sampling and defoliation schedule for 1981 treatments imposed on Florigraze rhizoma peanut.

Undefoliated control	Defoliation frequency					
	8 weeks		6 weeks		2 weeks	
	S†	D‡	S	D	S	D
	Date					
9 June		10 June		10 June		10 June
						26 June
9 July					12 July	11 July
			23 July	23 July		23 July
7 Aug.	4 Aug.	4 Aug.			5 Aug.	4 Aug.
						20 Aug.
5 Sept.			5 Sept.	6 Sept.	6 Sept.	6 Sept.
						18 Sept.
2 Oct.	2 Oct.	3 Oct.			3 Oct.	3 Oct.
			13 Oct.	14 Oct.		14 Oct.
31 Oct.					31 Oct.	1 Nov.
						12 Nov.
26 Nov.	26 Nov.		25 Nov.		25 Nov.	

†Sampling date (S).
‡Defoliation date (D).

Analyses for TNC were performed on shoot, primary and secondary rhizomes with the procedure outlined by Smith (1981) with the modifications reported by Valle-Melendez (1980) and Christiansen (1982). Nitrogen analyses were performed on the same plant fractions with a micro-Kjeldahl aluminum block digestor and a Technicon Autoanalyzer™ (Gallaher, 1975).

Primary and secondary rhizome weight data as well as percent TNC and N (organic matter basis) of all three plant fractions were fitted into regression equations. All defoliation frequencies were compared to the undefoliated control and among the defoliation frequencies. All statistical comparisons were carried out with General Linear Model procedure with time as continuous variable and defoliation frequencies as class variables (SAS Institute Inc., 1982).

Results and Discussion

Rhizome Growth

The growth of rhizomes for all defoliation treatments was plotted against time and is shown in Fig. 9. A regression analysis (Table 9) which included all defoliation treatments showed treatment differences and treatment x time effect, indicating differences among intercepts of the curves and differences between linear coefficients. Individual comparisons bewteen each defoliation treatment and the undefoliated control showed that only the linear coefficients of the 2-week and 6-week defoliation treatments were different from the linear coefficient of the control ($P = 0.0100$ and $P = 0.0482$, respectively). This indicated that the rhizome growth rates of the 2- and 6-week defoliation frequencies were lower than in the undefoliated plants. The

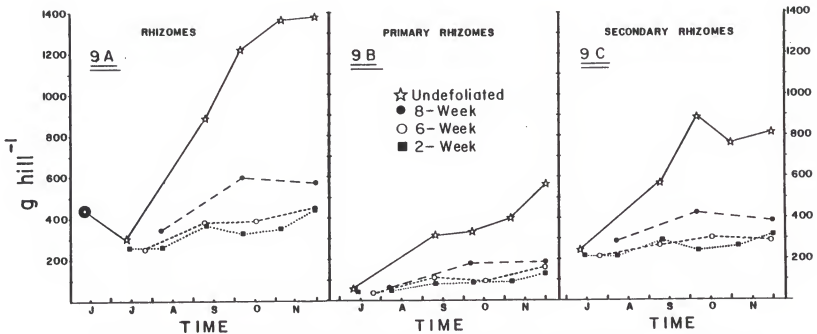

Fig. 9. Effect of defoliation treatments on growth of rhizomes in Florigraze rhizoma peanut.

Table 9. Regression analyses of complete data and individual comparisons between defoliation treatments for growth components of Florigraze rhizoma peanut.

	Comparisons of defoliation treatments†						
	0-2-6-8	0-2	0-6	0-8	2-6	2-8	6-8
	PR = F‡						
Rhizomes							
Time§	0.0001	0.0002	0.0002	0.0042	0.0103	0.0185	0.0571
TR¶	0.0001	0.0001	0.0001	0.0095	0.6427	0.0363	0.1977
TR x time	0.0208	0.0100	0.0482	0.1724	0.7233	0.4911	0.8071
Primary rhizomes							
Time	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
TR	0.0001	0.0001	0.0001	0.0013	0.2695	0.0010	0.1256
TR x time	0.0007	0.0015	0.0260	0.0887	0.2523	0.0413	0.5239
Secondary rhizomes							
Time	0.0012	0.0024	0.0056	0.0279	0.1175	0.1466	0.2643
TR	0.0001	0.0001	0.0015	0.0288	0.8091	0.0894	0.2471
TR x time	0.0983	0.0511	0.1656	0.2507	0.9252	0.7483	0.9032
Number of shoots							
Time	0.5848	0.7902	0.3893	0.6330	0.7889	0.8848	0.3434
$Time^2$	0.2333	0.2287	0.2830	0.3630	0.4506	0.6322	0.4329
TR	0.8949	0.8959	0.5295	0.5948	0.4514	0.4481	0.9411
TR x time	0.5288	0.4441	0.4299	0.6277	0.1111	0.3356	0.9172
TR x $time^2$	0.9722	0.7033	0.9785	0.8666	0.7811	0.6775	0.8757

†Frequency of defoliation in weeks.
‡Significance level.
§Time in days (Julian).
¶Intercept.

regression analyses also showed that the intercepts of the curves of all three defoliation frequencies were different from the undefoliated. Even though the 8-week frequency seemed to have similar rhizome growth rate to the undefoliated, the total growth was higher in the undefoliated ($P = 0.0095$). When comparisons were made among defoliated treatments, there was only significant difference between the intercepts of the 2-week and 8-week frequencies; indicating that all defoliation frequencies gave similar rhizome growth rates, although relative growth rates were higher in the 8-week frequency than in the 2-week frequency. The 6-week frequency was not different to either 8- or 2-week frequencies. The fitted curves (Table 10) give a better understanding of these results and clearly show the ranking of treatments. The ranking indicates that rhizome growth decreases as defoliation frequency increases. These results were expected since it had been reported that other tropical legumes reduced yields with increasing cutting frequencies (Jones, 1967; Bryan et al., 1971). Smith and Graber (1948) reported reduction on the size of coronal rhizomes in biennial sweet clovers (*Melilotus officinalis* Lam. and *Melilotus alba* Desr.) when defoliated early in the fall.

The separation of rhizomes into primary and secondary rhizomes did not show any particular change (Fig. 9) in the relationship reported in Chapter II for the undefoliated plants in the 1981 regrowth. This means that the secondary rhizomes continued to be the bulk of the rhizome mat, but the ratio of secondary to primary rhizomes decreased with time. The regression analyses to compare production curves of primary or secondary rhizomes, among defoliation treatments, gave essentially the same results and ranking that was reported for rhizome

Table 10. Mathematical equations to describe the effect or frequency of defoliation on the growth of rhizomes in Florigraze rhizoma peanut.

Component	Intercept	Coefficient
		time†
	--------- g $hill^{-1}$ day^{-1}	----------
Rhizome		
Undefoliated	-1034.59	7.51
8 week‡	- 48.16	2.03
6 week	- 40.40	1.53
2 week	- 30.19	1.17
Primary rhizomes		
Undefoliated	- 564.65	3.33
8 week	- 175.17	1.15
6 week	- 129.57	0.85
2 week	69.40	0.57
Secondary rhizomes		
Undefoliated	- 469.93	4.17
8 week	127.00	0.87
6 week	89.17	0.67
2 week	99.60	0.59

†Time in days (Julian).
‡Frequency of defoliation.

growth (Tables 9 and 10). Undefoliated plants produced more primary and secondary rhizomes than any defoliation treatment; and the 8-week frequency produced more of both rhizomes than the 2-week frequency. The dry-matter accumulation of the primary or secondary rhizomes of the 2-week and 6-week defoliation treatments were small and not different ($P = 0.2659$ and $P = 0.8091$ for primary and secondary rhizomes, respectively). However, more development was observed in the 6-week plants than in the 2-week plants. The regression coefficients (Table 10) show that defoliations every 2 or 6 weeks had a tendency to slightly increase rhizome grwoth ($\beta = 1.17$ g day^{-1} and $\beta = 1.53$ g day^{-1} for 2- and 6-week, respectively). The difference between them was a higher number of rhizomes in the 6-week frequency than in the 2-week. In the latter, rhizomes decreased in number but the remaining became thicker and rougher in texture; even secondary rhizomes increased in diameter. Under 6-week frequency, the plants produced secondary rhizomes and maintained the same morphology as the 8-week or undefoliated plants. It had been reported that rhizoma peanuts frequently defoliated developed rosette-like structures (Prine et al., 1981). The same thing was observed in this experiment in the 2-week defoliation treatment. Primary rhizomes developed into what seemed to be a taproot, but it only happened in deep-rooted primary rhizomes. Other primary rhizomes, without roots, remained connected to the "taproot-like" primary rhizomes but rarely branched. Most new shoots arose from the taproot, mostly vertically, although a few secondary rhizomes produced shoots. When plants from all defoliation treatments were visually compared to undefoliated plants, there was a clear reduction in area covered, except for the 8-week

defoliation frequency where plants kept spreading, although the amount of rhizomes per area seemed less than in the undefoliated plants.

