ROOT TUBERIZATION AND NITROGEN FIXATION

BY PACHYRHIZUS EROSUS (L.)

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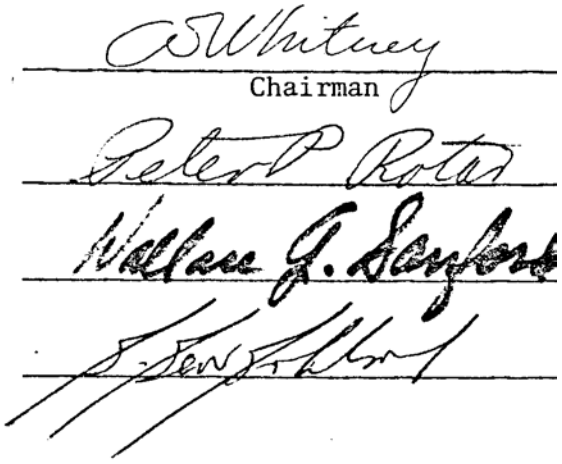

Chairman

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CHAPTER I

INTRODUCTION

Recently Pachyrhizus erosus (L.) (the Mexican yam bean) has been described as a legume of under-exploited potential in the tropics by the National Academy of Science (in press). Although root and tuber crops tend not to be agricultural export items (Leslie, 1967), this crop is currently exported from Mexico to the United States (Kay, 1973).

Earlier reports (Bautista and Cadiz, 1967; Kay, 1973) on the culture of this crop recommended use of nitrogenous fertilizers and failed to mention that this is a nodulated legume. More recently Marcarian (1978) recognized this as a symbiotic legume and considered the description of this crop's potential to fix nitrogen in the field to be a current research goal. This line of research could reduce the use of costly nitrogenous fertilizers.

The tuberous root of P. erosus is edible either raw or cooked. In Hawaii it is called the "Chinese potato" or the "chop suey yam" (Ezumah, 1970) and is raised on a back yard scale. Determining the yield potential, the optimal time to harvest and developing management techniques to increase yield and nutritive quality of this crop could serve to increase production in Hawaii, and potentially develop an agricultural export commodity at a time when production of sugar cane, the major crop in the islands, is proving unprofitable without subsidy from the federal government.

Increased production in developing tropical countries of this crop as an export commodity to the more developed countries would have two major consequences. Firstly, revenue would be generated in the producing countries. Secondly, just as more protein is needed in the diets of people in the lesser developed countries, so are less calories needed in

the diets of ever fattening affluent populations. If crispy snack foods can be processed from P. erosus, these would compete directly with far more fattening substitutes (cookies, potato chips, peanuts, etc.)

The intent of this thesis is to describe the sink capacities for assimilate and nitrogen of the various plant organs of P. erosus. The following investigations were undertaken:

1) Rhizobium strain testing, in which 23 strains of varying effectiveness were inoculated onto P. erosus grown in sterile, nitrogen free media. Included were treatments receiving chemical nitrogen and no Rhizobium applied. Across this gradient of symbiotic effectiveness dry weights, components of yield and nitrogen contents were compared.

2) Diurnal profiles in rates of acetylene reduction (symbiotic nitrogenase activity) for P. erosus at different stages of root tuberization.

3) Seasonal profiles on partitioning of dry matter and nitrogen between plant organs, weekly rates of acetylene reduction, and the effects of pod removal as a sink manipulation promoting root tuberization.

Pachyrhizus erosus is one of very few storage organ crops that are capable of symbiotic nitrogen fixation. Assimilate stored in the tuberous root may support nitrogen fixation, while at the same time nitrogen relations and symbiosis may affect root tuberization. If the extent of diurnal fluctuation in nitrogenase activity is not altered by increased root tuberization then the pattern of nitrogenase activity of tuberous-

rooted legumes is no different than that reported for nodulated legumes with fibrous roots. It is the intent of this thesis to describe the potential for root tuberization and nitrogen fixation by P. erosus.

CHAPTER II

LITERATURE REVIEW

Pachyrhizus erosus - Tropical Root Crop

Pachyrhizus erosus (L.) (Mexican yam bean) is one of few leguminous root crops. A hairy, twining herb native to Mexico and Central America, P. erosus is also cultivated in S.E. Asia (Purseglove, 1968), China, India (Deshaprabhu, 1966), and Hawaii. The lobed, turnip-shaped tuberous root is perennial, but P. erosus is generally cropped as an annual since the tuberous roots become fibrous with age. The root may be eaten raw, is mildly sweet and very crispy. After eating a sliced section some people unfamiliar with the "chop suey yam" might think this a fruit rather than a root. It is often used as a substitute for the Chinese water chestnut in oriental cooking. In 1973, Kay estimated the annual importation from Mexico to the United States to be 400 tons.

Tropical root and tuber crops, owing to their high bulk and relatively low value, tend not to be international trade items (Leslie, 1967). Even within tropical countries, root crops contribute much less to agricultural production than the acreage would otherwise indicate because root crops are often grown as a subsistence food and are not marketed. Root and tuber crops tend to be regarded as inferior foods, while cereals are often equated with civilization and progress. The motto of the United Nations Food and Agriculture Organization is "'Fiat Panis' - let there be bread" (Coursey and Haynes, 1970).

Because root crops tend to be high in carbohydrates and low in protein, vitamins and fats (Leslie, 1967), this bias is not entirely unjustified. The carbohydrate and protein content of P. erosus is even lower than that of yam, taro and sweet potato (Ezumah, 1970). Thus the roots from P. erosus would be a poor major staple for humans.

Additional constraints against expanding production of P. erosus in the tropics are the same as for other root crops. The scale of production tends to be quite small (Ezumah, 1970) and it is manually harvested (Bautista and Cadiz, 1967; Kay, 1973). Mechanical systems of planting and harvesting root crops have been developed (Jeffers, 1976) but due to the low value and small scale of production, initial inputs for increased production should be toward varietal improvement and expanded use of chemical fertilizers (Johnson, 1967).

Production of P. erosus by small farmers is encouraged by several cultural attributes of this crop. It is adapted to the very humid, hot tropics (Rachie and Roberts, 1974), although short term drought resistance is provided by the tuberous root. Insect and disease problems are infrequent (Bautista and Cadiz, 1967) due in part to the rotenone and pachyrhizid content of the shoots (Deshaprabhu, 1966). Tolerances to stress and pests allow for adequate yields under low input regimes. A practice easily affordable to small farmers raising P. erosus is that of flower and pod removal to promote root tuberization. Various authors report this to be a traditional practice (Deshaprabhu, 1966; Kay, 1973; NAS, in press) yet experimental results describing the consequences of depodding are not available. The young pods may be eaten after thorough boiling (Brucher, 1976).

Appendix (1) lists the average per acre yield, time to harvest, average price and gross return per acre for many root crops produced in Hawaii. No figures were available for P. erosus in the Statistics of Hawaiian Agriculture for 1977, although 11 other root and tuber crops were therein reported. When available in Hawaii, P. erosus retails for more than $.75 per pound. Assuming current price levels and a potential for export, P. erosus could offer gross returns comparable to alternative root crops in Hawaii.

P. erosus is typical of the major tropical root crops in that it is a nodulated legume, receiving benefit of nitrogen fixing Rhizobium bacteria (Figures 1a and 1b). Presently little is known about the Rhizobium

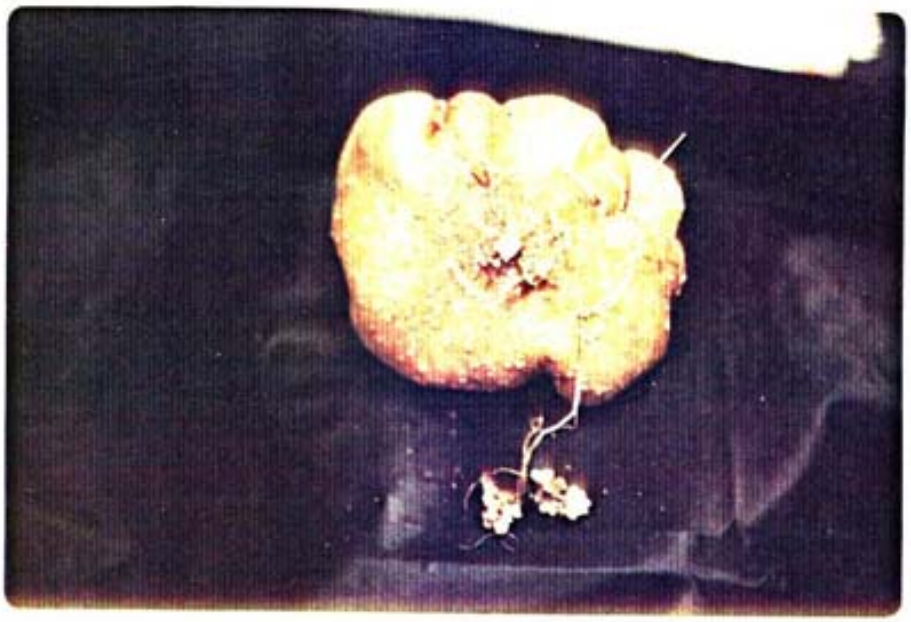
requirement or the potential of P. erosus to supply its nitrogen needs through symbiosis in the field (Marcarian, 1978). The role of legumes in farm ecology goes beyond directly providing nutrition or profit to producers. Through root nodule symbiosis, legumes act to restore and maintain the nitrogen status of the soil. The aerial portion of P. erosus contains much of the total plant nitrogen, and if reincorporated into the soil, would certainly prove of residual value.

Unfortunately, the shoots of P. erosus are poisonous and unusable as feed to ruminant animals. Deshaprabhu (1966) believes that horses accept this as a forage more readily than do cattle. He also noted that old and non-marketable roots are useful as fodder. The poisonous seeds of P. erosus are used as insecticides and fish poisons. The stems are said to render a fiber used in Fiji to make fish nets (Deshaprabhu, 1966). Despite the undesirability of P. erosus residue as animal food, this crop's acceptance as a food, the potential for export to temperate areas, the ability to fix atmospheric nitrogen, and the supplemental uses of non-marketable plant parts allow this crop to be considered as having under-exploited potential in the tropics.

Productivity and Partitioning of Carbohydrates in Root and Tuber Crops

Solar radiation levels determine the rate of dry matter accumulation in plants when other conditions are not limiting. Consequently, time to establishment of a full canopy after planting determines crop productivity (Loomis and Rapoport, 1976). Haynes et al. (1967) have well correlated the leaf area index and yield for several cultivars of yam (Dioscorea alata (L.)). The authors felt this is particularly significant since leaf area is alterable through management practices such as plant spacing, support, irrigation and fertilization. Net assimilation rate was also well correlated with storage organ yield during early stages of growth in yam; however, at later stages of

a)



b)

Figure 1. Tuberous root and root nodules of Pachyrhizus erosus a) attachment of large root nodule to root system. b) interior of root nodule, red region is the active bacteroidal zone

storage organ development, the immediate source of dry matter entering the tuber changes from strictly recent assimilate to plant translocate from the shoots (Degras, 1967). This is the onset of the "death by exhaustion" of the aerial parts described by Milthorpe (1967).

By necessity net productivity does influence yields, but the partitioning of assimilates between respiration, growth and storage result in an additional feature, unique to root and tuber crops (Loomis and Rapoport, 1976). The extent of root sink strength during the final stages of plant life greatly influences final yield in sugar beet (Beta vulgaris (L.)) (Das Gupta, 1969), potato (Solanum tuberosum (L.)) and Dahlia sp. (Loomis and Rapoport, 1976). It is not known if storage organs release growth inhibitors that act to mobilize substrate to that organ during late stages of growth (Loomis & Rapoport, 1976).

There are two basic patterns of storage organ accumulation, 1) balanced and 2) phasic partitioning. Balanced partitioning as represented in the sugar beet (Beta vulgaris) is relatively insensitive to the environment. Concentric cambia are formed early in ontogeny, roots and shoots develop synchronously (Mithorpe, 1967). In phasic partitioning rapid shoot and fibrous root growth precede storage organ initiation. Tuberization may be triggered by some aspect of the environment, followed by rapid predominance of the storage organ as a depository for assimilate (Loomis and Rapoport, 1976). Short days are known to regulate secondary thickening of roots in scarlet runner bean (Phaseolus coccineus (L.)), yam (D. alata), Jerusalem artichoke (Helianthus tuberous (L.)) (Garner and Allard, 1923) and winged bean Psophocarpus tetragonolobus (L.) DC) (Lawhead, 1978). Pachyrhizus erosus (L.) did not tuberize under a 14 hour photoperiod (Bautista and Cadiz, 1967), while other authors speculate that initiation of tuberous-root "bulking" in P. erosus is regulated through the photoperiod (Ezumah, 1979; Marcarian, 1978).

Torrey (1976) stressed the need of studies concerning hormone flow from the shoot to the root under different daylengths since the presence of cytokinin has been related to early secondary thickening of roots. Trapping of Golgi vesicles by microtubules along the primary xylem has been shown to be an early state in the secondary root thickening of alfalfa (Medicago sativa (L.)) (Maitra and Deepesh, 1971).

In conclusion, both external and internal factors are involved in plant growth and partitioning of assimilate into storage organs. Exact evidence of these factors for Pachyrhizus erosus is not currently available except an indication of a photoperiodic requirement for secondary root thickening.

The Acetylene/Ethylene Assay of Nitrogenase Activity

The acetylene reduction assay of nitrogen fixation has been shown to be sensitive, universal, and relatively simple (Hardy et al., 1968). Nitrogenase, the enzyme that reduces atmospheric nitrogen also reduces acetylene to ethylene, cyanide to methane and ammonia, N_20 to N_2 and water; to mention a few reactions. Using acetylene as a substrate for reduction results in sensitivity since only two electrons are required for each ethylene molecule produced while atmospheric dinitrogen requires 6 electrons for complete reduction.

The acetylene reduction technique was shown reliable for free living nitrogen fixing organisms, as well as with the root nodule symbiosis. Acetylene reduction, as measured by gas chromatography, is a less time-consuming technique than Kjeldahl analysis or ^{15}N assayed by mass spectrometry.

Bergersen (1970) compared rates of acetylene reduction and ^{15}N uptake of soybeans in nitrogen-free media. The ratio of acetylene reduced to

nitrogen fixed (C_2H_4:NH_3) ranged from 2.7 to 4.2. These observations do not invalidate the use of acetylene reduction to compare nitrogen fixing systems (nitrogenase enzyme activity); however, this work established that acetylene reduction is a poor quantitative measurement of exact amounts of nitrogen fixed.

Mague and Burris (1972) compared rates of acetylene reduction for intact soybean plants, decapitated root systems and detached nodules, finding activity ratios of 100/46/23 respectively. Water surfaces on the root nodules was shown to decrease activity. Hardy et al. (1973) comprehensively reported on the use of the acetylene/ethylene assay. It was found to have been useful in biochemical and physiological studies of the leguminous and non-leguminous symbiosis, soil, marine, rhizosphere, phylloplane and mammalian nitrogen fixing systems within five years of its development as a measurement of nitrogenase activity.

