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EFFECT OF ENVIRONMENTAL FACTORS AND MANAGEMENT PRACTICES ON NITROGEN FIXATION OF RHIZOMA PEANUT AND TRANSFER OF NITROGEN FROM THE LEGUME TO AN ASSOCIATED GRASS

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BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEDICATED TO MY WIFE SOCORRO AND
MY PARENTS JOSE AND ODETE

Embrapa

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EFFECT OF ENVIRONMENTAL FACTORS AND MANAGEMENT PRACTICES ON NITROGEN FIXATION OF RHIZOMA PEANUT AND TRANSFER OF NITROGEN FROM THE LEGUME TO AN ASSOCIATED GRASS

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This research was conducted to test the hypotheses that a) environmental factors (soil temperature and soil moisture) and management practices (defoliation and nitrogen fertilization) affect growth and nitrogen fixation of Florigraze rhizoma peanut (Arachis glabrata Benth.) and that b) during the establishment of rhizoma peanut the legume transfers symbiotically fixed nitrogen to an associated grass.

The field research indicates that the low soil temperatures that occur in early spring, late fall, and during the winter inhibited nodulation and nitrogen fixation of rhizoma peanut. Reduced soil moisture in late spring and early summer limited the development of new nodules and stimulated nodule senescence, thus reducing the capacity of the symbiotic system to fix nitrogen. The application of nitrogen fertilizer stimulated specific nitrogenase activity (micro moles C_2H_4 . h^{-1} . g nodule dry wt^{-1}) and total nitrogenase activity (n moles C_2H_4 . h^{-1} . $core^{-1}$) in pure stands of rhizoma peanut early in the growing season, but inhibited both nodulation and total nitrogenase activity of the legume during the summer and fall. In associations of rhizoma peanut with bermudagrass [Cynodon dactylon (L.) Pers.] there was no significant effect of nitrogen fertilization on nitrogenase activity of the legume. Defoliation of unfertilized stands of rhizoma peanut resulted in a pronounced decrease in nodulation and nitrogenase activity. The effect of

defoliation on nodulation of fertilized stands of rhizoma peanut was reduced because the plants were poorly nodulated and relied mostly on N fertilizer.

The acetylene reduction assay, in a gas flow-through system, shows that rhizoma peanut exhibits an acetylene-induced decline in rates of nitrogenase activity. Calculations of rates of nitrogenase activity of intact rhizoma peanut plants based on ethylene production at 30-min and 46-min after exposure to acetylene, underestimated the actual values by 36 to 46%. The utilization of disturbed rhizoma peanut plants also resulted in underestimated values of nitrogenase activity. Maximum rates of nitrogenase activity of detopped and shaken root systems were 35% of the maximum rates of intact plants of rhizoma peanut.

The data obtained in the greenhouse experiments indicate that transfer of nitrogen from establishing rhizoma peanut to bermudagrass accounted for up to 2% of the total nitrogen fixed by the legume. Defoliation of rhizoma peanut during establishment did not increase the amount of nitrogen tranferred from the legume to the associated grass. Repeated defoliations during the establishment of rhizoma peanut reduced its growth, thus slowing the establishment of the legume. Application of nitrogen fertilizer during the establishment of rhizoma peanut had a pronounced effect in increasing shoot and root growth and the establishment of the legume. During establishment rhizoma peanut competed strongly with associated bermudagrass for the available supply of nitrogen fertilizer.

CHAPTER I
INTRODUCTION

In 1984, the world population was 4.76 billion with the greatest growth occurring in the developing countries. The projections are that, even if the current measures of population control are successful, the world's population will more than double by the early decades of the next century (Halliday, 1985). The realities of undernourishment in developing countries impress scientists and politicians in all nations and are a sobering reminder that advances in food production continue to be frustrated by rampant population growth.

According to Brown (1985), topsoil is being depleted and lost from cropland in excess of soil formation at a rate of 25.4 billion tons per year. Desertification, as a result of land mismanagement and climatic changes, proceeds at an ever increasing rate. The prospect is alarming if we consider that the forest clearance : replanting ratio is 29:1 in tropical Africa, 10:1 in Latin America, and 5:1 in tropical Asia. Over 11 million hectares of tropical forests are being cut every year, a rate of deforestation that reduces this resource by 6 per cent in a decade (Brown and Wolf, 1985).

The tropics, defined as the area bounded by 30 degrees of latitude north and south, is a region of alarming statistics. It is where more than half of the world's population lives, but where only about one quarter of the world's food is produced. All those nations categorized as less developed countries are clustered in the tropics. The dominant sector of the economies of these countries is agriculture.

Most of the food in tropical developing countries is produced by subsistence farmers, whose resource base is so limited and whose physical access to supply markets is so restricted, that they cannot derive full benefits of the new technologies that rely mainly on purchased inputs. Traditional and modern farming systems in the tropics almost invariably include legumes and have done so for centuries.

Nitrogen is an essential element for all living organisms. Although nitrogen makes up four-fifths of the atmosphere and some 77,000 metric tons cover every hectare of the earth, it is not in a form that can be utilized by plants. Nitrogen is the most limiting nutrient for more crops and animals in more places than any other nutrient (Burton, 1976). Because of the need for this element, combined with the high costs or unavailability of fertilizers, nitrogen may be said to be the key factor, in addition to water in water deficient regions, that regulates the quantity and quality of food and that is responsible for undernutrition and malnutrition in many parts of the world (Alexander, 1984).

The difficulties of increasing the food supply are exacerbated by problems in producing nitrogenous fertilizers. The high cost of building fertilizer plants prevents many countries from producing the amounts of nitrogen required, while there is concern about the future supply of energy needed to manufacture these fertilizers. The inefficiency of nitrogen fertilizer utilization, potential groundwater pollution, denitrification losses, possible destructive effects of denitrification products on atmospheric ozone layer, and the costs of packaging, transport, storage, and application are limitations of the available technology (Hardy and Gibson, 1977).

The study of biological nitrogen fixation is an immensely important area of research. The main need for a better understanding of biological nitrogen fixation is the urgent requirement to increase agricultural production for a seemingly ever-expanding human population. Although all forms of biological nitrogen fixation ultimately contribute to such production, increases in the short-term are likely to depend on the greater and more efficient utilization of the legume-Rhizobium symbiosis. The grain and pulse legumes are the foremost in current research approaches aimed at increasing protein production, even though tropical and temperate forage legumes and green manure make significant contributions to animal production and the soil nitrogen supply, respectively.

Biological nitrogen fixation, used appropriately, has the potential to contribute significantly to sustainability of food production by subsistence farmers in Third World countries, reducing the production constraints facing them in the many nitrogen deficient soils of the

tropics. There is also an opportunity to alleviate the dependence of developing nations on costly nitrogen fertilizer.

Chapter II reports on field research conducted to test the hypotheses that a) environmental factors such as soil temperature and soil moisture limit nodulation and nitrogen fixation of 'Florigraze' rhizoma peanut (Arachis glabrata Benth.); b) application of nitrogen fertilizer to rhizoma peanut in pure stands, early in the growing season, increases rates of nitrogenase activity and nitrogen fixation; c) nitrogen fertilizer application in pure stands of rhizoma peanut, during the summer season, inhibits nodulation and nitrogen fixation; d) application of nitrogen fertilizer to rhizoma peanut in association with Tifton 'Hybrid-81' bermudagrass [Cynodon dactylon (L.) Pers.] has no effect on nitrogen fixation by the legume throughout the growing season; and e) nodule mass, nitrogenase activity, and nitrogen fixation of rhizoma peanut declines sharply following defoliation.

In Chapter III studies were conducted using a gas flow-through system to test the hypotheses that a) Florigraze rhizoma peanut exhibits an acetylene-induced decline in ethylene production and that b) the utilization of disturbed (detopped and shaken) root systems of rhizoma peanut in the acetylene reduction assay results in underestimated values of rates of nitrogenase activity.

Chapter IV reports on research conducted in the greenhouse to test the hypotheses that a) during the establishment of Florigraze rhizoma peanut it can transfer symbiotically fixed nitrogen to an associated grass; b) shoot removal increases the amount of nitrogen released by rhizoma peanut to the associated grass, but reduces the rate of establishment of the legume; c) application of nitrogen fertilizer increases the rate of growth and establishment of rhizoma peanut; and d) in legume-grass associations supplied with nitrogen fertilizer, rhizoma peanut competes strongly with the associated grass for the available mineral nitrogen.

CHAPTER II
SEASONAL VARIATION OF NODULATION AND NITROGENASE ACTIVITY OF *Arachis glabrata* Benth. AS AFFECTED BY SOIL TEMPERATURE, SOIL MOISTURE, COMBINED NITROGEN AND DEFOLIATION

The increasing ecological awareness throughout the world demands a better understanding of the earth's biological environment, an understanding of the inputs and outputs from all the habitats, and an appreciation of how alterations of any components of the existing ecosystems will affect all the other components.

Beginning in 1973, nitrogen prices escalated rapidly as a result of the rising cost of energy. This resulted in a great surge of interest and activity by biologists and agriculturalists in the field of dinitrogen fixation by the legume-*Rhizobium* symbiosis.

Symbiotic Nitrogen Fixation

Biological nitrogen fixation plays a significant role as a contributor of nitrogen to production in natural and agricultural habitats. Legumes have long been appreciated as contributors to soil fertility. However, as we stand at the threshold of an era of intensive scientific activity, the understanding of aspects of biological nitrogen fixation by legumes in association with the *Rhizobium* bacteria is far from complete.

Nitrogen fixation by biological means is the reduction of atmospheric nitrogen to ammonia by the enzyme complex nitrogenase (Brill, 1977). It is a unique process restricted to certain microorganisms. This dissertation will consider only the legume-*Rhizobium* symbiosis.

Nitrogen fixation in legumes occurs in small organs called root nodules. Common peanut (Arachis hypogeae L.) nodules are approximately spherical. The Rhizobium bacteroids are located inside the host-cell cytoplasm, enclosed by the peribacteroid membrane and the peribacteroid space. Symbiotic nitrogen fixation is a reaction that is highly dependent on energy in the form of ATP and low potential electrons supplied by $NADPH_2$. Tjepkema and Winship (1980) suggested that the actual energy requirement may be at least 24 moles of ATP per nitrogen molecule reduced. This energy is derived from nodule respiration which in turn requires both photosynthate for oxidation in the glycolytic and Kreb's pathways, and oxygen to act as a terminal electron acceptor in oxidative phosphorylation.

Approximately five molecules of oxygen are consumed by bacteroid oxidative phosphorylation for every molecule of nitrogen fixed (Tjepkema, 1971). Thus, the flow of oxygen into the nodule must be considerably higher than that of nitrogen. However, the nitrogenase complex (consisting of Mo-Fe and Fe proteins) is irreversibly inactivated by even traces of oxygen (Bergersen, 1962; Bergersen, 1971; Robson and Postgate, 1980). Therefore, in order for symbiotic nitrogen fixation to occur, large amounts of oxygen are required for respiration, yet nitrogenase must be protected from oxygen inactivation.

Nodule anatomy is one of the key elements of nitrogenase protection from oxygen inactivation (Goodchild, 1977; Sinclair et al., 1985; Tjepkema, 1971; Tjepkema and Yocum, 1973; Tjepkema and Yocum, 1974). Leghaemoglobin (an oxygen binding protein) facilitates oxygen flux to the Rhizobium bacteroids, stabilizing the oxygen tension at a concentration too low to inactivate the oxygen sensitive nitrogenase enzyme complex (Appleby, 1984). By placing the site of high respiratory demand and nitrogen fixation inside a diffusion barrier, the low oxygen environment necessary for nitrogenase activity is established (Weisz, 1986).

The Acetylene Reduction Technique

The observation that acetylene (C_2H_2) was an inhibitor of nitrogen fixation and that it was converted to ethylene (C_2H_4) by the nitrogen fixing enzyme nitrogenase (Dilworth, 1966; Schollhorn and Burris, 1967) led to the development of the acetylene reduction assay (Hardy et al., 1968; Stewart et al., 1967). This method provided a simple, inexpensive, rapid, and highly sensitive procedure for studies of the kinetics of nitrogen fixation (Herridge, 1982; Silver and Hardy, 1976).

The method has been widely applied and has resulted in a marked increase in studies on nitrogen fixation (Hardy et al., 1973; Turner and Gibson, 1980). It has proved to be particularly suitable for use under field conditions in detecting new nitrogen fixing systems and has made it possible to obtain valuable information on nitrogen fixing activities of nodulating legumes (Masterson and Murphy, 1980).

It is widely recognized that the development of the acetylene reduction assay was one of the greatest advances in nitrogen fixation research in recent times. Its application in the laboratory and field has provided a great stimulus to studies in the different areas of this subject. The increased use of this procedure in the future will allow advances which otherwise would not be possible (Masterson and Murphy, 1980).

Most time course studies on nitrogen fixation in the field have emphasized soybeans (Glycine max), peanuts, and peas [Pisum sativum (L.)], with some work done on clovers (Trifolium spp.). Time course studies on nitrogen fixation by forage legumes are important, since such information may lead to identification of limiting factors and definition of adequate management.

Factors Affecting Symbiotic Nitrogen Fixation

There are many factors that affect symbiotic nitrogen fixation. Although the physical features of the environment (temperature, light, oxygen, $C0_2$ levels, and moisture) are not readily changed, an understanding of environmental influences and the adoption of appropriate management practices can help overcome likely periods of stress or make the best use of conditions which are likely to occur. This research emphasized the effects of temperature, drought stress, nitrogen fertilizer application and defoliation on nitrogen fixation.

Temperature

Temperature has a marked effect on nearly all stages of the development and functioning of the legume-Rhizobium symbiosis. There is extensive literature on this topic covering many species of both tropical and temperate regions. The effects of root temperature, shoot temperature, diurnal variation, and temperature effects under greenhouse and field conditions have been studied (Gibson, 1976; La Favre and Eaglesham, 1986; La Favre and Eaglesham, 1987; Lie, 1981; Munévar and Wollum, 1982; Natakorn and Weaver, 1982; Sprent, 1979). In many parts of the world, temperature may be the major factor limiting the extent of the nitrogen fixation period: in some cases low temperatures are limiting, in other cases high temperatures.

Fyson and Sprent (1982) observed that at temperatures of 6°C and below, infection of Vicia faba L. roots by rhizobia, and subsequent nodule development, were severely retarded and final differentiation of bacteria into the nitrogen fixing form did not occur. According to Gates and

Silsbury (1987), nitrogen fixation in subterranean clover (Trifolium subterraneum) was greatest in swards growing at 10-15°C because net photosynthesis was highest in these comparatively cool environments.

According to Lie (1981), temperature operates mainly in a non-specific way through plant metabolic processes such as respiration, photosynthesis, transport, and transpiration. Weisz and Sinclair (in press) concluded that nitrogen fixation rates in field and hydroponically grown soybean root nodules were not affected by diurnal cycles in photosynthetically active radiation, but were strictly related to root temperature.

Nitrogenase activity, like all enzyme reactions, responds to temperature, even though the relationship is not always a simple one. Activity of this enzyme occurs over a wide range of temperatures with the optimum varying with species and variety of organisms. It is also altered by changes in the growing conditions (Dart and Day, 1971; Waughman, 1977). Dart and Day (1971) observed that the optimum temperature for maximum nitrogenase activity in several legumes was between 20 and 30°C, with the activity declining rapidly above and below the optimum temperature. Masterson and Murphy (1976) suggested 21°C as the optimum temperature for nitrogen fixation in Trifolium repens. They also found that soil temperature was the principal factor limiting nitrogen fixation by this species in the field. The literature indicates that incubation temperature is highly important in the acetylene reduction assay, especially if the results are to be related to in situ activity.

Schwitzer and Harper (1980) and Weisz (1986) observed that diurnal cycles in nitrogen fixation of soybeans were the result of changes in soil temperature and were not correlated with daily photosynthetically active radiation. Changes in soil temperature also resulted in proportional changes in the nodule gas permeability such that a linear correlation between temperature and acetylene reduction rates was maintained. Weisz (1986) indicated that gas permeability of soybean nodules is a dynamic parameter which can respond to changing environmental conditions and which appears to be under active physiological control. It was speculated that control of nodule gas permeability could be related to changes in turgor in the cells of the nodule

cortex. However, a mechanism which would trigger turgor changes in response to the oxygen concentration or temperature is yet unknown.

Drought Stress

Drought stress can be defined as the reduction of water and turgor potential of the plants to levels at which they interfere with normal plant growth and crop yield. Drought stress has a profound effect on nitrogen fixation in many legumes, with all stages of the symbiosis capable of being affected (Albrecht et al., 1980, 1984; Bennett and Albrecht, 1984; Engin and Sprent, 1973; Gibson, 1977; Sprent, 1972a,1976). There is little doubt that in many regions of the world, reduced water supply limits nitrogen fixation under field conditions.

Lower rates of nitrogen fixation can occur in several ways. Water deficiency can cause osmotic imbalance, impair oxygen diffusion in the nodule, and reduce nodule respiration with an accompanying reduction in nitrogen-fixing activity (Pankhurst and Sprent, 1975a, 1975b; Paterson et al., 1979). Transport of fixation products out of the nodule may also be depressed (Minchin and Pate, 1975; Sprent, 1972b, 1979). Pate et al. (1969) suggested that reduced rates of water movement out of the nodule during water stress may restrict export of fixation products, thus inhibiting nitrogen fixation through a feedback mechanism. Nitrogen fixation can be indirectly affected because of impaired supply of photosynthate from a stressed shoot system (Huang et al., 1975a, 1975b; Sprent, 1972b). Finn and Brun (1980) observed that CO_2 assimilation and photosynthate partitioning among plant parts was significantly changed by water stress. They reported that carbohydrate availability decreased by more than 50% in the stressed plants.

Bennett and Albrecht (1984), working with soybeans in a sandy soil, identified a very sensitive response in nitrogenase activity to reductions in nodule water potential, indicating that nodules surrounded by dry soil could desiccate to water potentials lower than those observed for

leaf tissue. Reduced rates of nitrogenase activity were observed as leaf water potential decreased and diffusive resistance increased. Rewatering restored activities to values equal to or above those of well watered plants even after water deficits had reduced midday nitrogen fixation to near zero for several days.

Sprent (1976), working with bean and white clover, observed maximum acetylene reduction at field capacity, with activity being reduced above and below this level. Murphy (1977) obtained similar results when measuring the effect of soil water content in acetylene reduction by white clover. Masterson and Murphy (1976) suggested that the optimum acetylene reduction by legumes is determined by the concomitant requirement of nodules for adequate supply of oxygen and water.

Ralston and Imsande (1982) reported that detached nodules lose an average 70% of apparent nitrogenase activity under 21% atmospheric oxygen pressure. According to Pankhurst and Sprent (1975a) oxygen movement into soybean nodules occurs initially by diffusion in air spaces in lenticel-like structures in the outer cortex. There is a layer of cells in the inner cortex which lacks intercellular air spaces. Tjepkema and Yocum (1974) and Sinclair and Goudrian (1981) suggested this layer in the inner cortex as the site of an oxygen diffusion barrier. Pankhurst and Sprent (1975a) observed that when soybean root nodules were subjected to drought stress the air spaces in the lenticels close as the cells in the outer cortex loose turgor. This led them to suggest that a decrease in nodule gas permeability would be associated with drought stress. This was supported by the data of Weisz et al. (1985) which indicated that drought stressed soybean nodules had significantly lower values of nodule gas permeability than did those on well watered plants. This would result in decreased oxygen concentration, inhibiting nitrogen fixation due to reduced oxydative phosphorylation. Weisz (1983) obtained a high correlation between acetylene reduction per unit surface area and nodule gas permeability which seems to corroborate this hypothesis. Weisz (1986) suggested that nodule gas permeability appears to play an active and essential role not only in protecting nitrogenase from oxygen inactivation, but also in controlling the rate of symbiotic nitrogen fixation in legumes.

Combined Nitrogen

Combined nitrogen is the total nitrogen (soil and fertilizer nitrogen) in the growth medium which can be utilized by legumes, in addition to or replacing symbiotically fixed nitrogen. The effects of combined nitrogen on the legume-Rhizobium symbiosis have been the subject of several studies in the last two decades. The extent of the influence of combined nitrogen under field conditions is not completely understood. A major difficulty arises when determining the natural level of available nitrogen in the soil, as it affects legume nodulation. It varies considerably and changes with time and depth. It is also dependent on such environmental factors as nitrification and denitrification rates, and the growth of root systems into root-free zones. The application of nitrogen fertilizer contributes to this problem (Gibson 1976, 1977).

Research results indicate that the relationship between combined nitrogen and nodulation is very complex (Bethlenfalvay and Phillips, 1978; Dart and Wildon, 1970; Gibson, 1976, 1977; Gibson and Pagan, 1977; Ham et al., 1976; Hamdi, 1976; Harper, 1974, 1976; Harper and Cooper, 1971; Harper and Gibson, 1984; Hera, 1976; Houwaard, 1980; Nutman, 1976; Ralston and Imsande, 1983; Rys and Phung, 1984; Sistachs, 1976; Subba Rao, 1976). In this context, level and placement of combined nitrogen, environmental conditions, host species, and bacterial strains, among other factors, must all be considered.

Davidson and Robson (1986) observed that mean dry weight per nodule and rates of acetylene reduction in white clover fell rapidly (2 to 3 days) during periods of exposure to high nitrate concentrations (> 7 mM N). They rose again, equally rapidly, when nitrate was withdrawn or was substantially reduced.

Freire (1984) reviewed the literature on the effects of mineral nitrogen in soil on the Rhizobium-legume symbiosis. He stated that the following conclusions could be drawn: (a) there is an inverse relationship between mineral nitrogen and symbiotic nitrogen fixation, a relationship

that may be explained by a competition for photosynthate; (b) positive responses to applied nitrogen may be obtained in stress conditions or during poor-growing seasons, at which time nodulation, N_2 fixation, or both would be inhibited; (c) although plants supplemented with mineral N at planting time receive a better start and are taller and greener because N_2 fixation starts 10 to 14 days after germination, inoculated plants recover rapidly so the yield is similar; (d) prolonged adverse environmental conditions may increase the influence of added nitrogen; (e) poor nodulation or nodulation by combinations of Rhizobium strain x cultivar that have low efficiencies may also account for the response to nitrogen fertilizer; (f) nitrogen fertilizer in the form of nitrate is more detrimental to nodulation than is ammonium; (g) the mechanisms which inhibit the symbiotic nitrogen fixation process in the presence of combined nitrogen are still not well understood; (h) deep placement of nitrogen fertilizer is less detrimental to nodule development than is uniform distribution; (i) in acid soils, rich in exchangeable Al and Mn, positive responses to nitrogen fertilizer may also occur.

According to Dart and Wildon (1970) nodule formation appears to be more severely inhibited by the presence of ammonium nitrate (NH_4NO_3) than either NH_4^+ or NO_3^- independently, under controlled conditions. However, Harper and Nicholas (1976) reported NO_3^- as being more inhibitory to nodulation than NH_4^+. Rys and Phung (1984) observed that ammonium had no effect on nodulation of white clover, while nitrate reduced nodulation to between $1/3$ to $1/2$ of the no nitrogen control. The combination of NH_4^+ and NO_3^- severely restricted nodule formation.

Munns (1968b) showed that nitrate utilization retards nodule development on Medicago sativa. High nitrate assimilation by field pea is reported to reduce nodule mass (Oghoghorie and Pate, 1971) and cause nodule senescence (Chen and Phillips, 1977). It has been suggested that nitrate may inhibit recognition between Rhizobium trifolii and the root hairs of white clover (Dazzo and Brill, 1978). Bauer (1981) indicated that nitrate may also affect the post-infection process. It is known that nitrate reduction produces hydroxyl ions, which in turn, may alter the plant-nodule metabolism and function (Raven and Smith, 1976; Imsande and Ralston, 1981).

There are reports of nitrogen fertilizer responses by tropical legumes which are not nodulated or are poorly nodulated. App and Eaglesham (1982) suggest that these fertilizer responses can be eliminated if those legumes are properly inoculated with an effective strain of rhizobia. Norman (1982) states that significant advances can also be made through a symbiosis more tolerant of high levels of mineral N, if this is physiologically possible.