In this experiment, three defoliations (8-week frequency) decreased rhizome production to about half of the undefoliated control, whereas 4 or 12 defoliations reduced it to one third of the control. These results suggest that if rhizome growth is desired, a more lenient (one defoliation) or no defoliation should be practiced. It should be pointed out, however, that visual observations of the plants early in 1982 did not indicate failures in regrowth in plants under the defoliation treatments of 1981. The canopies in all plants were closed very quickly, but the area covered reflected the defoliation treatments of the year before. These observations further indicated that defoliation will reduce rhizome production and spreading.

Total Nonstructural Carbohydrates (TNC)

Total nonstructural carbohydrates percentage of the organic matter was plotted against time for shoots, primary and secondary rhizomes (Figs. 10A, 10B, and 10C). In all three plant fractions, TNC was ranked in the same order: undefoliated > 8-week > 2-week > 6-week. A regression analysis (Table 11) showed that undefoliated plants had higher TNC in all three plant fractions than in any of the defoliation treatments. The comparisons between defoliation treatments showed that the 8-week frequency had higher TNC in shoots than the 6-week frequency. In primary and seocndary rhizomes, the 8-week frequency had higher TNC than both the 2- and 6-week frequencies. The 2- and 6-week frequencies had similar TNC in rhizomes but differed in shoots, although the 2-week frequency on the average had higher TNC than the 6-week frequency. The

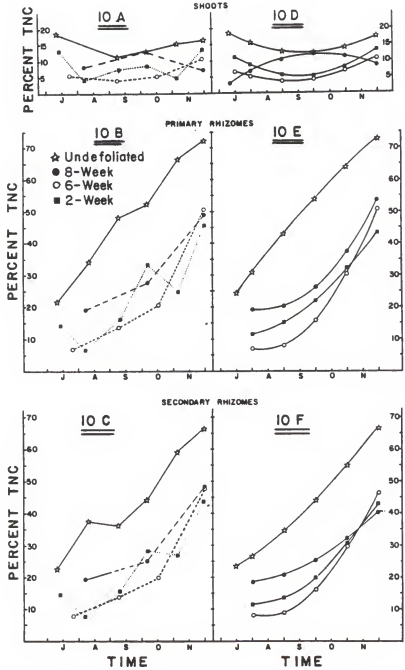

Fig. 10. Effect of defoliation treatments on TNC percent in plant parts of Florigraze rhizoma peanut. 10A, 10B, 10C = observed data. 10D, 10E, 10F = estimated data.

Table 11. Regression analyses of complete data and individual comparisons between defoliation treatments for TNC percent in plant parts of Florigraze rhizoma peanut.

Plant parts	Comparisons of defoliation treatments†						
	0-2-6-8	0-2	0-6	0-8	2-6	2-8	6-8
	PR = F‡						
Shoots							
Time§	0.0665	0.5396	0.0643	0.6297	0.0581	0.5525	0.0047
$Time^2$	0.0001	0.0001	0.0001	0.0392	0.0001	0.0078	0.5836
TR¶	0.0001	0.0001	0.0001	0.0001	0.0084	0.6796	0.0065
TR x time	0.2240	0.5805	0.1020	0.9688	0.2212	0.4096	0.0940
TR x $time^2$	0.0016	0.3638	0.9847	0.0004	0.3468	0.0002	0.0002
Primary rhizomes							
Time	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
$Time^2$	0.0002	0.1917	0.0074	0.9958	0.0001	0.0031	0.0001
TR	0.0001	0.0001	0.0001	0.0001	0.1304	0.0141	0.0001
TR x time	0.0143	0.0037	0.1301	0.2480	0.1888	0.8728	0.1170
TR x $time^2$	0.0006	0.0160	0.0001	0.0143	0.0944	0.5026	0.5441
Secondary rhizomes							
Time	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
$Time^2$	0.0001	0.0004	0.0001	0.2811	0.0001	0.0005	0.0001
TR	0.0001	0.0001	0.0001	0.0001	0.1762	0.0759	0.0070
TR x time	0.0007	0.0136	0.3174	0.0064	0.1476	0.0319	0.0005
TR x $time^2$	0.0556	0.1358	0.0183	0.7732	0.1416	0.4001	0.0549

†Frequency of defoliation in weeks.
‡Significance level.
§Time in days.
¶Intercept.

fitted curves for all treatments and the three plant fractions are shown in Figs. 10D, 10E, and 10F. All four treatments showed an increasing TNC accumulation trend, although the defoliated treatments presented a reduction of TNC at the beginning and an increasing rate afterwards. The highest reduction in rhizome TNC occurred at 2- and 6-week frequencies (Figs. 10B and 10C). In the 2-week frequency this reduction in TNC occurred after three defoliations whereas in the 6-week frequency it happened after one defoliation. It should be pointed out that, as compared to the undefoliated control, the 8-week frequency also reduced the rhizome TNC percentage after one defoliation. Since the 8-week had a higher TNC percentage than the 6-week frequency after one defoliation, the results indicated that plants needed about 8 weeks to recover their TNC level after one defoliation. Some reports in the literature indicate that TNC levels in grasses (Weinmann, 1947) and legumes (Smith, 1981; Barta, 1979) are reduced after defoliation. The extent of this TNC reduction depends on the degree of defoliation and the photosynthetic activity of the remaining tissue. It also depends on the time needed for the regrowth to photosynthesize enough assimilates to satisfy the demand for growth. The TNC contribution to regrowth in grasses occurs only between 2 to 18 days after defoliation (Davidson and Milthorpe, 1966; Sullivan and Sprague, 1943; Blaser et al., 1966).

In this experiment the short-term effects on rhizome TNC content after defoliation were not measured; however, the TNC percentage in initial rhizome samples and the weight of the plants (Fig. 9) indicated that TNC was used in regrowth. Furthermore, in the 6- and 8-week frequencies almost no leaf area was left after defoliation, because the

shoots were elongated enough that the first leaves were above cutting height (3.5 cm). The difference in TNC accumulation between 2- and 6-week frequencies may be due to the leaf area left after defoliation. After five defoliations, the shoots in the 2-week frequency grew almost parallel to the ground and leaves remained very small, so considerable leaf area remained close to the ground. Nevertheless, some material was removed at every mowing, thus, less TNC was mobilized for shoot growth. Conversely, in the 6- and 8-week frequencies, almost all leaf area was removed; therefore, some TNC must have been mobilized for initial shoot regrowth. Beltranena (1980) reported LAI of about 2.0, at the peak of the growing season, when Florigraze rhizoma peanut was defoliated every 2 weeks; LAI decreased from the end of August until October. Those leaf area measurements only included leaves above the 3.8 cm cutting height. Therefore, it could be speculated that a higher leaf area is attained at the end of 2 weeks. For the 6-week frequency, Beltranena's results show an LAI of about 3.5, indicating a slower recovery of LAI in the 6-week frequency; possibly because the leaf area under 3.8 cm cutting height was almost zero.

In rhizomes, all three defoliation frequencies had approximately the same TNC levels at the end of the year (50% in 8- and 6-week frequencies and 45% in 2-week frequency). The TNC percentage was higher in undefoliated plants (70%) than in defoliated. This difference in TNC accumulation between defoliated and undefoliated plants does not seem to explain the reduction in rhizome mass in defoliated plants, but it explains the excellent regrowth observed in 1982 on the plants defoliated during 1981.

Percent Nitrogen

Nitrogen percentage was also plotted against time and is presented in Figs. 11A, 11B, and 11C for shoots, primary rhizomes, and secondary rhizomes, respectively. In shoots, N percentage had a declining trend in all treatments. The ranking of N percentage was 2-week = 6-week > undefoliated > 8-week and it can be seen in Fig. 10D. The regression analysis (Table 12) showed that the intercepts of the lines were different, except between 2- and 6-week frequencies, indicating that more frequently defoliated plants maintained a higher N percentage in the shoots than the less frequently defoliated ones. This is in agreement with results commonly found in the literature for defoliated plants. In general, the N levels observed in shoots are lower than those reported by Breman (1980) and Beltranena (1980); however, they only included the plant material above cutting height. In this experiment, the samples included shoots cut at ground level; therefore, the stubble remaining below cutting height had a diluting effect. This explains the declining shoot N percent observed in all defoliation treatments, even though no dead stubble was included in any sample.