More recently in situ incubation in acetylene has been used to determine nitrogenase activity. Fishbeck et al (1973) working with soybean found that if the growth media was sufficiently porous, whole plant incubation did not result in significant differences from destructive incubation of nodulated roots. This in situ technique was used to measure diurnal changes in symbiotic nitrogenase activity. Since then other authors (Sinclair et al., 1978) have used the non-destructive acetylene reduction assay to compare acetylene/N_2 reduction rations, as well as plant species differences in nitrogen fixation. Periodic in situ assay did not disrupt growth processes of the many forage species that were compared.

Ruegg and Alston (1978) used in situ incubation to generate diurnal profiles of nitrogenase activity for glasshouse grown Medicago truncatula (Gaertn.). Significant diurnal fluctuation was observed over a two day cycle despite incubation in 10% acetylene for 30 or 60 minutes.

Productivity and Partitioning in Symbiotic Legumes

Under ideal field conditions light and temperature levels regulate plant productivity. Wilson et al. (1933) demonstrated that legume growth and symbiotic nitrogen accumulation were increased as the partial pressure of carbon dioxide was raised from .03% to 0.8%. Carbon dioxide is the substrate of photosynthetic productivity, just as light is the energy source. This experiment was the first strong indication that assimilate supply to the root nodules regulate rates of nitrogen fixation and the number, size and distribution of the root nodules. Later researchers, comparing ^{15}N accumulation of darkened and illuminated symbiotic legumes demonstrated the importance of light (and therefore recent assimilates) on the rate of nitrogen fixation (Lindstrom et al., 1952; Virtanen et al., 1955). Bach et al (1958) examined this directly using $^{14}CO_2$. During the photoperiod ^{14}C accumulated in the root nodules at twice the rate than at night. This work demonstrated the need of continued supply of photosynthate to the nodules to maintain maximum rates of nitrogen fixation. Lawrie and Wheeler (1973) correlated the rate of acetylene reduction with levels of labelled photosynthate in pea (Pisum sativum (L.)). The main sink within the nodules for assimilate was the bacteroidal areas. Later work by the same authors (Lawrie and Wheeler, 1975) with Vicia faba (L.) detected ^{14}C in the root nodules within 30 minutes of feeding the shoots $^{14}CO_2$. Ching et al (1975) related the decrease in ATP, sucrose, ATP/ADP ratio and nitrogenase activity to prolonged darkness for 1 day using 25 day old soybean. The energy balance of the nodules was dependent upon arrival of recent photosynthate.

Nitrogenase enzyme activity of temperate legumes is not greatly affected by incubation temperatures. Hardy et al (1968) equilibrated and then incubated nodulated roots of soybean at a range of temperatures.

Between 20° and 30° C there was no temperature effect on acetylene reduction, but a steady decrease was observed when root temperatures declined below 20° C.

Temperature strongly affects the supply of carbohydrates to the root nodules. Michin and Pate (1974) using pea (Pisum sativum) found that higher night temperatures resulted in a more pronounced decrease in N_2 fixation during the night. The authors speculated that nodule metabolism can utilize limited supplies of carbohydrate more efficiently for nitrogen fixation at lowered night temperature, since low night temperature reduces the rate of respiration more than the rate of nitrogen fixation. In the same study respiratory output was well correlated with nodule soluble carbohydrate.

Reports that changes in the rate of nitrogen fixation are more strongly correlated with air temperatures than with soil temperatures implies that temperature plays an indirect role on nodule function (Mague and Burris, 1972). Sloger et al (1975) found that for field-grown soybeans soil temperature varied less than nitrogenase activity throughout the day.

The effect of air temperatures on the rate of acetylene reduction varies between hosts and Rhizobium strains. Mes (1959) found that increasing day temperatures from approximately 20°C to either 25° or 27°C decreased nitrogen accumulation in the temperate legumes, Vicia sativa (L.) and Pisum sativum (L.). On the other hand, lowering day temperatures of tropical legumes, Arachis hypogaea and Stizolobium deeringianum Bort. depressed nitrogen accumulation. Similarly, Pate (1962) found that the symbiosis of Medicago tribuloides (Desr.) was more tolerant of higher temperatures, and that Vicia atropurpurea (Desf.) was more tolerant to lowered temperatures when the two species were compared. In general the symbiosis of tropical legumes are less sensitive to higher temperature regimes (27°-35°C) than are the temperate legumes.

Physiological Rhythms in Symbiotic Activity

Using a split shoot technique with Lupinus augustifolius (L.) in which one of the shoots was fed $^{14}CO_2$ and the other shoot was removed for collection of exudate, Greig, Pate and Wallace (1962) studied fluctuations in the amino content and radioactivity of the decapitated stem bleeding sap. The diurnal rhythm of temperature stimulated movement of labeled carbohydrate from the shoots. Specific activity of the amino fraction increased over several days, indicating continued radio labeled carbohydrate supply to the nodules after assimilation of $^{14}CO_2$. Plants maintained in constant temperature and darkness declined in $^{14}CO_2$ specific activity over time, translocation of carbohydrates from the shoot could not offset the depletion of root reserves. In this way both fluctuations of temperature and exposure to light were shown to stimulate nitrogen fixation.

Output of cations and amino compounds in the bleeding sap of nodulated Pisum arverense exhibited a diurnal rhythm with a maximum near noon and a minimum near midnight. Labeled amino acids were recovered after one hour of photosynthesis in $^{14}CO_2$ (Greig et al, 1962).

An endogenous component for rhythmic discharge of amino compounds was demonstrated for Lupinus augustifolius (L.) and Pisum arverense (Pate and Greig, 1964). This occurred for plants under normal light and prolonged darkness. The amplitude of the rhythm was increased by cold nights and warm days, which acted to time this rhythm.

Examination of the ultrastructure and functioning of the transport system to and from root nodules of Pisum arverense and Trifolium repens (L.) (Pate et al, 1969) indicated that normal source-sink processes are maintained with assimilate supply to the nodules, but that amino acid

export from the nodules was associated with active processes. Ultrastructural studies could not clearly define the export mechanism.

The differences in nitrogen fixation between fluctuating temperature/humidity regimes and constant temperature/humidity conditions were described by Minchin and Pate (1974) for P. sativum. Acetylene reduction, root respiration and nodule sugars increased during the photoperiod, while nodule soluble nitrogen decreased. The fluctuating environment stimulated overall growth and nitrogen fixation when compared to constant temperature/humidity. This was due in part to greater rates of nitrogen fixation under cooler night temperatures, resulting in less respiration during the dark period. This study included use of the acetylene reduction assay of nitrogenase activity. When these results were compared to bleeding sap estimates of the rate of nitrogen fixation, the results were in conflict. Bleeding sap flux greatly overestimated the extent of diurnal changes in nitrogen fixation because the products of nitrogen fixation were retained during the night, and not released until plants were rapidly transpiring during the next photoperiod. In this same study, more nitrogen was fixed during the night in the fluctuating temperature environment of 18°C day, 12°C night than during the photoperiod. The authors were not certain whether this is an artifact of growth cabinet conditions or if this applies to plants growing in some natural environments.

Examples of Diurnal Changes in Nitrogenase Activity

In most cases where diurnal fluctuation of nitrogenase activity has been observed, the maxima occurs near the period of maximum light intensity (Hardy et al., 1968). This has been demonstrated in the non-legumes Alnus Glutinosa and Myrica gale (Wheeler, 1969), and Casuarina sp. (Bond and

Mackintosh, 1975) as well as for quite a few legumes. Nitrogenase activity of field grown soybeans (Figure 2) consistently showed diurnal changes; however, the extent of these changes varied between two and three-fold (Sloger et al, 1975; Hardy et al, 1968) to five-fold (Mague and Burris, 1972). One published report (Ayanaba and Lawson, 1977) claims to have found no diurnal trend in the field, but when their results are plotted with other authors a trend does become evident. Some greenhouse (Fishbeck et al, 1973) and growth chamber (Mederski and Streeter, 1977) were compared to bleeding sap estimates of the rate of nitrogen fixation, the results were in conflict. Bleeding sap flux greatly overestimated the extent of diurnal changes in nitrogen fixation because the products of nitrogen fixation were retained during the night, and not released until plants were rapidly transpiring during the next photoperiod. In this same study, more nitrogen was fixed during the night in the fluctuating temperature environment of 18°C day, 12°C night than during the photoperiod. The authors were not certain whether this is an artifact of growth cabinet conditions or if this applies to plants growing in some natural environments.

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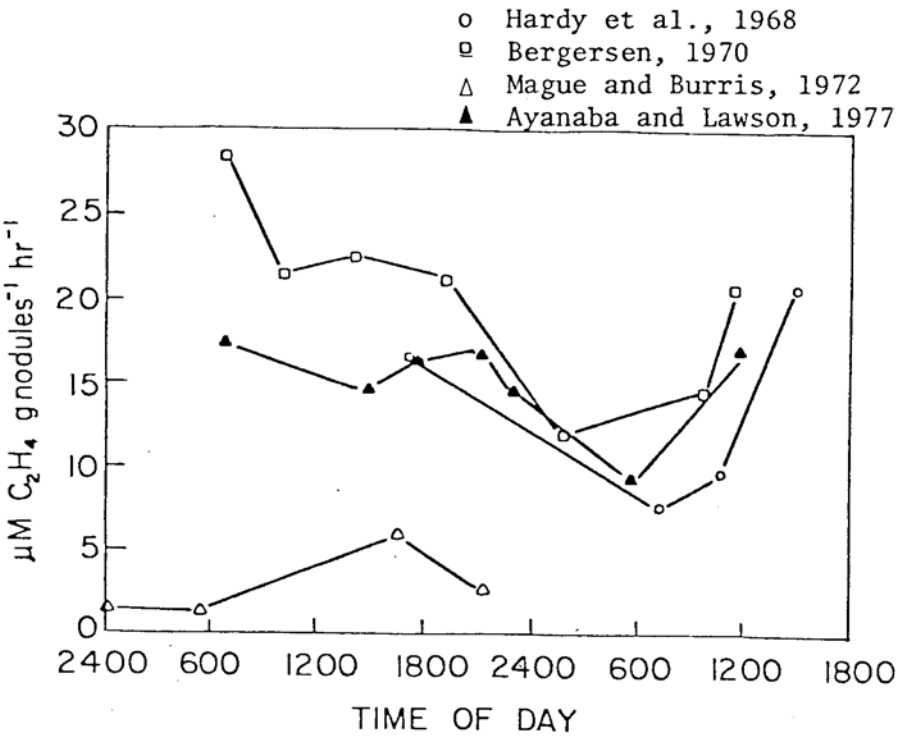


Figure 2. Diurnal changes in nitrogenase activity of field grown soybeans (Glycine max (L.) Merr.)

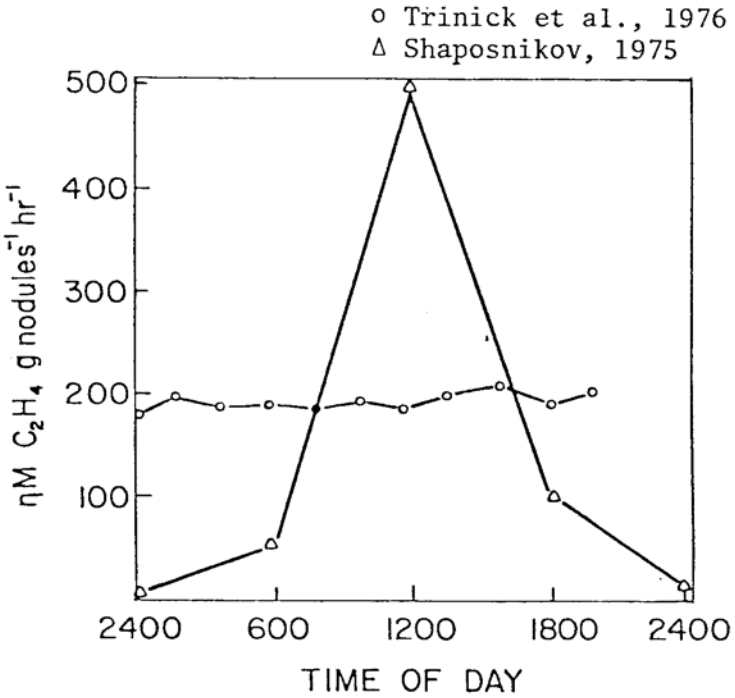


Figure 3. Conflicting reports of diurnal nitrogenase activity in Lupinus luteus (L.)

1972). One published report (Ayanaba and Lawson, 1977) claims to have found no diurnal trend in the field, but when their results are plotted with other authors a trend does become evident. Some greenhouse (Fishbeck et al, 1973) and growth chamber (Mederski and Streeter, 1977) investigations with soybean suggested considerably reduced diurnal changes with the maxima occurring nearing the end of the light period.

Descriptions of diurnal variation in acetylene reduction of field-grown Lupinus luteus (L.) by different authors are in conflict (Figure 3). Vegetative lupins had no significant differences in diurnal nitrogenase activity with no pronounced increase during the photoperiod (Trinick et al., 1976). The same field-grown species, sampled at the late bud stage by a different investigator (Shaposnikov, 1975) showed about a fifty-fold difference between the maxima and the minima. These tremendously different findings are hard to reconcile, despite differences in incubation techniques.

Growth room studies on P. sativum (Figure 4) indicated a less than two-fold difference between maximum and minimun nitrogenase activities. Again the maximum activity occurred during the end of the light period, or into the early dark period (Michin and Pate, 1974; Lawrie and Wheeler, 1973). Soluble carbohydrate levels in the nodules correlated well with changes in acetylene reduction (Michin and Pate, 1974). Prolonged darkness for 24 hours resulted in almost negligible nitrogenase activity. Longer periods of prolonged darkness also resulted in greater reduction of nitrogenase activity following reinitiation of the photoperiod (Lawrie and Wheeler, 1973). Lawrie and Wheeler (1976) later stated that peak activity often occurs at night.

Field-grown peanuts (Balandreau et al, 1974) displayed a strongly bimodal curve which the author concluded to be a product of climatic stress since the minima occurred at noon. Two cowpea cultivars (Ayanaba and

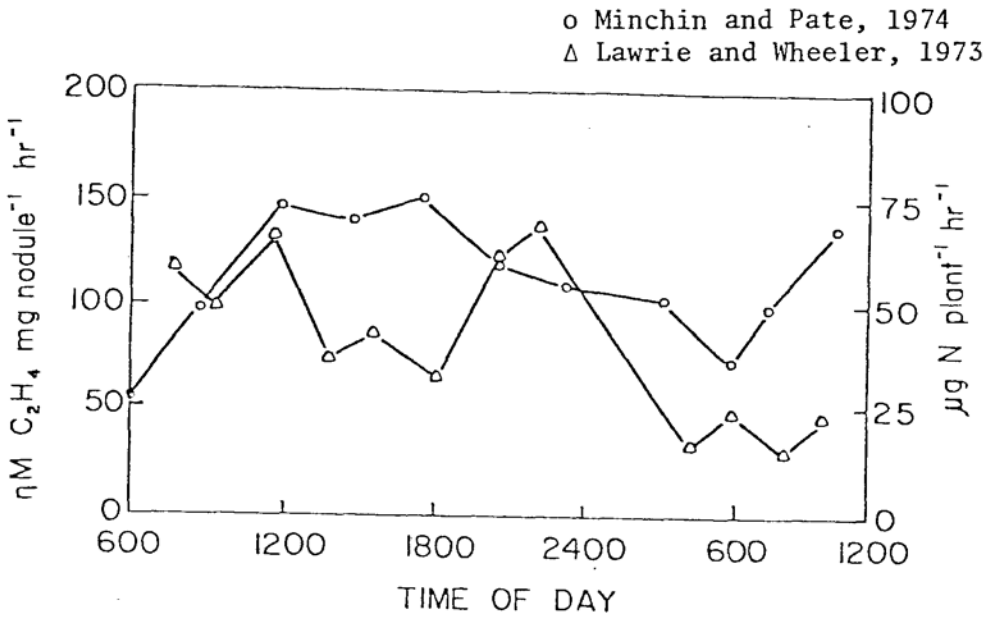


Figure 4. Diurnal nitrogenase activity of pea (Pisum sativum (L.)