Defoliation

Defoliation by removing topgrowth as in cutting or gazing, is a stress experienced periodically by forage legumes throughout the growing season. It is an integral part of the forage management system. There is extensive literature showing the effects of cutting or grazing on nodule activity. According to Gibson (1976, 1977) these effects range from severe, which is often accompanied by nodule shedding, through moderate, to no effect. The variation in the data may be due to the species studied, the conditions of the experiment (field or glasshouse), soil type, environment, and the severity and intensity of defoliation (Gibson, 1976, 1977; Henzell, 1981; Sprent, 1979).

Grazing, cutting, or treading results in the removal of the principal source of photosynthate necessary for continued nodule function and the development of new nodules (Gibson, 1976, 1977; Henzell, 1981; Pate, 1977). The decline in nitrogenase activity is generally considered to be due to a lack of photosynthate following removal of photosynthetizing tissue (Gordon et al., 1986; Halliday and Pate, 1976; Hardy and Havelka, 1976; Haystead and Marriott, 1978; Moustafa et al., 1969; Ryle et al., 1985b). However, when photosynthesis was suppressed by darkness, it had a delayed or a much less severe effect on nitrogenase activity as compared to defoliation (Halliday and Pate, 1976; Haystead et al., 1979; Masterson and Murphy, 1976; Minchin and Pate, 1973, 1974; Minchin et al., 1985a). Defoliation usually, but not inevitably (Haystead and

Marriott, 1978), causes a decrease in nitrogen fixation (Moustafa et al., 1969). The decline in nitrogen fixation following defoliation is concomitant with a reduction in specific nodule activity, nodule weight, nodule number, and nodule leghaemoglobin (Gibson, 1976, 1977; Graham and Chatel, 1983; Whiteman, 1970; Wilson, 1942).

Gibson (1976, 1977), reviewing the literature on this topic, states that (a) the ability of the nodules to survive depends on the severity of defoliation and the carbohydrate reserves that the plant can remobilize to produce new leaves and supply the nodules; (b) severe defoliation causes nodule loss; (c) renodulation depends on availability of carbohydrates and plant nitrogen; and (d) developing shoots, leaves, and roots compete with nodulation for these carbohydrates, the extent of the competition depending on the stimulus for the development of these organs by temperature and moisture.

Nitrogenase activity of white and subterranean clovers declined shortly after defoliation but recovered within 7 to 10 days (Chu and Robertson, 1974; Gibson, 1976; Moustafa et al., 1969; Sinclair, 1973). The results of Whiteman (1970) and Wilson (1942) suggest that changes in nodule weight may take 14 to 21 days to appear, although loss of leghaemoglobin may become evident within 7 days after defoliation.

Ryle et al. (1985a) observed that the immediate effects of defoliation of white clover were to curtail plant photosynthesis by 83 to 96%, respiration by 30 to 40%, and nitrogen fixation by more than 70%. The rate of recovery of nitrogen fixation during regrowth appeared to be more related to re-establishment of plant photosynthetic capacity than to any specific effect upon nodule mass or integrity.

Denison et al., (in press) stated that although photosynthate must eventually become limiting if photosynthesis is prevented by defoliation, the consequences of a photosynthate limitation to nodule respiration are not widely recognized. Whenever nodule respiration is limited by anything other than oxygen, the unused oxygen will accumulate and increase $p0_2$ inside the nodule, and nitrogenase will be inactivated. Therefore, nodules whose respiration is limited by photosynthate, presumably, also lack functional nitrogenase. Tjepkema and Yocum (1973) and

Denison et al. (in press) indicated that nodule respiration and specific nodule activity are normally limited by oxygen rather than photosynthate. This is consistent with the results obtained by Hartwig et al. (1987) which suggested that lack of oxygen at the site of nitrogen fixation, resulting from a dramatic increase in oxygen-diffusion resistance, is the main factor limiting nitrogenase activity following defoliation.

Seasonal Pattern of Nitrogen Fixation

Despite the extensive use of legumes as a nitrogen source in cropping systems, there is little knowledge of how nitrogen fixation varies with crop development, soil temperature, soil moisture, defoliation, combined nitrogen, age of stand, and with yielding ability for long-term, conventionally-managed stands of perennial forage legumes. Certainly, long-term measurement of nitrogen fixation is important in choosing the appropriate legume for use in intercropping, crop rotation, and mixed pastures, and for understanding the constraints to nitrogen fixation in crop communities.

The study of seasonal patterns of nitrogen fixation is relevant, especially when considering that pasture growth at the beginning and end of the growing season often limits animal production. An understanding of how the environment and management practices affect nitrogen fixation in perennial forage legumes is essential in defining a production system which maximizes yield and profit and, at the same time, guarantees the persistence and productivity of the legume over the years.

Arachis glabrata Benth.

Rhizoma peanut (Arachis glabrata Benth.) is a perennial forage legume native to South America. It is thought that this seedling 'Florigraze' was part of the material collected by W. Archer near Campo Grande in the state of Mato Grosso do Sul, Brazil in 1936 (Prine et al., 1981). It has branched, creeping underground rhizomes (root-stocks) producing short erect above ground shoots (Bogdan, 1977). Several introductions of rhizoma peanut have been studied and released to farmers for testing (Prine, 1964; Prine et al., 1981, 1986). Over two hundred genotypes of Arachis spp. have been collected and are being studied for their potential as forage crops in Brazil, Colombia, and the United States.

Rhizoma peanut is an excellent forage legume because of its high dry matter yields, excellent forage quality, and persistence. It can be used for grazing, hay, silage, and greenchop. Rhizoma peanut has also been used for soil protection along roads, as ground cover in citrus groves, and as an ornamental plant.

Saldivar (1983) in a growth study of Florigraze rhizoma peanut observed that rhizome growth paralleled shoot growth until September when shoot growth declined but rhizome growth continue to increase. Total nonstructural carbohydrate concentration in the rhizomes increased toward the end of the growing season and decreased early in the growing season. He also observed that rhizome growth decreased as frequency of defoliation increased. Defoliation led to lower total nonstructural carbohydrate accumulation and a decline in nitrogen concentration in the rhizomes.

Valentim (1985) observed that when nitrogen fertilizer was applied early in the growing season to establishing stands of rhizoma peanut, dry matter yields increased with increasing rates of nitrogen application (from 0 to 300 kg of N ha^{-1} $year^{-1}$). It was suggested that nitrogen fertilization occurred before the photosynthetic capacity had been established and the symbiotic

fixation system became effective. Therefore, the nitrogen fertilizer acted as an aid in the establishment of the legume, avoiding N deficiency during the early weeks of growth.

The current research was conducted in the field to test the hypotheses that a) environmental factors such as soil temperature and soil moisture limit nodulation and nitrogen fixation of Florigraze rhizoma peanut; b) application of nitrogen fertilizer to rhizoma peanut in pure stands, early in the growing season, increases rates of nitrogenase activity and nitrogen fixation; c) nitrogen fertilizer application in pure stands of rhizoma peanut during the summer season inhibits nodulation and nitrogen fixation; d) application of nitrogen fertilizer to rhizoma peanut in association with Tifton 'Hybrid-81' bermudagrass [Cynodon dactylon (L.) Pers.] has no consistent negative effect on nitrogen fixation by the legume throughout the growing season; and e) nodule mass, nitrogenase activity, and nitrogen fixation of rhizoma peanut declines sharply following defoliation. The objective of this study was to evaluate the seasonal variation of nodulation and nitrogenase activity of Florigraze rhizoma peanut in pure stands and in association with bermudagrass as affected by soil temperature, soil moisture, defoliation, and combined nitrogen.

Materials and Methods

General

The research was conducted on the University of Florida Agronomy Farm, Gainesville , Florida. The soil of the area is classified as Arredondo loamy fine sand (a loamy, siliceous, hyperthermic Grossarenic Paleudult).

The experiment was arranged as a split-plot in a randomized complete block design with three replications. The main plot treatments consisted of Florigraze rhizoma peanut in pure stands and the association of the legume with Tifton Hybrid-81 bermudagrass. The subplot treatments were three levels of nitrogen fertilization (0, 150, and 300 kg N ha^{-1} $year^{-1}$) and they

were randomized within main plots. Main plots were 3.7 x 13.7 m (50.7 m^2), separated by a 1.8 m clean tilled alley to prevent mixing of the species, and were equally divided into three subplots.

Land preparation began in 1983, and establishment was as described by Valentim et al. (1986). The experiment was evaluated from 1984 to 1986 for dry matter yield, forage quality, and botanical composition of the legume and grass in pure stands and in associations as affected by nitrogen fertilization.

In 1986, forage was harvested on 23 June 11 August and 21 October. Nitrogen fertilizer in the form of ammonium nitrate (335 g N kg^{-1} NH_4NO_3) was supplied in three equal applications in early spring (4 April), after the first harvest, and after the second harvest. The fertilizer was applied by hand in two passes over each subplot.

Nitrogen Fixation

Assessment of nitrogen fixation by rhizoma peanut was made by measuring acetylene reduction to ethylene in the field (Hardy et al., 1968) at approximately 2-week intervals from 2 Apr. 1986 to 26 Mar. 1987, for a total of 24 samplings (Table 1). The measurements were carried out on all treatments with three replications for each treatment, resulting in a total of 18 samples. Sampling in the field began around 1100 h EST and ended at approximately 1400 h EST. Soil temperature at a 5-to 10-cm depth was recorded at each sampling.

At each sampling, the topgrowth of the plants was clipped at 5 cm above the soil surface and removed. A soil core of the nodulated root system was removed to a 15-cm depth using a core sampler that was 10.2 cm in diameter. The root system was placed on a screen over a tray and gently separated from the soil. The nodulated root system was immediately placed in an incubation chamber consisting of a 500 mL Mason jar to which a metal lid was fitted, completely

Table 1 -- Sampling dates for the evaluation of nodulation and nitrogenase activity of Florigraze rhizoma peanut in pure stands and in association with bermudagrass, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987).

Sampling number	Sampling Dates	Observations
1	2 Apr. 1986	Plots were fertilized on 4 Apr.
2	18 Apr. 1986	
3	5 May 1986	
4	19 May 1986	
5	2 Jun. 1986	
6	16 Jun. 1986	Plots were defoliated on 23 Jun.
7	26 Jun. 1986	Plots were fertilized on 24 Jun.
8	10 Jul. 1986	
9	24 Jul. 1986	
10	7 Aug. 1986	Plots were defoliated on 11 Aug.
11	19 Aug. 1986	Plots were fertilized on 12 Aug.
12	2 Sep. 1986	
13	16 Sep. 1986	
14	30 Sep. 1986	
15	16 Oct. 1986	Plots were defoliated on 21 Oct.
16	6 Nov. 1986	
17	9 Dec. 1986	
18	23 Dec. 1986	
19	6 Jan. 1987	
20	20 Jan. 1987	
21	26 Jan. 1987	
22	10 Feb. 1987	
23	26 Feb. 1987	
24	26 Mar. 1987	

sealing the chamber. A hole was bored in the lid and a rubber septum fitted, allowing the addition and removal of gas by syringe.

The reaction was initiated by replacing 10% (v/v) of the gas phase in the incubation chamber with acetylene (50 mL). Incubation was at ambient temperature out of direct sunlight, and soil temperature and barometric pressure were recorded. During the incubation period, 0.1 mL gas samples were extracted at 30 and 60 min with a gas-tight syringe. Ethylene and acetylene concentrations were measured with a gas chromatograph (Varian Series 3700) fitted with a flame ionization detector and equipped with a 2.8-m column packed with Poropac R. Nitrogen was used as the carrier gas. Ethylene production was calibrated against standard curves. Rates of nitrogenase activity were calculated from linear regression lines of ethylene production (corrected for temperature and pressure) fitted to the time sequence measurements.

After the incubation period, the nodules were detached from the root systems, dried to a constant weight and nodule dry weight determined. Specific nitrogenase activity was calculated by dividing nitrogenase activity of each sample by the respective dry weight of the nodules.

Gravimetric Soil Water

Soil samples were collected from the cores, consisting of the entire 15-cm depth to determine moisture. The samples were placed in tin containers, capped, transported to the laboratory, and weighed. The samples were then thoroughly dried for 24 h at 100°C and dry weight of the soil recorded. Soil water is expressed as a percentage of soil dry weight.

Distribution of the Root System in the Soil Profile

On 23 Sep. 1986 pure stands of rhizoma peanut were sampled to determine the distribution of the root system within the soil profile, and to measure nodulation and specific nitrogenase activity at different soil depths. The sampling procedure consisted of clipping the topgrowth of the plants at 5 cm above the soil surface and removing a soil core (10.2 x 15 cm) with the nodulated root system. The soil core was divided into three 5-cm sections according to depth of the soil profile (0-5, 5-10, and 10-15 cm depth). Each section was placed in a screen over a tray and the root system gently separated from the soil. There were four replications for each soil depth, resulting in 12 samples.

Evaluation of nodulation, specific nitrogenase activity, and soil moisture were as described for the previous experiment. After the incubation period, the nodules were detached from the roots. Both nodules and roots were dried to a constant weight and dry weight recorded. Specific nitrogenase activity was calculated by dividing nitrogenase activity of each sample by the respective dry weight of the nodules.

Analysis of variance was performed using the General Linear Model procedure of SAS (SAS Institute Inc., 1982). Comparison of treatment means of nodule dry weight, specific nitrogenase activity, and total nitrogenase activity for each sampling period was performed using Duncan's Multiple Range Test.

Results

At the beginning of the experiment (2 Apr. 1986) soil temperature was 22°C, but increased thereafter to 28 to 30°C, and remained within this range throughout the summer and early fall (until 30 Sep. 1986). In October, soil temperature started to decrease, reaching the lowest level (12°C) in the months of January and February, and then rising to 22°C at the end of March 1987. Soil temperatures were unseasonably warm during the fall of 1986 and early winter of 1987 (Fig.1).

Soil moisture fluctuated throughout the experimental period, but generally remained between 2.9 and 10%. Reduced rainfall during May, June, and September resulted in soil moisture dropping to 2.9, 4.6, and 3.4%, respectively (Fig. 2). Moderate to severe dry periods in late spring and early summer are a common occurrence in North-Central Florida. However, with the exception of these three dry periods, levels of soil moisture did not appear to be limiting growth of rhizoma peanut and bermudagrass throughout the experimental period.

A pure stand of rhizoma peanut was sampled on 23 Sep. 1986 to evaluate the distribution of the root system, nodulation, nitrogenase activity, and soil moisture levels at different soil depths. It was observed that 95% of the roots and rhizomes, and 90% of the nodules were found within the top 10 cm of the soil. Specific nitrogenase activity was higher at 6 to10 cm soil depth. However, there was not a marked difference in rates of nitrogenase activity between 0 to 5 and 5 to10 cm soil depth. Specific nitrogenase activity at 10 to15 cm soil depth was markedly lower than that found at 0 to10 cm soil depth. Soil moisture was lower at 0 to 5 cm, but increased gradually with increasing soil depth (Table 2).

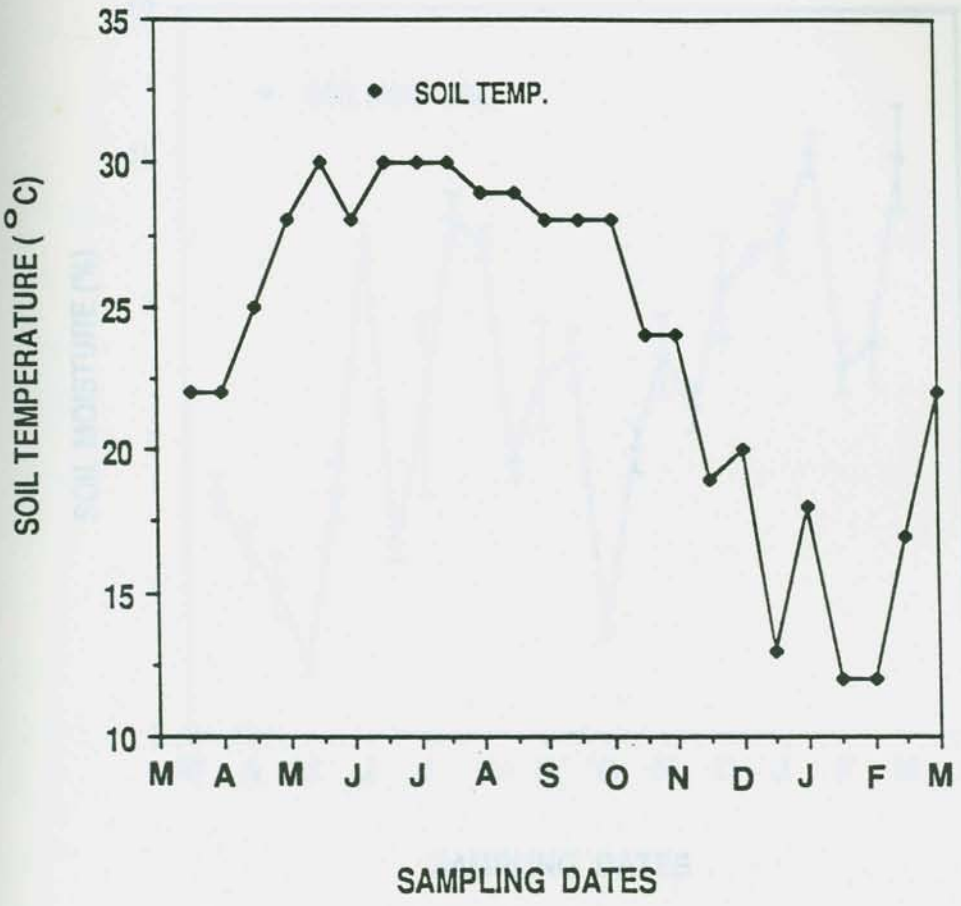

Fig. 1 - Mean soil temperature at 0 to 15-cm depth in the experimental plots of Florigraze rhizoma peanut in pure stands and in associations with bermudagrass (from 2 Apr. 1986 to 26 Mar. 1987).

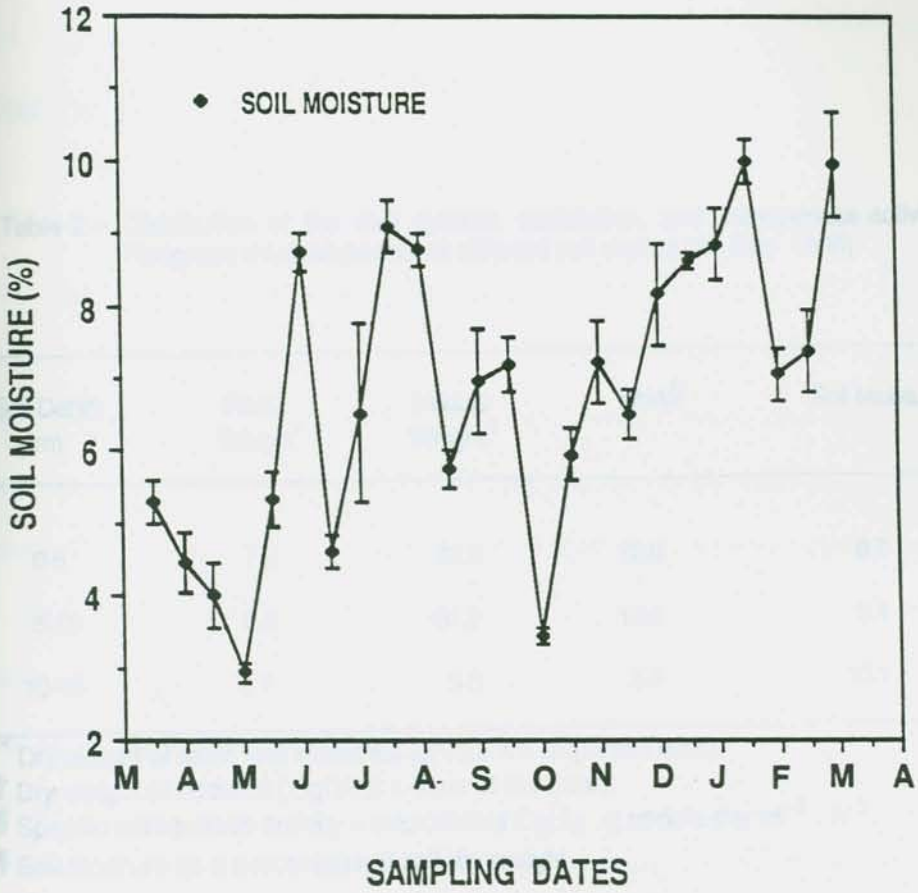

Fig. 2 - Soil moisture at 5-to 10 -cm depth in the experimental plots of Florigraze rhizoma peanut in pure stands and in associations with bermudagrass (from 2 Apr. 1986 to 26 Mar. 1987). Bars indicate the standard error of the means.

Table 2 -- Distribution of the root system, nodulation, and nitrogenase activity of Florigraze rhizoma peanut at different soil depths (23 Sep. 1986).

Soil Depth (cm)	Root Weight*	Nodule Weight†	SNA§	Soil Moisture¶
0-5	7.3	22.8	12.8	8.7
5-10	5.6	61.2	13.4	9.1
10-15	0.7	9.3	8.4	10.1

* Dry weight of roots and rhizomes (g/10.2 x 5 cm of soil core).
† Dry weight of nodules (mg/10.2 x 5 cm of soil core).
§ Specific nitrogenase activity = micromoles C_2H_4 . g nodule dry wt^{-1} . h^{-1}.
¶ Soil moisture as a percentage of soil dry weight.

Seasonal Variation in Nodulation

Rhizoma Peanut in Pure Stands

In pure stands of rhizoma peanut, which were fertilized with 0 and 50 kg ha^{-1} of nitrogen on 4 April, nodule mass fluctuated during the months of April and May without any marked changes. However, application of 100 kg ha^{-1} of N resulted in a decrease in nodule dry weight. In early June, there was a significant ($P<0.05$) increase in nodule mass of the treatments which received 50 kg ha^{-1} of N when compared with stands of the legume fertilized with 100 kg of N ha^{-1}. From July until November the nodule mass of unfertilized stands of rhizoma peanut was significantly ($P<0.05$) higher when compared with the treatments which received N fertilizer, except following defoliation of the legume. Between December 1986 and January 1987, rhizoma peanut fertilized with nitrogen had a slightly lower nodule mass when compared with stands of the legume which did not receive nitrogen. During February of 1987, the nodule dry weight of rhizoma peanut which did not receive N fertilizer in the previous growing season was significantly ($P<0.05$) higher than the nodule dry weight of the stands of the legume which were fertilized with nitrogen. Defoliation of rhizoma peanut on 23 June resulted in a decrease in nodule dry weight of all treatments. Nodule mass of unfertilized stands of rhizoma peanut recovered to preharvest levels within 14 days of defoliation. However, the stands of the legume which were fertilized with 50 to100 kg ha^{-1} of N on 24 June, did not recover their nodule mass until 45 days after defoliation (Table 3 and Fig. 3).

A second defoliation of pure stands of rhizoma peanut on 11 August, had no immediate effect on nodule mass over all levels of fertilization. However, nodule mass of stands of the legume fertilized with 300 kg N ha^{-1} $year^{-1}$declined shaprly on 2 September. Nodule dry weight of unfertilized rhizoma peanut continue to increase reaching its maximum level on 2 September,

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Table 3 -- Seasonal variation in nodule dry weight (g) of Florigraze rhizoma peanut in pure stands, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 March 1987).

Sampling Dates	Nitrogen Level (kg ha^{-1} $year^{-1}$)		
	0	150	300
	g x 10^{-3} . $core^{-1}$		
2 Apr. 1986†	38 a*	41 a	42 a
18 Apr. 1986	45 a	41 a	23 a
5 May 1986	32 a	46 a	18 a
19 May 1986	49 a	33 a	20 a
2 Jun. 1986	40 ab	47 a	11 b
16 Jun. 1986†¶	70 a	45 a	44 a
26 Jun. 1986	37 a	34 a	14 a
10 Jul. 1986	64 a	27 b	18 b
24 Jul. 1986	52 a	17 b	12 b
7 Aug. 1986†¶	65 a	37 b	39 b
19 Aug. 1986	79 a	36 a	41 a
2 Sep. 1986	178 a	49 ab	7 b
16 Sep. 1986	81 a	26 b	12 b
30 Sep. 1986	99 a	26 b	8 b
16 Oct. 1986¶	90 a	14 b	16 b
6 Nov. 1986	69 a	14 b	26 b
9 Dec. 1986	87 a	23 a	27 a
23 Dec. 1986	72 a	41 a	60 a
6 Jan. 1987	32 a	28 a	11 a
20 Jan. 1987	39 a	28 a	8 a
26 Jan. 1987	77 a	38 a	33 a
10 Feb. 1987	75 a	59 ab	22 b
26 Feb. 1987	54 a	39 ab	23 b
26 Mar. 1987	89 a	54 a	49 a

* Means in the same row followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Application of nitrogen fertilizer on 4 Apr., 24 Jun., and 12 Aug. 1986.