In primary rhizomes there were also significant differences between treatments (Table 12) but the ranking was undefoliated > 2-week > 6-week = 8-week (Fig. 11E). In secondary rhizomes the differences between undefoliated plants and all defoliation treatments were more marked, and there was no difference among N percent in all three defoliation frequencies (Table 12). These results, on one side, point out that primary rhizomes are a less active metabolic center since levels of N are in general lower than in secondary rhizomes.

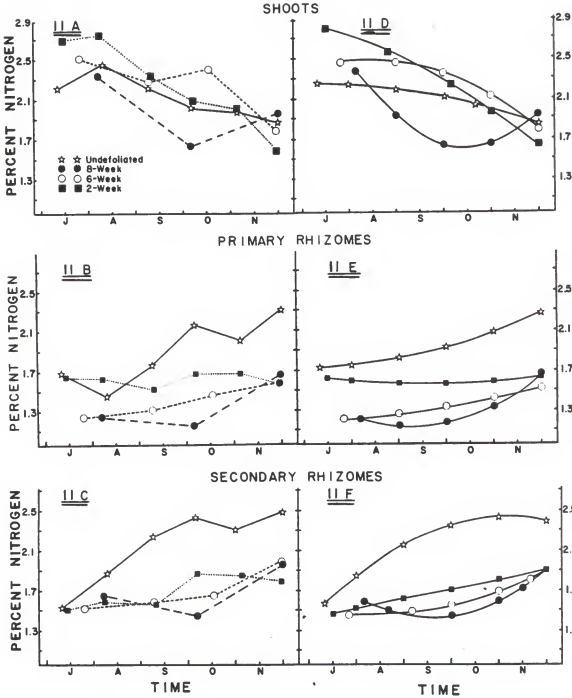

Fig. 11. Effect of defoliation treatments on N percent in plant parts of Florigraze rhizoma peanut
11A, 11B, 11C = observed data.
11D, 11E, 11F = estimated data.

Table 12. Regression analyses of complete data and individual comparisons between defoliation treatments for N percent in plant parts of Florigraze rhizoma peanut.

Plant parts	Comparisons of defoliation treatments†						
	0-2-6-8	0-2	0-6	0-8	2-6	2-8	6-8
	PR = F‡						
Shoots							
Time§	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
$Time^2$	0.7655	0.1515	0.0020	0.0665	0.1312	0.0141	0.3028
TR¶	0.0001	0.0004	0.0012	0.0224	0.4054	0.0045	0.0012
TR x time	0.0001	0.0001	0.1332	0.0742	0.0163	0.0526	0.6521
TR x $time^2$	0.0001	0.7806	0.0896	0.0001	0.4455	0.0005	0.0001
Primary rhizomes							
Time	0.0001	0.0129	0.0001	0.0006	0.2742	0.4475	0.0001
$Time^2$	0.0608	0.3594	0.1966	0.1497	0.4004	0.0916	0.0089
TR	0.0001	0.0001	0.0001	0.0001	0.0014	0.0061	0.5649
TR x time	0.0859	0.0384	0.3613	0.5617	0.1815	0.1834	0.6375
TR x $time^2$	0.7279	0.8609	0.8173	0.3542	0.9602	0.2913	0.0297
Secondary rhizomes							
Time	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
$Time^2$	0.2819	0.0139	0.0307	0.0005	0.4754	0.3103	0.0137
TR	0.0001	0.0001	0.0001	0.0001	0.1743	0.2572	0.7997
TR x time	0.0211	0.0121	0.0198	0.0240	0.9162	0.5046	0.3187
TR x $time^2$	0.0002	0.0162	0.0009	0.0001	0.3870	0.0477	0.1785

†Frequency of defoliation in weeks.
‡Significance level.
§Time in days (Julian).
¶Intercept.

On the other hand, differences in N percentage due to different treatments can more easily be detected in primary rhizomes than in secondary ones. Most nodules were found in secondary rhizomes or in short primary roots arising from secondary rhizomes; consequently, more N in secondary rhizomes would be expected. Data in Figs. 11E and 11F clearly show that more N was mobilized from primary rhizomes than from secondary ones. It can also be seen that the 2-week frequency maintained a rather constant N percentage in primary rhizomes, indicating that N demand in other tissues was being supplied by other sources. The N percent in rhizomes reported by Adjei and Prine (1976) was 1.91%. It was very comparable to the values ranging from 1.87 to 1.96% reported by Breman (1980) for several defoliation treatments. Breman's results came from rhizome samples taken at the end of the year, whereas the results of Adjei and Prine were obtained with 3-month old rhizomes. In the experiment reported herein, very similar values (1.84-2.0%) were found in secondary rhizomes of defoliated plants at the end of the year; whereas in primary rhizomes they were lower (1.64%). In undefoliated plants N varied from 1.60 to 2.36% for July and November, respectively. In the experiments reported in Chapter III it was found that N in rhizomes was 2.24 and 2.36% in 1980 growth and 2.56 and 2.33% in 1981 growth for July and November, respectively.

In 1981, rhizome peanut depended almost entirely on N fixation since no N fertilizer had been added for at least two growing seasons. In Chapter III it was postulated that the growth of undefoliated plants during the second growing season had been hampered by either a lack of

N or by reduction in growth potential in the plants. The comparison of undefoliated and defoliated plants in regard to TNC and N percentage does not clarify the problem because it gives evidence in both directions. However, it gives a perspective of the relationships between TNC accumulation, N availability and plant growth. In rhizomes of undefoliated plants, TNC and N percent (Figs. 10E, 10F, and 11F) increased throughout the experimental period, indicating that enough carbohydrates were available for N fixation. It also indicated that photosynthesis and N fixation were occurring at a faster rate than growth; therefore, TNC and N accumulated. In defoliated plants two situations seem to prevail. First, plants defoliated every 2 weeks increased percent TNC in rhizomes with a steady but slow increase in N, although the N level remained rather low throughout most of the growing season. The stress on these plants stopped growth and development of rhizomes. Hence, the small increment of weight in both primary or secondary rhizomes was probably due to TNC and somewhat to N accumulation. Likewise, the initial reduction in weight might have been due to TNC and N removal, along with death of some secondary rhizomes. The second situation is given by 6- and 8-week frequencies. In both frequencies, N remained at a lower percentage than both 2-week and undefoliated plants, while percent TNC increased like in 2-week frequency plants. This suggested that more growth was occurring in these two frequencies; in fact, the rhizomes looked more developed than those of the 2-week frequency. These observations suggested that TNC and N accumulation could not account for the slight weight increment in the 6-week frequency and the weight increment in the

8-week frequency. The explanation of these results may have been found in the dry matter percent of the rhizomes but unfortunately it was not measured.

Defoliation of legumes often results in reduction of N fixation, due to reduction in the supply of sugars to the nodules. In this respect, Whiteman (1970) reported reduction in size and number of nodules, with a subsequent reduction in yield of 'Siratro' [Macroptilium atropurpureum (D.C.) Urb.] with increasing cutting frequencies. Jones (1974) with the same species found that plant density and yield increased linearly with cutting interval. In this experiment, all defoliation treatments reduced biomass production with increased frequency of defoliation. Table 13 shows the weight of shoots per hill for every sampling date and defoliation frequency. These results indicated taht the 6- and 8-week frequencies produced almost as much as the undefoliated plants. Although no real measurement of yield was taken, an estimation of yield in the 2-week frequency suggested that it was not appreciably lower than with longer frequencies and in undefoliated plants. The corollary of these results is that plants modified their growth pattern when put under stress.

The number of shoots (Fig. 12) did not show a significant difference (Table 9), although the general trend indicated that all defoliation frequencies promoted early increase in shoot numbers which may have been responsible for the drop in N percent in primary rhizomes. It also indicated that a 2-week frequency caused an early decline in shoot numbers. Since the importance of N in bud development and shoot formation in grasses is recognized (Jewiss, 1972b), it seems that the

Table 13. Weight of shoots per plant for defoliation treatments at sampling dates in Florigraze rhizoma peanut.

Undefoliated		Defoliation frequency					
		8 week		6 week		2 week	
Date	Weight†	Date	Weight	Date	Weight	Date	Weight
9 Juy	304			23 July		12 July	180
7 Aug.	---‡	4 Aug.	519			5 Aug.	161
5 Sept.	908			5 Sept.	301	6 Sept.	187
2 Oct.	1177	2 Oct.	341	13 Oct.	141	3 Oct.	149
31 Oct.	940					31 Oct.	115
26 Nov.	826	26 Nov.	103	25 Nov.	118	25 Nov.	53

†g $hill^{-1}$.
‡Lost sample.