Lawson, 1977) also showed two peaks in acetylene reduction activity during the course of the day, despite the unimodal nature of temperature and light levels. The daytime nitrogenase peak tended to be much larger than the dark period peak. In the same investigation, cowpea variety TVu 1190 sampled at eight weeks showed a four-fold difference between maximum and minimum activities. The trend in nitrogenase activity was unimodal with a maxima near noon.

All of the previous examples of diurnal changes in nitrogenase activity deal with annuals. It is possible that some perennials with different assimilate storage organs (e.g., tuberous roots) and which lack strictly determinate reproductive sinks, could display very attenuated diurnal patterns.

Source-Sink Manipulations in Legumes

That carbohydrate supply regulates rates of N_2 fixation is supported by observed changes in nitrogen fixation following photosynthetic source-sink manipulations. Pod removal of soybeans resulted in increased nodulation and root weight (Loong and Lenz, 1974) indicating that more carbohydrates reached the root system. Total plant weight was increased by 70% and 100% pod removal. Lawn and Brun (1974) established a range of source-sink ratios by depodding, defoliating, shading and providing supplementary light to soybean. Treatments designed to enhance carbohydrate supply to the nodules increased the rates of acetylene reduction and numbers of nodules. Treatments that limited carbohydrate supply reduced N_2 fixation and nodule numbers. The authors speculated that the decrease in N_2 fixation during podfill was related to competition for carbohydrates from the developing pods. Mondal et al. (1978) showed that removal of pods decreased photosynthetic rates slightly, and that starch accumulated in leaves as a

result of pod removal. Starch accumulation in leaf tissues is thought to shade chloroplasts, thereby lowering photosynthesis. Pod removal did not prevent a dramatic decrease in photosynthetic efficiency of leaves about 40 days after flowering despite the leaves remaining green. Plant weights or nitrogen fixation were not reported in this study.

Ciha and Brun (1978) found that depodding resulted in lowered rates of dry matter accumulation, but total plant weights were similar because of increased leaf duration in depodded plants. Depodding resulted in an increase of nonstructural carbohydrates in the leaves and petioles, primarily due to starch accumulation.

Continuous flower removal in pea (P. sativum) resulted in an increase in total plant acetylene reduction, nodule specific activity and total nodule weight (Lawrie and Wheeler, 1974). Similar results were obtained by Bethlenflavay et al. (1978); depodding of pea (P. sativum) increased rates of acetylene reduction, nodule mass and total plant nitrogen when plants were harvested after 60 days. Leaf removal decreased the previously mentioned parameters.

In conclusion, photosynthetic source sink manipulations designed to increase carbohydrate supply to the roots consistently increase rates of symbiotic nitrogen fixation. Total plant production does not necessarily reflect this increase in nitrogen fixation because sink capability becomes limiting as increased nitrogen fixation and vegetative vigor are not completely substitutable sinks compared to podfill. Hormonal imbalances resulting from pod removal, and consequent changes in plant metabolism and morphology complicate interpretation of these research findings. Also, many authors do not report changes in root weight as a result of depodding. Both of these species that have been described are temperate annuals.

Different plant responses to depodding could be expected among tropical perennials. Three perennial Desmodium spp. did not show any

relationship between the development of reproductive structures and root nodules (Whiteman, 1970). The effects of pod removal and partial defoliation on root tuberization have been described for Psophorcarpus tetragonolobus (Bala and Stephenson, 1978; Herath and Fernandex, 1978). Bala and Stephenson (1978) found no significant differences in tuberous root weight after 15 weeks of plant growth and seven weeks of periodic flower removal. After 20 weeks there was an approximate six-fold increase in tuberous root dry weight in response to deflowering. Herath and Fernandez (1978) compared the effects of flower and young pod removal and of vegetative pruning on four lines of P. tetragonolobus. After five months of growth, flower and young pod removal had increased the dry weight of tuberous roots three-fold while vegetative priming had slightly decreased root weight when compared to the control.

Rhizobium Strain Requirements

Establishing effective Rhizobium strains for P. erosus has received very little attention. Early studies on effective cross inoculation groupings within the "cowpea miscellany" did not include Pachyrhizus spp. hosts, nor were host isolates included among the Rhizobium strains evaluated (Burrill and Hansen, 1917; Walker, 1928; Allen and Allen, 1939). As the study of the legume symbiosis and rhizobiology became more diversified, Pachyrhizus spp. remained overlooked as a nodulated legume. Currently the Nitragin Company, commercial inoculant producers, markets rhizobia for P. erosus. These cultures were obtained in Thailand, and have not been extensively compared to host isolates from the area of origin, Southern Mexico (J. C. Burton, personal communication).

Recently Marcarian (1978) has identified P. erosus as an economic plant well adapted to stress conditions of the humid, lowland tropics. She

has determined this crop's potential to provide nitrogen through symbiosis in the field as a current research need. Before this can be done, highly effective isolates for P. erosus must be identified.

Root nodules similar to those formed on P. erosus were described by Spratt (1919) as the Viceae type nodule. It is elongated with a well defined apical meristem. The nodule branches and may form very large clusters as with Vicia faba (L.) and Stizolobium sp. The bacteroidal zone remains continuous as the nodule develops (Figure 1b) as opposed to bacteroidal zones which separate into distinct areas adjacent to vascular tissue.

Establishing known effective Rhizobium strains for a legume host is an essential beginning to further studies, including the host's symbiotic potential in the field. Exploratory tests of this nature should be Rhizobium strain intensive, particularly if the cross inoculation grouping of a host is unknown, and if host root nodules or site soils are not available (Burton, 1977).

CHAPTER III

THE *RHIZOBIUM* AFFINITIES OF *PACHYRHIZUS EROSUS* (L.)

INTRODUCTION

Pachyrhizus erosus (Mexican yam bean) is a tuberous-rooted legume which has been identified as a plant adapted to hot, wet tropical stress conditions (Rachie and Roberts, 1974; Marcarian, 1978), and is considered a legume of under-exploited potential by the National Academy of Science (in press). Although it has low nutritive qualities compared to other root and tuber crops (Ezumah, 1970; Evans et al, 1977), it is appreciated for its crispness and mild sweetness when eaten raw. When cooked, it may be considered a substitute for the chinese water chestnut (Kay, 1973). Although it is presently exported from Mexico to the U.S. (Kay, 1973) there is much potential to develop improved varieties and cultural systems.

Published accounts of Mexican yam bean culture (Bautista and Cadiz, 1967; Kay, 1973) recommend application of nitrogenous fertilizers and fail to mention that this is a nodulated legume. Inoculated *P. erosus* grown at Paia, Maui (NifTAL Project site) in the field without application of chemical nitrogen yielded 27 metric tons/ha within 15 weeks (Chapter V). This is comparable to most other tropical root and tuber crop yields even under moderate levels of nitrogen fertilization. Marcarian (1978) suggests that the potential of this crop to provide its nitrogen requirement through the root nodule symbiosis is a basic research need.

Before field comparisons of yields from inoculated and nitrogen fertilized legumes should be conducted, the *Rhizobium* strain requirement of a given legume must be evaluated. The purpose of this research was to identify effective *Rhizobium* strains for *P. erosus*, to draw inferences concerning the effective cross inoculation group to which this species belongs, and to

compare the growth and nitrogen contents of symbiotic and nitrogen fertilized P. erosus.

MATERIALS AND METHODS

The technique of establishing legumes in a sterile, nitrogen free media inoculated with various strains of Rhizobium makes it possible to rank strains by effectiveness. One liter "Leonard jar" assemblies (Vincent, 1970) were employed using a vermiculite filled upper container which was connected by a cotton wick to a two liter reservoir filled with full strength Broughton and Dilworth solution (1971). These assemblies were sterilized by autoclaving at 121°C and 15 psi for 45 minutes.

Seeds of P. erosus (Tpe-1 from IITA) were treated in concentrated sulfuric acid for five minutes, then repeatedly rinsed in sterilized water. These were germinated on to water agar, selected for uniformity, planted two per vessel and inoculated with two ml. of a turbid suspension of the intended rhizobia (= 2 x 10^9 rhizobia/ml). Twenty three strains from the NifTAL culture collection were compared in this fashion (Table 1) after being raised in yeast extract-Mannitol broth (Vincent, 1970). Two uninoculated controls (zero N and 70 ppm N - supplied as KNO_3) were included. The "Leonard jars" were placed in the glasshouse in a randomized complete block design with 25 treatments replicated three times. After 60 days all treatments were harvested and nodule observations taken. Shoots and roots were separated and oven dried. Selected treatments were analyzed for total nitrogen by a colorimetric technique (Mitchell, 1972).

RESULTS AND DISCUSSION

The dry matter yield, percentage nitrogen in tissues and total nitrogen content of the roots and shoots revealed a wide range of symbiotic effectiveness for the Rhizobium strains tested (Table 1, Table 2, Figure 5, and Appendix 2). The most vigorous of the symbiotic treatments did not produce as much dry matter as the nitrogen-supplied control, but assimilated more total nitrogen. The percentage nitrogen in the tuberous roots of the control treatments (zero nitrogen and 70 ppm N) was lower than in most of the symbiotic treatments (Table 2 and Appendix 2).

The proportion of total dry matter in the tuberous root was not influenced by nitrogen source or symbiotic effectiveness (Tables 2 and 4, Appendix 2). Long dark periods promote secondary thickening of P. erosus (Bautista and Cadiz, 1967; Kay, 1973) and other legumes (Garner and Allard, 1923). Since these plants were grown during short days, it is assumed that partitioning of assimilate was a photoperiodic effect and, therefore, independent of nitrogen nutrition over the ranges tested. However, the proportion of total plant nitrogen in the tuberous root was related to the symbiotic effectiveness of the Rhizobium strain. This is not surprising since nitrogen availability was limiting plant growth. Nitrogen storage in the tuberous roots depended on the nitrogen status of the plant as a whole (Tables 2 and 4).

Certain Rhizobium strains associated with legumes common to the natural habitat of P. erosus varied in their ability to nodulate and fix nitrogen. Isolates from Phaseolus vulgaris (L.) and Leucaena leucocephala (L.) did not nodulate P. erosus. A R. lupini strain (Tal 1102) established a partially effective symbiosis resulting in low tissue nitrogen concentrations, but relatively high dry matter accumulation. Tal 22 and Tal 731, belonging to the Phaseolus lunatus-Canavalia subgrouping of the "cowpea miscellany" were only partially effective.

Two Rhizobium strains widely used in commercial inoculum for many

Table 1

Rhizobium Strain Designation, Source and Rating on *P. erosus*

TAL number[1]	Original host	Laboratory and designation[2]	Rating[3]
734	*Crotalaria juncea*	CIAT 276	H
727	*Calopogonium caeruleum*	CIAT 493	H
1000	*Arachis hypogaea*	NifTAL	P
657	*Pachyrhizus sp.*	UMKL 82	P
651	*Calopogonium mucunoides*	UMKL 44	P
744	*Dolichos sp.*	CIAT 111	P
22	*Phaseolus lunatus*	NifTAL	P
648	*Psophocarpus tetragonolobus*	UMKL 57	P
731	*Canavalia sp.*	CIAT 273	P
761	*Indigofera hirsuta*	CIAT 576	P
1102	*Lupinus alba*	Allen 807	P
848	*Pithecellobium jiringa*	UMKL 67	P
794	*Stylosanthes hamata*	CIAT 543	I
169	*Vigna unguiculata*	Nit 176A22	I
187	*Sphenostylis stenocarpa*	Nit 143A1	I
819	*Clitoria laurifolia*	UMKL 28	I
309	*Dolichos africanus*	Syd. U. CB 756	I
82	*Leucaena leucocephala*	NifTAL	NI
43	*Voandzeia subterrania*	IITA	I
742	*Desmodium heterophyllum*	CIAT 80	I
182	*Phaseolus vulgaris*	NifTAL	NI
849	*Pithecellobium jiringa*	UMKL 68	NI
656	*Pachyrhizus sp.*	UMKL 81	I

[1] Strains are ranked according to total dry matter production after 60 days of growth (see Appendix 2).

[2] Source laboratories
CIAT - Centro International de Agricultura Tropical, Cali, Columbia (P.H. Graham); Allen - O.N. Allen collection, Madison, Wisconsin (Mrs. E. Allen); Nit - Nitragin Company, Milwaukee, Wisconsin (J. C. Burton); Syd. U. - Department of Microbiology, University of Sydney, Sydney, Australia (J. M. Vincent); UMKL - Department of Genetics and Cellular Biology, University of Malaya, Kuala Lampur, Malaysia; NifTAL - NifTAL Project, University of Hawaii, Paia, Maui; IITA - International Institute of Tropical Agriculture, Ibadan, Nigeria (arrived at NifTAL as dried nodules).

[3] Rating from Table 2.