¶ Plants were defoliated on 23 Jun., 11 Aug., and 21 Oct. 1986.

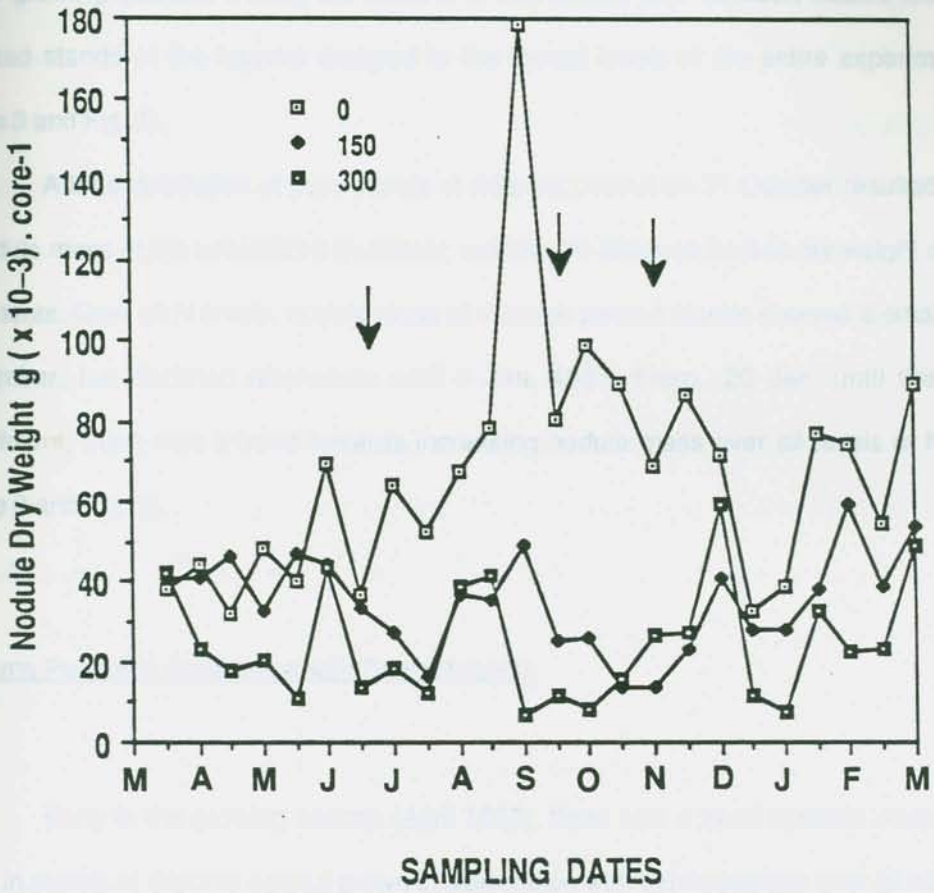

Fig. 3 - Seasonal variation in nodule dry weight (g) of Florigraze rhizoma peanut in pure stands fertilized with 0, 150, and 300 kf of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987). Arrow indicates defoliation followed by N fertilization.

with a sharp decline thereafter. Throughout the month of August, nodule dry weight of the treatments which received N fertilization remained at levels comparable to those at the beginning of the growing season. During the months of September and October, nodule mass of the N fertilized stands of the legume dropped to the lowest levels of the entire experimental period (Table 3 and Fig. 3).

A third defoliation of pure stands of rhizoma peanut on 21 October resulted in a decline of nodule mass of the unfertilized treatment, but had no effect on nodule dry weight of N fertilized treatments. Over all N levels, nodule mass of rhizoma peanut stands showed a small recovery in December, but declined afterwards until 6 Jan. 1987. From 20 Jan. until the end of the experiment, there was a trend towards increasing nodule mass over all levels of N fertilization (Table 3 and Fig. 3).

Rhizoma Peanut in Association with Bermudagrass

Early in the growing season (April 1986), there was a trend towards increasing nodule mass in stands of rhizoma peanut grown in association with bermudagrass over all nitrogen levels. There was a decline in nodule dry weight of all treatments at the beginning of May, but nodule mass of unfertilized stands recovered shortly after. However, treatments which received 50 and 100 kg of N ha^{-1} on 4 April, continued to show a decrease in nodule dry weight until 45 and 59 days after fertilization, respectively. Nodule mass of unfertilized stands of rhizoma peanut associated with bermudagrass decreased on 16 June. Defoliation of the grass-legume associations on 23 June resulted in a sharp decrease in nodule dry weight of rhizoma peanut stands with no N fertilization. However, defoliation had no negative effect in the nodule mass of N fertilized rhizoma peanut. Nodule dry weight of these treatments continued to increase until the end of July and beginning of August, decreasing thereafter. Nodule mass of unfertilized stands of

the legume grown in association with bermudagrass was recovered within 14 to 28 days after defoliation, but decreased markedly afterwards (Table 4 and Fig. 4).

A second defoliation of the grass-legume associations on 11 August, had no immediate effect on nodule mass of unfertilized rhizoma peanut. However, nodule dry weight increased sharply thereafter, reaching its maximum level of the entire experimental period on 30 September. A third defoliation of the legume-grass associations on 21 October, reflected in a marked decrease in nodule mass of the unfertilized treatment, but had no consistent effect on treatments which received nitrogen. Nodule dry weight of the legume in associations which did not receive nitrogen continued to decline until it reached its lowest level on 6 Jan. 1987. The treatments which were fertilized with N continued to show low nodule dry weight throughout the fall of 1986 and most of the winter season of 1987. There was a slight trend towards increasing nodule mass from 20 January until the end of the experiment on 26 Mar. 1987 (Table 4 and Fig. 4).

Throughout most of the experimental period, nodule mass of unfertilized stands of rhizoma peanut-bermudagrass associations was consistently higher than in treatments which were fertilized with nitrogen. During the later part of the winter and early in the spring of 1987, nodule dry weight from the unfertilized treatment was also slightly, but consistently higher than that from the fertilized treatment (Fig. 4).

Seasonal Variation in Nitrogenase Activity

Rhizoma Peanut in Pure Stands

At the beginning of the experiment, specific nitrogenase activity of pure stands of rhizoma peanut was low over all levels of N fertilization. However, it was significantly ($P<0.05$) higher in the treatment that received 300 kg of N ha^{-1} in the previous growing season than in the

Table 4 -- Seasonal variation in nodule dry weight (g) of Florigraze rhizoma peanut in association with bermudagrass, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987).

Sampling Dates	Nitrogen Level (kg ha^{-1} $year^{-1}$)		
	0	150	300
	g x 10^{-3} . $core^{-1}$		
2 Apr. 1986†	44 a*	28 a	29 a
18 Apr. 1986	60 a	54 a	47 a
5 May 1986	39 a	22 a	44 a
19 May 1986	51 a	17 a	29 a
2 Jun. 1986	94 a	32 a	17 a
16 Jun. 1986†¶	75 a	40 a	18 a
26 Jun. 1986	45 a	41 a	22 a
10 Jul. 1986	74 a	41 a	40 a
24 Jul. 1986	92 a	58 a	47 a
7 Aug. 1986†¶	38 a	35 a	56 a
19 Aug. 1986	38 a	38 a	39 a
2 Sep. 1986	73 a	36 a	10 a
16 Sep. 1986	53 a	21 a	12 a
30 Sep. 1986	121 a	20 a	20 a
16 Oct. 1986¶	120 a	33 a	8 a
6 Nov. 1986	73 a	20 a	9 a
9 Dec. 1986	73 a	18 a	28 a
23 Dec. 1986	69 a	24 a	12 a
6 Jan. 1987	15 a	23 a	20 a
20 Jan. 1987	60 a	19 a	21 a
26 Jan. 1987	43 a	28 a	19 a
10 Feb. 1987	62 a	32 a	38 a
26 Feb. 1987	64 a	18 a	54 a
26 Mar. 1987	69 a	42 a	21 a

* Means in the same row followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Application of nitrogen fertilizer on 4 Apr., 24 Jun., and 12 Aug. 1986.

¶ Plants were defoliated on 23 Jun., 11 Aug., and 21 Oct. 1986.

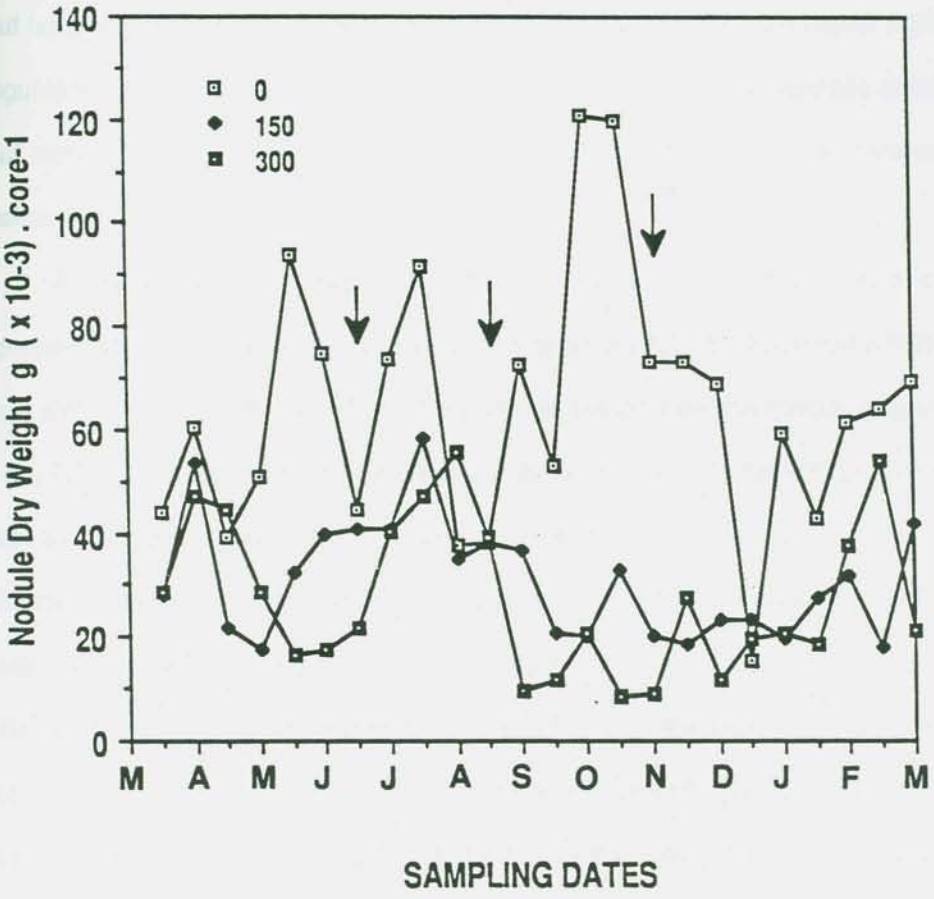

Fig. 4 - Seasonal variation in nodule dry weight (g) of Florigraze rhizoma peanut in association with bermudagrass, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987). Arrow indicates defoliation followed by N fertilization.

treatment which did not receive N fertilizer. In early May, there was a small decline followed by a sharp rise in rates of nitrogenase activity of N fertilized rhizoma peanut, reaching its maximum level for the entire experimental period on 16 June. On 19 May, rates of nitrogenase activity of rhizoma peanut fertilized with 300 kg of N ha^{-1} $year^{-1}$ was significantly ($P<0.05$) higher than in stands of the legume which received 0 and 150 kg of N ha^{-1} $year^{-1}$. Rates of nitrogenase activity of rhizoma peanut with no N fertilizer reached a peak at the beginning of June and started to decline thereafter (Table 5 and Fig. 5).

A defoliation of the legume on 23 June resulted in a sharp decrease in specific nitrogenase activity of all treatments. Rates of nitrogenase activity recovered within 14 and 28 to 42 days after defoliation for unfertilized and fertilized rhizoma peanut stands, respectively. On 10 July, the rates of nitrogenase activity were significantly ($P<0.05$) higher in unfertilized rhizoma peanut than in the treatments which received N fertilization. A second defoliation on 11 August had the most marked effect on specific nitrogenase activity of all treatments, reducing rates equal or below 0.5 micromoles C_2H_4 . h^{-1} . g nodule dry wt^{-1}. Specific nitrogenase activity of unfertilized rhizoma peanut recovered within 14 to 28 days after defoliation, reaching the highest rate for the entire growing season on 16 September. Specific nitrogenase activity of rhizoma peanut fertilized with 150 kg of N ha^{-1} $year^{-1}$ followed a similar pattern rising to rates comparable to those achieved earlier in the growing season. However, specific nitrogenase activity of rhizoma peanut which received 300 kg of N ha^{-1} $year^{-1}$ was slow to recover and did not rise to the levels achieved earlier in the season. On 30 September, rates of nitrogenase activity of the treatments which were fertilized with 0 and 150 kg of N ha^{-1} $year^{-1}$ declined markedly, but recovered thereafter (Table 5 and Fig. 5).

A third defoliation of pure stands of the legume resulted in the decline of specific nitrogenase activity of all treatments. However, the rates of nitrogenase activity did not recover following defoliation, with the exception of a small increase in the treatment which received 300 kg of N ha^{-1} $year^{-1}$. Specific nitrogenase activity continued to decline towards the end of the fall of 1986 until it reached levels close to zero at the end of January and early February 1987.

Table 5 -- Seasonal variation in rates of nitrogenase activity of Florigraze rhizoma peanut in pure stands, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987).

Sampling Dates	Nitrogen Level (kg ha^{-1} $year^{-1}$)		
	0	150	300
	— micromoles C_2H_4 . h^{-1} . g nodule dry wt^{-1} —		
2 Apr. 1986†	0.1 b*	0.2 b	0.6 a
18 Apr. 1986	0.8 b	1.1 ab	1.6 a
5 May 1986	0.8 a	0.5 a	0.8 a
19 May 1986	2.2 b	1.9 c	2.8 a
2 Jun. 1986	2.6 a	3.8 a	2.0 a
16 Jun. 1986†¶	2.3 a	4.8 a	5.0 a
26 Jun. 1986	0.5 a	0.6 a	0.6 a
10 Jul. 1986	5.2 a	1.5 b	2.0 b
24 Jul. 1986	3.9 a	3.5 a	4.2 a
7 Aug. 1986†¶	3.9 a	4.3 a	3.0 a
19 Aug. 1986	0.5 a	0.2 a	0.2 a
2 Sep. 1986	2.2 a	0.7 a	0.4 a
16 Sep. 1986	6.9 a	4.6 a	0.4 a
30 Sep. 1986	1.9 a	2.1 a	1.0 a
16 Oct. 1986¶	3.8 a	4.4 a	2.7 a
6 Nov. 1986	2.4 a	3.8 a	1.8 a
9 Dec. 1986	1.7 a	3.3 a	2.7 a
23 Dec. 1986	1.2 b	2.1 ab	2.8 a
6 Jan. 1987	0.5 a	0.7 a	0.6 a
20 Jan. 1987	0.4 b	0.8 a	0.5 ab
26 Jan. 1987	0.1 a	0.5 a	0.2 a
10 Feb. 1987	0.1 a	0.2 a	0.3 a
26 Feb. 1987	0.8 a	1.0 a	2.3 a
26 Mar. 1987	1.2 a	1.4 a	1.8 a

* Means in the same row followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Application of nitrogen fertilizer on 4 Apr., 24 Jun., and 12 Aug. 1986.

¶ Plants were defoliated on 23 Jun., 11 Aug., and 21 Oct. 1986.

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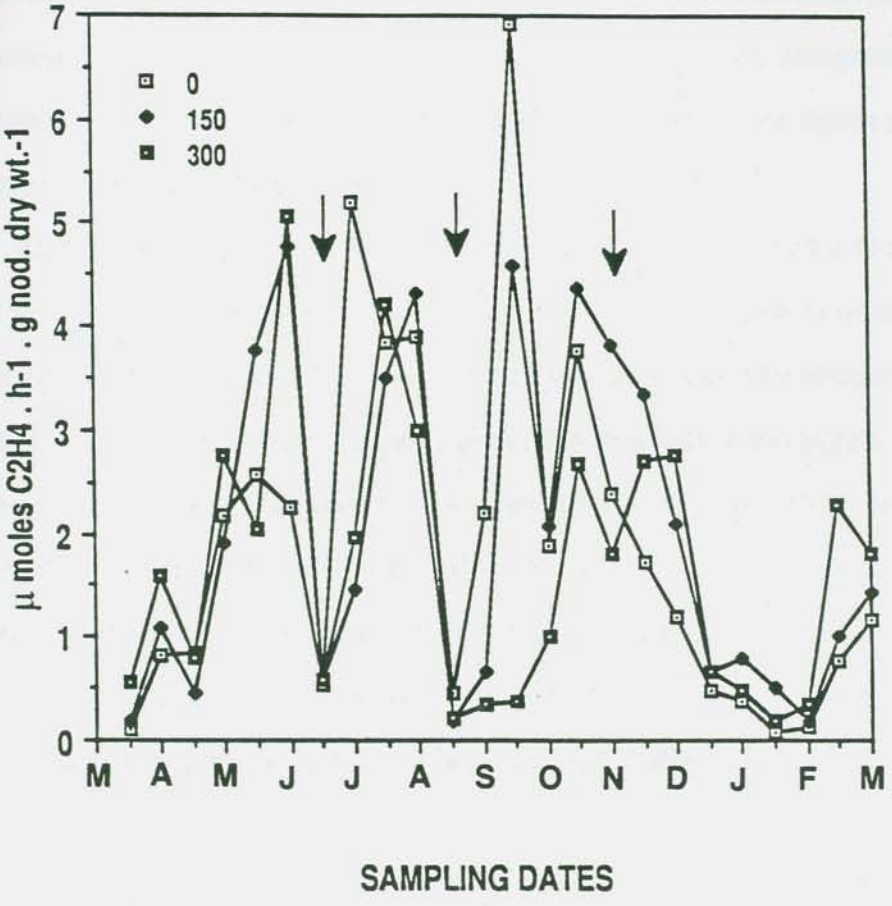

Fig. 5 - Seasonal variation in rates of nitrogenase activity of Florigraze rhizoma peanut in pure stands, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987). Arrow indicates defoliation followed by N fertilization.

Thereafter, a trend towards increasing rates of nitrogenase activity was observed until the end of the experiment on 26 March. Later in the fall of 1986 and during the winter and early spring of 1987, there was a slight superiority in rates of nitrogenase activity of N fertilized rhizoma peanuts when compared with unfertilized stands. On 23 December, specific nitrogenase activity of unfertilized rhizoma peanut was significantly ($P<0.05$) lower than in the stands of the legume which were fertilized with 300 kg of N ha^{-1} $year^{-1}$ (Table 5 and Fig. 5).

Early in the spring of 1986, total nitrogenase activity (activity in 10.2 x 15 cm of the core sample) was significantly ($P<0.05$) higher in stands of rhizoma peanut which were fertilized with 300 kg of N in the previous growing season. From June to November, total nitrogenase activity of unfertilized rhizoma peanut was significantly ($P<0.05$) higher than in the stands of the legume which received N fertilization, except on 19 Aug. and 30 Sep. On 6 Jan. 1987, total nitrogenase activity of rhizoma peanut fertilized with 150 kg of N in the previous growing season was significantly ($P<0.05$) higher than in stands of the legume which were supplied with 300 kg of N. Over all levels of N fertilization, there was no difference in total nitrogenase activity of pure stands of rhizoma peanut during late winter and early spring of 1987 (Table 6 and Fig. 6).

Rhizoma Peanut in Association with Bermudagrass

Early in the growing season, specific nitrogenase activity of rhizoma peanut grown in association with bermudagrass over all nitrogen levels was low (below 1.0 micromole C_2H_4 . h^{-1} . g nodule dry wt^{-1}), but showed a trend towards increasing rates as the season progressed. There was a small decline in rates of nitrogenase activity of fertilized stands at the beginning of May, followed by a consistent rise. Specific nitrogenase activity of stands of the legume associated with the grass, fertilized with 300 kg of N ha^{-1} $year^{-1}$, reached a peak on 19 May, while rates of stands which received 0 and 150 kg of N ha^{-1} $year^{-1}$ peaked on 16 June (Table 7 and Fig. 7).

Table 6 -- Seasonal variation in total nitrogenase activity of Florigraze rhizoma peanut in pure stands, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987).

Sampling Dates	Nitrogen Level (kg ha^{-1} $year^{-1}$)		
	0	150	300
	nmoles C_2H_4 . h^{-1} . $core^{-1}$		
2 Apr. 1986†	4 b*	10 ab	18 a
18 Apr. 1986	37 a	45 a	60 a
5 May 1986	34 a	21 a	14 a
19 May 1986	107 a	62 a	55 a
2 Jun. 1986	109 a	182 a	25 a
16 Jun. 1986†¶	178 a	180 a	160 a
26 Jun. 1986	13 ab	31 a	6 b
10 Jul. 1986	334 a	37 b	42 b
24 Jul. 1986	200 a	55 b	57 b
7 Aug. 1986†¶	250 a	150 b	111 b
19 Aug. 1986	27 a	8 a	6 a
2 Sep. 1986	267 a	34 b	2 b
16 Sep. 1986	565 a	86 b	5 b
30 Sep. 1986	177 a	67 ab	10 b
16 Oct. 1986¶	338 a	53 b	48 b
6 Nov. 1986	159 a	47 b	23 b
9 Dec. 1986	176 a	57 a	70 a
23 Dec. 1986	85 a	85 a	136 a
6 Jan. 1987	15 ab	18 a	7 b
20 Jan. 1987	14 a	26 a	4 a
26 Jan. 1987	5 a	6 a	4 a
10 Feb. 1987	10 a	12 a	9 a
26 Feb. 1987	38 a	35 a	58 a
26 Mar. 1987	120 a	78 a	53 a

* Means in the same row followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Application of nitrogen fertilizer on 4 Apr., 24 Jun., and 12 Aug. 1986.

¶ Plants were defoliated on 23 Jun., 11 Aug., and 21 Oct. 1986.

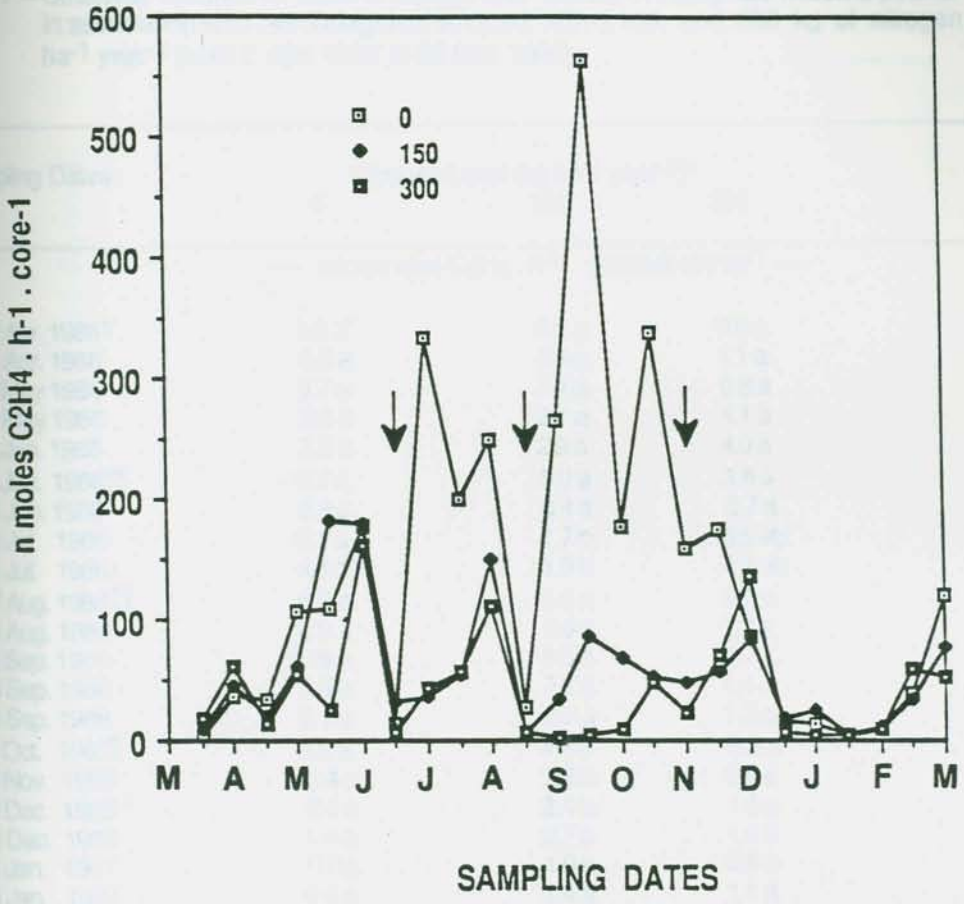

Fig. 6 - Seasonal variation in total nitrogenase activity of Florigraze rhizoma peanut in pure stands, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987). Arrow indicates defoliation followed by N fertilization.