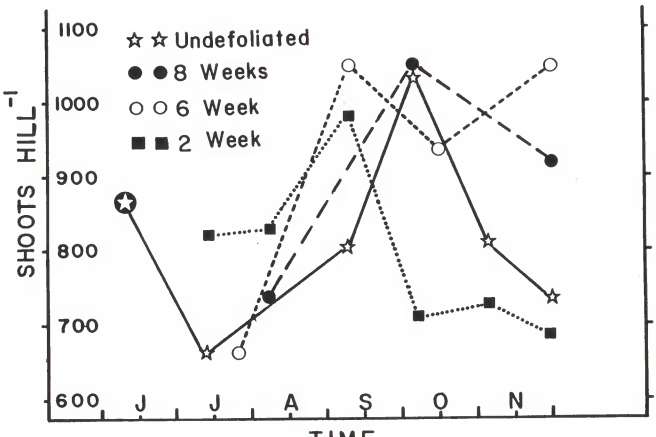

Fig. 12. Effect of defoliation treatments on the number of shoots in Florigraze rhizoma peanut.

drought experienced early in 1981 lowered the levels of N in the plants, and impaired later shoot formation. This in turn reduced potential growth but not photosynthesis; therefore, TNC accumulated. Defoliation with longer intervals reduced rhizome growth but, relatively it increased the production of forage. In other words, the proportion of rhizome to top growth was changed in favor of the top growth. At least during one growing season, no defoliation treatment reduced the rhizome mass. This finding explains the observed excellent persistence of Florigraze rhizoma peanut, and it has particular relevance since it can almost guarantee its survival under the most stressful conditions. It had been suggested by Breman (1980), that rhizome production paralleled forage yield. The results in this experiment indicate that any defoliation frequency would change the ratio of rhizome growth to forage growth. Therefore, for establishment purposes, rhizomes should be given preference. These results confirm the observations of Prine et al. (1981) who stated that no defoliation should be practiced during the first year to improve establishment. In a better year, probably N levels would be higher than in this experiment and growth would be higher, with lower TNC levels. Rhizoma peanut is a tropical plant; therefore, with the onset of cooler temperatures in the autumn, growth is reduced more than photosynthesis. The results in Chapter II indicated that it changed its partitioning and assimilates were translocated to the rhizome system (Chapter III). Defoliated plants in this study do not confirm that hypothesis, probably because the stress and lack of N also inhibited bud formation and development in rhizomes.

Summary

Plants require active meristematic tissue and/or nutrient reserves to initiate regrowth after winter or after defoliation. Defoliation may alter plant morphology. The objectives of this study were to quantify the effect of frequency of defoliation upon the growth and development of rhizomes, shoot formation and accumulation of TNC and N. A field experiment was conducted in 1980 and 1981, 18 km west of Gainesville, FL. Florigraze rhizoma peanut was planted in 1980. In 1981 three frequencies of defoliation (2, 6, and 8 weeks) were randomly assigned to 91 hills. In addition to this, undefoliated plants also planted in 1980 were used as control. Samples were taken from every defoliation treatment according to a sampling schedule to record weight of rhizomes and shoots. Samples were also taken for TNC and N analyses. The growth results were fitted to a linear model, TNC and N results were fitted to a quadratic model. All data were statistically analyzed with regression analyses.

Rhizome growth decreased as frequency of defoliation increased. The number of shoots was not significantly altered by frequency of defoliation. Defoliation stimulated shoot growth, and changed the ratio of shoots to rhizomes. This was also reflected in lower TNC accumulation in defoliated than in undefoliated plants. The 6- and 8-week defoliation frequencies decreased N percent in rhizomes indicating that N was mobilized for shoot production. The 2-week defoliation stopped rhizome growth, although TNC accumulation took place. The latter probably accounted for the slight increase in weight of the rhizome of plants under 2-week defoliation frequency. The 6- and 8-week defoliation

frequencies did not stop rhizome spreading. Secondary rhizomes maintained the same proportions observed with primary rhizomes in undefoliated plants. These results suggested that more growth can be expected in defoliated plants if enough N is available. Nitrogen percent increased in all defoliation treatments towards the end of the growing season; however, a deficiency at the beginning might have reduced the growth potential of the plants.

CHAPTER V

EFFECT OF GRINDING METHOD ON *in vitro* ORGANIC MATTER DIGESTIBILITY OF FORAGES

Introduction

Precise estimation of forage quality with laboratory procedures greatly facilitates the evaluation of forages. Digestibility of forages is an important parameter of forage quality (Raymond, 1969). Tilley and Terry (1963) proposed a two-stage technique that attempts to simulate the digestive process in ruminants. This in vitro procedure, with a number of modifications, has become the most widely accepted method of forage evaluation because it predicts *in vivo* digestibility with more accuracy than other procedures (Barnes, 1981; Minson, 1981a). The usefulness of the technique is based on the recognition of its limitations. If the sources of variation are not properly controlled, interpretation of results may be impossible or useless. Rumen inoculum is the main source of variation in the technique itself. Therefore, it has been suggested that comparison among a given set of forage samples should be made with the same batch of rumen fluid (Clark and Mott, 1960). Baumgardt et al., 1962, recommended the inclusion of a standard forage in every laboratory run to account for variability among batches. Another source of variation is the length of incubation time (Troelsen and Hanel, 1966; McLeod and Minson, 1969). Special reference is made by McLeod and Minson (1969) as to the particle size of forage samples since they found that coarsely ground samples were digested *in vitro* less rapidly than

finely ground ones. Worrell (1982) found variability among forage species for the effect of particle size in vitro digestibility of cell wall, using only one fermentation time. However, McLeod and Minson (1969) indicated that the effects of particle size tended to disappear when incubation time was increased. Grinding of samples for digestibility analyses at the University of Florida's Animal Nutrition Laboratory is usually performed with a Model 3 Wiley Mill and 1.0-mm screen (J. E. Moore. Personal communication). However, grinding of similar forage samples with the same screen size in a Udy Cyclone mill, the particle size appeared to be smaller and more uniform than with the Wiley mill. Therefore, the objectives of this study were to determine the effects of mill type and screen size upon the in vitro organic matter digestibility (IVOMD) of rhizoma peanut and nine selected grasses, and upon variability in the prediction of in vivo organic matter digestibility in tropical grasses.

Materials and Methods

A laboratory experiment conducted in 1982 at the University of Florida's Animal Science Nutrition Laboratory, in Gainesville, FL. Forage material of Florigraze rhizoma peanut (*Arachis glabrata* Benth.) was obtained from a previous field experiment conducted during 1980 and 1981. The plant material represented four stages of maturity of undefoliated plants, for 1980 and three stages of maturity for 1981 (Table 14). For every sampling, three composite samples of rhizoma peanut forage were separated into leaf and stem; therefore, three samples of leaves and three of their corresponding stems represented each stage of maturity. The samples had been dried at 70 C for 36 hrs,

Table 14. List of grasses and sampling dates for Florigraze rhizoma peanut.

No.	Grass Samples†		Regrowth
			- weeks-
I	Paraguay 22 bahiagrass	(*Paspalum notatum* Flügge)	4
II	Paraguay 22 bahiagrass	(*Paspalum notatum* Flügge)	6
III	Paraguay 22 bahiagrass	(*Paspalum notatum* Flügge)	8
IV	Argentina bahiagrass	(*Paspalum notatum* Flügge)	2
V	X124-4 digitgrass	(*Digitaria* spp.)	4
VI	Survenola digitgrass	(*Digitaria* X *umfolozi* Hall)	8
VII	Survenola digitgrass	(*Digitaria* X *umfolozi* Hall)	6
VIII	X124-4 digitgrass	(*Digitaria* spp.)	2
IX	Coastcross 1 bermudagrass	(*Cynodon dactylon* Pers.)	2

Legume Samples‡

Sampling Dates

	1980		1981
I	19 July	V	9 July
II	4 Sept.	VI	5 Sept.
III	17 Oct.	VII	31 Oct.
IV	11 Dec.		

†One composite sample per grass (from two field res.).

‡Three composite samples per date (from ten field reps. in 1980 and 7 field reps. in 1981)

and ground in a Model 3 Wiley mill (4-mm screen) and stored in Whirlpak bags. Details on field sampling procedures and compositing of samples were reported in Chapters II and III. From each leaf or stem sample, four 5-g subsamples were weighed and stored in capped plastic bottles.

In addition to the rhizoma peanut samples, nine grasses (Table 14) of known in vivo digestibility were selected for this study (Abrams, 1980). The selection was based on results reported by Worrell (1982) (Table 15). All grasses had higher in vitro NDF digestibility, when reground in an Intermediate Wiley mill with a 475-μm screen, than subsamples of forage previously ground through a 4-mm screen in a Model 3 Wiley mill.