Table 2. Properties of Pachyrhizus erosus in response to symbiotic effectiveness and nitrogen form

grouping	number of isolates	total dry matter	total nitrogen	tissue nitrogen roots	tissue nitrogen shoots	proportion in tuberous roots dry matter	proportion in tuberous roots nitrogen
		g	mg	%			
Highly effective[1/]	2	8.14-8.83	201.4	1.60	4.09	64-70	50
Partially effective	10	3.55-6.67	52.7-179.5	0.67-1.91	2.83-4.57	53-73	33-44
Ineffective [2/]	8	1.17-2.60	9.1-58.5	0.28-1.31	1.35-4.17	57-70	32-37
Non-infective [3/]	3	1.46-1.53	nd [4/]	nd	nd	63-78	nd
Uninoculated	na[5/]	1.46	11.3	0.52	1.33	68	46
+N (70 ppm N as KNO_3)	na	10.79	132.1	0.60	2.66	69	34

1/ dry weight of the tuberous root not significantly different from the +N treatment at the 95% confidence level

2/ not significantly different from the uninoculated treatment at the 95% confidence level

3/ no nodules observed

4/ not determined

5/ not applicable

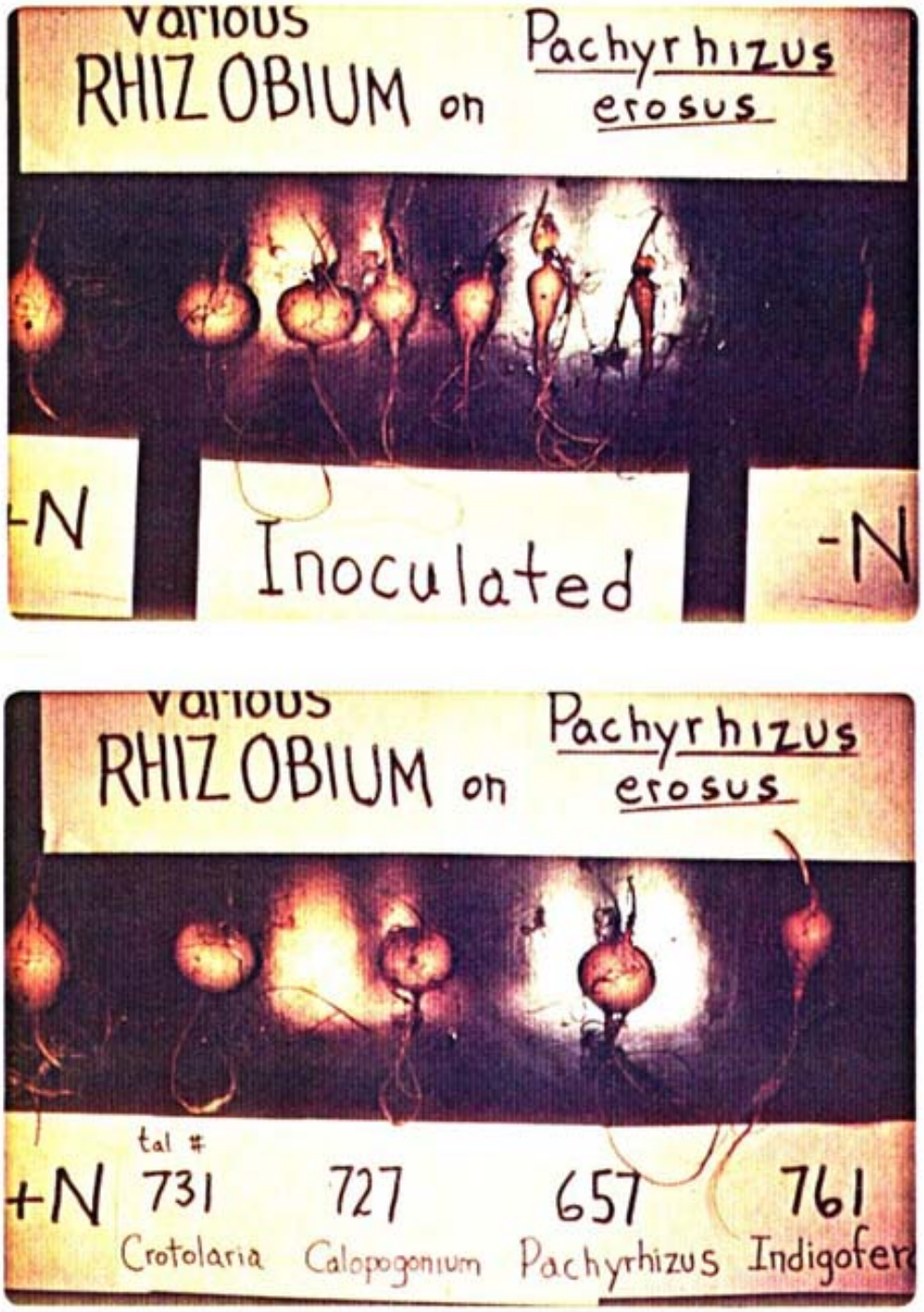


Figure 5. Tuberous root size and shape as a function of Rhizobium strain effectiveness

tropical legumes belonging to the broad "cowpea miscellany", Tal 309 (CB756) and Tal 169 (Nit 176A22) were also only partially effective. The percent nitrogen in the plant tissues was high, but total dry matter and nitrogen accumulation was less than 50% of that obtained with the best strains. Many small, ineffective nodules resulted from inoculation with Tal 742, an isolate from Desmodium heterophyllum DC., a widely distributed Desmodium sp. with a reputation of specificity. The nitrogen demand of this ineffective nodule sink resulted in very low concentrations of nitrogen in the tuberous roots (0.28%).

The effectiveness of host isolates-from Pachyrhizus sp. was quite variable. Tal 656 produced nodules that were completely ineffective while Tal 657 was among the better strains. Both of these strains were collected from the same site in Malaysia. Thus it appears likely that ineffective nodulation must frequently occur in the field.

The most effective strain was a fast growing isolate from Crotalaria juncea (L.). Walker (1928) classified this species as belonging to a separate cross inoculation group from the broad "cowpea miscellany" and other Crotolaria spp. Allen and Allen (1939) reported that C. juncea and C. spectablis Roth. were nodulated by a wide range of "cowpea type" rhizobia, but did not report the effectiveness of the nodules formed. The fact that a strain from C. juncea was the most effective among the diverse strains tested deserves the attention of additional studies to determine whether or not P. erosus and C. juncea belong to the same effective cross inoculation group.

Nodulation seldom occurred on the taproot of P. erosus, rather the early secondary roots were nodulated. Effective nodules were elongate and branching. The active bacteroidal region of the nodules was continuous (Viceae type, after Spratt, 1919), and migrated as the nodule elongated (Figure 1b) with the oldest region of the nodule interior

turning green, but not decomposing with age. Based on the size and longevity of root nodules observed in lengthier pot studies and in the field, the nodules of P. erosus may be functionally perennial. However, the earliest nodules to form on the root system are spacially displaced and crushed or are severed from the roots as the storage organ expands (see Figure 15).

Total plant nitrogen was highly correlated with plant dry weight and also with tuberous root nitrogen (Table 3). Tissue nitrogen concentrations of the shoots and roots were significantly correlated with symbiotic effectiveness, but at a lower level of confidence. This is in agreement with the findings of Duhigg et al (1978) when individuals of a single alfalfa cultivar (Medicago sativa (L.) cv. "Mesilla") were compared for their ability to fix nitrogen.

As was mentioned previously, various authors (Bautista and Cadiz, 1967; Ezumah, 1970) have speculated that root tuberization of P. erosus is regulated by the photoperiod, as it is with Phaseolus coccineus (L.) (Garner and Allard, 1923). Our data seems to support this speculation since the proportion of total dry matter partitioned into the tuberous root was constant irrespective of plant nitrogen nutrition (Table 4). The low levels of nitrogen in the tuberous root of the nitrate supplied treatment suggests that nitrate reduction occurs largely in the shoots, and that the reduced nitrogen is not readily partitioned into the tuberous root. The extent of nitrogen accumulation in the tuberous root may therefore be related to the form in which the nitrogen is supplied to the plant. The fact that some Rhizobium strains (i.e., Tal 309, Tal 656) which have low symbiotic effectiveness resulted in high nitrogen concentrations in the tuberous root support this observation.

Table 3. Regression coefficient matrix of plant dry weight and nitrogen parameters.

Parameter	simple correlation coefficient for linear regression			
	total plant nitrogen	% nitrogen in shoot	% nitrogen in root	total root nitrogen
	r			
total plant dry weight	.97***	.65*	.69*	.97***
total plant nitrogen	--	.76*	.82*	.99***
% nitrogen in shoot	--	--	.91***	.70*
% nitrogen in root	--	--	--	.79**

*, **, *** indicate the 95%, 99% and 99.9% confidence levels respectively.

Table 4. Regression matrix comparing relative effectiveness and tuberous root characters.

Relative [1/] effectiveness	Proportion of plant attribute in the tuberous root	
	Dry weight	Nitrogen
Dry weight basis	.17	.88**
Total nitrogen basis	.02	.89**

** indicates the 99% confidence level.

[1/] relative effectiveness is the value observed for a given symbiotic treatment divided by the maximum observed symbiotic treatment.

SUMMARY

The National Academy of Science called attention to the Mexican yam bean (*P. erosus*) as an "under-exploited" legume. Recommendations for cultivation of this tuberous root crop include fertilization with nitrogen, suggesting ignorance of, or inadequacy of, the nitrogen contribution from this legume's association with *Rhizobium*. Twenty-three strains of *Rhizobium* of widely differing origins were used to inoculate *P. erosus* (Tpe-1 from IITA, Nigeria). Growth of inoculated *P. erosus* plants in Leonard jar culture was compared to uninoculated plants receiving no combined nitrogen and uninoculated plants receiving combined nitrogen (70 ppm N as KNO_3) in the rooting medium. *P. erosus* was nodulated by 20 out of the 23 strains of *Rhizobium* but formed highly effective symbiotic associations with only two strains. The best strains had been isolated originally from *Crotolaria juncea* and *Calopogonium caeruleum*. Strains from *Arachis hypogaea* and *Pachyrhizus tuberous* also proved moderately effective. The results suggest that there is a potential to increase field performance of *P. erosus* through inoculation with superior strains of *Rhizobium* at the time of sowing. The best strain (TAL 734) produced 80% of the dry matter observed in the combined nitrogen treatment. Partitioning of dry matter between the root and shoot was not affected by strain of *Rhizobium* nor source of nitrogen (symbiotic vs combined). The most effective strain increased the nitrogen content of the tuberous root three-fold over the uninoculated control and in the case of an ineffective strain (TAL 742) the nitrogen content was actually below that of the control (0.28% vs 0.52% N).

CHAPTER IV

DIURNAL CHANGES IN SYMBIOTIC NITROGENASE ACTIVITY OF THE TUBEROUS-ROOTED LEGUMES *PACHYRHIZUS EROSUS* (L.) AND *PSOPHOCARPUS TETRAGONOLOBUS* (L.) DC.

INTRODUCTION

Under field conditions, symbiotic nitrogenase activity as measured by the acetylene reduction technique fluctuates diurnally. This has been observed in *Glycine max* (L.) Merr. (Hardy et al., 1968; Mague and Burris, 1972; Sloger et al., 1975; Ayanaba and Lawson, 1977), *Arachis hypogaea* (L.) (Balandreau et al., 1974) and *Vigna unguiculata* (L.) Walp. (Ayanaba and Lawson, 1977). Glasshouse studies indicate this is also the case in non-leguminous symbiosis (Wheeler, 1969; Bond and Mackintosh, 1975) as well as in the rhizosphere of field grown rice (*Oryza sativa*) (Balandreau et al., 1974).

Fluctuations in symbiotic nitrogenase activity observed in the field result from changes in light intensity and temperature (Mague and Burris, 1972). The former regulates photosynthate supply, the latter affects basal metabolic rates of photosynthetic utilization for both host and microsymbiont. Sloger et al. (1975) found that during cloudy days diurnal fluctuation in the symbiotic nitrogenase activity of field grown soybean was greatly reduced. Also average specific nitrogenase activity was significantly correlated with average air temperature but not with average soil temperature, thus the rate of nitrogen fixation is dependent upon the temperature of the photosynthetic organs rather than that of the root nodule environment. Bimodal profiles have been accounted to midday vapor pressure deficit in cowpea (Ayanaba and Lawson, 1977) and to reduction of atmospheric humidity around peanut (Balandreau et al., 1974). Both of these bimodal observations occurred in the tropics.

The concept that carbohydrate supply to the nodules acts as the regulator of nodule activity has been reviewed by Pate (1976). During the photoperiod, not only is more nitrogenase activity resulting from increased photosynthate arriving from the shoot, but nodule soluble carbohydrates and insoluble starch pools are being replenished (Minchin and Pate, 1974). Consequently, the magnitude of carbohydrate supply differences between photo and dark periods is not necessarily reflected in the products of nitrogen fixation or measured nitrogenase activity. Minchin and Pate (1974) have demonstrated this in the growth room using pea (Pisum sativum (L.)) grown in fluctuating day, night temperatures and humidities. More nitrogen fixation took place during the dark period than the photoperiod when temperatures were 12°C and 18°C respectively.

Tuberous-rootedness may greatly alter carbohydrate supply patterns to the root nodules since shoot translocates must pass through a taproot with increasing assimilate demand. At the same time soluble carbohydrates stored in the tuberous root may be available to the energy demand of the root nodules at night. Reports concerning diurnal changes in symbiotic nitrogenase activity of tuberous-rooted legumes are not found in the literature. Consequently a glasshouse experiment was conducted to describe the diurnal patterns in acetylene reduction using Pachyrhizus erosus (Mexican yam bean) and Psophocarpus tetragonolobus (Winged bean). Later growthroom, glasshouse, and field studies sought to elucidate possible relationships between root tuberization and observed patterns in symbiotic nitrogenase activity using Pachyrhizus erosus as a host rather than Psophocarpus tetragonolobus because of the former's more rapid secondary root thickening.

MATERIALS AND METHODS

Experiment 1. Diurnal pattern of Psophocarpus tetragonolobus and Pachyrhizus erosus.

Two winged bean lines (Tpt-1 and Tpt-3) and a Mexican yam bean line (Tpe-1) from the International Institute of Tropical Agriculture, Ibadan, Nigeria, were tested for acetylene reduction at varying times of day. Seeds were surface sterilized in 30% household bleach for six minutes, rinsed in .01N HCl for 5 minutes followed by five rinses with sterilized distilled water. Seeds were germinated on sterile water agar petri dishes. Two-liter pots were filled with vermiculite, planted with three seeds per pot and connected to a sterile subirrigation system modified after Weaver (1975). The nutrient solution used was a modification of Broughton and Dilworth solution (1971) adjusted to pH 6.8 in which 2.5% Fe chelate was substituted for the .02M iron citrate stock solution. After emergence plants were thinned to one plant per pot and inoculated with 1.0 ml. of yeast mannitol broth containing appropriate rhizobia (= 10^9 cells/ml). The pots were arranged in a randomized complete block design with five replicates.

Thirty-eight days after planting, plants were sampled for acetylene reduction in a non-destructive fashion using 20 liter plastic incubation vessels injected with to acetylene and incubated for one half hour (Figures 6 and 7). Sampling was at selected intervals not less than four hours apart. Between incubations, plants were removed from the larger vessels and exposed to moving air. Samples were stored in 10 ml. vacuum tubes and measured for ethylene production by injection into a Varian Aerograph gas chromatograph containing a "Poropak-R" column. Results were expressed as U moles ethylene produced per plant per hour.

Experiment 2. Diurnal changes using different plant propagules of P. erosus.

A second glasshouse experiment tested the extent of which plants resultant from seeds versus those propagated from tuberous roots show diurnal

fluctuation in symbiotic nitrogenase activity. Seeds were treated with concentrated sulfuric acid for five minutes and rinsed several times in sterile, deionized water. Fresh tuberous roots weighing approximately 1.2 kg. were selected from the field, decapitated non-tuberous roots removed, and thoroughly washed. Both propagule types were planted in 20 liter pots containing a mixture of vermiculite and peralite (1:1 v/v). Tuberous roots were planted one per pot, seeds at two per pot. These pots were watered daily with 1.0 liter of the nutrient solution previously described. Upon emergence plants originating from seed were either inoculated with 1.0 ml. of a turbid suspension of Tal 657 or with 1.0 ml. of that suspension diluted 100-fold with quarter strength nutrient solution. The plants from tuberous roots were inoculated with the same 100-fold dilution. Inoculum was diluted to assure that rhizobia would be well distributed around the exterior of the large tuberous root. The design was a randomized complete block with five treatments and four replicates. After 46 days, all plants were sampled for acetylene reduction destructively by incubation of fibrous root systems in 2.0 liter vessels injected with 5.0% acetylene. Ethylene was determined by gas chromatography as previously described at either 0200 or 1400 hours.