Table 7 -- Seasonal variation in rates of nitrogenase activity of Florigraze rhizoma peanut in association with bermudagrass, fertilized with 0,150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987).

Sampling Dates	Nitrogen Level (kg ha^{-1} $year^{-1}$)		
	0	150	300
	micromoles C_2H_4 . h^{-1} . g nodule dry wt^{-1}		
2 Apr. 1986†	0.2 a*	0.2 a	0.6 a
18 Apr. 1986	0.5 a	0.8 a	1.1 a
5 May 1986	0.7 a	0.7 a	0.8 a
19 May 1986	2.3 a	2.1 a	4.1 a
2 Jun. 1986	3.5 a	2.9 a	4.0 a
16 Jun. 1986†¶	3.7 a	5.0 a	3.6 a
26 Jun. 1986	0.4 a	0.4 a	0.7 a
10 Jul. 1986	8.1 a	1.7 b	6.5 ab
24 Jul. 1986	4.4 a	1.9 b	4.2 ab
7 Aug. 1986†¶	6.2 a	5.8 a	2.8 a
19 Aug. 1986	0.9 a	0.5 a	0.2 a
2 Sep. 1986	10.9 a	8.5 a	0.7 a
16 Sep. 1986	5.6 a	7.2 a	1.4 a
30 Sep. 1986	2.1 a	2.9 a	1.2 a
16 Oct. 1986¶	3.0 a	4.8 a	3.9 a
6 Nov. 1986	2.4 a	2.8 a	8.0 a
9 Dec. 1986	2.0 a	2.4 a	1.6 a
23 Dec. 1986	1.4 a	3.7 a	1.4 a
6 Jan. 1987	0.6 a	1.0 a	0.8 a
20 Jan. 1987	0.6 a	1.4 a	1.1 a
26 Jan. 1987	0.2 a	0.3 a	0.4 a
10 Feb. 1987	0.2 a	0.3 a	0.3 a
26 Feb. 1987	1.1 a	7.0 a	0.7 a
26 Mar. 1987	1.7 a	2.3 a	4.4 a

* Means in the same row followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Application of nitrogen fertilizer on 4 Apr., 24 Jun., and 12 Aug. 1986.

¶ Plants were defoliated on 23 Jun., 11 Aug., and 21 Oct. 1986.

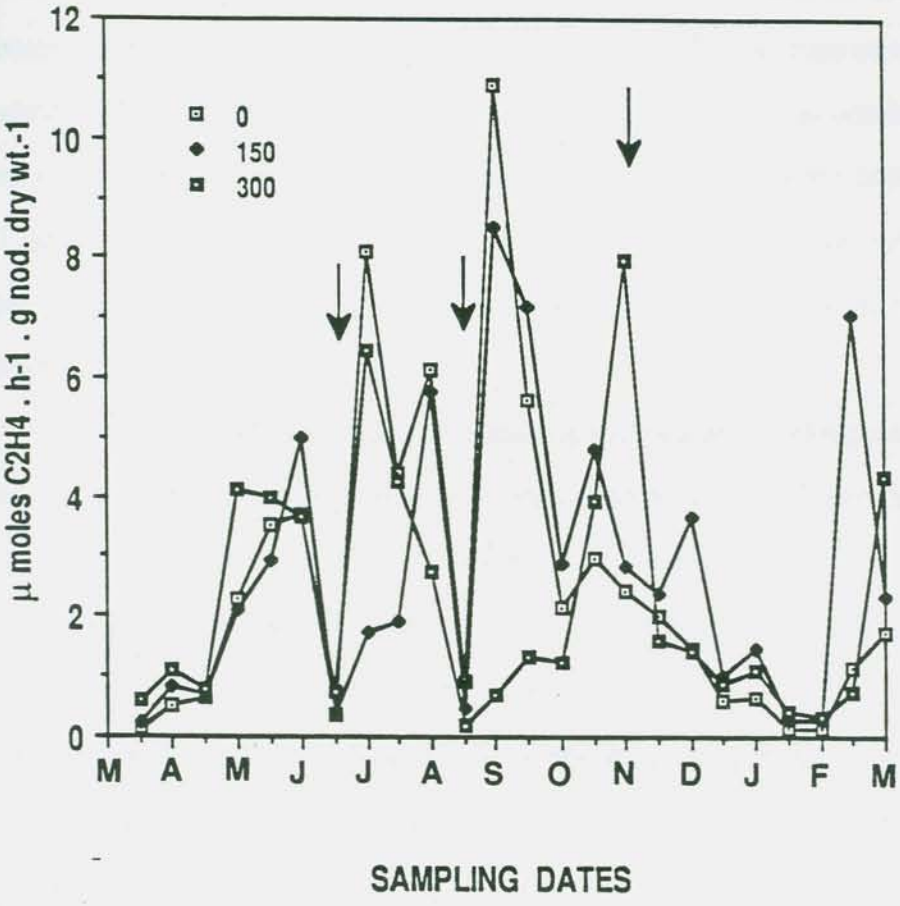

Fig. 7 - Seasonal variation in rates of nitrogenase activity of Florigraze rhizoma peanut in association with bermudagrass, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987). Arrow indicates defoliation followed by N fertilization.

A first defoliation of the legume-grass associations on 23 June resulted in a sharp decline in rates of nitrogenase activity of all treatments. Specific nitrogenase activity of treatments which received 0 and 300 kg of N ha^{-1} $year^{-1}$ recovered within 14 days, while the rates of the treatments fertilized with 150 kg of N ha^{-1} $year^{-1}$ did not recover until 42 days after defoliation and were significantly ($P<0.05$) lower when compared with rates of nitrogenase activity of unfertilized rhizoma peanut. Specific nitrogenase activity of stands fertilized with 0 and 300 kg of N ha^{-1} $year^{-1}$ reached a second peak in early July, decreasing thereafter. However, while the rates of nitrogenase activity of the former recovered afterwards, the latter continued to decline until the beginning of August (Table 7 and Fig. 7).

A second defoliation on 11 August drastically reduced rates of nitrogenase activity of all treatments. Specific nitrogenase activity of the stands which received 0 and 150 kg of N ha^{-1} $year^{-1}$ recovered within 14 days after defoliation, but declined sharply thereafter. Rates of nitrogenase activity of stands which received 300 kg of N ha^{-1} $year^{-1}$ were slow to recover, rising to levels comparable to those achieved earlier in the growing season only at the beginning of November. A third defoliation on 21 October resulted in a small decrease in rates of nitrogenase activity of the treatments which were fertilized with 0 and 150 kg of N ha^{-1} $year^{-1}$, but had no effect on stands fertilized with 300 kg of N ha^{-1} $year^{-1}$ (Table 7 and Fig. 7).

After the third defoliation of the stands, even though there were some cycles of rise and decline, there was a consistent decrease in rates of nitrogenase activity of all treatments until they reached their lowest level of the entire experimental period in late January early February of 1987. Thereafter, there was a trend towards increasing rates of nitrogenase activity until the end of the experiment at the end of March, except for a sharp decrease in specific nitrogenase activity of the treatment which received 150 kg of N ha^{-1} in the previous growing season (Table 7 and Fig. 7).

Specific nitrogenase activity was slightly higher in the fertilized treatments during early spring, most of the fall of 1986 and winter and early spring of 1987. During the summer, rates of nitrogenase activity of the unfertilized treatment was only slightly higher than those of the treatments which received nitrogen fertilizer (Table 7). On 5 May, total nitrogenase activity was

significantly ($P<0.05$) higher in stands of rhizoma peanut fertilized with 300 kg of N ha^{-1} $year^{-1}$ than in unfertilized stands of the legume. On 30 Sep., total nitrogenase activity was significantly ($P<0.05$) higher in unfertilized stands of rhizoma peanut than in the treatments which received N fertilization. Over all levels of N fertilization, there was no difference in total nitrogenase activity (activity in the 10.2 x 15 cm of the core sample) of stands of rhizoma peanut grown in association with bermudagrass in early spring of 1986 and winter and early spring of 1987 (Table 8 and Fig. 8).

Discussion

Effect of Temperature on Nodulation and Nitrogenase Activity

When 'Florigraze' rhizoma peanut was grown in pure stands and in association with bermudagrass, with no N fertilizer applied, nitrogenase activity followed a pattern similar to that of soil temperature, except when drought stress and defoliation reduced the activity of the enzyme. At the beginning of the experiment, soil temperature (Fig. 1) appeared to be limiting nodulation and nitrogenase activity of rhizoma peanut, both in pure stands and in associations, over all levels of nitrogen fertilization.

Nodule dry weight and rates of nitrogenase activity increased and reached the highest levels for the entire growing season as soil temperature increased to 28 to 30°C in late-spring and during the summer(Figs. 3 to 8). Gibson (1977, 1980), in reviews of the effects of root temperature on nitrogen fixation, observed that the optimum day and night root temperatures for N_2 fixation by tropical legumes seem to be in the range of 28 to 32°C and 23 to 25°C, respectively.

Saldivar (1983), in a growth analysis of rhizoma peanut, observed that little shoot development occurred early in the growing season. Shoots presented a growth curve

Table 8 -- Seasonal variation in total nitrogenase activity of Florigraze rhizoma peanut in association with bermudagrass, fertilized with 0,150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987).

Sampling Dates	Nitrogen Level (kg ha^{-1} $year^{-1}$)		
	0	150	300
	nmoles C_2H_4 . h^{-1} . $core^{-1}$		
2 Apr. 1986†	4 a*	9 a	5 a
18 Apr. 1986	32 a	39 a	77 a
5 May 1986	14 b	25 ab	48 a
19 May 1986	79 a	64 a	90 a
2 Jun. 1986	270 a	100 a	70 a
16 Jun. 1986†¶	254 a	209 a	112 a
26 Jun. 1986	6 a	3 a	5 a
10 Jul. 1986	511 a	132 a	148 a
24 Jul. 1986	341 a	202 a	221 a
7 Aug. 1986†¶	226 a	214 a	296 a
19 Aug. 1986	33 a	20 a	8 a
2 Sep. 1986	546 a	43 a	2 a
16 Sep. 1986	283 a	110 a	12 a
30 Sep. 1986	129 a	54 b	13 b
16 Oct. 1986¶	431 a	157 a	31 a
6 Nov. 1986	200 a	32 a	14 a
9 Dec. 1986	134 a	44 a	59 a
23 Dec. 1986	103 a	57 a	18 a
6 Jan. 1987	8 a	16 a	11 a
20 Jan. 1987	26 a	13 a	18 a
26 Jan. 1987	6 a	8 a	7 a
10 Feb. 1987	10 a	9 a	14 a
26 Feb. 1987	42 a	50 a	33 a
26 Mar. 1987	138 a	130 a	112 a

* Means in the same row followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Application of nitrogen fertilizer on 4 Apr., 24 Jun., and 12 Aug. 1986.

¶ Plants were defoliated on 23 Jun., 11 Aug., and 21 Oct. 1986.

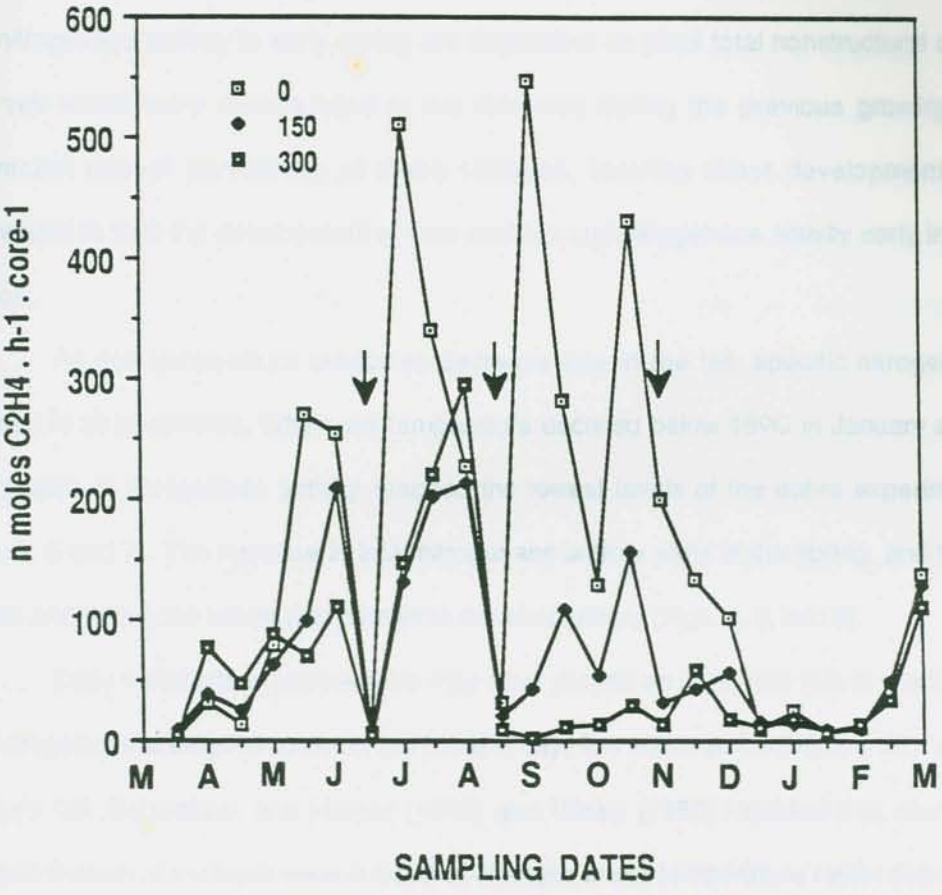

Fig. 8 - Seasonal variation in total nitrogenase activity of Florigraze rhizoma peanut in association with bermudagrass, fertilized with 0, 150, and 300 kg of nitrogen ha^{-1} $year^{-1}$ (from 2 Apr. 1986 to 26 Mar. 1987). Arrow indicates defoliation followed by N fertilization.

characterized by a rapid increase in growth from June to September, followed by a decrease thereafter, as air temperature started to decline. Shoot and root growth, nodule development, and nitrogenase activity in early spring are dependent on plant total nonstructural carbohydrate reserves which were accumulated in the rhizomes during the previous growing season. A differential rate of partitioning of these reserves, favoring shoot development, may have contributed to limit the development of new nodules and nitrogenase activity early in the growing season.

As soil temperature started to decrease late in the fall, specific nitrogenase activity declined in all treatments. When soil temperature declined below 15°C in January and February 1987, rates of nitrogenase activity reached the lowest levels of the entire experimental period (Figs. 1, 5 and 7). The increase in total nitrogenase activity early in the spring and the decline in late fall and during the winter was parallel to soil temperature (Figs. 1, 6, and 8).

Daily variations in temperature may have played an important role in limiting nodulation and nitrogenase activity. In north-central Florida, days are warm and nights are cool in early spring and late fall. Schweitzer and Harper (1980) and Weisz (1986) reported that diurnal cycles in nitrogen fixation of soybean were a result of changes in soil temperature rather than a function of daily fluctuations in photosynthetic active radiation. Changes in soil temperature also reflected in proportional changes in nodule gas permeability such that a linear correlation between temperature and acetylene reduction rates was maintained. Weisz (1986) concluded that the change in gas permeability associated with soil temperature was likely the result of an active mechanism regulating turgor pressure in the cortical cells.

Nodulation of rhizoma peanut in pure stands, over all nitrogen levels, and of unfertilized stands of the legume in association with bermudagrass was moderately affected by the decrease in soil temperature from 28 to 30°C, to 20°C in the fall. However, reduced soil temperatures had no marked effect on nodulation of fertilized stands of the rhizoma peanut in association with bermudagrass. Nodule dry weight of rhizoma peanut declined gradually until the first week of January 1987, when soil temperature reached 13°C. Thereafter, there was a tendency towards

the development of new nodules, resulting in increased nodule mass until the end of the experiment, even though soil temperature declined to 12ºC in February.

Saldivar (1983) reported decreasing rates of shoot growth as opposed to increasing rates of rhizome growth in the fall, suggesting that photosynthate was being translocated to the rhizomes. The cessation of growth in the shoots with concurrent growth in the rhizomes led to the conclusion that even though air temperature was affecting shoot formation, soil temperature was not affecting rhizome formation. The existence of a lag time between the decline in air temperature and the corresponding decline in soil temperature, associated with the accumulation of nonstructural carbohydrates in the rhizomes towards the end of the growing season, may have contributed to maintainance of a nodule mass in the root system of the legume during the cool season, even though the rate of nitrogenase activity was drastically reduced in February 1987, when minimum soil and air temperatures reached 10ºC and -6ºC, respectively (Table 1-A). The existing nodule mass may play an critical role in supplying plants with symbiotically fixed nitrogen during early shoot development at the beginning of the growing season and until an effective photosynthetic apparatus and fixation system are established. This is especially important at the beginning of the growing season when remobilization of the carbohydrate reserves are directed preferentially towards the development of the photosynthetic capacity of the plants. It has been reported that nitrogen concentration in the rhizomes of Florigraze rhizoma peanut declined at the beginning of the growing season, during a period of rapid growth, and increased towards the end of the year, as plant growth was limited by low temperatures (Saldivar, 1983).

Nitrogen fixation and accumulation during the cool season (fall and winter), even at reduced rates of nitrogenase activity, may contribute to increase the level of nitrogenous compounds which are available for regrowth at the beginning of the growing season. However, in north-central Florida the occurrence of drought stress during spring and early summer and low soil and air temperatures during the fall and winter of the previous growing season hamper the capacity of the symbiotic fixation system to supply nitrogenous compounds in the amounts required for growth and accumulation in the root system. This would explain the occurrence of

nitrogen deficiency early in the growing season in establishing stands of rhizoma peanut and established stands subjected to temperature and water stress.

Effect of Soil Moisture on Nodulation and Nitrogenase Activity

This experiment was conducted on sandy soils which often dry rapidly in the surface layers. It was observed that approximately 95% of the roots and rhizomes and 90% of the nodules of rhizoma peanut were located within 10 cm of soil surface (Table 2). This distribution of rhizoma peanut nodules in the soil profile made nodulation and nitrogenase activity more susceptible to the fluctuations in soil moisture which occurred throughout the experimental period.

Soil moisture reached the lowest level of the entire experimental period on 19 May 1986 (Fig. 2). However, the drought stress to which rhizoma peanut was subjected at that time did not have a marked effect on nodulation and nitrogenase activity, since both processes had been limited by low soil temperatures (Figs. 3 to 8). The effects of a second period of drought stress experienced by rhizoma peanut in late June and early July were confounded with the effects of defoliation. On 30 September, as soil moisture declined below 3.5%, there was a drastic decrease in specific nitrogenase activity of rhizoma peanut in pure stands and in association with bermudagrass fertilized with 0 and 150 kg of N ha^{-1} $year^{-1}$. However, it had no effect on specific nitrogenase activity of both pure stands and associations fertilized with 300 kg of N ha^{-1} $year^{-1}$ (Figs. 5 and 7). Reduced soil moisture at this time had no effect on nodulation of rhizoma peanut (Figs. 6 and 8). This occurred as a compound effect of declining soil and air temperatures, previous defoliations, and nitrogen fertilizer application which inhibited nodule development and stimulated nodule senescence.

It has been reported in the literature that nodules can control oxygen diffusion into their tissue (Minchin et al.,1983a) and that changes in the environment can result in adjustment to the

magnitude of this resistance (Minchin et al., 1985b, 1986). Durand et al. (1987) suggested that water stress exerts an influence on nitrogenase activity which is independent of the rate of photosynthesis; it acts directly on nodule activity through increases in the resistance to oxygen diffusion to the bacteroids. Weisz (1986) observed that nodule gas permeability appears to play an active and essential role not only in protecting nitrogenase from oxygen inactivation, but also in controlling the rate of symbiotic nitrogen fixation in legumes.

The decline in specific nitrogenase activity of Florigraze rhizoma peanut under drought stress seems to corroborate the observations and the hypothesis of Pankhurst and Sprent (1975a). According to that hypothesis, the decline in specific nitrogenase activity of rhizoma peanut under drought-stressed conditions is associated with the closure of the air spaces in the lenticels as the cells of the outer cortex lose turgor. This would increase the resistance to oxygen diffusion, and inhibit nitrogen fixation due to reduced oxidative phosphorylation. Weisz et al. (1985) obtained similar results with drought stressed soybean nodules.

There was a varied response of nodulation and specific nitrogenase activity of rhizoma peanut to periods of drought stress during the growing season. The data suggest that this was due to interactions with soil and air temperatures, stage and rate of plant growth, carbohydrate reserves, and the level of plant dependence on symbiotic nitrogen fixation versus combined nitrogen.

Effect of Combined Nitrogen on Nodulation and Nitrogenase Activity

Application of nitrogen (150 to 300 kg N ha^{-1} $year^{-1}$) in the form of NH_4N0_3 severely restricted nodule formation of Florigraze rhizoma peanut in pure stands throughout most of the summer and fall of 1986 and late winter of 1987, resulting in a nodule mass equal or lower than that present at the beginning of the growing season (Fig. 3). This is in agreement with results

obtained by Dart and Wildon (1970) and Rys and Phung (1984) who stated that nodule formation is severely inhibited by the application of ammonium nitrate. The presence of ammonium nitrate in the rhizosphere also delayed the recovery of the nodule mass following defoliation and application of N fertilizer. Application of nitrogen fertilizer to stands of rhizoma peanut in association with bermudagrass resulted in a moderate, but not significant decline in nodulation as compared with unfertilized stands (Fig 4). Nitrogen uptake by bermudagrass in association reduced the effect of nitrogen on nodulation of the legume.

Nitrogen fertilizer application in pure stands of rhizoma peanut resulted in a moderate increase in specific nitrogenase activity during the spring and early summer. However, there was no difference in rates of nitrogenase activity of N fertilized associations of rhizoma peanut with bermudagrass during the same period (Figs. 5 and 7). During the summer, rates of nitrogenase activity of unfertilized stands of rhizoma peanut, both in pure stands and in association with bermudagrass, tended to be higher when compared with nitrogen fertilized stands (Figs. 5 and 7). However, total nitrogenase activity was markedly higher in unfertilized stands of rhizoma peanut than in N fertilized treatments (Figs. 6 and 8).

Following defoliation, rates of nitrogenase activity of unfertilized stands of rhizoma peanut in pure stands and in association increased faster and reached a higher peak as compared with N fertilized stands. Following the second defoliation on 11 August, the recovery of rates of nitrogenase activity was markedly delayed in pure stands of rhizoma peanut and in associations with bermudagrass fertilized with 300 kg of N ha^{-1} $year^{-1}$. In the fall, specific nitrogenase activity of stands of rhizoma peanut was moderately increased by nitrogen fertilization (Figs. 5 and 7).