To select the grasses in Table 15, three ranges were established based on the differences in NDF digestibility between 4-mm and 475 mμ. The average digestibility differences were 0.5, 2.1 and 5.0 percentage units for small, medium, and large differences, respectively. Forage production details for the grasses were reported by Abrams (1980). The grass samples had been ground (Model 3 Wiley mill, 4-mm screen) and stored in plastic containers under controlled temperature and humidity (J. E. Moore. Personal communication). Twelve 5-g subsamples were taken from each grass sample and stored in capped plastic bottles.

Grass and legume subsamples were randomly assigned to four grinding treatments with three replications (Table 16). The grinding treatments were 1) Udy cyclone mill 0.5-mm screen, 2) Intermediate Wiley mill, 0.5-mm screen, 3) Udy cyclone mill 1.0-mm screen, 4) Standard (Model 3) Wiley mill 1.0-mm screen. The 0.5-mm screen of the Intermediate Wiley mill was modified by replacing the original screen

Table 15. Differential IVNDFD[†] in relation to grinding treatment (Worrell, 1981).

Group[‡]	Grass[§]		Grinding Treatments 4 mm	475 µm	Difference
			---------- IVNDFD (%) ----------		
	I	Par. -4	49.0	54.0	5.0
1	II	Par. -6	50.1	55.2	5.1
	III	Par. -8	48.1	53.3	5.2
	IV	Arg. -2	53.0	55.9	2.9
2	V	'X124-4'-4	62.3	64.1	1.8
	VI	Sur. -8	52.7	54.4	1.7
	VII	Sur. -6	57.3	58.2	0.9
3	VIII	'X124-4'-2	71.1	71.5	0.4
	IX	CC1-2	55.0	55.3	0.3

[†]In vitro Neutral Detergent Fiber Digestibility

[‡]Group numbers denote large (1), medium (2), and small (3) IVNDFD differences.

[§]See Table 14 for grass identification.

Table 16. Diagram of the laboratory experiment for grasses and legume.

Forage Sample		4 subsamples	4 Grinding Treatment			3 Incubation Time
			Mill	Screen Size	Sub-subsamples	hours
Any Grass[†] Sample		1	(1) Udy	0.5 mm	3	24
		1	(2) Wiley	0.5 mm	3	48
		1	(3) Udy	1.0 mm	3	72
		1	(4) Wiley	1.0 mm	3	
Any Legume[†] Sample	Leaf	1	(1) Udy	0.5 mm	3	24
		1	(2) Wiley	0.5 mm	3	48
		1	(3) Udy	1.0 mm	3	72
		1	(4) Wiley	1.0 mm	3	
	Stem	1	(1) Udy	0.5 mm	3	24
		1	(2) Wiley	0.5 mm	3	48
		1	(3) Udy	1.0 mm	3	72
		1	(4) Wiley	1.0 mm	3	

[†]This example illustrates one of three laboratory runs.

with a standard 0.5-mm screen of the Udy cyclone mill (144 holes cm^{-2}). The subsamples were randomly ground and returned to the containers. A timer was attached to the Wiley mills to grind the samples 2 and 4 minutes in the Intermediate and Model 3 mills, respectively.

The IVOMD analysis was performed on all samples in three laboratory runs, confounding the treatment replications with runs. In each run, three sub-subsamples were taken from every subsample and randomly assigned to three lengths of fermentation in stage 1 of the standard *in vitro* procedure used at the Nutrition Laboratory (Moore and Mott, 1974). The fermentation times were 24, 48, and 72 hours. The entire run was started the same day, and 24 hours later, the first sub-subsamples (24 hours incubation) were acidified, and at 72 hours the third sub-subsamples were acidified. The second stage of the procedure (acid-pepsin) lasted 48 hours in all cases. Therefore, recovery of organic matter was carried out in three consecutive days. A diagram showing how each sample was processed and evaluated in the laboratory is presented in Table 16. Two standard forages (low and high quality) were included in each run.

In grasses and legume statistical analysis were done with analysis of variance and General Linear Model procedures (SAS, Institute Inc., 1982).

Results and Discussion

The low and high quality standards included in every run had 42.2% (S.D. = 2.22) and 63.8% (S.D. = 2.19) IVOMD, respectively, at 48 hours incubation. The variation came from the second run (39.7 and 61.31%

IVOMD for low and high quality, respectively). These results confirmed the observations of Clark and Mott (1960) and Bawmgardt et al. (1962) who stated that variation should be expected from batch to batch. It should be pointed out, however, that at 72 hours incubation (no 72 hours samples were included in the first run) the differences due to batch seem to disappear (52.5 and 71.1; 52.2 and 71.6% IVOMD, for low and high quality in second and third laboratory runs).

The results of the analyses of variance, for all forage samples, for grasses, and for legume samples are presented in Table 17. In the analysis of variance, that included all forage samples (grass and legume), there were significant grinding-treatment and forage x grinding-treatment effects on IVOMD. The separation of grasses from legume samples indicated that, in grasses, the interaction forage x grinding-treatment remained significant, whereas in legume samples the same interaction was non-significant ($P = 0.0819$). The IVOMD means per grinding treatment (Table 17) indicated that grasses and legume samples responded differently to grinding treatments. In grasses, higher IVOMD was obtained with 0.5-mm screen than with 1.0-mm screen. In legume samples, higher IVOMD was obtained with Udy mill than with Wiley mills regardless of screen-size; although 0.5-mm screen gave also higher IVOMD than 1.0-mm screen within mill-type. These results indicated that the responses of grasses were related more to the size of the screen than to the type of mill; while the responses of the legume samples were related to both mill-type and size of screen. This might be the reason why part of the variation

Table 17. Analyses of variance for all forages, grasses, and legume samples.

	All Forages PR=F			Grasses PR=F			Legume PR=F		
	D.F.	IVOMD	DM	D.F.	IVOMD	DM	D.F.	IVOMD	DM
Run	2	0.0011*	0.0223	2	0.0001*	0.0382*	2	0.0759	0.0365*
Forage	22	0.0001*	0.0001*	8	0.0001*	0.0001*	13	0.0001*	0.0001*
Treatment	3	0.0001*	0.0001*	3	0.0001*	0.0001*	3	0.0001*	0.0001*
For. x Treat.	66	0.0001*	0.0350*	24	0.0031*	0.3348	39	0.3173	0.0819
Incubation	2	0.0001*		2	0.0001*		2	0.0001*	
Inc. x For.	44	0.0001*		16	0.0001*		26	0.0220*	
Inc. x Treat.	6	0.0024*		6	0.0008*		6	0.1699	
Inc. x For. x Treat.	132	0.9979		48	0.8936		78	0.9999	

	Treatment Means					
Treatments	IVOMD	DM	IVOMD	DM	IVOMD	DM
0.5-mm Udy	57.51	92.30	52.53	92.74	61.03	92.01
0.5-mm Wiley	56.52	90.32	52.26	90.45	59.26	90.24
1.0-mm Udy	55.74	91.58	49.23	92.06	59.92	91.25
1-0-mm Wiley	53.89	90.46	47.10	90.81	58.26	90.24

* Statistically significant.

among forage samples was removed by separating the grasses from the legume samples.

The results in Table 17 also show that there were differences in dry-matter content among forage samples which was expected because of the differences in maturity among forage samples. The analyses of variance (Table 17) show that separating the forage samples, into grasses and legume samples, eliminated the forage x grinding-treatment interactions. The means of dry-matter content in grasses and legume samples are presented in Table 17. These results indicated that the dry matter contents in grasses and legume subsamples, ground in the Udy mill, were consistently higher (1 to 2 percentage units) than in subsamples ground in the Wiley mills. The loss of moisture caused by Udy mill is probably due to the air that drives the ground forage off the grinding chamber into the collection bottle. More moisture was lost with 0.5-mm screen than with 1.0-mm screen, probably because of the increased surface area in the fine-ground samples. In Wiley mills no difference was observed in dry-matter content.

Since the response to grinding-treatments of legumes and grasses were different, they will be discussed separately.

Grasses

The grinding treatment effects (mill and screen-size) on IVOMD were plotted against incubation time for each individual grass (Fig. 13). The analyses of variance, Table 18, showed that the separation of grasses by genus (*Paspalum* and *Digitaria*) eliminated the forage x grinding-treatment interaction. The same thing was observed when the grasses were separated into the three groups reported in Table 15.

Fig. 13. Effect of grinding treatment and incubation on IVOMD of nine grasses. (See Table 14 for grass identification). Dotted line denotes *in vivo* organic matter digestibility.