Experiment 3. Nitrogenase patterns in field grown P. erosus.

Seeds of Pachyrhizus erosus (Tpe-1) were scarified, inoculated with a peat carrier containing 6.3×10^7 rhizobia of strain Tal 657 per seed, and planted in a randomized complete block design with four replications. Row spacing was 75 cm. with four seeds planted per meter of row (53,333 plants/ha). Bagasse at 0.6% dry weight basis had been recently incorporated to reduce available nitrogen in the soil (a Typic Haplustoll, elevation 100 m.). Basal levels of potassium, magnesium phosphorus (as treble super phosphate), iron and molybdate were added. Plants were watered every other

Figure 6. Vessels and plants for non-destructive acetylene reduction assay in the greenhouse

Figure 7. Pachyrhizus erosus (Tpe-1) at the time of sampling for non-destructive acetylene reduction

day prior to emergence and once weekly thereafter.

Light intensity was measured as μ einsteins/m^2/sec. on a Li-Cor quantum meter but the measurements do not represent light levels during the exact time of sampling. Air temperatures were measured inside the canopy using a shaded bulb thermometer. Soil temperatures were recorded at the 15 cm. depth, temperatures inside the incubation vessels were monitored by insertion of a thermometer through the rubber septum of an unincubated control vessel.

Acetylene reduction activity was determined for four plants one meter of row) in each plot by incubating root samples for one hour with 5% acetylene in 2.0 liter vessels and immediately analyzing for ethylene by gas chromatography. Vessels were immediately placed into a shaded, insulated container equilibrated at 27°C. These were transferred within 25 minutes to the laboratory where the samples were maintained at 27°C prior to injection into the gas chromatograph. Alternate rows were sampled at different stages of root tuberization. Young, non-tuberous plants were harvested after three weeks, mildly swollen taprooted plants after seven weeks and turnip shaped tuberous-rooted plants after twelve weeks. At the later samplings, multiple vessels per plot were required due to the large size of roots. Vigna unguiculata (cv. California Blackeye) was planted into rows vacated by the week 3 sampling. Rates of acetylene reduction were compared at different times of day to tuberous-rooted P. erosus when both species were at the early pod stage.

Experiment 4. Effect of prolonged darkness on nitrogenase level.

P. erosus was grown from seed in the glasshouse for 15 weeks using the method described in Experiment 2 in a completely randomized design with three replicates. These plants were then moved into a growth room with a

12-hour photoperiod of 160 μ einsteins/m^2/sec. of photosynthetically active radiation. Constant leaf temperatures were maintained at 31.4± 0.3°C by evaporative cooling of the air during the day and supplemental heating at night. After an acclimatization period of two weeks, plants were destructively sampled for acetylene reduction during the normal light and dark periods. Following this, prolonged darkness was initiated and samples were taken 8, 32 and 174 hours into the prolonged darkness period.

RESULTS

Experiment 1.

Nitrogenase levels at various times during the day are given in Table 5. At the time of sampling, all of the plants had developed tuberous roots. Total acetylene reduction activity of both tuberous-rooted legume species increased rapidly during the morning and did not decline until late afternoon or early evening (Table 5). Air temperatures were better correlated with nitrogenase activity than were root temperatures (Table 6). These environmental factors are discussed further under Experiment 3.

Experiment 2.

The acetylene reduction activity per unit of nodule weight (nitrogenase specific activity) of plants propagated from transplanted tuberous roots varied to the same extent as those started from seed (Table 7). The concentration of rhizobia in the inoculant broth did not affect nodule mass or nitrogenase activity of those plants raised from seed when Rhizobium numbers were held constant (Table 8). Despite initial shoot dormancy, dry matter increase was greatest in plants developed from tuberous roots. Later work indicated that dormancy in tuberous roots of P. erosus could be overcome by short term acetylene incubation (Appendix 5). The

Table 5. Daily nitrogenase levels of two tuberous-rooted legume species.

Time	Winged bean		Yam bean	Temperature	
	Tpt-1	Tpt-3	Tpe-1	air	pot
	—μ mole ethylene·plant^{-1}·hr^{-1}—			——°C——	
0850	22.6	26.2	8.0	24.3	23.4
1250	30.9	28.8	10.4	33.5	26.5
1700	29.7	27.2	11.5	28.1	29.1
2100	27.5	22.9	8.62	23.8	25.0
0300	18.3	21.8	8.14	21.8	23.0
LSD.05	5.23	n.s.	1.53		

Table 6. Nitrogenase activity as affected by root and air temperature.

Legume		simple correlation coefficient of acetylene reduction vs: Root temperature	Air temperature
Winged Bean	Tpt-1	.82	.84
	Tpt-3	.63	.89*
Yam Bean	Tpe-1	.75	.98**

*, ** indicate the 95% and 99% levels respectively.

Table 7. Specific activity (μ moles ethylene g nodules^{-1} hour^{-1}) of Pachyrhizus erosus root nodules as a function of progagule and sampling time of day.

Sampling time of day	Propagule		
	Seed, concentrated inoculum	Seed, dilute inoculum	tuberous root dilute inoculum
	μ moles·g^{-1} ·hr^{-1}		
1400	177.9	161.7	191.9
0400		115.5	124.0

Bayes LSD (.05) = 50.8

Table 8. Components of yield increase of *Pachyrhizus erosus* as a function of propagule

Propagule	Total Weight	Percentage in component parts		
		Shoots	Roots	Nodules
	g	%		
Seed	1.97	55	38	6
Tuberous root	5.78	77	22	1
t test probability	>.95	>.95	>.95	>.999

Note: *Pachyrhizus erosus* Tpe-1, planted March 9, 1978, harvested April 24, 1978

first roots to emerge from the tuberous root were very fleshy, non-branching and were not infected by rhizobia until they attained several cm. in length.

Experiment 3.

The extent of fluctuations in specific nitrogenase activity in field grown P. erosus did not change with different stages of root tuberization (Figure 8 and Table 9). During week twelve, specific activity levels were reduced compared to earlier observations. After three weeks of growth, nitrogenase activity was very highly correlated with the solar radiation levels (r=.906*) and to a lesser extent with air temperature (r=.800) and soil temperature (r=.776).

Experiment 4.

Symbiotic nitrogenase activity persisted through 174 hours of prolonged darkness, maintaining a level equal to 40% of that during the normal dark period (Table 10). This is discussed later in the text. Under constant temperature and relatively low light levels there were no significant changes in acetylene reduction between the normal dark period and the photoperiod, indicating the importance of fluctuating environment on symbiotic nitrogenase activity.

DISCUSSION

Of the approximately 290 nodulated genera belonging to the Papilionatae, very few have tuberous roots. It is more likely that tuberous roots developed on nodulated root systems than vice versa. Just as Lawn and Brun (1974) have shown that source-sink manipulations affect rates of nitrogen fixation in soybean, root tuberization would also be

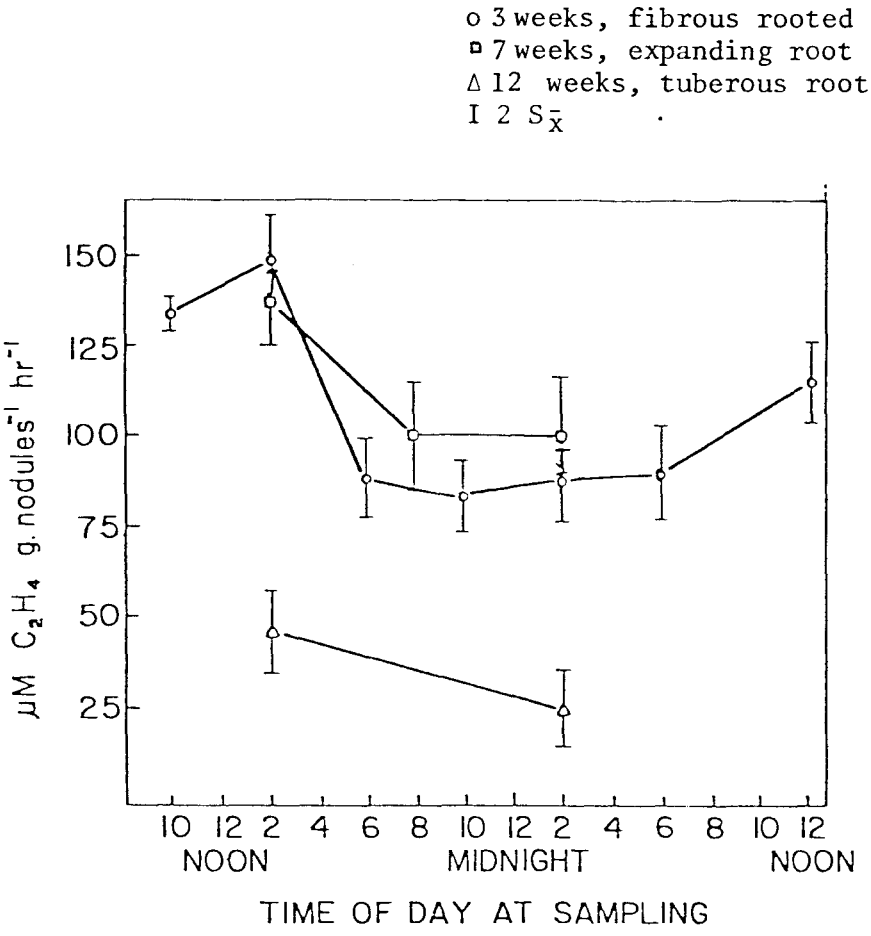


Figure 8. Diurnal changes in symbiotic nitrogenase activity of field grown Pachyrhizus erosus at different states of tuberous-rootedness

Table 9. Acetylene reduction values and root tuberization data during weeks 3, 7 and 12 of field grown *Pachyrhizus erosus*

Week	Specific activity		Root DW	Root/Total	Root N concentration	Stage of root tuberization
	Max.	Min.				
	—μ moles ethylene—		—g—	—— % ——	—— % ———	
3	147.7	83.9	0.06	10.5	1.30	non tuberous
7	137.5	100.4	1.27	12.8	1.89	beginning to swell
12	13.3	6.86	9.66	30.0	1.63	turnip shaped
LSD (0.05)					0.30	

Table 10. Effect of prolonged darkness on symbiotic nitrogenase activity (acetylene reduction) for 17 week old P. erosus.

		μ moles ethylene · g nodule^{-1} · hour^{-1}
normal photoperiod		49.3 ± 11.4
normal dark period		45.2 ± 10.2
prolonged darkness	8 hours	41.6 ± 6.7
	32 hours	29.9 ± 12.2
	174 hours	18.2 ± 2.3

ave. of 3 replicates ± S.E. mean

expected to compete with the root nodules for supply of assimilates. Yet if nitrogen supply is limiting plant growth, it is possible that stored carbohydrate from the tuberous root could help satisfy the energy demands of root nodules, particularly during the dark period when activity is normally lowered. Another possibility could be that all of the carbohydrate supply to the root nodules is regulated through the tuberous root as it develops. Such regulation of nodule assimilates through the tuberous root could have the effect of attenuating diurnal changes in nodule activity.

The extent of diurnal fluctuation in symbiotic nitrogenase activity as affected by root tuberization has been examined in two ways: a) different propagules (taproot vs. tuberous root) and b) different stages of tuberous root development over time. Diurnal changes in nitrogenase specific activity were highly significant but were not altered by the degree of tuberous-rootedness in either case. The field grown tuberous-rooted legumes reported here and annual crop legumes have been shown to be similar in their ratios between maximum and minimum activity values (Table 11).

The applicability of the non-destructive method of sampling was confirmed by the fact that greenhouse grown plants increased in nitrogenase activity between 0900 and 1300 hours by 400, which was identical to the results obtained in Experiment 2 when sampled destructively (Table 11).

In Experiment 2 the type of propagule did not affect the ratio of maximum and minumum nitrogenase activities (Tables 7 and 11) when root nodules of the same age were compared on plants with very different root systems.

That no root nodules formed on the first roots to emerge from the tuberous root may be related to the morphology of the roots which were

Table 11. Ratio of the maximum and minimum observed nitrogenase activities for some field grown and tuberous-rooted legumes.

Crop	Max:Min Ratio	Time of Maxima	Comments	Author
Reports in literature				
Peanut	2.3:1	*	field	Belandreau et al, 1974
Soybean	2.8:1	1600	field, early bloom	Hardy et al., 1968
	5.0:1	1700	field	Mague and Burris 1972
	1.8:1	1600	field, 4 weeks	Sloger et al., 1972
	3.2:1	1200	field, 5 weeks	Sloger et al., 1972
	2.8:1	1200	field, 8 weeks	Sloger et al., 1972
	3.0:1	1700	field, 9 weeks	Sloger et al., 1972
	4.3:1	1400	field, 12 weeks	Sloger et al., 1972
	2.0:1	1000	field, 13 weeks	Sloger et al., 1972
	1.7:1	1000	41 days	Ayanaba and Lawson, 1977
	2.0:1	1700	56 days	Ayanaba and Lawson, 1977
Cowpea	1.7:1	*	field TVU 1190 35 days	Ayanaba and Lawson, 1977
Experimental results				
Cowpea	1.9:1	1400	field, California Blackeye, 8 weeks	
Winged Bean				
Tpt-1	1.7:1	1200	glasshouse, tuberous-rooted	
Tpt-3	1.3:1	1200	glasshouse, tuberous-rooted	
Mexican Yam Bean				
Tpe-1	1.4:1	1500	glasshouse, tuberous-rooted	
	1.8:1	1400	field, 3 weeks, non-tuberous root	
	1.4:1	1400	field, 7 weeks, developing tuberous roots	
	1.9:1	1400	field, 12 weeks, well developed tuberous roots	
	1.1:1	n.a.	growth room, constant temperature	
	1.5:1	1400	glasshouse, 35 days, from tuberous root	
	1.4:1	1400	glasshouse, 35 days from seed	

* indicates bimodal trend
n.a. indicates not applicable

fleshy, lacked fibrous secondary roots and appeared to serve primarily for plant support. This resistance to early infection may also be related to the superior nitrogen status of tuberous roots. The average tuberous root propagule contained more than 1500 mg N (120 g. x 1.3%N dry weight basis) whereas a seed contained only 9 mg N (0.2 g. x 4.1%N). Evans et al (1977) have shown that a considerable portion of the nitrogen in the tuberous root of P. erosus is comprised of free amino acids and non-amino nitrogenase compounds, and these compounds are probably available to support new growth of roots and shoots.

The nodules of P. erosus were elongate and multi-branched. It was observed that as the nodules aged, a decreased portion of the total nodule mass was actively bacteroidal tissue. At the same time the rapidly expanding tuberous root spacially displaced the nodules and their connective roots. If this loss of functional nodules were to shift the plant into a less N-sufficient mode, then this should result in less carbohydrate utilization in the shoots and additional storage of carbohydrates in the tuberous roots. Selected increments of root nodule displacement can be said to alter the plant's nitrogen relations such to promote continued root tuberization.