The stimulatory effect of nitrogen fertilization on rates of nitrogenase activity of rhizoma peanut in pure stands during the spring, fall, and winter seasons seems to indicate the existence of other strong sinks (shoot, root, and rhizome growth) competing for photosynthate and carbohydrate reserves stored in the rhizomes. Since the application of nitrogen does not seem to affect the existing nodule mass, even though it severely inhibits formation of new nodules during the summer, it stimulates photosynthesis early in the growing season, increasing the availability of

photosynthate for plant growth and nitrogen fixation. In legume-grass associations the effect of nitrogen in stimulating growth and nitrogen fixation by rhizoma peanut early in the growing season is drastically reduced due to more efficient uptake of fertilizer N by bermudagrass. The inhibition of rhizoma peanut nodulation during the summer by nitrogen fertilizer is also drastically minimized due to nitrogen uptake by bermudagrass in association with the legume.

The early advantage of N-fertilized stands of rhizoma peanut was short-lived and was more than compensated for by increased nodule formation and higher rates of nitrogenase activity in unfertilized stands of the legume later in the growing season. However, in establishing stands of rhizoma peanut and under conditions of moderate drought stress, which are common in north-central Florida, an application of nitrogen fertilizer early in the growing season may result in a significant increase in rate of establishment and dry matter yield. This hypothesis is corroborated by the data of Valentim (1985) who observed that N application resulted in an increase in dry matter yield and reduced the establishment period of a pure stand of rhizoma peanut.

Effect of Defoliation on Nodulation and Nitrogenase Activity

Defoliation of Florigraze rhizoma peanut in pure stands on 23 June resulted in nodule senescence and decay. The decrease in nodule dry weight was more severe in the treatments fertilized with 0 and 300 kg of N ha^{-1} $year^{-1}$, reducing nodule mass to levels comparable to those present at the beginning of the growing season (Fig. 3). In stands of rhizoma peanut associated with bermudagrass this first defoliation drastically reduced nodule mass of the unfertilized treatment (Fig. 4). However, it had no effect on nitrogen fertilized treatments in which nodule formation had already been inhibited by the combined nitrogen. A second defoliation on 11 August had no marked effect on nodule mass of rhizoma peanut in pure stands and in associations over all levels of nitrogen fertilization. A third defoliation on 21 October resulted in

severe and moderate reductions of nodule mass of unfertilized stands of rhizoma peanut in association with bermudagrass and in pure stands, respectively. However, it had no effect on nodule dry weight of N-fertilized treatments (Figs. 3 and 4).

Specific nitrogenase activity of Florigraze rhizoma peanut in pure stands and in associations over all N levels was severely reduced by defoliations on 23 June and 11 August. A third defoliation on 21 October resulted in a moderate decrease in rates of nitrogenase activity of rhizoma peanut in pure stands over all levels of nitrogen fertilization and in the associations with bermudagrass which were fertilized with 0 and 150 kg of N ha^{-1} $year^{-1}$. However, it had no effect on specific nitrogenase activity of the legume-grass associations which received 300 kg of N ha^{-1} $year^{-1}$ (Figs. 5 and 7).

Defoliation results in the removal of the main source of photosynthate necessary for continued nodule function and development of new nodules. Gibson (1976, 1977) observed that the ability of the nodules to survive depends on the severity of defoliation and the carbohydrate reserves that the plant can remobilize to produce new leaves and supply the nodules. Saldivar (1983) reported that carbohydrate reserves in the rhizomes of rhizoma peanut decreased at the beginning of the growing season as they were remobilized for shoot, root, rhizome and nodule development. The removal of rhizoma peanut shoots at the beginning of the summer drastically reduced the supply of photosynthate. Consequently, the limited carbohydrate reserves were preferentially remobilized to re-establish the photosynthetic capacity of the plants. This resulted in nodule senescence and decay and a severe decline in specific nitrogenase activity. A second defoliation in August had a similar effect on both nodulation and nitrogenase activity since the plants were in a phase of rapid growth and the carbohydrate reserves had not been replenished. Thus, the stimulus for the development of shoots, roots and rhizomes given by favorable conditions of temperature and moisture reflected in an advantage for these organs in the competition with nodulation for the limited supply of carbohydrate reserves.

As Saldivar (1983) reported, air temperatures decline during the fall limiting shoot growth, but plants continue to photosynthesize. Since there is a lag of about 90 days between

the decline in air and soil temperatures, the product of photosynthesis during this period is directed toward root and rhizome growth, nitrogen fixation, and accumulation of carbohydrate reserves in the rhizomes. Consequently, a third defoliation on 21 October resulted in a moderate decrease in both nodulation and nitrogenase activity, since carbohydrate reserves in the plants were adequate for shoot development and to maintain the existing nodule mass and moderate rates of nitrogenase activity.

The reduced supply of photosynthate due to defoliation limits the availability of substrate for oxidative phosphorylation in the nodule. The decline in respiration due to a limited supply of photosynthate to the nodule leads to oxygen accumulation and increase of $p0_2$ inside the nodule and nitrogenase is inactivated. However, Weisz (1986) and Hartwig et al. (1987) observed that nodule gas permeability is variable and seems to be under active physiological control. Thus, following defoliation, there seems to be a dramatic increase in oxygen diffusion resistance, resulting in the decline of the supply of oxygen at the site of nitrogen fixation at the same time that shoot removal reduces photosynthate supply to the nodules.

CHAPTER III
EVALUATION OF NITROGENASE ACTIVITY OF INTACT AND DISTURBED PLANTS OF Arachis glabrata Benth. USING A GAS FLOW-THROUGH SYSTEM

The development of the acetylene reduction assay (Hardy et al., 1968) provided a simple, rapid, inexpensive, and highly sensitive procedure for studies of nitrogen fixation by the legume-Rhizobium symbiosis. The assay has been widely applied and has resulted in a marked increase in research on nitrogen fixation (Hardy et al., 1973; Hopmans et al., 1982; Sprent, 1979; Turner and Gibson, 1980). The procedure applied in different studies involved the use of intact or detopped nodulated root systems, attached or detached nodules, closed or open systems, and short- or long-term assays under very different environmental conditions.

A basic assumption of the acetylene reduction assay is that the change in substrate from nitrogen to acetylene and the change in the final product from ammonia to ethylene does not affect the rate of nitrogenase activity . However, Haystead et al. (1980) and Minchin et al. (1982) using closed circulatory gas systems showed a marked decrease in respiration rates of nodulated white clover roots and detached pea nodules in the presence of acetylene. Minchin et al. (1982) observed that under these conditions the cumulative curve of ethylene production was non-linear over a 30-to 60-min period.

Minchin et al. (1983b), studying both attached nodulated roots and detached nodules of several legumes, using a gas flow-through system, reported a decrease in nitrogenase activity in the presence of acetylene with a concurrent reduction in respiration. Ryle et al. (1986) observed that exposure of nodulated white clover root systems to a 10% acetylene gas mixture resulted in a sharp peak in rate of ethylene production after 1.5 to 2.5 min. Subsequently, rate of ethylene production declined rapidly before stabilizing after 30 to 60 min at a rate about 50% of that initially observed. Davidson and Robson (1986) obtained similar results. Minchin et al. (1983a) suggested that this decline occurs in two phases, followed by a small recovery. When acetylene was removed, nitrogenase activity and respiration returned to pre-exposure values. Based on

current N^{15} studies Minchin et al. (1983b) concluded that the peak value, and not the plateau reached later, most nearly represents the true rate of nitrogen fixation.

An acetylene-induced effect on diffusivity of the nodule to oxygen was also observed by Ralston and Imsande (1982), Sinclair and Goudrian (1981) and Weisz et al. (1985). Minchin et al. (1983a) and De Visser and Poorter (1984) suggested a possible feedback inhibition of nitrogenase activity, following cessation of ammonia production when the nitrogenase reduces substrates other than nitrogen.

Witty et al. (1984) argued that if diffusion resistance remained constant, the acetylene-induced decline in nitrogenase activity and respiration would lead to an increase in internal oxygen concentration which would inactivate nitrogenase. However, Sheehy et al. (1983) observed that nitrogenase in white clover (Trifolium repens) nodules was not damaged when oxygen concentration in the rhizosphere was increased from 21 to 80 per cent.

Sheehy et al. (1983) and Witty et al. (1984) suggested that this decrease in the rate of ethylene production in the presence of acetylene was due to a gradual increase in the diffusion resistance of the nodules, resulting in a decreased oxygen flux from the atmosphere to the bacteroids. At present, the nature of the variable resistance is unknown, but it may involve the distribution of leghaemoglobin within the structures of the cells of legume root nodules (Sheehy and Bergersen, 1986) and variations in the length of a water filled section of the diffusion pathway (Minchin et al., 1985). This zone could be formed by cell layers devoid of intercellular air spaces (Tjepkema, 1984) or water within narrow intercellular pores (Sheehy et al., 1985).

The acetylene-induced decrease in ethylene production and respiration is not uniform or universal to all legumes. It can vary with cultivar, Rhizobium strain, plant age and the pre-assay environment (Minchin et al., 1983a, 1983b). Tjepkema and Winship (1980), using a flow-through system for soybean nodule clusters, observed the absence of the acetylene-induced decline in ethylene production in USA bred cultivars. This is in agreement with the results of Mederski and Streeter (1977).

Embrapa

Minchin et al. (1986) claimed substantial errors in the standard acetylene reduction assay performed in closed vessels containing detopped-shaken nodulated root systems of soybeans and white clover. They observed that regardless of whether flow-through or closed systems were used, the acetylene reduction assay of detopped-shaken root systems did not measure nitrogenase activities which existed prior to the assay. Hansen et al. (1987), working with Acacia alata R. Br., A. extensa Lindl., and A. pulchella R. Br., obtained similar results. However, near maximal rates of ethylene production were maintained over 180 min of exposure of intact plants of A. extensa and A. pulchella to acetylene.

Further investigations into the possibility of devising simple and accurate procedures for using acetylene to assay symbiotic systems which display a decline in ethylene production are required (Minchin et al., 1983a). Flow-through systems must be employed to determine whether an acetylene-induced decline occurs. If a decline is exhibited, then the short duration maximum rate plateau can be accurately determined only by the use of a flow through system in intact plants (Minchin et al., 1983b, 1986).

This research was conducted using a gas flow-through system to test the hypotheses that a) Florigraze rhizoma peanut exhibits an acetylene-induced decline in ethylene production and that b) the utilization of disturbed (detopped and shaken) root systems of rhizoma peanut in the acetylene reduction assay result in underestimated values of rates of nitrogenase activity. The objective of this study was to evaluate nitrogenase activity of intact and disturbed plants of rhizoma peanut using the acetylene reduction assay in a gas flow-through system.

Materials and Methods

Plant Material

'Florigraze' rhizoma peanut (Arachis glabrata Benth.) plants were grown from 7.5 cm rhizome cuttings taken from an established stand on 22 Dec. 1986. The plants were grown in 250 cm^3 Erlenmeyer flasks with two ports for supply and return of gas, and drainage of excess moisture. A 2.5 cm layer of pea gravel was placed at the bottom of the Erlenmeyer flasks to allow the passage of a gas stream through the root system of the plants and to facilitate drainage of excess moisture. The Erlenmeyer flasks were completely covered with aluminum foil to avoid light penetration, and filled to $^3/_4$ of their capacity with soil from the established field of rhizoma peanut as the source of the inoculum of the Rhizobium bacteria. Air space in each Erlenmeyer flask was 125 cm^3. Two cuttings were planted in each container. The plants were grown in a naturally-illuminated glasshouse receiving 1200 micro E m^{-2} sec^{-1} PAR (photosynthetic active radiation), with day/night temperature regimes of 30/20°C. All plants received Jensens nitrogen-free nutrient solution (Vincent, 1970) throughout the growth period. Water was provided in adequate amounts to avoid drought stress. These plants were utilized for assays of nitrogenase activity of nodulated root systems of intact plants. Plants used for assaying nitrogenase activity of detopped and shaken nodulated root systems were taken from a pure stand of rhizoma peanut established in the field in 1983, as described in the previous chapter. These plants received 2000 micro E m^{-2} sec^{-1} PAR, with day/night temperature regimes of 30/20°C, and were grown under rainfed conditions.

Disturbance Treatments

The experiment was arranged in a completely randomized design. Nitrogenase activity assays were performed on 28 May 1987 on six plants of rhizoma peanut for each treatment. Two treatments were applied: (a) intact plants, and (b) plants were detopped and the root system shaken free of soil. In treatment (a), 5 min prior to the initiation of the assay, the nodulated root systems of intact plants were sealed into the Erlenmeyers flasks where they were grown using a rubber stopper split in half. The shoot systems were sealed into a central hole with Mortex® sealing compound. With treatment (b), 5 min prior to the initiation of the assay a large swath of rhizoma peanut was dug in the field, trying to maintain the plants intact. Immediately before the initiation of the assay, the shoots of the plants were clipped and the nodulated root system shaken free of soil and then sealed into the erlenmeyers using rubber stoppers. Both treatments used assay vessels with a total volume of 250 cm^3 and an estimated air space of 125 cm^3.

Apparatus for Measurement of Nitrogenase Activity

The apparatus used to obtain measurements of nitrogenase activity of nodulated root systems of either intact or disturbed plants was a gas flow-through system designed based on work done by Minchin et al. (1983). Nitrogenase activity was measured by passing a gas stream through the nodulated root systems of the experimental material and determining ethylene production in the effluent gas. The equipment consisted of a gas supply and mixing system, an assaying system, and a gas flow control system.

The gases were supplied by an acetylene (C_2H_2) cylinder and an air pump controlled with regulator valves and flow meters to produce a gas stream of 1000 $cm^3 . min^{-1}$ consisting of

10% C_2H_2, 19% 0_2, and 71% N_2. The gas stream, sparged through 2000 mL of water contained in a 6000 cm^3 Erlenmeyer flask, was humidified and thoroughly mixed. The excess gas mixture was released to the atmosphere.

A uniform flow rate of the gas mixture into the assay vessels was maintained by a vacuum pump controlled with a regulator valve and a flow meter. The vacuum pump was connected with the gas mixing system through the assay vessels, using rubber tubes. The flow rate of the gas mixture (250 cm^3 min^{-1}) into the assay vessels was set to twice the air space to facilitate rapid equilibration within the vessels and minimize errors due to mixing time while preventing excessive dilution of ethylene in the effluent gas stream. After sampling, the effluent gas was dehumidified in a 500 cm^3 side-arm Erlenmeyer flask filled to $^1/_2$ its capacity with Dryrite. The effluent gas was then filtered in through glass wool, before reaching the vacuum pump.

The assaying system consisted of 250 cm^3 Erlenmeyer flaks (assay vessels) with two ports for supply and return of the gas. The assay vessels were held tightly in a ring stand to facilitate operation of the system. Samples of the effluent gas (0.5 cm^3) for determination of ethylene production were taken through the rubber tube section located at the outlet of the assay vessels, using 1 cm^3 gas-tight syringes.

Nitrogenase Measurements

Samples of the effluent gas were taken at 15-second intervals during the first 2 min of incubation. Thereafter, samples were taken at 30-, 60-, 120-, and 240-second intervals until 8, 16, 26, and 46 min after the beginning of the incubation period, respectively. Concentrations of ethylene and acetylene were measured with a gas chromatograph (Varian Series 3700) fitted with a flame ionization detector and equipped with a 2.8 m column packed with Poropac R®. Nitrogen was used as the carrier gas. Ethylene production was calibrated against standard curves. Rates of

ethylene production (nitrogenase activity) were calculated from the product of concentration and flow rate, normally 250 cm^3 min^{-1}.

After the incubation period, the nodules were detached from the root systems, dried to a constant weight and nodule dry weight determined. Specific nitrogenase activity was calculated by dividing nitrogenase activity of each sample by the respective dry weight of the nodules. Analysis of variance was performed using the General Linear Model procedure of SAS (SAS Institute Inc., 1982). Statistical significance was determined using the F test.

Results

Effect of Acetylene on Rates of Nitrogenase Activity

When nodulated root systems of intact plants of rhizoma peanut were exposed to 10% acetylene gas mixture, the concentration of ethylene in the effluent gas increased sharply during the initial 3 min mixing time (period taken for the acetylene concentration in the assay vessels to rise to its equilibrium value). Subsequently, the rate of ethylene production (nitrogenase activity) declined rapidly before reaching a steady state between 10 to 14 min. There was a second phase decline in nitrogenase activity of smaller proportions between 15 and 30 min, followed by a small recovery between 30 and 46 min. after exposure to acetylene. The subsequent declines from the maximum rate of nitrogenase activity were 37, 46, and 36%, during 14, 30, and 46 min after exposure to acetylene, respectively (Fig. 9).

Nodulated root systems of rhizoma peanut plants detopped, shaken free of soil and exposed to a 10% C_2H_2 gas mixture showed a sharp increase in nitrogenase activity during the first 5 min of exposure. Subsequently, there was a gradual decline from the maximum rate of

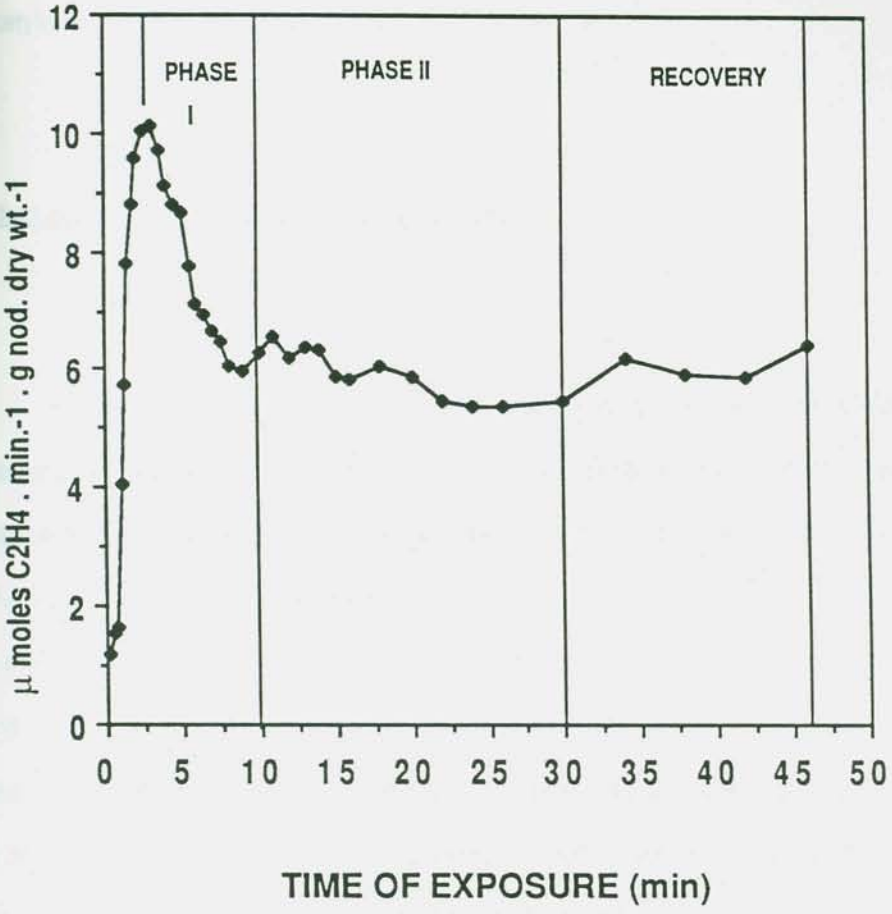

Fig. 9 - Rates of nitrogenase activity (ethylene production) of intact plants of Florigraze rhizoma peanut (28 May 1987).

nitrogenase activity until 46 min after exposure. The subsequent declines from the maximum rate of nitrogenase activity were 44, 48, and 53%, during 15, 30, and 46 min after exposure to acetylene, respectively (Fig. 10).

Effect of Disturbance on Nitrogenase Activity

Detopping rhizoma peanut plants and shaking the soil from nodulated root systems of rhizoma peanut resulted in a sharp decrease in maximum rate of nitrogenase activity compared with intact plants systems. Specific nitrogenase activity of intact plant systems was significantly ($P<0.05$) higher than in detopped and shaken plant systems throughout the 46 min of exposure of the plant systems to acetylene (Table 9). The maximum rate of nitrogenase activity of detopped and shaken root systems was 35% of the maximum rate of intact plant systems. The rate of nitrogenase activity of detopped shaken roots systems was 34 and 26% of the rate of intact plants at 30 and 46 min after exposure to acetylene, respectively. Detopping and shaking the soil from the root systems of rhizoma peanut affected the extent of the acetylene-induced decline and the response pattern of nitrogenase activity to acetylene exposure. This resulted in a one phase gradual decline from the maximum rate of nitrogenase activity of detopped and shaken root systems until 46 min after exposure, compared with a two-phase decline followed by a small recovery in nitrogenase activity of intact root systems (Fig. 11).

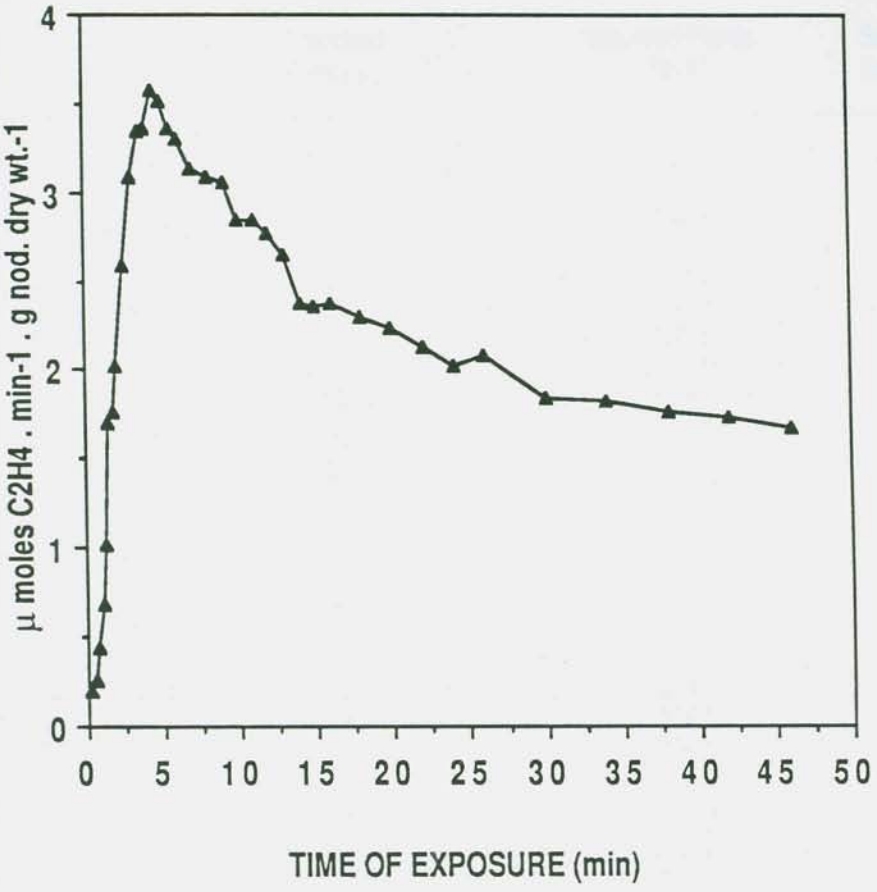

Fig. 10 - Rates of nitrogenase activity (ethylene production) of detopped, shaken nodulated root systems of Florigraze rhizoma peanut (28 May 1987).

Table 9 -- Means and standard error of the means of rates of nitrogenase activity (ethylene production) of intact and disturbed plant systems of Florigraze rhizoma peanut (28 May 1987).