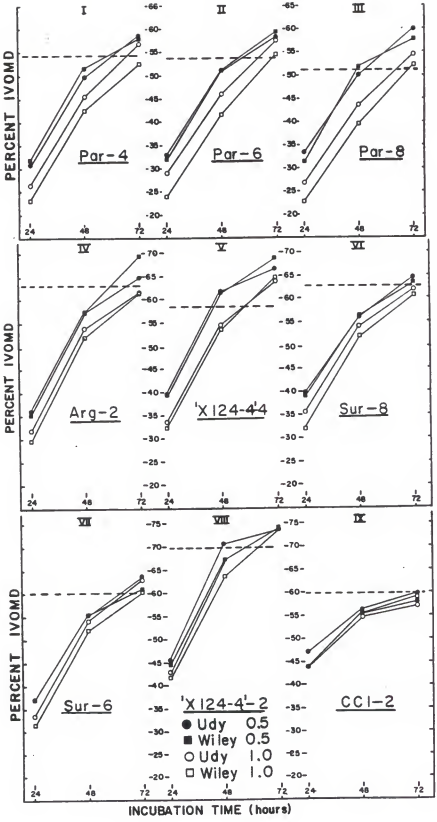
I
II
III
IV
V
VI
VII
VIII
IX
PERCENT IVOMD
Par-4
Par-6
Par-8
Arg-2
'X 124-4'-4
Sur-8
Sur-6
'X 124-4'-2
CCI-2
Udy 0.5
Wiley 0.5
Udy 1.0
Wiley 1.0
INCUBATION TIME (hours)

Table 18. Analyses of variance for grasses separated by genus, and for legume samples separated into leaf and stem.

	All Grasses		Paspalum		Digitaria	
	D.F.	IVOMD	D.F.	IVOMD	D.F.	IVOMD
Run	2	0.0001*	2	0.1147	2	0.0023
Grass	8	0.0001*	3	0.0001*	3	0.0001*
Treat.	3	0.0001*	3	0.0001*	3	0.0001*
Grass x Treat.	24	0.0031*	9	0.3912	9	0.4014
Incubation	2	0.0001*	2	0.0001*	2	0.0001*
Inc. x Grass	16	0.0001*	6	0.0213*	6	0.0023*
Inc. x Treat.	6	0.0008*	6	0.0736	6	0.0457
Inc. x Grass x Treat.	48	0.8936	18	0.4973	18	0.8739

	All Legume Samples		Leaf Samples		Stem Samples	
	D.F.	IVOMD	D.F.	IVOMD	D.F.	IVOMD
Run	2	0.0759	2	0.5987	2	0.0956
Leg	13	0.0001*	6	0.0009*	6	0.0001*
Treat.	3	0.0001*	3	0.0001*	3	0.0001*
Leg. x Treat.	39	0.3173	18	0.0071*	18	0.6590
Incubation	2	0.0001*	2	0.0001*	2	0.0001*
Inc. x Leg.	26	0.0220*	12	0.0588	12	0.3969
Inc. x Treat.	6	0.1699	6	0.2969	6	0.6192
Inc. x Leg. x Treat.	78	0.9999	36	0.9986	36	0.9922

* Statistically significant.

At first it was thought that these results were due to a loss in sensitivity, in the analyses of variance, caused by the reduction of degrees of freedom to test the interaction. However, the random formation of groups with the nine grasses showed that the interaction could be significant with three grasses. These observations suggested that grasses with similar characteristics (genetic or IVNDFD) responded to grinding treatments in the same form. The results in Table 18 also show that the interaction incubation-time x grinding-treatment disappeared when the grasses were separated by forage genus; the interaction incubation-time x forage remained significant. These results indicated that grasses responded differently to incubation time regardless of grinding treatment. This kind of response was expected since the grasses were selected within a wide range of digestibilities; and it has been reported that there is variation in rates of *in vitro* digestion within and between forage species (Gill et al., 1969; Smith et al., 1972).

The results of the regression analyses to compare the effects of grinding, as a function of incubation time, on IVOMD of the nine grasses are presented in Table 19. There were no statistical differences among the rates of digestion of grinding treatments, within grasses; and only Coastcross-1 bermudagrass resulted in no grinding-treatment effects (P = 0.1804). These results indicate that grinding treatments did not increase the rate of digestion after 24 hours. Therefore, the largest effect of grinding must have occurred within 24 hours. In this experiment, the grass responses to incubation can be classified into three categories. Category 1 includes *Paspalum*

Table 19. Effect of grinding treatment and incubation time on IVOMD by grass, and IVOMD means by grass by treatment.

Source of variation	D.F.	Grasses I Par.-4	II Par.-6	III Par.-8	IV Arg.-2	V 'X124-4'-4	VI Sur.-8	VII Sur.-6	VIII 'X124-4'-2	IX CCI-2
		PR = F†								
Run	2	0.0029	0.5165	0.5423	0.3426	0.1085	0.0358	0.3536	0.0745	0.3355
Treat	3	0.0001	0.0001	0.0001	0.0006	0.0001	0.0001	0.0293	0.0210	0.1804
Inc	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
I^2	1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
I * T	3	0.2790	0.2395	0.3376	0.3643	0.4717	0.3157	0.3185	0.4758	0.5217
I^2*T	3	0.5022	0.3620	0.0377	0.8967	.5463	0.7572	0.9750	0.1964	0.8890
MSE	22									
Treatments		IVOMD MEANS %								
Udy (.5 mm)		46.4 (2)	47.5 (2)	47.9 (1)	52.7 (2)	55.8 (2)	53.0 (1)	52.1 (1)	63.4 (1)	53.9 (1)
Wiley (.5 mm)		47.1 (1)	47.6 (1)	47.0 (2)	54.0 (1)	56.3 (1)	52.7 (2)	51.2 (2)	62.1 (2)	52.4 (2)
Udy (1.0 mm)		43.0 (3)	44.4 (3)	41.7 (3)	49.1 (3)	50.6 (3)	50.4 (3)	50.4 (3)	61.6 (3)	51.9 (4)
Wiley (1.0 mm)		39.4 (4)	40.0 (4)	38.2 (4)	47.7 (4)	50.0 (4)	48.0 (4)	48.1 (4)	59.7 (4)	52.9 (3)

† Significance level.

species (Figs. 13-I, 13-II, 13-III, and 13-IV) which showed a nearly linear response to incubation time. The IVOMD at 24 hours was about 30%; and increasing the incubation from 48 to 72 hours had a small decrease in the rate of digestion. Category 2 includes digitgrasses (Figs. 13-V, 13-VI, 13-VII and 13-VIII) which showed a response until 48 hours and then digestion rate declined between 48 and 72 hours. The IVOMD at 24 hours varied between 35 and 45% depending on maturity. In Category 3 Coastcross-1 bermudagrass stands alone (Fig. 13-IX). It showed a rapid rate of digestion within 24 hours, with little additional digestion between 24 and 48 hours. The IVOMD at 24 hours was similar to the 2-week regrowth of X124-4 digitgrass; and increasing incubation from 48 to 72 hours slightly increased IVOMD.

The IVOMD means for grinding treatments (Table 19) show that, except for Coastcross-1 bermudagrass, grinding with 0.5-mm screen increased IVOMD regardless of mill. Although, IVOMD with 1.0-mm screen was higher with the Udy mill than with Wiley mill. The differences between 0.5 and 1.0-mm screens can be as large as 8 percentage units (Fig. 13-III), or only 2 percentage units (Fig. 13-VII). In most grasses there was less than one percentage unit between Udy (0.5) and Wiley (0.5) mills. Conversely, with 1.0-mm screens there were increments of up to 4 percentage units when grinding with Udy compared to the Wiley mill. The responses to grinding treatment followed the pattern adopted as selection criteria for the grasses (Table 15). This confirmed the results of Worrell (1981) who found variability among grasses in their response to <u>in vitro</u> NDF digestibility when finely ground (475 mμ) vs coarsely ground (4-mm).

The results in this experiment indicated that low quality forages have low initial rates of digestion, but they can be altered by decreasing the particle size. Medium and high quality forages responded very little to fine-grinding indicating high initial rates of digestion. McLeod and Minson (1969) showed that mean in vitro digestibility of four tropical grasses was increased by reducing the particle size of the sample. In the same study they found that increasing length of incubation time increased digestibility and reduced the magnitude of differences caused by particle size. In this study there was a tendency to reduce grinding-treatment differences at 72 hours incubation, but in only the 2-week regrowth of X124-4 digitgrass (Fig. 13-VIII) were the differences completely eliminated

Legume

The results of grinding-treatment effects (mill and screen size) were plotted against plant maturity and incubation time (Fig. 14). Since there were marked differences between leaf and stem IVOMD, both plant fractions were plotted separately on the same figure. The results in Table 18 show that the separation of legume samples into leaf and stem brought up a significant forage x grinding-treatment interaction in the leaf fraction. This separation also eliminated the interaction incubation-time x forage. These results indicated that IVOMD in leaves had the same responses to grinding treatments and to incubation than stems.