No root nodules form on the upper taproot of P. erosus, rather the earliest nodules occur on secondary roots. Perhaps tuberous-rootedness in legumes has been selected as a host countermeasure against excess nodulation.

Environmental factors also acted to lower nodule specific activity. Rainfall of 122 mm. was recorded in the 8 days prior to the final sampling date. Waterlogging is known to disrupt nodule function (Mague and Burris, 1972; Minchin and Summerfield, 1976). Soil fauna occasionally attacked root nodules. However, predation upon one section of a multi-branched nodule did not seriously disrupt other sections of that same nodule.

Both light and air temperature were well correlated with measured enzyme activity. The multiple correlation for the equation

$$Y = 270 - 0.58\ X_1 - 5.84\ X_2 + 2.2\ X_3$$

was significant to the 950 level (R = .928) where X_1 = air temperature (°C), X_2 = soil temperature (°C), X_3 = photosynthetically active radiation in micro einsteins·m^{-2}·sec^{-1} and Y = predicted nitrogenase specific activity. Light intensity accounted for most of the variation in nitrogenase levels (r = .908).

Fitting the observed diurnal acetylene reduction values from week three to a Fourier periodic curve (Figure 8) generated the equation

$$Y = 102.8 + 24.6 \cos (cs) + 14.1 \sin (cx)$$

where x is an observed time and c is a constant equal to 360° divided by 24, the number of units in the diurnal cycle. In this case r = .83 and is significant at the 950 level. The predicted maxima (from θtan) occurs at 1200 hours.

Another interpretation of the week three diurnal profile is a two phase linear "sawtooth." Using this model levels remained depressed or increased very slightly throughout the dark period (r = 0.93, b = 0.67 μ moles ethylene·g $nodules^{-1}$ hr^{-1}). With resumption of the photoperiod, nitrogenase levels increased steadily until the mid-afternoon (r = 0.86, b = 5.94). The two phase linear ("sawtooth") interpretation was less significant than either the multiple linear or the periodic interpretations, owing to loss of degrees of freedom. Also there were no sampling points between mid-afternoon and early evening, consequently an important phase of the cycle was not described.

The extent of diurnal fluctuation in symbiotic nitrogenase activity of field grown P. erosus at three stages of root tuberization is compared to that of Vigna unguiculata in Table 12. Attenuation of diurnal changes in nitrogenase with increased root tuberization would be evident in the

interaction term. This was not the case; significant changes in activity at different times of day for both species at all three sampling dates and stages of root tuberization were observed, and the interaction term was not significant in any of these situations.

That nitrogenase activity continued through 174 hours of prolonged darkness is impressive but cannot be taken as direct evidence of tuberous root support of root nodules. Other investigators (Hardy et al., 1968) have found that legumes without tuberous roots also continue to fix nitrogen during prolonged darkness periods. Investigations directly comparing tuberous-rooted and non-tuberous legume cultivars' abilities to fix nitrogen would be facilitated if non-tuberous lines of Pachyrhizus erosus (L.) were known and available. Other species which could provide these comparisons would include Vigna unguiculata vs. V. vexillata (L.) Benth. and different root types of Psophocarpus tetragonolobus . Although much of the carbohydrate in the tuberous root of Pachyrhizus erosus is in the form of soluble sugars and could presumably be mobilized to support symbiotic nitrogen fixation, these assimilates do not seem to provide any buffering effect for maintaining nitrogen fixation at a constant rate throughout the day.

The knowledge of daily nitrogenase activity profiles does have the advantage of providing a better basis for selecting sampling times of day that result in minimal variance. Diurnal patterns generated in the glasshouse and field indicate that nitrogenase activity increases rapidly during the morning and stays relatively constant throughout the later morning and early afternoon for both of the tuberous-rooted tropical legumes tested. Quantitative description of the fluctuation in daily nitrogenase activity as measured by acetylene reduction also permits the extropolation of daily nitrogenase activity from single timepoint observations. This had been done for peanut (Balandreau et al., 1974), soybeans (Bezdicek et al.,

Table 12. Fluctuation in nitrogenase specific activity for *V. unguiculata* and *P. erosus*

Species	Age	Nitrogenase specific activity at 0200 hours	1400 hours
	—weeks—	— μ moles C_2H_4 g nodules^{-1} hr^{-1} —	
V. unguiculata	8	55.9	90.0
P. erosus	3	89.3	116.8
P. erosus	7	101.1	137.5
P. erosus	12	25.7	45.6

ANOVA	df	F [1/]
Legumes	3	16.8***
Time of day	1	11.3**
Legumes x Times	3	0.2 n.s.

1/ ** P > 0.95; *** P > 0.99

1978) and alfalfa (Duhigg et al, 1978). The later two authors included mention of this in their materials and methods, indicating the importance of diurnal variation in symbiotic nitrogenase activity as a methodology study.

In conclusion, the sink capacity of the root nodules as measured by acetylene reduction in tuberous-rooted, symbiotic legumes appears to be as dependent upon recent photosynthate as are normal rooted legumes. Sampling for maximum acetylene reduction activity should be undertaken during the late morning and early afternoon.

SUMMARY

Two tropical tuberous-rooted legume species, Pachyrhizus erosus (L.) (Mexican yam bean) and Psophocarpus tetragonolobus (L.) DC. (winged bean) fluctuate in their daily nitrogenase levels as measured by acetylene reduction. Additional investigations compared the extent of diurnal fluctuation to increased root tuberization. Root tuberization does not alter the daily nitrogenase profile observed in seedlings of P. erosus. Nodule activity of the Mexican yam bean continues through 174 hours of prolonged darkness.

CHAPTER V

ACCUMULATION AND DISTRIBUTION OF DRY MATTER AND NITROGEN IN PACHYRHIZUS EROSUS (L.)

INTRODUCTION

Total plant growth, and the partitioning of that production into the storage organ are basic factors in the yield of tuberous roots attainable in Pachyrhizus erosus (Mexican yam bean). Description of the patterns of tuberous root growth and nitrogen accumulation over time are not available for P. erosus, even though this plant has been identified as adapted to the stress conditions of the humid tropics (Rachie and Roberts, 1974) and is considered to be a tropical legume of under-exploited potential by the National Academy of Science (in press).

P. erosus is a symbiotic legume, yet the use of nitrogenous fertilizers has been recommended in its culture (Bautista and Cadiz, 1967; Kay, 1974) without mention of nodulation. Marcarian (1978) stated that the potential of this crop to fix nitrogen in the field is not currently known.

Flower and pod removal have been shown to increase root weight in another tuberous-rooted perennial legume, Psophocarpus tetragonolobus (L.) DC. (Bala and Stephenson, 1978; Harath and Fernandez, 1978). Flower removal is practiced with P. erosus under traditional cropping systems (Deshaprabhu, 1966; Kay, 1973; NAS in press) but no detailed description of the response of the plant to this source-sink manipulation is available.

In this experiment, inoculated P. erosus was grown without the addition of chemical nitrogen. Plants were harvested at selected intervals and evaluated for 1) accumulation and partitioning of dry matter

and nitrogen, 2) nodulation and nitrogen fixation, and 3) the effect of periodic flower removal on root tuberization and plant growth.

MATERIALS AND METHODS

A field experiment was carried out at the NifTAL site near Paia, Hawaii on a Typic Haplustoll soil with a pH of approximately 6.0. Bagasse at 0.6% dry weight basis was incorporated to reduce available nitrogen in the soil. Basal levels of potassium, phosphorus (as treble super phosphate), magnesium, iron and molybdate were tilled into the soil prior to planting.

Seeds of P. erosus (Tpe-1) originally from the International Institute of Tropical Agriculture, Ibadan, Nigeria were scarified in concentrated sulfuric acid for five minutes, followed by repeated rinses with tap water. These were then inoculated with a peat carrier containing 6×10^7 rhizobia per seed of strain Tal 657 (=UMKL 82, an isolate obtained from the University of Malaya) and planted in a randomized complete block design with four replications on August 8, 1978 (Figure 9). Row spacing was 75 cm with four seeds per meter of row (53,333 plants per hectare). Plants were watered every day prior to emergence, and once weekly thereafter.

Plants were harvested at selected intervals, divided into components, and oven dried at 65°C. Acetylene reduction activity of the root nodules was determined for four plants (one meter of row) in each plot by incubating root samples for 1 hour at 27°C with 5.0% acetylene in 2.0 liter vessels and immediately analyzing for ethylene using a Varian aerograph gas chromatograph containing a "Poropak-R" column. Results were expressed as µ moles ethylene evolved per plant per hour. Root nodules were separated from the root and oven dried. Nitrogen

Figure 9. Field experiment at the NifTAL Project site, Pachyrhizus erosus 5 weeks after emergence, Vigna unguiculata had been recently planted in rows vacated by the week 3 sampling

determinations were made by digestion in sulfuric acid followed by a colorimetric determination of ammonium after the technique of Mitchell (1972).

In each block, 5 meter sections of row were chosen at random for flower removal treatment. All inflorescences were removed each week from these row sections beginning 10 days after first bud and continuing until harvest. These plants and comparable controls were then harvested at 15 weeks and fresh and dry weights and nitrogen content were determined for shoots, flowers and pods, and tuberous roots. Total dissolved solids from the supernatant of crushed, centrifuged tuberous roots were measured with an American optical hand held refractometer.

RESULTS AND DISCUSSION

Dry Matter Accumulation.

The growth of different plant components over time is given in Figure 10. Rapid shoot growth preceded storage organ accumulation which in turn preceded inflorescence development and podfill. This pattern of storage organ "bulking" is similar to that of potato (*Solanum tuberosum* (L.)), yams (*Dioscorea alata* (L.)) and cassava (*Manihot esculenta* (Crantz.)) described by Milthorp (1967) and Loomis and Rapoport (1976). *P. erosus* thus follows the *phasic* pattern of storage organ accumulation in which early vegetative growth is characterized by predominance of shoot and fibrous root growth. Storage organ "bulking" begins later in the growth cycle of the plant and may require environmental induction (Loomis and Rapoport, 1976). For example, *P. erosus* did not tuberize during 14 hour photoperiods (Kay, 1973) but tuberized readily in Hawaii, especially under short days. *Phaseolus coccineus* (L.) is another example which is known to have a short day requirement for initiation of secondary root thickening (Garner and Allard,

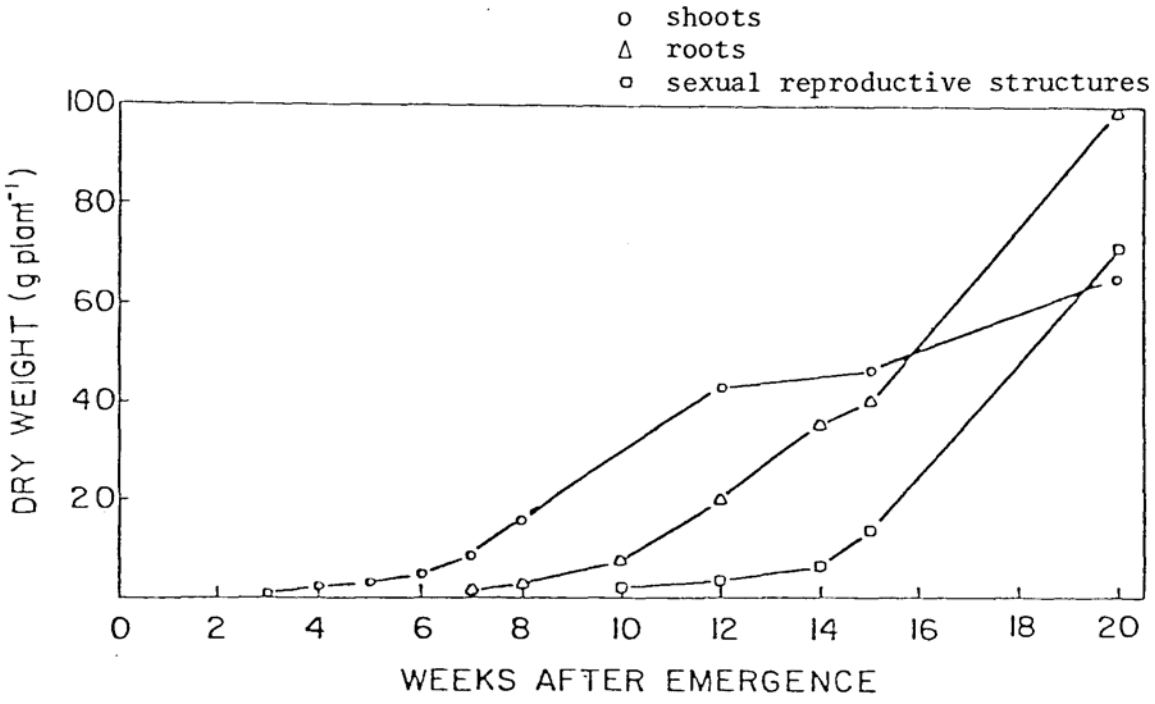


Figure 10. Dry matter distribution of field grown Pachyrhizus erosus over time follows phasic partitioning

1923). The change to storage organ growth, and later to podfill, was dramatic. At seed maturity the aerial portions of P. erosus senesce (Deshaprabhu, 1966). This is the "death by exhaustion" described by Milthorp (1967) of potato except that instead of nutrient and assimilate migration to one sink, there are two strongly competitive sinks: the tuberous roots and the reproductive organs.

Once established, the reproductive structures and the storage organ competed equally for assimilate despite the positional advantage of the pods. P. erosus appears to be unique in that two strong sinks are in operation simultaneously and are in strict competition with one another, rather than the situation in which assimilate from the tuberous roots is used to support seed development as in the case of radish (Raphanus sativus L.) or sugar beet (Beta vulgaris). The tuberous root of P. erosus is the only perennial feature of the plant in its natural life cycle. If the tuber is left in the ground, some carbohydrates and nutrients stored in the tuberous root are eventually used to re-establish new vegetative shoots after the aerial portion of the plant dies. Tuberous-rootedness thus indirectly provides an additional opportunity for seed production the next season.

Nitrogen Accumulation and Tissue Concentration.

While the storage organ and the sexual reproductive sinks may compete equally for carbon (Figure 10), podfill is a stronger sink for nitrogen than is the tuberous root (Figure 11). Plant nitrogen, whether symbiotic or absorbed, passes through the tuberous root as it is translocated upwards in the xylem to the aerial portion of the plant. It may be concluded that even though the tuberous root has a positional advantage for nitrogen

accumulation, it is at a competitive disadvantage for nitrogen compared to the developing pods and seeds.

The pattern of nitrogen accumulation in the vegetative shoot is similar to that of dry matter. There is a phase or rapid nitrogen accumulation; then a plateau is established, followed by diversion of nitrogen to the rapidly expanding tuberous root. Initially, nitrogen accumulation in the reproductive parts lags behind that of the storage organ, but later nitrogen accumulation is faster in the developing pods and seeds (figure 11). After twenty weeks of growth, 205 kg/ha of nitrogen had accumulated in the crop. Of this, 37%, 23% and 40% were found in the vegetative shoots, the tuberous roots and the sexual reproductive structures, respectively.