Time (min)	Intact Plants SNA†	Standard Error (±)	Disturbed Plants SNA†	Standard Error (±)
0.25	1.18 a*	0.35	0.20 b	0.50
0.50	1.57 a	0.44	0.26 b	0.09
0.75	1.63 a	0.56	0.44 b	0.13
1.00	4.05 a	0.63	0.69 b	0.26
1.25	5.74 a	0.90	1.01 b	0.27
1.50	7.80 a	0.47	1.71 b	0.43
1.75	8.82 a	0.56	1.76 b	0.38
2.00	9.60 a	0.53	2.02 b	0.43
2.50	10.06 a	0.42	2.59 b	0.47
3.00	10.12 a	0.63	3.08 b	0.52
3.50	9.74 a	1.07	3.34 b	0.56
4.00	9.15 a	1.04	3.36 b	0.52
4.50	8.80 a	1.29	3.57 b	0.63
5.00	8.66 a	1.16	3.51 b	0.64
5.50	7.76 a	0.80	3.37 b	0.64
6.00	7.12 a	0.93	3.30 b	0.68
6.50	6.95 a	0.71	3.22 b	0.71
7.00	6.66 a	0.69	3.14 b	0.68
7.50	6.48 a	0.77	3.11 b	0.59
8.00	6.07 a	0.78	3.08 b	0.61
9.00	5.98 a	0.82	3.06 b	0.64
10.00	6.28 a	1.03	2.84 b	0.62
11.00	6.56 a	1.13	2.84 b	0.65
12.00	6.22 a	0.84	2.76 b	0.70
13.00	6.38 a	0.92	2.65 b	0.69
14.00	6.34 a	0.91	2.37 b	0.68
15.00	5.90 a	0.83	2.36 b	0.62
16.00	5.86 a	0.77	2.37 b	0.68
18.00	6.05 a	0.82	2.29 b	0.69
20.00	5.90 a	0.73	2.24 b	0.69
22.00	5.46 a	0.48	2.13 b	0.69
24.00	5.39 a	0.57	2.02 b	0.64
26.00	5.37 a	0.58	2.08 b	0.61
30.00	5.46 a	0.52	1.84 b	0.64
34.00	6.21 a	0.73	1.83 b	0.79
38.00	5.93 a	0.49	1.77 b	0.57
42.00	5.89 a	0.51	1.73 b	0.91
46.00	6.45 a	0.32	1.68 b	0.74

† Specific nitrogenase activity = micromoles C_2H_4 . h^{-1} . g nodule dry wt^{-1}.

* Means of intact and disturbed plant systems in the same row followed by the same letter are not significantly different at the 5% level of probability.

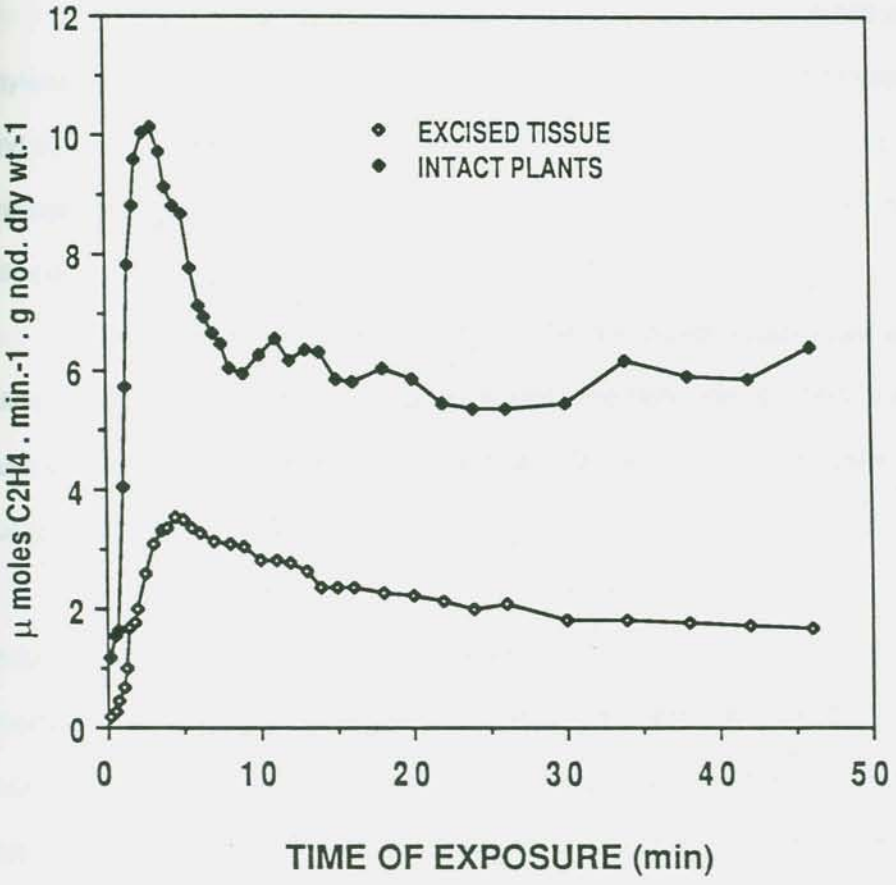

Fig. 11 - Effect of detopping and shaking nodulated root systems free of soil on rates of nitrogenase activity of Florigraze rhizoma peanut (28 May 1987).

Discussion

The sharp increase in nitrogenase activity in the initial minutes after exposure to acetylene occurs during a period of equilibration of the gas mixture within the assay vessels (Figs. 9 and 10). Minchin et al. (1983) reported that this initial increase is observed with all symbiotic combinations, independent of whether or not they show a subsequent acetylene-induced decline in nitrogenase activity.

Intact plants of 'Florigraze' rhizoma peanut showed a two-phase acetylene-induced decline in nitrogenase activity followed by a small recovery (Fig. 9). This is in agreement with results obtained by Minchin et al. (1982, 1983a). Minchin et al. (1983b) observed an acetylene-induced reduction in respiration rates concurrent with the decrease in nitrogenase activity.

Ralston and Imsande (1982) and Weisz et al. (1985) observed that exposure of nodulated root systems of intact plants to acetylene, induced a decline in diffusivity of the nodule to oxygen. It was suggested by Sheehy et al. (1983) and Witty et al. (1984) that this decrease in the rate of nitrogenase activity in the presence of acetylene was due to a gradual increase in the diffusion resistance of the nodules, resulting in a reduction of the oxygen flux from the atmosphere to the bacteroid.

Denison et al. (in press) observed that oxygen supply to the bacteroids depends on nodule gas conductance of the cortex. According to Davis (1984) the time lag for response to changes in the external gas mixture shows evidence that conductance is not unlimited. However, Denison et al. (in press) argue that the relationship between conductance and the time lag is not a simple one, since the latter depends both on nodule volume and internal porosity. Even though the nature of the variable resistance has not yet been conclusively established, it may be affected by the distribution of leghaemoglobin within the structure of cells of legume root nodules (Sheehy and Bergersen, 1986) and variations in the length of a water filled section of the diffusion pathway.

The observation of an acetylene-induced decline in nitrogenase activity of intact Florigraze rhizoma peanut plants indicates that rates of nitrogenase activity of this legume calculated based on ethylene production at 30 and 46 min after exposure to acetylene, may be underestimated by at least 36 to 46%. The magnitude of this decline may vary with changes in temperature, levels of water stress, and plant management (harvesting, grazing, and fertilization), among other factors. The pre-assay rates of nitrogenase activity are better represented by the maximum values, rather than the mean or final rates.

The removal of shoots and shaking off of the soil from the nodulated root system of rhizoma peanut plants, which are common practices in the standard acetylene reduction assay, resulted in significantly lower rates of nitrogenase activity of disturbed plant systems when compared with intact plant systems throughout the entire time of exposure to acetylene. Maximum rates of nitrogenase activity of detopped and shaken roots systems was 35% of the maximum rate of intact plants (Fig. 11). This is in agreement with the data of Minchin et al. (1986) who observed substantially greater oxygen diffusion resistances of nodules on detopped and shaken roots than for those on intact plants. They also reported that when oxygen concentration was increased to 60% the rate of nitrogenase activity of detopped and shaken root systems was higher than the maximum rate under 21% oxygen, indicating that the nitrogenase enzyme complex had not been seriously damaged by shoot removal and shaking the root system free of soil.

In detopped and shaken root systems, rates of nitrogenase activity declined, after reaching the maximum (Fig. 10). This decline reflected an increase in diffusion resistance due not only to acetylene exposure but also due to shoot removal and shaking the root system free of soil. Thus, while a small recovery in rates of nitrogenase activity was observed in intact plants between 30 and 46 min after exposure, the decline in rates of detopped and shaken root systems was gradual but constant, the final rate of nitrogenase activity being only 26% of that of intact plants.

These data, as those of Minchin et al. (1986), indicate that the accurate application of the acetylene reduction assay for nitrogen fixation studies requires the utilization of intact plants in a

gas flow-through system. This system allows the determination of maximum rates of ethylene production, prior to the acetylene-induced decline, which most nearly reflect the pre-assay rates of nitrogenase activity.

CHAPTER IV
EFFECT OF NITROGEN FERTILIZER AND DEFOLIATION ON GROWTH AND NITROGEN FIXATION OF FLORIGRAZE RHIZOMA PEANUT AND TRANSFER OF NITROGEN IN A RHIZOMA PEANUT-BERMUDAGRASS ASSOCIATION

Nitrogen availability usually is an important factor limiting productivity in agricultural systems. In most instances, legumes overcome this limitation through the symbiotic fixation of nitrogen by Rhizobium bacteria associated with the plant. Understanding how various plant genotypes, Rhizobium strains, and management practices can influence symbiotic nitrogen fixation under differing environmental conditions is a challenging agronomic problem. In many cases, a better understanding of how available soil nitrogen is used at the same time that nitrogen fixation occurs is essential, since the productivity of various legume culture systems can be affected by N assimilation patterns. In mixed pastures, any soil nitrogen not used by the legume component can contribute to increase dry matter production of the companion grass, thus increasing total forage yield (Phillips et al., 1986).

Competition for Mineral Nitrogen by Legumes and Grasses in Associations

According to Davidson and Robson (1985a, 1985b) the relationships between uptake of mineral N, symbiotic nitrogen fixation, and N transfer in mixed grass-clover swards are complex but essential to the understanding of the system as a whole. The response of nodulated legumes to combined nitrogen is complicated by the fact that it involves the interaction of two separate processes (nitrate or ammonium uptake and nitrogen fixation) and two organisms (the host

legume and the Rhizobium bacteria). In legume-grass associations the complexity of the system is increased by the relative competitive ability of the species for nitrogen and other resources such as light, moisture, and other nutrients.

Nitrogen fixation by legumes is of major importance in maintaining soil N levels of many agricultural systems. One of the factors influencing N_2 fixation is soil nitrogen concentration. Small amounts of soil nitrogen appear to be necessary to ensure nodulation and to help early growth of the legume (Dart and Wildon, 1970; Lie, 1974; Munns, 1968a, 1968b), whereas high concentrations may inhibit fixation (Alston and Graham, 1982; Dean and Clark, 1977; Haystead and Marriott, 1979). Oghoghorie and Pate (1972) observed that during early growth a legume derives its nitrogen supply mainly from the soil. When soil inorganic N is depleted and when symbiotic N_2 fixation is at a maximum, fixed nitrogen is directed disproportionately to the tops, and especially to the developing seeds. Symbiotically fixed N accumulates preferentially in the shoots and nodules while most root nitrogen comes from the soil (Oghoghorie and Pate, 1971). According to Vallis (1978) the partitioning of the uptake of mineral nitrogen between grass and legume in associations may be dependent on the proportions of grass and legume and on the characteristics of the species, especially plant vigor and seasonal growth patterns.

Pasture legumes appear to be less effective than grasses in absorbing mineral nitrogen. Allos and Bartolomew (1955) and Walker et al. (1956) reported that the uptake of fertilizer nitrogen in pure stands is typically 50 to 80% of that taken up by grasses. They observed that this differential N uptake by grasses and legumes does not appear to be directly related to comparative weights of the root systems or rates of growth. In mixed pastures the legumes are widely assumed to obtain only a small proportion of the available mineral nitrogen (Walker et al., 1954; Whitehead, 1970). However, many exceptions occur (Davies, 1964; Simpson, 1965; Willoughby, 1954) in which the legume clearly competes with the grass for available soil N. Vallis (1978) suggested that in older pastures, in which the supply of mineral nitrogen has been increased as a result of recycling of symbiotically fixed N, i.e. by nitrogen transfer, competition

could occur without reducing N uptake by the grass in the association to a level below that of the grass in pure stands.

Evaluations of uptake of mineral N by grass-legume associations using ^{15}N show that where the supply of nitrogen is the main factor limiting grass growth, the grass obtains most of the available nitrogen. The grass absorbed 90 to 95% of available N in associations of Chloris gayana with Stylosanthes humilis (Vallis et al., 1967), Lolium perenne with Trifolium repens (Walker et al., 1956), and 60 to 70% of that taken up by C. gayana with Macroptilium atropurpureum (Henzell et al., 1968).

Transfer of Legume Nitrogen to Associated Grasses

Nitrogen transfer in legume-grass associations consists of the movement of N from the legume to the associated grass. This process is especially important because it represents an input of N from a legume to an associated non-leguminous plant (Agboola and Fayemi, 1972; Burton et al., 1983; Vallis et al., 1967; Wahua and Miller, 1978a). The inclusion of a legume in a pasture mixture commonly, but not invariably, increases the nitrogen uptake of the grass component. There are a number of possible pathways by which nitrogen can enter the grass and legume components of grass-legume associations. The legume obtains its nitrogen from the atmosphere by symbiotic nitrogen fixation and from the soil. The grass obtains its nitrogen from the soil and from the associated legume (Vallis et al., 1967). The transfer of symbiotically fixed nitrogen from the legume to the associated grass may occur through several pathways. These pathways include a) direct excretion of soluble nitrogen compounds from living plants; b) sloughing of nodules and root system decay; c) leaching from leaves; d) decomposition from fallen leaves; and e) animal excreta (Burton et al., 1983; Vallis, 1978).

Butler and Bathurst (1956) observed that the essential conditions (long cool days with low light intensity) required for the transfer of symbiotically fixed nitrogen to associated grasses through the excretion of soluble amino acids from the living root systems of the legumes are so exacting that it is likely to be a very rare occurrence. They considered the nodules to be a more important source of nitrogen because of their higher N concentration. According to Elmore and Jackobs (1986) the prerequisites for transfer of symbiotically fixed nitrogen from legumes to associated grasses include low soil nitrogen and ideally, different N utilization peaks. Nodulation of the legume and adequate environmental conditions for nitrogen fixation are also essential (Wahua and Miller, 1978b). Whitney and Kanehiro (1967) conducted experiments involving sequential perfusion of root systems of legume and grass plants grown in pumice cinders. They reported that soluble nitrogen released from the roots of three tropical legumes was generally between one to three percent, with the maximum reaching nine percent, of the nitrogen fixed. Several studies indicate that the sum of all forms of transfer of nitrogen from intact root systems of actively growing legumes is only a small proportion of the total nitrogen fixed (Henzell, 1962; Henzell et al., 1968; Simpson, 1965; Vallis et al., 1967; Whitney and Kanehiro, 1967). Whitehead (1970) also observed that very little transfer of legume nitrogen occurs in the year of establishment of associated pastures. The results of Whitney and Kanehiro (1967) indicate that leaching of soluble nitrogen from living leaves of legume plants appears to result in significant contributions to total nitrogen transfer.

Transfer of nitrogen from legumes to non-legumes is thought to occur during the growth of the legume and may partly replace N fertilizers. Estimates of transfer range from 26 to 154 kg of N ha-1, depending upon species, botanical composition, and length of the growth period (Agboola and Fayemi, 1972; Bland, 1967; Cowling and Locker, 1967; Johansen and Kerridge, 1979; Simpson, 1976). In general, significant transfer of fixed nitrogen from a legume to an associated grass only appears to occur where the legume has been damaged or has reached maturity (Broadbent et al., 1982; Henzell et al., 1968; Vallis et al., 1977). Boote (1976) suggested that senescence of roots is accelerated by stress factors such as frequent defoliation. Whitehead

(1983) reported that frequent defoliation and drought over a 60-day period reduced root mass of white clover by 85 and 40%, respectively.

In intensively-grazed pastures, the nitrogen from legume shoots which returns to the soil passes predominantly via the dung and urine of grazing animals. An alternate and in some cases more important pathway is the release of legume N from decomposition of roots and nodules in response to defoliation, shading, and low temperatures (Simpson, 1976). Rates of nitrogen fixation decreased rapidly when clover plants were defoliated (Chu and Robertson, 1974; Halliday and Pate, 1976; Moustafa et al., 1969). Chu and Robertson (1974) observed an almost immediate increase in the rate of decomposition of roots and nodules of white clover subjected to defoliation and shading. Heichel and Henjum (1985) observed that evidence of nitrogen transfer from alfalfa (Medicago sativa L.), white clover (Trifolium repens), and birdsfoot trefoil (Lotus corniculatus L.) to reed canarygrass (Phalaris arundinacea L.) was obtained often during regrowth, following herbage harvest.

According to Haystead and Marriott (1978), in field situations, direct transfer of symbiotically fixed nitrogen from white clover to an associated grass is small, accounting for less than six percent of the grass nitrogen as opposed to about 27% in the pots. Simpson (1976) and Stewart and Chesnutt (1974) suggested that, under field conditions, the amount of nitrogen which is transferred from white clover to an associated grass tends to be more closely related to the growth of the legume in the previous year than to current production. Vallis et al. (1967) observed no underground transfer of legume nitrogen to an associated grass. Simpson (1965) reported transfer of legume N to an associated grass only after several harvests. Vallis et al. (1977) using ^{15}N failed to detect transfer of legume N to an associated grass. Haystead and Marriott (1979) found no evidence of transfer of fixed nitrogen from defoliated clover to the associated grass until harvest at 105 days. Morris et al. (1986) investigated the ability of arrowleaf clover (Trifolium vesiculosum Savi) to transfer biologically fixed N to ryegrass (Lolium multiflorum Lam.) and bermudagrass. They concluded that no appreciable transfer of nitrogen from the legume to the grasses occurred during the active growing period of the grasses and arrowleaf clover.

Simpson (1976) found that little symbiotically fixed N was transferred by subterranean clover to associated grasses directly during the growing season. Ledgard et al. (1985) suggested that most of the nitrogen transfer must occur during and after plant senescence. They reported significant transfer of legume N to an associated grass in a pot experiment, but found no measurable N transfer under field conditions. It was argued that this may have been due to the different cultural conditions of the experiments. In the pots, the root volume was restricted and there was a greater concentration of the two root systems than in the field. Consequently, any nitrogen released by the legume was more likely to be absorbed by adjacent grass roots in the pots than in the field.

Willey (1979a, 1979b) reported nitrogen contributions from intercropped legumes to non-legumes of up to 40 kg ha^{-1}. Elmore and Jackobs (1986) observed that nitrogen accumulation in sorghum [Sorghum bicolor (L.) Moench.] was increased by association with soybean, through N transfer, and that closer proximity of sorghum and soybeans enhanced this beneficial effect. Eaglesham et al. (1981) found that nitrogen transfer contributed 52% of maize (Zea mays L.) nitrogen in a maize-cowpea [Vigna unguiculata (L.) Walp.] intercrop. However, not all intercrop studies provide evidence for N transfer (Bunpromma and Mabbayad, 1978; Kitamura and Nishimura, 1979).

Whitney et al. (1967) reported that centro (Centrosema pubescens) fixed 270 kg of N ha^{-1} in pure stands and 120 kg of N ha^{-1} when grown in association with grasses. Some transfer of nitrogen from centro to the grasses was observed during a 6-month growing period, amounting to 6 to11% of the N fixed. Desmodium intortum fixed 380 kg of N ha^{-1} $year^{-1}$ and 5% or less of this was transferred to the associated grasses. The nitrogen transfer observed apparently involved N released by both the aerial and underground portions of the legume plants. Reynolds (1982) reported that apparent nitrogen fixation for six tropical legumes (Calopogonium mucunoides, Centrosema pubescens, Pueraria phaseoloides, Macroptilium atropurpureum, Desmodium heterophyllum, Mimosa pudica, and Vigna luteola) ranged from 31 to 136 kg of nitrogen ha^{-1} $year^{-1}$. Apparent nitrogen transfer from the legumes to the associated grasses

ranged from 5 to 23 kg ha^{-1} $year^{-1}$ with tall guinea grass (Panicum maximum) and 13 to 42 kg ha^{-1} $year^{-1}$ with cori grass (Brachiaria miliiformis).

Haystead and Marriott (1978, 1979) showed that 6 to 12% of the nitrogen content of perennial ryegrass (Lolium perenne L.) was derived from white clover in association in the field. Broadbent et al. (1982) observed that up to 79% of the N content of 'Wimmera' annual ryegrass (Lolium rigidum L.) was transferred from ladino white clover. Ledgard et al. (1985), in a pot experiment, reported a transfer of 2.2% of subterranean clover (Trifolium subterraneum L.) nitrogen to associated annual ryegrass over 29 days, but found no N transfer to ryegrass from either subterranean clover or alfalfa during a 36-day field study.

Ismaili and Weaver (1986, 1987) using ^{15}N observed that when Panicum coloratum L. was grown in association with Macroptilium atropurpureum D. C. Urb. in a 50:50 mixture, with low concentrations of mineral nitrogen present, siratro assimilated approximately 40% of the mineralized N, while the grass assimilated the remainder. Biologically fixed nitrogen transfer from siratro accounted for 12 to 14% and 5% of the total grass nitrogen respectively for the low and high rates of N fertilization. Brophy et al. (1987) reported that in associations of alfalfa and birdsfoot trefoil with reed canarygrass the grass derived a maximum of 68% of its nitrogen from alfalfa and 79% from trefoil. This represented 13% of the nitrogen fixed by alfalfa and 17% of that fixed by birdsfoot trefoil. Their results indicate that significant nitrogen transfer occurred and that the amount of N transferred was dependent on interspecies distance and legume-grass ratio.

This research was conducted in greenhouse to test the hypotheses that a) during the establishment of Florigraze rhizoma peanut, the legume transfers symbiotically fixed nitrogen to bermudagrass grown in association; b) removal of shoots (defoliation) increases the amount of nitrogen released by rhizoma peanut to the associated grass, but reduces the rate of establishment of the legume; c) application of nitrogen fertilizer increases the rate of growth and establishment of rhizoma peanut; and d) in legume-grass associations supplied with nitrogen fertilizer, rhizoma peanut competes strongly with the associated grass for the available mineral nitrogen. The objective of this study was to evaluate the effect of nitrogen fertilizer and defoliation

on growth and nitrogen fixation of Florigraze rhizoma peanut and nitrogen transfer from the legume to an associated grass.

Materials and Methods

The research was conducted in glasshouses of the United States Department of Agriculture-USDA/ARS, University of Florida and Institute of Food and Agricultural Sciences-IFAS, located at the Agronomy Farm, Gainesville, Florida, from March to November 1986.

The experimental design was a randomized complete block with six replications. The treatments consisted of: 1) Florigraze rhizoma peanut; 2) Tifton Hybrid-81 bermudagrass; 3) rhizoma peanut + bermudagrass; 4) rhizoma peanut + Nitrogen (N); 5) bermudagrass + N; 6) rhizoma peanut + bermudagrass + N; 7) rhizoma peanut (shoots were not removed until the end of the experiment) + bermudagrass. Experiment 1 was planted on 25 Mar., and the treatments imposed on 6 Jun. and evaluated until 24 Oct. 1986. Experiment 2 was planted on 21 May, and the treatments imposed on 23 Aug. and evaluated until 7 Nov. 1986.

The plants were grown in naturally illuminated glasshouses, receiving 1200 micro E m^{-2} sec^{-1} PAR (midday), with day/night temperature regimes of 30/20°C. Rhizoma peanut and bermudagrass were planted in containers made of Poly Vinyl Chloride (PVC) of 10 cm in diameter and 30 cm long to allow for unrestricted vertical growth of the root systems of the grass and legume during the experimental period. A PVC cap, with a hole in the center for drainage was sealed to the bottom part of the containers. To facilitate drainage of the nutrient solution and avoid loss of sand a 10-cm diameter disk of plastic screen (2 mm mesh) was placed at the bottom of the containers followed by a 3-cm layer of pea gravel and another 10-cm diameter disk of screen. The containers were filled with 1.7 kg of coarse yellow sand previously washed with tap water. A

sample of the sand was collected for nitrogen analysis. A 3-cm layer of pea gravel was placed at the top of the containers to prevent the growth of blue green algae.