The regression analyses (Table 20) showed grinding-treatment effects in both years for the leaf and stem fractions. In the legume, like the grasses, there were no grinding-treatment differences in rates

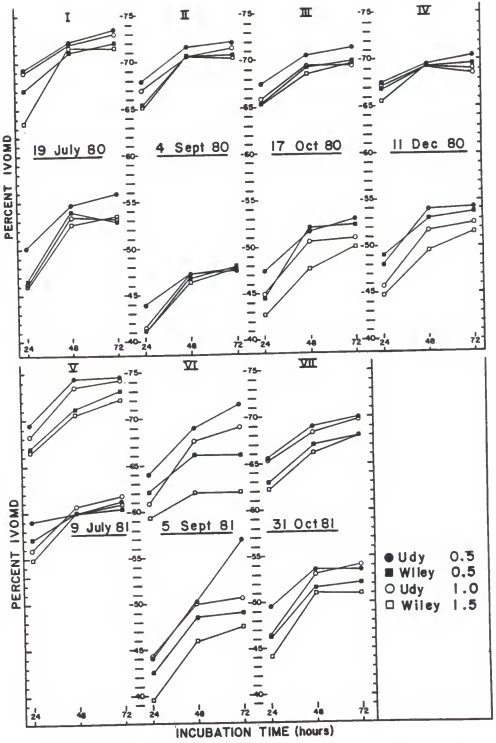

Fig. 14. Effect of grinding treatment and incubation on IVOMD of seven samples of Florigraze rhizoma peanut. (See table 14 for legume sample identification).

of digestion. Also, there were no grinding-treatment differences for linear and quadratic coefficients of plant maturity. These results indicated that grinding treatments affected IVOMD as in the grasses; that is, the effect of grinding occurred within 24 hours, and further incubation did not show an increase in the rates of digestion due to grinding treatments. The magnitude of the responses, however, was much smaller in the legume than in the grasses. The ranges of variation seldom exceeded five percentage units. This is comparable to the results found for high quality grasses (Figs. 13-VIII, 13-IX). The response of legume samples to incubation time was also small, increasing incubation time from 24 to 48 hours yielded about 5 percentage units increase in IVOMD whereas increasing from 48 to 72 hours increased IVOMD less than 2 percentage units. These trends were similar in both leaves and stems. These results indicated that about 90% of the digestion occurs within 24 hours. Similar results were reported by Troelsen and Hanel (1966) who found that alfalfa (Medicago sativa L.) IVOMD reached a plateau at 24 hours, increasing only 3.2 percentage units when the incubation time was extended to 48 hours and 0.5 percentage units from 48 to 96 hours. In the same study, Troelsen and Hanel (1966) found that wheat straw (Triticum aestivum L.) IVOMD did not reach a plateau at 96 hours.

Since the objectives of this study were to determine the effects of grinding treatment, the effects of legume age will not be discussed here. The ranking of treatment means (Table 20) shows that with the exception of the 1980 leaf fraction, the Udy mill gave higher IVOMD than the Wiley mills. The reason for this is not completely understood. This response did not occur in the grasses, suggesting that

Table 20. Regression analyses per year and plant fraction for treatments and incubation effects upon IVOMD of Florigraze rhizoma peanut, and treatment means by year and plant fraction.

	1980			1981		
	D.F.	Leaf P = F	Stem P = F	D.F.	Leaf P = F	Stem P = F
Run	2	0.8223	0.0099	2	0.0029	0.0027
Treat	3	0.0001	0.0002	3	0.0001	0.0324
Inc	1	0.0001	0.0001	1	0.0001	0.0001
I^2	1	0.0001	0.0001	1	0.0005	0.0133
I × T	3	0.2328	0.8600	3	0.2191	0.5975
$I^2 \times T$	3	0.3484	0.6596	3	0.6067	0.8717
AGE	1	0.0001	0.7442	1	0.0001	0.0001
A^2	1	0.0199	0.0001	1	0.0001	0.0001
I × A	1	0.0031	0.9546	1	0.4298	0.9908
A × T	3	0.1215	0.2700	3	0.4939	0.8578
$A^2 \times T$	3	0.6688	0.8913	3	0.4973	0.0550
MSE	121			85		

Treatments	Treatment Means†			
Udy (.5 mm)	70.3 (1)	50.6 (1)	69.7 (1)	54.0 (1)
Wiley (.5 mm)	69.3 (2)	48.8 (3)	67.0 (3)	52.0 (3)
Udy (1.0 mm)	68.9 (3)	49.7 (2)	68.5 (2)	53.0 (2)
Wiley (1.0 mm)	68.4 (4)	47.7 (4)	65.6 (4)	51.4 (4)

†Means of three incubation times.

the mode of grinding of the mills produced different textures (particle size distribution) in the samples of legumes. Chenost (1966) reported grass differences in ease of pulverization as measured by electrical power. Therefore, it is possible that Florigraze rhizoma peanut is more fragile than the grasses included in this study, so when it is processed through a Udy mill, it is pulverized. The answer to this problem may be found by categorizing the textures of the legume and grass samples run through the mills. The dry matter content in legume and grass subsamples ground in the Udy mill were consistently higher (1 to 2 percentage units) than in subsamples ground in the Wiley mills. These observations indicated that some moisture was lost with the Udy mill, especially with the 0.5 mm screen. The grasses in this study also lost moisture, when ground with a Udy mill, in the same magnitude as the legume. However, the IVOMD results did not show the same responses as the legume. Therefore, these results suggest that ease of physical degradation was a major determinant in the response of the legume to grinding. These results also point out that the observed in vivo performance (Prine et al., 1981) of Florigraze rhizoma peanut may be due to ease of degradation, both physical and chemical.

The IVOMD percentages of the nine grasses were correlated for each grinding treatment and incubation time to the in vivo data (Abrams, 1980) of the same grasses. Prediction equations were generated for organic matter digestibility (OMD) and organic matter intake (OMI) (Table 21). The results in Table 21 show that incubation for 24 hours

Table 21. Regression and correlation coefficients for prediction of Organic Matter Digestibility (OMD) and Organic Matter Intake (OMI) from IVOMD of nine grasses with four grinding treatments and with three incubation times.

Inc	Screen	Mills							
		Udy				Wiley			
		β	Intercept	r	$s_{y \cdot x}$	β	Intercept	r	$s_{y \cdot x}$
hrs	- mm -	IVOMD vs OMD							
24	0.5	0.72	32.0	0.70	4.3	0.89	26.2	0.78	3.8
	1.0	0.64	37.6	0.71	4.3	0.53	42.5	0.73	4.1
48	0.5	0.82	12.7	0.95	1.8	1.03	1.3	0.95	1.9
	1.0	0.76	19.2	0.95	1.9	0.70	24.5	0.94	2.0
72	1.0	0.99	-3.8	0.88	2.8	0.84	5.7	0.89	2.7
	0.5	0.88	5.5	0.90	2.6	0.82	10.1	0.97	1.6
		IVOMD vs OMI							
	0.5	0.55	24.2	0.75	3.4	0.67	17.1	0.74	3.5
	1.0	0.51	28.4	0.75	3.4	0.47	31.6	0.75	3.4
	0.5	0.59	17.7	0.61	4.1	0.48	24.8	0.60	4.1
	1.0	0.54	22.3	0.65	3.9	0.50	25.1	0.69	3.7
		($\bar{X}$ IVOMD)† vs OMD							
	0.5	1.03	5.3	0.94	2.1	1.12	0.6	0.97	1.5
	1.0	0.88	15.8	0.92	2.3	0.75	24.0	0.92	2.3
		($\bar{X}$ IVOMD) vs OMI							
	0.5	0.68	19.5	0.73	3.5	0.71	18.2	0.72	3.6
	1.0	0.59	26.2	0.72	3.6	0.51	31.0	0.74	3.5

†Mean of three incubation times.

gave realtively low correlation coefficients regardless of grinding treatment. Incubation for 48 hours gave high correlation coefficients in all grinding treatments; there was a slight tendency in the Udy mill to reduce the standard error of the estimate ($s_{y.x}$). The 72 hours incubation gave medium to high correlation coefficients; and particularly important was the correlation between IVOMD with 1.0-mm Wiley and OMD, because it was the highest correlation coefficient obtained in this set of comparisons. The explanation to this can be found in Fig. 13; 48 hours incubation for all grinding treatments and almost all grasses, consistently underestimated in vivo digestibility. At 72 hours most grinding treatments overestimated in vivo digestibility but 1.0-mm Wiley seemed to be closer to the target at 72 hours than any other grinding treatment. In spite of the apparent better estimation of in vivo digestibility at 72 hours, the prediction equations with 48 hours incubation gave lower standard errors of estimate. Similar results were reported by McLeod and Minson (1969). Data reported by Hartadi (1980) for the same grass samples used in this study generated similar correlation coefficients to the reported herein. Hartadi (1980) reported a standard error of estimate of 3.3 using 78 grass samples.