The phasic pattern of partitioning into the shoots followed by accumulation of nutrients in the roots was reflected in the tissue nitrogen concentrations (Figure 12). The nitrogen concentration in the shoots increased steadily until the fifth week following emergence. At this time shoot nitrogen concentrations reached a plateau and nitrogen then began to accumulate in the roots. When flowering was initiated, both shoot and root nitrogen concentrations decreased significantly. The percentage concentration of nitrogen in the reproductive parts also decreased as the nitrogen was steadily diluted during peduncle development. At the final harvest much of the plant stem tissue was associated with the peduncles.

It is important to note that during the period of storage organ "bulking" (after week 8) the nitrogen concentration of the root storage organ remained relatively constant. Consequently the time of harvest did not greatly affect the percentage of nitrogen in the tuberous root.

Nodulation and Nitrogen Fixation.

Despite addition of 0.6% bagasse and consequent raising of the soil carbon-nitrogen ratio, the products of nitrogen fixation, as estimated by

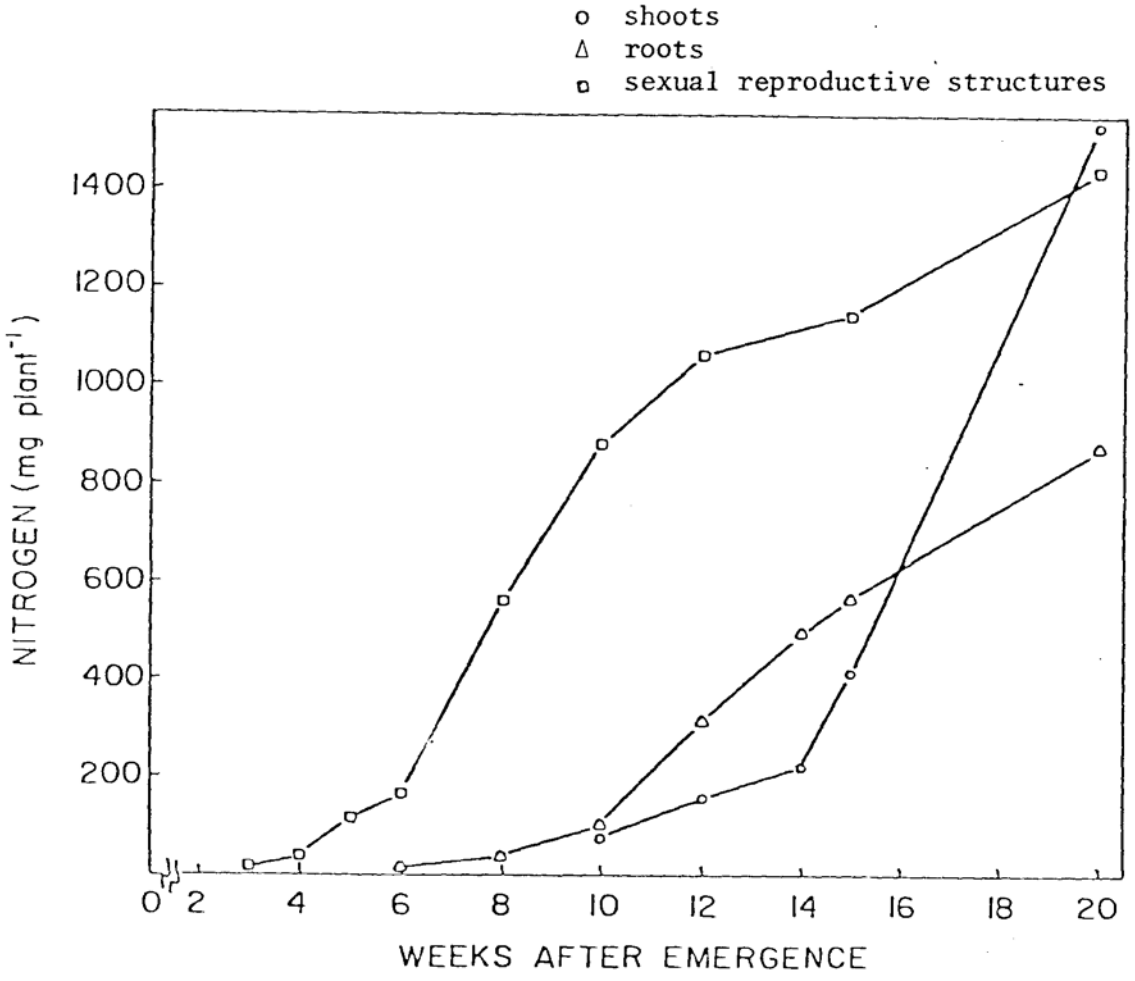


Figure 11. Nitrogen accumulation of the components of total yield over time, podfill is a strong sink for available nitrogen

acetylene reduction, contributed only a fraction of the total plant nitrogen. The soil at the experimental site was a Typic Haplustoll and may be considered quite fertile. Various molar ratios of acetylene-reduced to nitrogen-fixed have been described; generally these fall between 2.7 and 4.2 for soybean (Bergersen, 1970). Assuming the ideal ratio of three acetylene molecules to each molecule of atmospheric nitrogen reduced, and compensating for diurnal fluctuations in nitrogenase activity (Chapter IV), the proportion of symbiotically-fixed nitrogen to total plant nitrogen was calculated to be 9.2%, 4.5%, and 1.8% at weeks 3.5, 6.5 and 12, respectively (Figure 13). However, total nodule recovery was probably incomplete during the latest sampling.

Because this was a site specific observation, additional studies must be conducted before the genetic potential of P. erosus to provide its nitrogen requirement through the root nodule symbiosis can be determined.

The nodule mass per plant (Figure 14a) and the nodule specific activity (μ moles ethylene ·g nods^{-1} ·hour^{-1})(Figure 14b) indicate that between weeks three and eight, nodule mass increased linearly (r=0.98) from 0.10 to 0.52 grams of nodules per plant. Between weeks eight and twelve, both nodule mass and nitrogen fixation decreased. Standing water resulting from heavy rains and consequent waterlogging probably was responsible for most of this decrease. This is in accord with observed reduction in nodulation of Vigna unguiculata due to waterlogging (Minchin and Summerfield, 1976). Also, increases in tuberous root diameters as "bulking" proceeded resulted in the necrosis of many of the root nodules as these nodules were spacially displaced and connective tissues severed by the expanding tuberous root (Figure 15).

Nitrogenase specific activity did not vary greatly between weeks three and seven, despite changes in nodule and root morphology that took place at that time (Figure 16). However, the same factors which reduced nodule mass, particularly oxygen stress, probably acted to reduce nodule specific activity

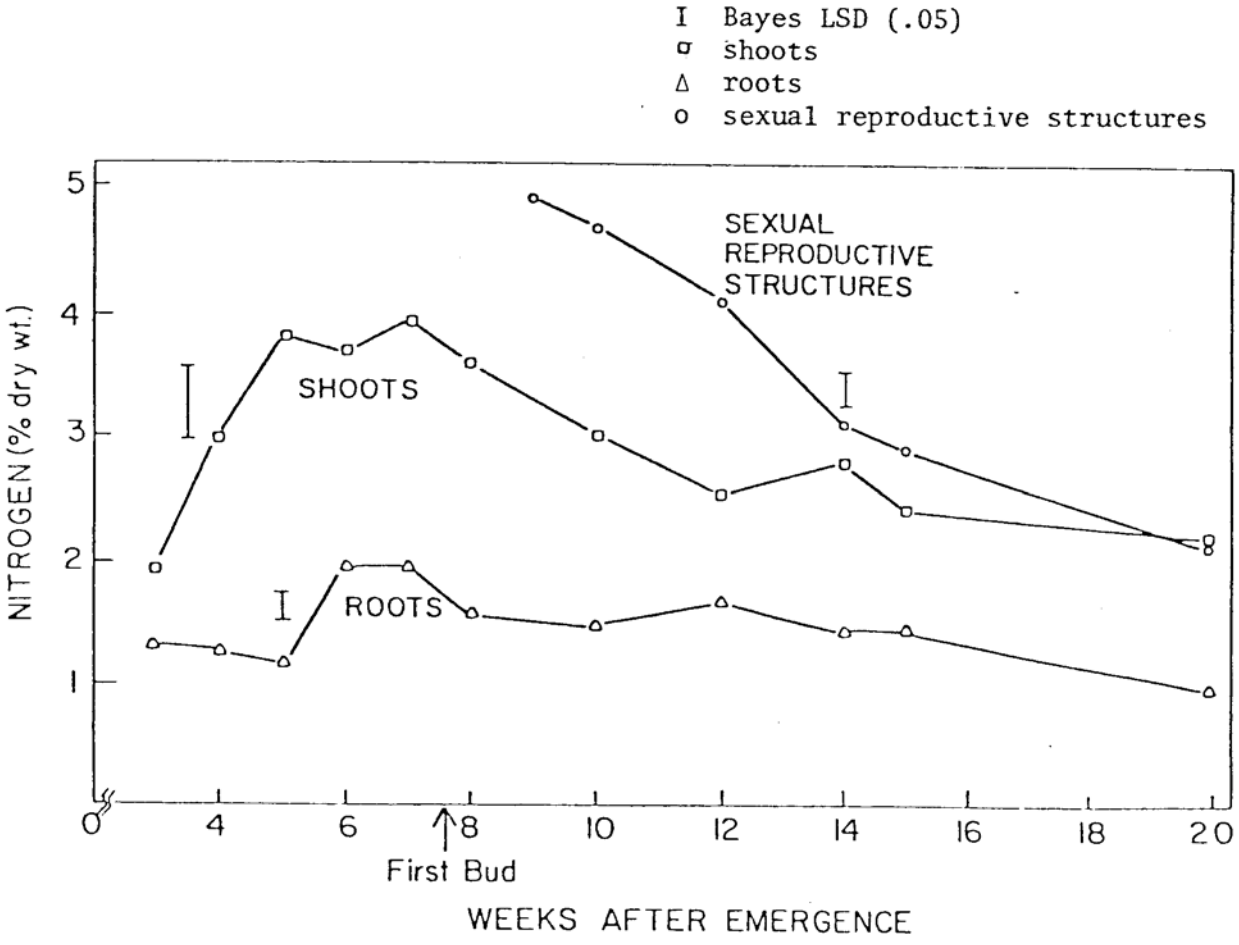


Figure 12. Percentage nitrogen in the tissues of plant components over time

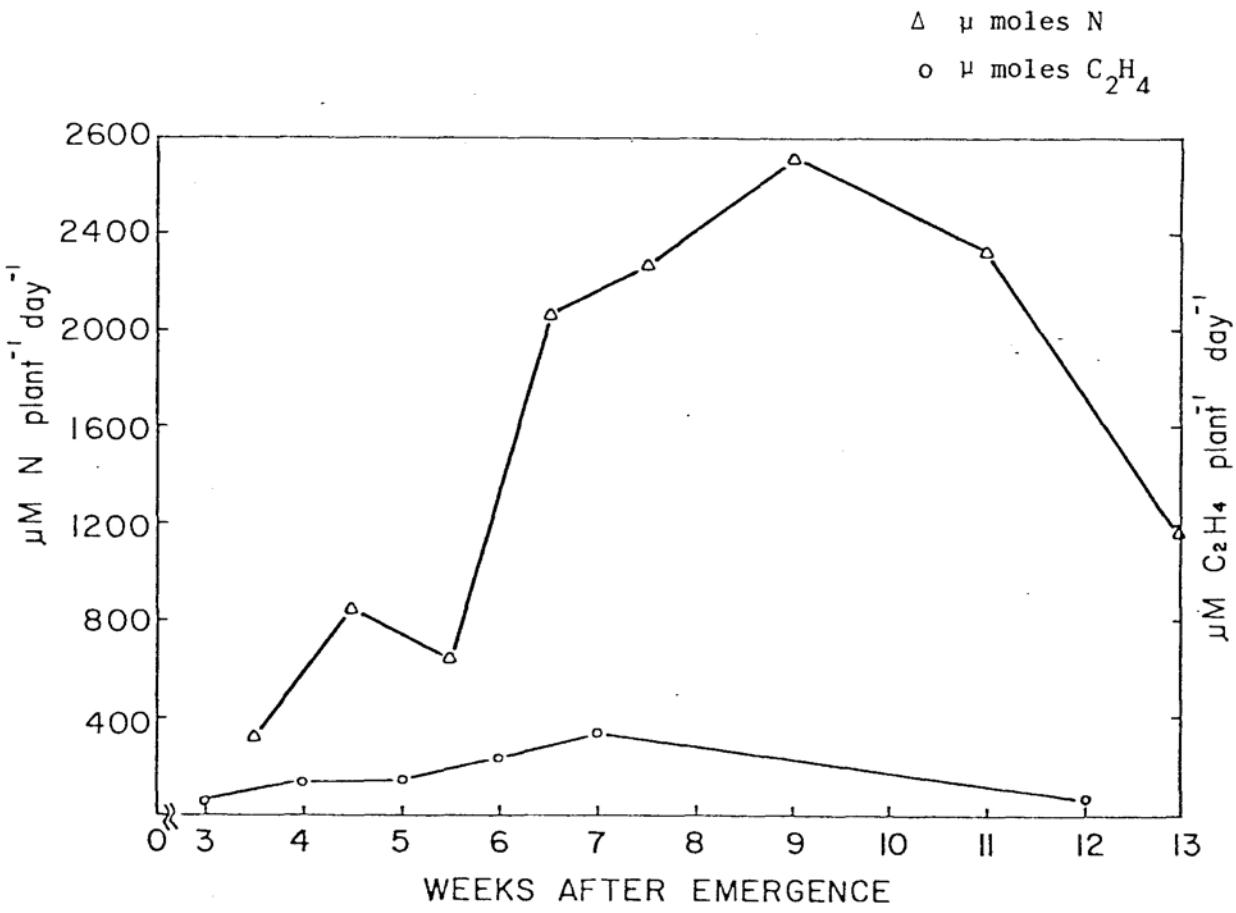


Figure 13. Rates of nitrogen accumulation and acetylene reduction by field grown Pachyrhizus erosus over time

I Bayes LSD (.05)