A wooden rack structure was built to support the plant containers and to allow collection and cycling of the nutrient solution. An 800 mL beaker was placed below each container to collect the leachate. The beakers were covered with aluminum foil to prevent light from reaching the nutrient solution, thus stimulating growth of blue green algae. Prior to planting, a trial was run to determine the water holding capacity of the sand filled containers. The mean water holding capacity of the sand filled containers was 500 mL. Nutrient solution (Hoagland and Arnon,1950) was prepared and the pH was adjusted, prior to application, to a range between 6 and 6.5 using a 10% solution of NaOH.

Rhizomes of rhizoma peanut and bermudagrass were taken from the field and cut in pieces approximately 7.5 cm long. The rhizoma peanut cuttings were moistened in water and inoculated with the cowpea cross-inoculation group of Rhizobium bacteria. Samples of the grass and of the inoculated legume cuttings were saved for nitrogen analysis. Rhizoma peanut and bermudagrass were planted separately with 5 cuttings per container. Two weeks after planting, density was reduced to 3 plants per container.

During the first 4 weeks after planting, while the grass cuttings were rooting they received 500 mL of nutrient solution per week, containing 100 ppm of nitrate nitrogen (adapted from Hoagland and Arnon, 1950). The grass shoots were cut back to a height of 3 cm, 4 weeks after planting. From 4 to 10 weeks (in Experiment 1) and 4 to 13 weeks (in Experiment 2) after planting, the grass received N-free nutrient solution. The legume received N-free nutrient solution during the first 10 and 13 weeks after planting, respectively in Experiments 1 and 2.

Evaluations began on 6 Jun. and 23 Aug. 1986, respectively in experiments 1 and 2. The shoots of the N-starved grass plants were cut back to a 3-cm height. Samples of the grass roots, the shoots and roots of the legume, and the sand were collected for N analysis. From this point until the end of the experiment, the grass and legume in treatments 1, 2, 3, and 7 received 500 mL of N-free nutrient solution per container per week. Samples of the N-free nutrient

solution were collected for N analysis. The grass and legume in treatments 4 and 5 received 500 mL of a nutrient solution containing 100 ppm of nitrate nitrogen. The grass and legume in treatment 6 received 500 mL each, of a nutrient solution containing 100 ppm of nitrate nitrogen. The leachate was collected and cycled daily. In treatments 3, 6, and 7, the nutrient solution was exchanged daily between the grass and the legume containers of each treatment. Water lost by plant uptake and evapotranspiration was replaced with distilled water in sufficient amounts to avoid drought stress. The nutrient solution was replaced weekly and samples of the spent solution were collected for nitrogen analysis.

The shoots of the grass and legume were harvested to a 3-cm height at 4- and 5-week intervals, respectively in Experiments 1 and 2, with the exception of rhizoma peanut in treatment 7 which was not harvested until the end of the experiment. The plant material was air dried for 48 h at 65°C and dry weight determined. Senesced leaves of rhizoma peanut which occurred only in non-defoliated rhizoma peanut were collected at 2-week intervals, dried, weighed, and saved for nitrogen analysis.

At the end of the experiment the shoots were harvested, and the roots were carefully separated from the sand. The shoots and roots were dried and their weight determined. Samples of the sand in each treatment were collected for nitrogen analysis. The total dry weight of shoots accumulated throughout the experimental period and the roots were ground in a Spex mill to pass a 1-mm screen. The nitrogen concentration of the planting tissue was analyzed by the procedure used at the Agronomy Forage Evaluation Support Laboratory at Gainesville, using an aluminum block digestion of the plant material (Gallaher et al., 1975) followed by automated colorimetric determination of total nitrogen by a Technicon Autoanalyzer. Two plant standards were included with every group of samples. The values for dry weight and nitrogen yield of the planting material (cuttings) was subtracted from the respective values of the root systems of the legume and the grass. The values of dry weight, nitrogen concentration, and nitrogen yield of senesced leaves of non-defoliated rhizoma peanut were added to the correspondent values of shoots of the non-

defoliated legume, since the layer of gravel did not allow direct contact of these leaves with the growth medium, thus preventing decomposition.

Samples of the sand were ground in a Hammer mill to pass a 0.5-mm screen to ensure complete oxidation of organic matter within small aggregates. Total nitrogen content in the sand and spent nutrient solution was analyzed by the procedure described by Nelson and Sommers (1972). Inorganic forms of nitrogen in the sand and nutrient solution were analyzed by the procedure described by Keeney and Nelson (1982).

Analysis of variance was performed using the General Linear Model procedure of SAS (SAS Institute Inc., 1982). Comparison of the treatment means of dry matter yield, nitrogen concentration, and nitrogen yield of shoot and roots of rhizoma peanut and bermudagrass was performed using Duncan's Multiple Range Test.

Results

The nitrogen analysis of the nutrient solution, revealed no detectable amounts of nitrogen released by rhizoma peanut in the spent solutions. Analysis of the nitrogen concentration in the sand at the beginning and at the end of the experiments showed no significant addition of nitrogen to the sand at the end of the experiments.

Shoot Growth of Rhizoma Peanut and Bermudagrass

Stands of rhizoma peanut and bermudagrass in Experiments 1 and 2, which were supplied with 100 ppm of nitrate nitrogen per week, produced higher ($P< 0.05$) shoot dry matter yields than stands of the legume and of the grass which received N-free nutrient solution (Tables

10 and 11). In Experiment 1, when 100 ppm of nitrate nitrogen was supplied in sequence to containers of rhizoma peanut and bermudagrass, it resulted in significantly ($P< 0.05$) lower shoot dry matter yield of the legume and of the grass as compared to stands of rhizoma peanut and bermudagrass which received 100 ppm of nitrogen individually (Table 10). In Experiment 2, the application of 100 ppm of nitrogen, in sequence, to containers of the legume and grass reduced shoot dry matter yield of bermudagrass ($P< 0.05$), but had no significant effect on shoot dry weight of rhizoma peanut when compared respectively, with containers of the grass and the legume which received 100 ppm of N individually (Table 11).

In both Experiments, application of N-free nutrient solution in sequence to rhizoma peanut and bermudagrass had no effect on shoot dry weight of either species when compared with containers of the grass and legume which received N-free nutrient solution individually. Shoot dry weight of bermudagrass was not significantly affected by the sequential application and daily interchangeably cycling of N-free nutrient solution in the legume and grass containers, independent of the defoliation treatment imposed on the legume (Tables 10 and 11).

Nitrogen Concentration in the Shoots of Rhizoma Peanut and Bermudagrass

In Experiment 1, the application of 100 ppm of nitrogen to containers of rhizoma peanut and bermudagrass, individually or in sequence, had no significant effect on nitrogen concentration of rhizoma peanut and bermudagrass shoots. Nitrogen concentration of rhizoma peanut and bermudagrass in these treatments was higher ($P< 0.05$) than in the treatments where N-free nutrient solution was applied individually and in sequence (Table 12).

In Experiment 2, the nitrogen concentration of rhizoma peanut and bermudagrass shoots supplied with 100 ppm of nitrate nitrogen individually was significantly higher ($P< 0.05$) than the nitrogen concentration of the grass and legume supplied with 100 ppm of N in

Table 10 -- Dry matter (DM) yield of shoots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 20-week period (4 harvests). Experiment 1 (from 6 Jun. to 24 Oct. 1986).

Treatments	Dry Matter Yield	
	Grass	Legume
	g container^{-1}	
1 - Rhizoma peanut	-	9.6 c*
2 - Bermudagrass	1.8 c	-
3 - Rhizoma peanut + Bermudagrass	-	11.0 c
	2.5 c	-
4 - Rhizoma peanut + N	-	26.3 a
5 - Bermudagrass + N	62.9 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	18.0 b
	48.9 b	-
7 - Rhizoma peanut† + Bermudagrass	-	12.8 c
	2.2 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 11 -- Dry matter (DM) yield of shoots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 12-week period (3 harvests). Experiment 2 (from 23 Aug. to 7 Nov. 1986).

Treatments	Dry Matter Yield	
	Grass	Legume
	g container^{-1}	
1 - Rhizoma peanut	-	11.2 b*
2 - Bermudagrass	0.6 c	-
3 - Rhizoma peanut + Bermudagrass	-	12.1 b
	0.7 c	-
4 - Rhizoma peanut + N	-	16.2 a
5 - Bermudagrass + N	17.3 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	15.2 a
	14.6 b	-
7 - Rhizoma peanut† + Bermudagrass	-	9.9 b
	0.8 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 12 -- Nitrogen concentration of shoots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 20-week period (4 harvests). Experiment 1 (from 6 Jun. to 24 Oct.. 1986).

Treatments	Nitrogen Concentration	
	Grass	Legume
	g kg^{-1}	
1 - Rhizoma peanut	-	21.6 b*
2 - Bermudagrass	7.2 b	-
3 - Rhizoma peanut + Bermudagrass	-	22.6 b
	7.1 b	-
4 - Rhizoma peanut + N	-	26.3 a
5 - Bermudagrass + N	11.2 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	25.5 a
	10.2 a	-
7 - Rhizoma peanut† + Bermudagrass	-	15.4 c
	7.4 b	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

sequence. There was no significant difference in nitrogen concentration of rhizoma peanut shoots supplied with N-free nutrient solution individually and the legume supplied with 100 ppm of nitrate nitrogen individually or in sequence with bermudagrass. The nitrogen concentration of rhizoma peanut shoots fertilized with 100 ppm of N individually was higher ($P< 0.05$) than that of the legume which received N-free nutrient solution in sequence with bermudagrass. Nitrogen fertilized shoots of bermudagrass had a higher ($P< 0.05$) nitrogen concentration than that of the grass which received N-free nutrient solution (Table 13).

In both experiments, the nitrogen concentration in the shoots of non-defoliated rhizoma peanut was significantly ($P< 0.05$) lower when compared with defoliated shoots of the legume which did not receive nitrogen fertilization. Sequential application and daily interchangeable cycling of N-free nutrient solution in the grass and legume containers had no significant effect on nitrogen concentration of bermudagrass, independent of the defoliation treatment applied to rhizoma peanut (Tables 12 and 13).

Nitrogen Yield in Shoots of Rhizoma Peanut and Bermudagrass

Application of 100 ppm of nitrate nitrogen to rhizoma peanut and bermudagrass individually resulted in significantly higher ($P< 0.05$) nitrogen yield in the shoots of both species when compared with shoots of the grass and legume supplied with nitrogen in sequence. Nitrogen yield of rhizoma peanut and bermudagrass shoots fertilized with 100 ppm of nitrogen was significantly higher ($P< 0.05$) when compared with the shoots of the grass and legume which received N-free nutrient solution (Tables 14 and 15). In Experiment 1, there was no significant difference on nitrogen yield of non-defoliated rhizoma peanut and that of the legume supplied with N-free nutrient solution and harvested at 5 week intervals (Table 14). In Experiment 2, rhizoma peanut supplied with N-free nutrient solution and defoliated at 4 week intervals produced

Table 13 -- Nitrogen concentration of shoots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 12-week period (3 harvests). Experiment 2 (from 23 Aug. to 7 Nov. 1986).

Treatments	Nitrogen Concentration	
	Grass	Legume
	g kg^{-1}	
1 - Rhizoma peanut	-	28.7 ab*
2 - Bermudagrass	9.5 c	-
3 - Rhizoma peanut + Bermudagrass	-	26.9 b
	9.2 c	-
4 - Rhizoma peanut + N	-	31.2 a
5 - Bermudagrass + N	25.0 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	27.7 b
	19.2 b	-
7 - Rhizoma peanut† + Bermudagrass	-	20.6 c
	8.5 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 14 -- Nitrogen yield of shoots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 20 -week period (4 harvests). Experiment 1 (from 6 Jun. to 24 Oct.1986).

Treatments	Nitrogen Yield	
	Grass	Legume
	mg container^{-1}	
1 - Rhizoma peanut	-	204 c*
2 - Bermudagrass	13 c	-
3 - Rhizoma peanut + Bermudagrass	-	249 c
	18 c	-
4 - Rhizoma peanut + N	-	684 a
5 - Bermudagrass + N	704 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	456 b
	500 b	-
7 - Rhizoma peanut† + Bermudagrass	-	197 c
	16 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

significantly higher ($P< 0.05$) nitrogen yield than non-defoliated rhizoma peanut (Table 15). Nitrogen yield of bermudagrass was not significantly affected by the sequential application and daily cycling of N-free nutrient solution in the legume and grass containers, independent of the defoliation treatment imposed on the legume (Tables 14 and 15).

Root Growth of Rhizoma Peanut and Bermudagrass

In Experiments 1 and 2, the root dry weight of non-defoliated rhizoma peanut was significantly greater ($P< 0.05$) than in the treatments where the legume was defoliated, independent of level of nitrogen supplied in the nutrient solution. There was no significant difference in dry weight of roots of rhizoma peanut which were supplied with 100 ppm of nitrate nitrogen individually or in sequence with bermudagrass. Root dry weight of defoliated rhizoma peanut was not significantly affected by cycling the N-free nutrient solution in the same legume container or in sequence with a bermudagrass container. There was a significant ($P< 0.05$) difference in dry weight of roots of bermudagrass supplied with 100 ppm of nitrogen individually and root dry weight of the grass which received nitrogen in sequence with rhizoma peanut. Application of 100 ppm of nitrate nitrogen to bermudagrass resulted in significantly ($P< 0.05$) higher root dry weight than when the N-free nutrient solution was supplied to bermudagrass containers individually or in sequence with rhizoma peanut containers (Tables 16 and 17).

In Experiment 1, when 100 ppm of nitrogen was applied individually to rhizoma peanut, root dry weight was significantly ($P< 0.05$) increased in comparison with the dry weight of roots of the legume which received N-free nutrient solution. There was no significant difference in root dry weight of rhizoma peanut supplied with nitrogen in sequence with bermudagrass and the legume which received N-free nutrient solution. Root dry weight of bermudagrass was significantly increased when the N-free nutrient solution was cycled in sequence with defoliated rhizoma

Table 15 -- Nitrogen yield of shoots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 12-week period (3 harvests). Experiment 2 (from 23 Aug. to 7 Nov.1986).

Treatments	Nitrogen Yield	
	Grass	Legume
	g kg^{-1}	
1 - Rhizoma peanut	-	322 c*
2 - Bermudagrass	6 c	-
3 - Rhizoma peanut + Bermudagrass	-	325 c
	7 c	-
4 - Rhizoma peanut + N	-	504 a
5 - Bermudagrass + N	370 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	423 b
	272 b	-
7 - Rhizoma peanut† + Bermudagrass	-	204 d
	7 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 16 -- Dry matter (DM) yield of roots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 20-week period (4 harvests). Experiment 1 (from 25 Mar. to 24 Oct. 1986).

Treatments	Dry Matter Yield	
	Grass	Legume
	g container^{-1}	
1 - Rhizoma peanut	-	3.6 c*
2 - Bermudagrass	10.8 d	-
3 - Rhizoma peanut + Bermudagrass	- / 14.6 c	3.9 c / -
4 - Rhizoma peanut + N	-	8.2 b
5 - Bermudagrass + N	59.0 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	- / 50.0 b	5.5 bc / -
7 - Rhizoma peanut† + Bermudagrass	- / 11.0 d	14.1 a / -

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 17 -- Dry matter (DM) yield of roots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 12-week period (3 harvests). Experiment 2 (from 21 May to 7 Nov. 1986).

Treatments	Dry Matter Yield	
	Grass	Legume
	g container^{-1}	
1 - Rhizoma peanut	-	5.6 d*
2 - Bermudagrass	2.4 c	-
3 - Rhizoma peanut + Bermudagrass	-	6.3 cd
	2.5 c	-
4 - Rhizoma peanut + N	-	6.8 bc
5 - Bermudagrass + N	7.7 b	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	7.1 b
	8.3 a	-
7 - Rhizoma peanut† + Bermudagrass	-	13.1 a
	2.4 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

peanut. There was no significant difference in root dry weight of bermudagrass which received N-free nutrient solution individually or in sequence with non-defoliated rhizoma peanut (Table 16).

In Experiment 2, root dry weight of rhizoma peanut supplied with 100 ppm of nitrogen in sequence with bermudagrass was significantly ($P< 0.05$) higher than the legume which received N-free nutrient solution individually or in sequence with bermudagrass. There was no significant difference in root dry weight of rhizoma peanut supplied with 100 ppm of nitrogen individually and the legume supplied with N-free nutrient solution in sequence with bermudagrass. Rhizoma peanut supplied with N-free nutrient solution individually, produced significantly ($P< 0.05$) lower root dry weight than the legume which received 100 ppm of nitrogen in individual containers. Root dry weight of bermudagrass was not significantly affected by cycling the N-free nutrient solution in sequence with non-defoliated and defoliated rhizoma peanut containers (Table 17).

Nitrogen Concentration in the Roots of Rhizoma Peanut and Bermudagrass

In the first experiment, there was no significant difference in nitrogen concentration in the roots of rhizoma peanut supplied with 100 ppm of nitrate nitrogen individually or in sequence with bermudagrass. Roots of the legume which received nitrogen had a significantly ($P< 0.5$) higher nitrogen concentration than the roots of the legume which received N-free nutrient solution, independent of the defoliation treatment imposed. Non-defoliated rhizoma peanut had a significantly ($P< 0.05$) higher nitrogen concentration in the roots than the legume which was defoliated at 5 week intervals and supplied with N-free nutrient solution. Nitrogen concentration in the roots of bermudagrass supplied with 100 ppm of nitrogen individually and in sequence with rhizoma peanut was not significantly different. The nitrogen concentration in the roots of bermudagrass was significantly ($P< 0.05$) increased by the application of 100 ppm of nitrogen

individually or in sequence with rhizoma peanut resulted when compared with nitrogen concentration in the roots of the grass supplied with N-free nutrient solution (Table 18).

In Experiment 2, the nitrogen concentration in the roots of non-defoliated rhizoma peanut was significantly ($P< 0.05$) higher than in the roots of the defoliated legume which received 100 ppm of nitrate nitrogen and N-free nutrient solution in individual containers or in sequence with bermudagrass. There was no significant difference in nitrogen concentration in the roots of rhizoma peanut which received 100 ppm of nitrogen in individual containers or in sequence with bermudagrass containers. Roots of rhizoma peanut supplied with 100 ppm of nitrogen in individual containers had a significantly ($P< 0.05$) higher nitrogen concentration than the root of the legume which was defoliated and supplied with N-free nutrient solution individually or in sequence with the grass. There was no significant difference in nitrogen concentration in the roots of rhizoma peanut which received nitrogen individually and the roots of the legume which was defoliated and received N-free nutrient solution (Table 19).

In the second experiment, the application of 100 ppm of nitrate nitrogen to bermudagrass in individual containers resulted in a significantly ($P< 0.05$) higher nitrogen concentration in the roots of the grass when compared with the roots of bermudagrass supplied with nitrogen in sequence with rhizoma peanut. The nitrogen concentration in the roots of bermudagrass supplied with nitrogen was significantly ($P< 0.05$) higher than in the roots of the grass supplied with N-free nutrient solution, independent of whether the nutrient solution was cycled in individual containers or in sequence with rhizoma peanut (Table 19).

Nitrogen Yield in the Roots of Rhizoma Peanut and Bermudagrass

Nitrogen yield in roots of non-defoliated rhizoma peanut was significantly ($P< 0.05$) higher than in the roots of the defoliated legume, independent of the level of nitrogen application

Table 18 -- Nitrogen concentration of roots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 20-week period (4 harvests). Experiment 1 (from 25 Mar. to 24 Oct.. 1986).

Treatments	Nitrogen Concentration	
	Grass	Legume
	---------- g kg^{-1} ----------	
1 - Rhizoma peanut	-	9.7 c*
2 - Bermudagrass	3.2 b	-
3 - Rhizoma peanut + Bermudagrass	-	10.3 c
	2.5 b	-
4 - Rhizoma peanut + N	-	13.8 a
5 - Bermudagrass + N	6.0 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	13.6 a
	5.8 a	-
7 - Rhizoma peanut† + Bermudagrass	-	11.5 b
	3.0 b	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 19 -- Nitrogen concentration of roots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 12-week period (3 harvests). Experiment 2 (from 21 May to 7 Nov. 1986).

Treatments	Nitrogen Concentration	
	Grass	Legume
	g kg^{-1}	
1 - Rhizoma peanut	-	17.3 c*
2 - Bermudagrass	4.4 c	-
3 - Rhizoma peanut	-	15.9 c
+		
Bermudagrass	3.6 c	-
4 - Rhizoma peanut + N	-	19.4 b
5 - Bermudagrass + N	9.8 a	-
6 - Rhizoma peanut + N	-	17.7 bc
+		
Bermudagrass + N	7.8 b	-
7 - Rhizoma peanut†	-	22.5 a
+		
Bermudagrass	4.4 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

in both experiments. There was no significant difference in nitrogen yield in the roots of defoliated rhizoma peanut supplied with N-free nutrient solution in individual containers and in sequence with bermudagrass. Bermudagrass supplied with 100 ppm of nitrate nitrogen individually and in sequence with rhizoma peanut had significantly ($P< 0.05$) higher nitrogen yield in the roots than bermudagrass which was supplied with N-free nutrient solution in individual containers or in sequence with the legume. Sequential application and daily interchangeable cycling of N-free nutrient solution in the grass and legume containers had no significant effect on nitrogen yield in the roots of bermudagrass, independent of the defoliation treatment applied (Tables 20 and 21).

In the first experiment, nitrogen yield in the roots of rhizoma peanut and bermudagrass which received 100 ppm of N in individual containers was significantly ($P< 0.05$) higher than in the roots of the legume and of the grass which were supplied with N in sequence. Nitrogen yield in the roots of rhizoma peanut defoliated at 5-week intervals and supplied with N individually or in sequence with the grass was significantly ($P< 0.05$) higher than in the roots of the legume defoliated and supplied with N-free nutrient solution in individual containers or in sequence with bermudagrass (Table 20).

In the second experiment, the nitrogen yield in the roots of rhizoma peanut and bermudagrass which were supplied with 100 ppm of nitrogen in individual containers and in sequence, was not significantly different (Table 21). Rhizoma peanut supplied with nitrogen in individual containers had significantly ($P< 0.05$) higher nitrogen yield in the roots than the legume which was supplied with N-free nutrient solution individually or in sequence with bermudagrass. There was no significant difference in nitrogen yield in the roots of the legume when N-containing and N-free nutrient solutions were cycled in sequence with bermudagrass (Table 21).

Table 20 -- Nitrogen yield of roots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 20-week period (4 harvests). Experiment 1 (from 25 Mar. to 24 Oct.1986).

Treatments	Nitrogen Yield	
	Grass	Legume
	mg container^{-1}	
1 - Rhizoma peanut	-	35 d*
2 - Bermudagrass	34 c	-
3 - Rhizoma peanut + Bermudagrass	-	39 d
	36 c	-
4 - Rhizoma peanut + N	-	114 b
5 - Bermudagrass + N	349 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	75 c
	290 b	-
7 - Rhizoma peanut† + Bermudagrass	-	162 a
	33 c	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Table 21 -- Nitrogen yield of roots of Florigraze rhizoma peanut and Tifton Hybrid-81 bermudagrass in a greenhouse experiment, over a 12-week period (3 harvests). Experiment 2 (from 21 May to 7 Nov. 1986).

Treatments	Nitrogen Yield	
	Grass	Legume
	g kg^{-1}	
1 - Rhizoma peanut	-	96 d*
2 - Bermudagrass	10 b	-
3 - Rhizoma peanut + Bermudagrass	-	101 cd
	9 b	-
4 - Rhizoma peanut + N	-	133 b
5 - Bermudagrass + N	70 a	-
6 - Rhizoma peanut + N + Bermudagrass + N	-	125 bc
	64 a	-
7 - Rhizoma peanut† + Bermudagrass	-	291 a
	11 b	-

* Means in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's Multiple Range Test.

† Rhizoma peanut was not defoliated until the end of the experiment and includes senesced leaves.

Discussion

Effect of Nitrogen Fertilizer on Growth of Rhizoma Peanut

Application of 100 ppm of nitrate nitrogen to rhizoma peanut in individual containers resulted in 174 and 45% increase in shoot dry matter, 22 and 9% increase in nitrogen concentration, and 235 and 56% increase in nitrogen yield, respectively in Experiments 1 and 2, when compared with the defoliated legume which received N-free nutrient solution in individual containers. When nitrogen was supplied to the legume in sequence with bermudagrass it increased shoot dry weight of the legume by 64 and 26%, nitrogen concentration by 13 and 3%, and nitrogen yield by 83 and 30%, respectively in Experiments 1 and 2, over that of defoliated rhizoma peanut which was supplied with N-free nutrient solution in sequence with the grass (Tables 10 to 15).