The correlations of grinding treatments and incubation times with OMI followed the same pattern as the correlations with OMD. There were not marked differences among grinding treatments in the prediction of OMI from IVOMD. The incubation at 24 and 72 hours reduced the correlation coefficients as compared to 48 hours incubation.

The mean IVOMD of three incubation times ($\bar{X}$ IVOMD) was also regressed against OMD and OMI. The correlation coefficients obtained were very similar to the ones obtained with 48 hours incubation.

The results in this study indicated that for comparison purposes all forage samples must be ground in the same mill and also that the grinding technique must be standardized if prediction of in vivo data is required. Weller (1973) found that crude protein was a good predictor of OMD, with a small number of samples; however, the prediction failed when a large number of samples was involved. In this experiment, only nine grasses were studied, and even though they represented different quality forages (Moore et al., 1981) more forage species are needed to corroborate these findings. The results in Figs. 13-VII and 14-IX for Survenola digitgrass, and Coastcross-1 bermudagrass, respectively, gave conclusive evidence that 48 hours incubation does not give an indication of the "average" digestibility of forage particles that occurs in the rumen of animals. Histologic work by Akin et al. (1977) has shown that the extent of digestion in tissues varies a great deal.

Summary

Digestibility of forages is an important parameter of forage quality. Its precise estimation through laboratory procedures facilitates the evaluation of forages. A laboratory experiment was conducted at the University of Florida's Animal Science Nutrition Laboratory in 1982. The objectives were to compare Florigraze rhizoma peanut (leaf and stem) to nine grasses as to the effect of grinding treatments and

incubation time (stage I) upon the IVOMD, and to explore the possibility of reducing variability in the prediction of OMD from IVOMD.

Grinding treatments were 1) Udy Cyclone mill (0.5 mm screen), 2) Wiley mill (0.5-mm screen), 3) Udy mill (1.0-mm), 4) Wiley mill (1.0-mm). Experimental design was a split-plot with three replications (laboratory runs). Grass and legume subsamples were weighed (5 g) and ground (4 treatments). Three sub-subsamples were randomly allocated to three incubation times (24, 48, and 72 hours). In every laboratory run, all incubations were started the same day and recovered on three consecutive days. Results were statistically analyzed with analyses of variance and linear regression.

All grasses and legume samples responded to grinding treatments. In grasses 0.5-mm grinding increased IVOMD regardless of mill. In the legumes samples 0.5-mm grinding increased IVOMD also, but 1.0-mm Udy gave higher IVOMD than 0.5-mm Wiley. These results indicated differences in ease of degradation between grass and legume samples. In both grasses and legume samples, Udy mill decreased the moisture content in samples. Grasses responded differently to incubation time, three categories of responses were summarized. The first one included grass samples in the genus Paspalum; the second included grass samples in the genus Digitaria; and the third included Coastcross-1 bermudagrass. Grinding effects were large in the first group (>10 percentage units); medium in the second (<10 percentage units); and little in the third (<5 percentage units). The response of Florigraze rhizoma peanut to incubation time was very small, with a pattern similar to the third group of grasses, but at a much higher digestibility. The response

to grinding was very small (<5 percentage units) for leaf and stem. The response of grasses and legume samples indicated that grinding increased the rate of digestion within 24 hours.

The $s_{y \cdot x}$ for prediction of OMD from IVOMD were lowest with fine-grinding and 48 hours incubation and also with medium-grinding and 72 hours incubation, low $s_{y \cdot x}$ were also obtained with $\bar{X}$ IVOMD (mean of three incubation times) and fine-grinding.

CHAPTER VI

GENERAL DISCUSSION

The growth of Florigraze rhizoma peanut was studied for 2 years (growth and regrowth) with a replication of the growth phase in another year and different field. Laboratory analyses were performed in plant parts for TNC and N. In 1981 three frequencies of defoliation were imposed on 1-year-old plants and their growth monitored. Forage samples were compared to nine grasses of known in vivo organic matter digestibility as to the effect of four grinding treatments and three incubation times on IVOMD.

Dry matter accumulation in plant parts indicated that rhizome growth paralleled shoot growth until September when shoot growth declined and rhizome growth continued to increase. The rates of growth in rhizomes were still increasing which indicated that net photosynthetic rates were still above the compensation point. In these studies it was not completely understood what caused rhizomes to grow more than shoots. Rhizome formation preceded shoot formation. Therefore, it would have been expected to observe an increment of shoots as well; however, this was not observed. The observations of 2 years indicate that in the growth phase (first year) rhizome growth from September on was not only related to an increase in the size of rhizomes. The increase was also related to rhizome formation and development. At this time, shoots emerged and remained small or died. These shoots were

easily observed in the periphery of the plants. Shoots emerging within the canopy were also observed; most of them did not develop and usually died within 2 weeks.

These observations suggested that above ground temperature was limiting shoot growth. The underground growth was not affected as much probably because temperature gradients in the soil do not drastically change early in the autumn. In the 1981 regrowth, these trends were very similar although the extent of changes was altered. It was suggested that rhizome development was possibly hampered by a deficiency of N, which reduced the potential of rhizome and shoot growth. Although this hypothesis remains to be tested, it was formulated indirectly from the following observations: 1) In summer and autumn, rhizomes in the 1981 regrowth appeared thicker, although they were not counted. 2) The N concentration in shoot and rhizome tissues was rather low as compared to the 1980 and 1981 growth experiments. 3) The analyses of TNC indicated that the accumulation of TNC accounted for about 30% of the total weight increment. This comparison was made with the weight reached by rhizomes in the first year of growth, which had only about 40% TNC. 4) The reduction in shoot growth was rather drastic and relatively early in the growing season, indicating low rates of shoot replacement. Therefore, leaf senescence was probably responsible for low rhizome growth rates in 1981 regrowth.

In addition to the above mentioned observations, it can be added that defoliation of plants reduced the allocation of dry matter to rhizomes, indicating that rhizome growth is enhanced by the absence of shoot growth. Defoliation stimulated the plants to produce shoots,

therefore, rhizome production was reduced. On the other hand, in defoliated plants, TNC accumulation did not occur in the same proportion as in plants in the 1981 regrowth. This stimulation was observed in the removal of N from the rhizomes defoliated every 6 or 8 weeks. Therefore, one could expect to find lower levels of TNC in defoliated plants with no N starvation. These plants would produce more shoots, and probably more rhizomes; the latter depending on the frequencies of defoliation. In extreme cases of stress, like in continuously defoliated plants, rhizome production and development can be stopped, although TNC and N will accumulate in the remaining tissues. This experiment did not involve observation of defoliation on several consecutive years; therefore, questions on rhizome longevity effects, on subsequent regrowths cannot be answered. However, it is clear that Florigraze rhizoma peanut can withstand the expected abuse in a dry year.

The observations in these studies suggest that in some years Florigraze rhizoma peanut may need N fertilizer at the beginning of the growing season, although this consideration must be studied on economical basis. From the standpoint of establishment, it would probably increase the rate of spreading, which in turn may reduce pasture maintenance costs. Adjei and Prine (1976) reported that rhizoma peanut did not respond to N; however, the results herein do not disagree with those observations because those observations were not made on extremely dry years.

The comparison of Florigraze rhizoma peanut with nine tropical grasses of various quality degrees indicated that Florigraze rhizoma

peanut stands among the best quality forages available for North and Central Florida. *In vivo* data had already indicated high organic matter intake. The results herein indicated that part of the reason for this may be the high rates of digestion as observed with *in vitro* procedure. The responses of Florigraze to grinding treatments and incubation times are similar to those reported for high quality temperate legumes.

The responses of Florigraze to defoliation indicated that persistence will not be directly affected, and the quality comparisons highlighted some of the reasons of the observed performance of Florigraze rhizoma peanut. Therefore, the results in this study confirmed and helped to explain some of the morphological and physiological reasons which make rhizoma peanut a species with great potential for the tropical areas.

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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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