When nitrogen was applied to individual containers of rhizoma peanut it resulted in 128 and 21% increase in root dry weight, 42 and 12% increase in nitrogen concentration, and 226 and 38% increase in nitrogen yield, respectively in the first and second experiments, when compared with rhizoma peanut which received N-free nutrient solution in individual containers. Application of nitrogen to the legume in sequence with bermudagrass, increased root dry weight of the legume by 41 and 13%, nitrogen concentration by 32 and 11%, and nitrogen yield by 92 and 24%, respectively in experiments 1 and 2, when compared with rhizoma peanut supplied with N-free nutrient solution in sequence with bermudagrass (Tables 16 to 21).

The data indicates that during the establishment of rhizoma peanut, the capability of the symbiotic fixation system to supply adequate amounts of fixed nitrogen is not compatible with the growth potential of the legume. Limited nitrogen supply can be one of the main factors resulting in

the long period (2 to 3 years) required for adequate establishment of rhizoma peanut as reported by Prine (1964) and Prine et al. (1981, 1986). Valentim et al. (1986) observed a beneficial effect on dry matter yield due to application of nitrogen fertilizer early in the growing season to establishing stands of rhizoma peanut. They suggested that nitrogen fertilizer acted as an aid in avoiding N deficiency during early weeks of growth before the symbiotic fixation system became effective. The data in Chapter II show that application of nitrogen fertilizer early in the growing season significantly increased specific nitrogenase activity of rhizoma peanut in pure stands.

Effect of Defoliation on Growth of Rhizoma Peanut

In the first experiment, shoot dry weight of non-defoliated rhizoma peanut was 33 and 16% higher than in the legume which was defoliated at 5-week intervals and received N-free nutrient solution in individual containers and in sequence with bermudagrass, respectively (Table 10). In the second experiment, shoot dry weight of non-defoliated rhizoma peanut decreased 12 and 18% in relation to the legume which was defoliated at 4-week intervals and received N-free nutrient solution in individual containers and in sequence with bermudagrass, respectively (Table 11).

The different response of shoot dry weight of rhizoma peanut to defoliation in the two experiments was due to planting date and length of the establishing period allowed before the defoliation treatments were imposed. The period of time allowed for the rhizoma peanut plants to establish was shorter in the first (73 days) than in the second experiment (94 days). In the first experiment, planting material was taken from the field in early spring, while the plants were still dormant. The first emerging buds and fully expanded leaf could be observed only 14 and 22 days after planting, respectively. The legume plants in this experiment had a low growth rate during the entire establishment period, resulting in reduced photosynthetic capacity. When defoliated (108

days after planting), the limited supply of food reserves accumulated during establishment were gradually depleted in the process of re-establishing the photosynthetic apparatus, thus reducing shoot growth in comparison with the non-defoliated treatment.

In the second experiment, the planting material was taken in late spring when the plants were actively growing. The first emerging buds and the first fully expanded leaf could be observed 3 and 8 days after planting , respectively. The rhizoma peanut plants in this experiment had a high growth rate throughout most of the experimental period. When defoliated (112 days after planting), it stimulated branching and the development of new leaves, resulting in higher photosynthetic capacity, thus increasing shoot growth in comparison with non-defoliated rhizoma peanut.

In both experiments the nitrogen concentration in the shoots of non-defoliated rhizoma peanut was significantly lower when compared with the legume which was defoliated at 4- to 5-week intervals and received N-free nutrient solution individually or in sequence with bermudagrass (Tables 12 and 13). Nitrogen yield in the shoots of the non-defoliated legume was lower than in rhizoma peanut which was defoliated at 4- to 5-week intervals and supplied with N-free nutrient solution in individual containers or in sequence with the grass (Tables 14 and 15). The lower nitrogen concentration and nitrogen yield in the leaves of non-defoliated rhizoma peanut when compared with the defoliated legume which received N-free nutrient solution was likely due to increased leaf senescence, lower leaf-stem ratio, and higher cell wall constituents in the older stems.

Root dry weight of non-defoliated rhizoma peanut was 292 and 134% higher than in the defoliated legume which received N-free nutrient solution, respectively in the first and second experiment. When compared with the legume which received 100 ppm of nitrate nitrogen in individual containers, the root dry weight of non-defoliated rhizoma peanut was 72 and 93% higher, respectively, in Experiments 1 and 2 (Tables 16 and 17).

Nitrogen concentration in the roots of non-defoliated rhizoma peanut was 19 and 12% higher in experiment 1, and 30 and 42% higher in experiment 2 than in the defoliated legume which received N-free nutrient solution in individual containers and in sequence with bermudagrass, respectively (Tables 18 and 19). Nitrogen yield in the roots of non-defoliated rhizoma peanut was 363 and 315% higher in the first experiment, and 203 and 188% higher in the second experiment than in the defoliated legume which received N-free nutrient solution in individual containers and in sequence with bermudagrass, respectively. When compared with the legume which was supplied with 100 ppm of nitrate nitrogen in individual containers and in sequence with bermudagrass, the nitrogen yield of non-defoliated rhizoma peanut was 42 and 116% higher in the first experiment and 119 and 134% higher in the second experiment, respectively (Tables 20 and 21).

The data show that repeated defoliation of rhizoma peanut during establishment resulted in a drastic reduction in the development of roots and rhizomes. This suggests that after defoliation, the food reserves accumulated in the root system were remobilized to re-establish the photosynthetic apparatus of the plants. In the non-defoliated treatment the legume continued to accumulate food reserves in the root system, even though leaf senescence and reduced leaf area contributed to reduce photosynthesis later in the experimental period. Application of nitrogen fertilizer reduced the effect of defoliation on root and rhizome development. However, the interval between defoliations (4 to 5 weeks) was not long enough to allow the plants a complete recovery of the food reserves. Consequently, rhizoma peanut supplied with nitrogen and repeatedly defoliated during establishment had a significantly lower root dry weight and nitrogen yield when compared with non-defoliated rhizoma peanut.

Competition for Mineral N by Rhizoma Peanut and Bermudagrass

In the first experiment, when 100 ppm of nitrate nitrogen was applied to rhizoma peanut in sequence with bermudagrass there was a decrease of 32 and 22% in the shoot dry weight of the legume and the grass respectively when compared with the grass and legume which received nitrogen in individual containers (Table 10). In the second experiment, the application of nitrogen to rhizoma peanut in sequence with bermudagrass resulted in 6 and 16% decrease in shoot dry weight of the legume and the grass respectively when compared with rhizoma peanut and bermudagrass which received 100 ppm of N in individual containers (Table 11). Plant competition for mineral N reduced nitrogen concentration in the legume and grass by 3 and 9% in the first experiment and 11 and 23% in the second experiment when compared with the grass and legume which were individually fertilized with the same nutrient solution (Tables 12 and 13). Nitrogen yield in the legume and grass supplied with nitrogen in sequence decreased 33 and 29% in the first experiment and 16 and 26% in the second experiment when compared with the grass and legume which received 100 ppm of nitrogen in individual containers (Tables 14 and 15).

In Experiment 1, the grass and legume which received 100 ppm of nitrogen in sequence had a root dry weight 33 and 15% lower, respectively, when compared with rhizoma peanut and bermudagrass which were supplied with nitrogen in individual containers (Table 16). In the second experiment, root dry weight of the grass and the legume which were supplied with nitrate nitrogen in sequence, increased 4 and 8%, respectively when compared with rhizoma peanut and bermudagrass which received 100 ppm of N in individual containers (Table 17). Nitrogen concentration in the roots of the legume and the grass fertilized with N in sequence declined 1 and 3% in the first experiment and 9 and 29% in the second experiment when compared with the rhizoma peanut and the grass which received 100 ppm of nitrogen in individual containers (Tables 18 and 19). Nitrogen yield in the roots of rhizoma peanut and bermudagrass

supplied with 100 ppm of nitrogen in sequence decreased 34 and 17% in the first experiment and 6 and 9% in the second experiment when compared with the legume and grass which received 100 ppm of N in individual containers (Tables 20 and 21).

The data show that rhizoma peanut competes strongly with bermudagrass for available mineral nitrogen during establishment. The effect of plant competition for mineral nitrogen on shoot and root growth of the grass and the legume was more pronounced in the first experiment than in the second. Rhizoma peanut plants in the second experiment were allowed a longer establishment period before the treatments were imposed. In addition, daylength was longer during establishment of the plants in the second experiment. This extended daylength probably allowed more photosynthetic activity, resulting in increased accumulation of food reserves in the roots and rhizomes of the legume. Rhizoma peanut plants grew more vigorously in the second experiment and had a better developed and well nodulated root system at the beginning of the experimental period than in the first experiment. Consequently, rhizoma peanut plants in the second experiment may have been less dependent on nitrogen fertilizer. This hypothesis seems to be supported by the data in Table 17 which shows that when 100 ppm of nitrate nitrogen was supplied in sequence to rhizoma peanut and bermudagrass in Experiment 2, the root dry weight of both species was higher than when the same level of nitrate nitrogen was applied to the grass and legume individually. This indicates that application of 100 ppm of fertilizer nitrogen to individual containers of rhizoma peanut stimulated shoot development to an extent that a concomitant reduction in root and rhizome development occurred.

Nitrogen Transfer From Rhizoma Peanut to Bermudagrass

When N-free nutrient solution was cycled in sequence in defoliated and non-defoliated rhizoma peanut and bermudagrass containers in increased shoot dry matter yield of the grass 39

and 22% in the first experiment and 17 and 33% in the second experiment, respectively when compared with the grass which received N-free nutrient solution individually (Tables 10 and 11). Nitrogen concentration in the shoots of the grass which received N-free nutrient solution in sequence with defoliated rhizoma peanut declined 1 and 3%, respectively in the first and second experiments in comparison with the grass which was supplied with N-free nutrient solution individually. When N-free nutrient solution was cycled in sequence in containers of non-defoliated rhizoma peanut and bermudagrass, it increased the nitrogen concentration in the shoots of the grass by 3% in the first experiment and decreased it by 10% in the second experiment when compared with the grass supplied with N-free nutrient solution in individual containers (Tables 12 and 13).

Nitrogen yield in the shoots of the grass which received N-free nutrient solution in sequence with defoliated and non-defoliated rhizoma peanut increased by 38 and 23% in the first experiment and 17% in the second experiment, respectively when compared with the grass which received N-free nutrient solution in individual containers (Tables 14 and 15).

Root weight of bermudagrass which received N-free nutrient solution in sequence with defoliated rhizoma peanut increased 38 and 4%, respectively in the first and second experiments when compared with the grass supplied with the same nutrient solution individually. Cycling the N-free nutrient solution in the grass and in undefoliate rhizoma peanut increased root dry weight of the grass by 4% in the first experiment but had no effect on root dry weight of the grass in the second experiment when compared with the grass which was supplied with N-free nutrient solution in individual containers (Tables 16 and 17). Nitrogen concentration in the roots of the grass which received N-free nutrient solution in sequence with defoliated rhizoma peanut declined 22 and 18%, respectively in the first and second experiments when compared with the grass which was supplied with N-free nutrient solution in individual containers. When the N-free nutrient solution was cycled in the grass in sequence with non-defoliated rhizoma peanut it decreased nitrogen concentration in the roots of the grass by 6% in the first experiment but had

no effect in the second experiment when compared with nitrogen concentration of the grass which received N-free nutrient solution in individual containers (Tables 18 and 19).

Nitrogen yield in the roots of the grass which was supplied with N-free nutrient solution in sequence with defoliated rhizoma peanut increased 6% in the first experiment but declined 10% in the second experiment when compared with the grass which was supplied with N-free nutrient solution individually. When N-free nutrient solution was cycled in sequence in bermudagrass and non-defoliated rhizoma peanut, nitrogen yield in the roots of the grass declined 3% in the first experiment and increased 10% in the second experiment when compared with the grass which received the same nutrient solution in individual containers (Tables 20 and 21).

Nitrogen transfer from defoliated and non-defoliated rhizoma peanut to bermudagrass which received N-free nutrient solution in sequence with the legume, accounted, respectively, for 13 and 4% of the grass nitrogen in the first experiment and 0 and 11% of the grass nitrogen in the second experiment. Total nitrogen transfer from defoliated and non-defoliated rhizoma peanut to bermudagrass receiving N-free nutrient solution in sequence accounted for 0 to 2% of the total nitrogen fixed by the legume during the establishment. The small transfer of fixed nitrogen from rhizoma peanut to bermudagrass under greenhouse conditions suggests two hypotheses: a) rhizoma peanut does not release significant amounts of fixed nitrogen during establishment even when subjected to defoliation; b) during establishment of rhizoma peanut, the legume reassimilates much of the underground nitrogen released due to root and nodule decay and sloughing of roots as they penetrate the soil during root growth.

In non-defoliated rhizoma peanut which was supplied with N-free nutrient solution in sequence with bermudagrass, there was considerable leaf senescence in the legume (Table 22). These senesced leaves did not contribute nitrogen to the grass due to the layer of gravel which did not allow decomposition to occur. These senesced leaves had the potential to transfer 155 and 383% more nitrogen than the total N content in the shoots and roots of bermudagrass grown

Table 22 -- Dry matter (DM) yield, nitrogen concentration, and nitrogen yield of senesced leaves of non-defoliated Florigraze rhizoma peanut receiving N-free nutrient solution in sequence with Tifton Hybrid-81 bermudagrass in greenhouse experiments in 1986.

Experiment	Dry Matter Yield	Nitrogen Concentration	Nitrogen Yield
	g container-1	g kg-1	mg container-1
Experiment 1	4.7	16.2	76
Experiment 2	3.5	19.9	69

in sequence with non-defoliated rhizoma peanut, respectively, in the first and the second experiments (Table 22).

The data on leaf senescence indicate decomposition of senesced leaves and stems as the potential major pathway of transfer of symbiotically fixed nitrogen from rhizoma peanut to associated grasses. Transfer of legume nitrogen in pastures of rhizoma peanut in association with grasses under intensive grazing is likely to occur in a shorter period of time and to be significantly greater due to nitrogen from shoots which are returned to the soil via the dung and urine of the grazing animals.

The symbiosis requires a very specific and close cooperation between the legume and the Rhizobium bacteria. In addition, symbiotic nitrogen fixation is a high energy requiring process. During the process of development of the symbiosis, nature has selected for higher efficiency of both the Rhizobium in utilizing the energy supplied by the legume in the form of photosynthate, and the legume in utilizing the biologically fixed nitrogen supplied by the Rhizobium in the form of amino acids. The data in this study suggest that during establishment, rhizoma peanut does not transfer significant amounts of symbiotically fixed nitrogen to an associated grass. In nature, undisturbed legumes growing under favorable conditions are not likely to transfer significant amounts of symbiotically fixed nitrogen. Unfavorable climatic conditions (drought, flooding, and freezing soil tempratures), and management practices (grazing and cutting) may significantly increase nitrogen transfer from the established stands of a legume to associated nonlegumes as a result of the recycling of nitrogen through the grazing animal, leaf senescence, and root and nodule decay. With the exception of the nitrogen recycled through the grazing animals, most legume nitrogen is likely to be available to the associated nonlegumes only in the following growing season.

CHAPTER V
CONCLUSIONS

This research was conducted to test the hypotheses that a) environmental factors (soil temperature and soil moisture) and management practices (defoliation and nitrogen fertilization) affect growth and nitrogen fixation of Florigraze rhizoma peanut; b) rhizoma peanut exhibits an acetylene-induced decline in ethylene production; c) the utilization of disturbed (detopped and shaken) root systems of rhizoma peanut in the acetylene reduction assay result in underestimated values of rates of nitrogenase activity; d) during the establishment of rhizoma peanut the legume transfers symbiotically fixed nitrogen to an associated grass; and e) in grass-legume associations supplied with nitrogen fertilizer, rhizoma peanut competes with the associated grass for the available supply of mineral nitrogen.

In Chapter II the effects of soil temperature, soil moisture, nitrogen fertilizer, and defoliation on seasonal variation of nodulation and nitrogenase activity of rhizoma peanut in pure stands and in association with bermudagrass were studied in the field. The data indicate that low soil temperatures that occurred in early spring, late fall, and during the winter had a pronounced effect in inhibiting nodulation and nitrogen fixation of rhizoma peanut. Reduced soil moisture in late spring and early summer contributed to limit the development of new nodules and stimulated nodule senescence, thus reducing the capacity of the symbiotic system to fix nitrogen. Under these circumstances, the application of nitrogen fertilizer early in the growing season stimulated specific nitrogenase activity (micromoles $C_2H_4 \cdot h^{-1} \cdot$ g nodule dry wt^{-1}) and total nitrogenase activity (nmoles $C_2H_4 \cdot h^{-1} \cdot core^{-1}$) of pure stands of rhizoma peanut. Application of N fertilizer in pure stands of rhizoma peanut during the summer and fall inhibited both nodulation and total nitrogenase activity, but had no consistent negative effect on specific nitrogenase activity. In associations of rhizoma peanut with bermudagrass the beneficial effect of nitrogen application on nitrogenase activity early in the spring, and the negative effect on both nodulation and total

nitrogenase activity during the summer and fall, did not occur due to a more efficient uptake of mineral N by the associated grass, which rapidly reduced the levels of N fertilizer available in the rhizosphere for uptake by the legume. Defoliation of unfertilized stands of rhizoma peanut at any time during the growing season had a depressing effect on nodulation and nitrogenase activity. The effect of defoliation on nodulation of fertilized stands of rhizoma peanut was reduced because the plants were poorly nodulated and relied mostly on N fertilizer.

In Chapter III, the acetylene reduction assay in a gas flow-through system was used to test the hypotheses that a) rhizoma peanut exhibits an acetylene induced decline in rates of nitrogenase activity and b) the utilization of disturbed (detopped and shaken) root systems of rhizoma peanut in the acetylene reduction assay results in underestimated values of rates of nitrogenase activity. The data support both hypotheses. They indicate that calculations of rates of nitrogenase activity of intact rhizoma peanut plants based on ethylene production at 30 and 46 min after exposure to acetylene underestimated the actual values by 36 to 46%. The utilization of rhizoma peanut plants detopped and shaken free of soil in assaying rates of nitrogenase activity resulted in pronouncedly underestimated values. Maximum rates of nitrogenase activity of detopped and shaken root systems were only 35% of the maximum rates of intact plants of rhizoma peanut. These data suggest that the accurate application of the acetylene reduction assay for nitrogen fixation studies requires the utilization of intact plants in a gas flow-through system. Such a system allows the determination of maximum rates of ethylene production, prior to the acetylene-induced decline, which most nearly reflect the pre-assay rates of nitrogenase activity.

In Chapter IV, greenhouse studies were conducted to test the hypotheses that a) during the establishment of Florigraze rhizoma peanut, the legume transfers symbiotically fixed nitrogen to an associated grass; b) removal of shoots (defoliation) increases the amount of nitrogen released by rhizoma peanut to bermudagrass in association, but reduces the rate of growth of the legume; c) application of nitrogen fertilizer increases the rate of growth and

establishment of rhizoma peanut; and d) in legume-grass associations supplied with nitrogen fertilizer, rhizoma peanut competes with bermudagrass for the available mineral nitrogen.

The data indicate that during the establishment of rhizoma peanut, the transfer of nitrogen from the legume to bermudagrass was minimal and accounted for up to 2% of the total nitrogen fixed by the legume. In the field, the major pathways of nitrogen transfer from rhizoma peanut to associated grasses are likely to be the recycling of nitrogen through grazing animals, senesced leaves and decaying roots and nodules. The legume nitrogen recycled through the grazing animal (urine and dung) may become available to the associated grass within the same growing season. Leaf senescence and root and nodule decay occur to a greater extent in late fall and during the winter. The nitrogen from this source would be available to the associated grasses only in the following growing season.

This study shows that defoliation of rhizoma peanut during the establishment did not increase the amount of nitrogen transferred from the legume to the associated grass. The data indicate that repeated defoliations during the establishment of rhizoma peanut reduced the growth of its shoots and roots, thus slowing the establishment of the legume. A single defoliation in mid- to late summer may stimulate branching and increase the photosynthetic capacity of the plants, thus increasing the rate of establishment of rhizoma peanut. Application of nitrogen fertilizer during establishment had a pronounced effect in increasing shoot and root growth and establishment of rhizoma peanut. The data show that during establishment, rhizoma peanut was a strong competitor with associated bermudagrass for the available supply of nitrogen fertilizer.

These studies show that low soil temperatures and water stress severely limit nitrogenase activity and nodulation of rhizoma peanut. They also indicate that nitrogen deficiency occurs in establishing stands of rhizoma peanut and established stands under stress conditions. The knowledge achieved in these studies can be used to develop management practices to overcome likely period of stress or make the best use of favorable conditions which are likely to occur. Selection of genotypes which are drought tolerant and adapted to lower soil temperatures may help to overcome adverse environmental conditions. The observation that rhizoma peanut is

a strong competitor with non-legumes for available mineral nitrogen, suggests that the application of nitrogen fertilizer during the establishment of rhizoma peanut in pure stands and in association with grasses may be a viable alternative to increase growth of the legume and reduce the period of time needed to achieve its complete establishment.

APPENDIX

Table A-1 -- Soil temperature (5- to 10-cm depth) and air temperature (40 cm above ground) in the experimental plots from 2 Apr. 1986 to 26 Mar. 1987.

Sampling Dates	Soil Temperature		Air Temperature	
	Max.	Min.	Max.	Min.
	°C			
2 Apr. 1986	24	19	33	10
18 Apr. 1986	24	19	27	8
5 May 1986	28	22	33	30
19 May 1986	29	26	18	26
2 Jun.1986	32	28	37	21
16 Jun.1986	29	26	34	21
26 Jun.1986	32	28	38	23
10 Jul. 1986	32	28	37	23
24 Jul. 1986	32	27	34	22
7 Aug.1986	32	26	36	21
19 Aug.1986	30	28	35	23
2 Sep.1986	30	26	32	21
16 Sep.1986	30	26	33	20
30 Sep.1986	30	26	34	21
16 Oct. 1986	26	22	26	12
6 Nov.1986	24	23	30	18
9 Dec.1986	20	18	29	17
23 Dec.1986	20	19	20	12
6 Jan. 1987	14	12	22	4
20 Jan. 1987	18	17	20	9
26 Jan. 1987	13	12	17	7
10 Feb. 1987	14	10	18	-6
26 Feb. 1987	18	16	22	11
26 Mar. 1987	22	21	28	20

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BIOGRAPHICAL SKETCH

Judson Ferreira Valentim was born on 23 January 23 1957, in the village of Barra do Cuieté, Conselheiro Pena, State of Minas Gerais, in southeast Brazil. He is the eighth of thirteen children. His father is José Ferreira Valentim and his mother Odete Barbosa Valentim. He is married to Maria Socorro Nobre Dantas Valentim.

He completed high school at the Colégio Estadual Augusto Meira, in Belém, State of Pará, in 1974. In 1978, he received his Bachelor of Science degree in Engenharia Agronômica from the Faculdade de Ciências Agrárias do Pará (FCAP). From 1976 to 1979, while attending FCAP, he worked as a trainee at the Centro de Pesquisa Agropecuária do Trópico Umido (CPATU) and at the Secretaria de Agricultura do Estado do Pará (SAGRI/PA). In 1979, he joined the staff of the Unidade de Execução de Pesquisa de Ambito Estadual de Rio Branco (UEPAE/Rio Branco) of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), in the State of Acre, in the Amazon Region. From 1979 to 1983, he conducted research on forage evaluation and establishment and management of legume-grass pastures.

In 1983, he was granted a scholarship by EMBRAPA to pursue graduate training. In 1985, he received a Master of Science degree in agronomy from the University of Florida, Gainesville, Florida. He is currently a Ph. D. candidate at the University of Florida. Upon completion of his graduate work, he will return the the State of Acre, in the Brazilian Amazon, where he will continue to conduct research with tropical legumes.

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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