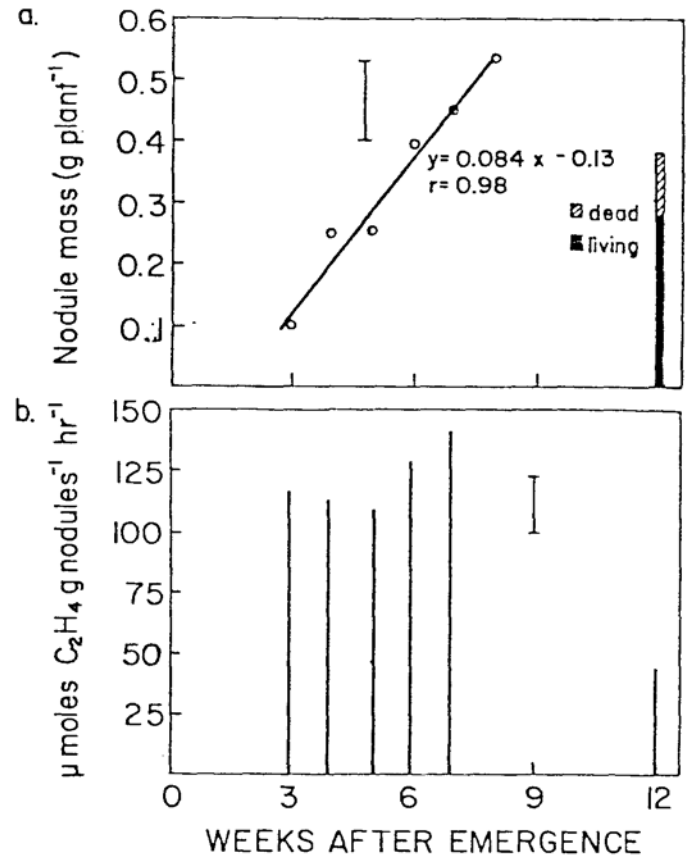


Figure 14. Nodule mass (a) and specific nitrogenase activity (b) of field grown Pachyrhizus erosus over time

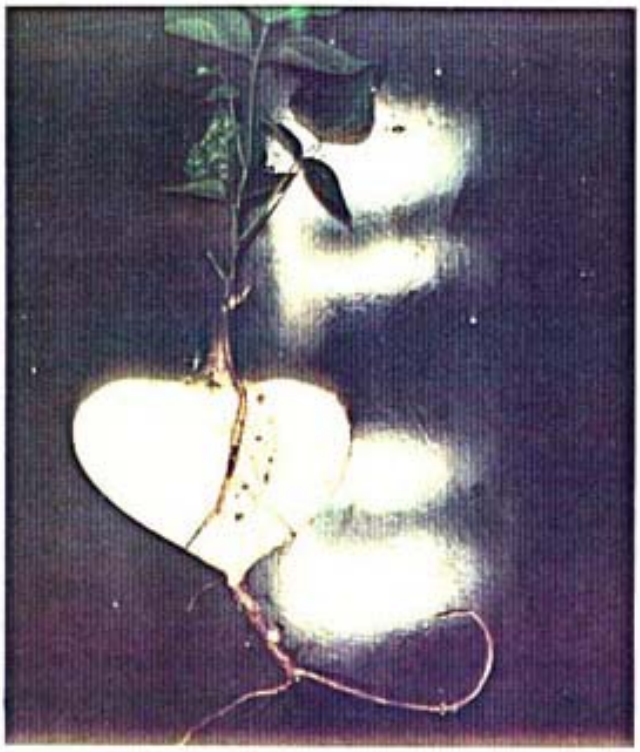


Figure 15. Spacial displacement of the early root nodules of Pachyrhizus erosus by the tuberous root

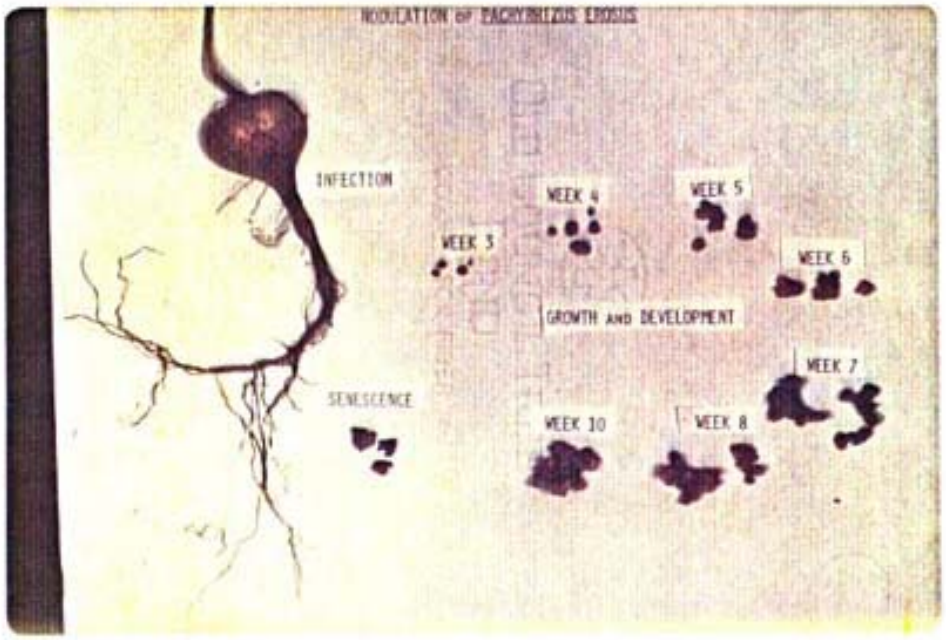


Figure 16. Root nodule growth and development of field grown Pachyrhizus erosus

during the week 12 observation.

Effects of Pod Removal.

In P. erosus root tuberization and seed development were competitive and substitutable sinks. Total dry weight and tuberous root weight were increased by flower bud removal (Table 13, Figures 17 and 18). The increase in dry weight of tuberous roots due to flower removal was greater than the weight of the reproductive parts of normal plants, indicating that some fraction of assimilate that was diverted from pod fill promoted increased vegetative vigor, which in turn resulted in increased dry weight of tuberous roots (Table 13).

Substituting a more efficient sink for a less efficient one increased net assimilation in sugar beet (Beta vulgaris (L.)) grafting experiments (Thorn and Evans, 1964), as well as in graft combinations between two subspecies of Beta vulgaris, chard and sugar beet (Loomis et al, 1976). Root tuberization may be a more efficient sink than seed development in P. erosus, accounting for this increased dry matter when pods were removed. Increased root tuberization in response to periodic flower removal has been documented by Bala and Stephenson (1978) and Herath and Fernandez (1978) in Psophocarpus tetragonolobus, another tuberous-rooted legume.

Flower removal of P. erosus resulted in significant increases in total nitrogen accumulation in the roots (+ 3.0 kg N/ha) and shoots (+ 22.5 kg N/ha). Root nodule activity could not be assayed at this late stage of root development, and since the root system of the deflowered treatments was larger than that of the control, the effects of increased nitrogen fixation and uptake of soil nitrogen could not be separated. In soybean, pod removal has also been shown to result in increased root weight (Loong and Lenz, 1974) as well as increased nitrogen fixation (Lawn and Brun, 1974).

The nitrogen percentage of the tuberous roots of P. erosus was not

Table 13. Effects of inflorescence removal on field grown *Pachyrhizus erosus* [1/]

	Treatment		
	Flowers Removed	Control	t test [2/]
DRY WEIGHT			
Total per plant (g.)	117.3	100.9	0.1
Tuberous root per plant (g.)	65.3	39.1	**
Proportion in tuberous root (% of total)	55.7	39.4	***
Flowers and pods per plant (g.)	0.37	14.4	3/
NITROGEN			
Total per plant (mg.)	2604	2126	*
Total in tuberous root (mg.)	988	567	**
Proportion in tuberous root (% of total)	37.9	26.6	**
Content in tuberous root (%)	1.51	1.42	ns
Content in shoot (%)	2.93	2.50	*4/
DISSOLVED SOLIDS			
Total per tuberous root (g.)	38.5	23.9	*
% of tuberous root dry weight	58.3	60.0	ns
FRESH WEIGHT CHARACTERS			
Weight per tuberous root (g.)	688.3	448.8	**
Water content (%)	90.3	91.0	*
Symmetrical tuberous root (%)	93.7	100	*
Multiple tuberous roots per plant (%)	5.3	3.5	ns
Cracked tuberous root at harvest (%)	44.0	39.3	ns

1/ *Pachyrhizus erosus*, Tpe-1 from IITA, planted at 53,333 plants/ha, 50% emerged-August 17, 1978, first bud - October 9, 1978, pruned weekly - October 19 to November 22, 1978, harvested - November 28, 29 (seeds not mature)

2/ 0.1, *, **, *** indicate the 90%, 95%, 99% and 99.9% confidence levels, respectively

3/ not tested, obviously different

4/ one of four replicates not included

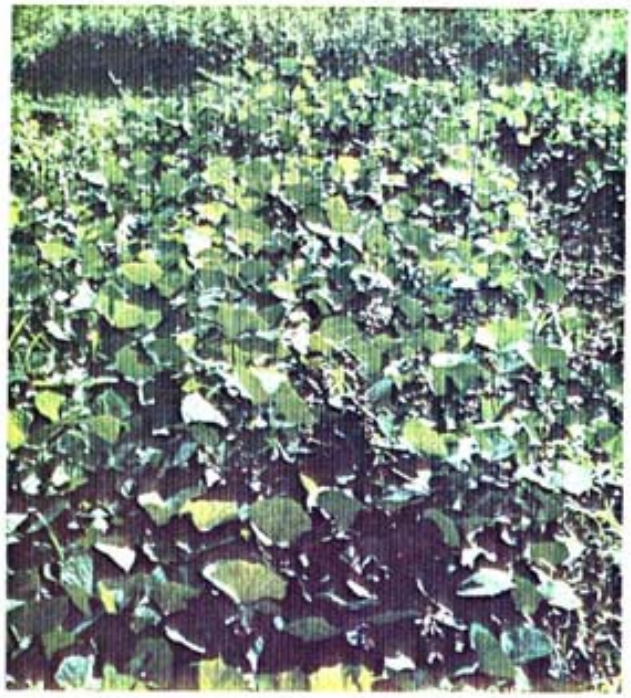


Figure 17. Effects of flower removal on field grown Pachyrhizus erosus, flowers removed (left), control (right)

Figure 18. Effects of deflowering Pachyrhizus erosus

significantly altered by deflowering; therefore, unlike the nitrogen contained in the shoots, the nitrogen stored in the tuberous roots was not available to the demands of later podfill. The nitrogen percentage of the tuberous root declined somewhat at initiation of the inflorescences, but remained constant thereafter (Figure 12).

Deflowering, and consequent lack of assimilate diversion into the reproductive sink, did not influence the concentration of total dissolved solids in the tuberous root (Table 13). However, both total dry matter and total dissolved solids per tuberous root were increased as a function of increased tuberous root weight. This could be an attractive and easy management practice since most of the inflorescenses of P. erosus rise well above the leaf canopy of unstaked plants.

The slight decrease in the moisture content of the tuberous roots that resulted from deflowering was significant. However, this 0.7% difference does not nearly offset the increase in tuberous root yield.

Lateral symmetry of the tuberous roots was decreased by deflowering. This could affect the efficiency of mechanical harvesting schemes, but since these schemes do not presently exist, this is of small consequence. Much more serious would be loss of market appeal due to irregular tuberous root shape.

Neither the frequency of multiple tuberous roots per plant nor the cracking of tuberous roots were related to flower removal. Multiple tuberous roots per plant probably resulted from branching of the young tap root apex. Since this occurred prior to flowering, the removal of those flowers would not result in extra multiple tuberous roots.

Cracking of the tuberous roots (Figure 19) was associated with heavy rainfall and standing water. During the eight days prior to harvest, 122 mm. of rainfall was recorded. Pronounced lenticil development (Figure 20) preceded the cracking, indicative of poor oxygen relations in the plant

root. Cracking served to raise the ratio of root surface area to mass. Exposed interiors of the tuberous roots callous quickly, but there was some growth of saphophatic fungi. Planting on raised beds should reduce the problem of tuberous root cracking under wet soil conditions.

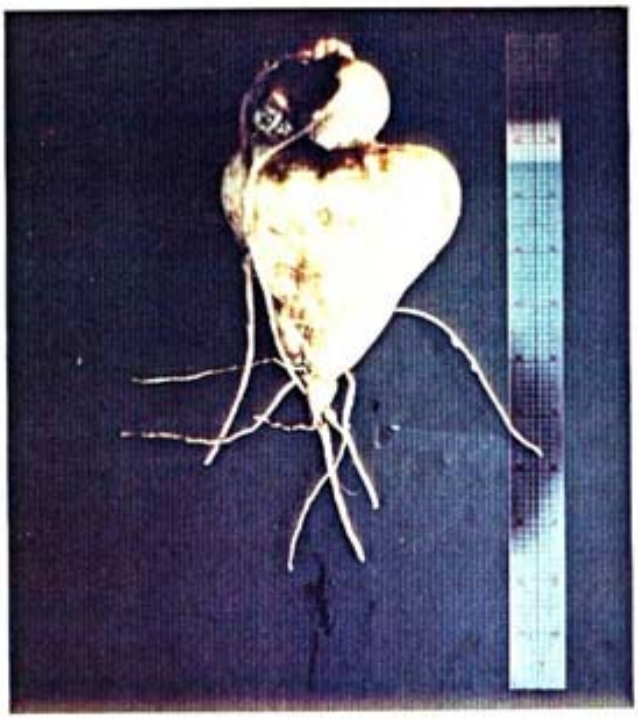
Cracking did reduce the proportion of marketable tuberous roots in the deflowered treatment but total marketable yield per unit area was still increased (Table 14). Total tuberous root and total marketable tuberous root yield was increased by 16.2 metric tons/ha and 6.1 metric tons/ha, respectively.

In conclusion, the benefits of deflowering P. erosus as a photosynthetic source-sink manipulation in the field increased yield and nitrogen accumulation. Problems associated with such treatment were decreased root symmetry and moisture content. These are relatively minor compared to the 33% yield increase of marketable tuberous roots.

SUMMARY

Pachyrhizus erosus demonstrated phasic partitioning of dry matter and nitrogen into shoots, followed by tuberous roots, followed by reproductive structures. The nitrogen content of the tuberous root remained constant during the period of root enlargement. P. erosus was shown to be a nodulated legume, but its genetic potential to meet its nitrogen requirement through symbiosis could not be determined in the present study. Deflowering P. erosus resulted in increased root tuberization and nitrogen accumulation and should be considered as a field scale management practice for the production of tuberous roots of this legume.

a)



b)

Figure 19. Extremes of tuberous root cracking
a) minor cracking of secondary tuberous root
b) extreme cracking

Figure 20. Prolific lenticel development on the tuberous root of deflowered treatment (left), control (right)

Table 14. Fresh tuberous root yields after 15 weeks as affected by inflorescence removal.

Parameter	Fresh yield of tuberous roots	
	Control	Flowers removed
	metric tons/ha	
Total yield	26.9	43.1
Marketable yield	18.7	24.8

CHAPTER VI

THESIS SUMMARY

Several aspects of symbiotic nitrogen fixation and root tuberization of Pachyrhizus erosus (L.) (Mexican yam bean) were examined in the growth room glasshouse and field at the NifTAL Project site, Paia, Maui.

A wide spectrum of Rhizobium isolates were capable of nodulating P. erosus but only two of the twenty three strains examined were able to establish highly effective symbiotic relationships when compared to the nitrogen supplied control. The best symbiotic treatments assimilated more nitrogen but less total dry matter than did the nitrate treatment.

The nitrogen nutrition of the plant did not affect the proportional partitioning of dry matter into the tuberous root during short days but the tissue concentration of nitrogen in the tuberous root was influenced by both the form of available nitrogen and the effectiveness of the root nodule symbiosis.

The extent of diurnal fluctuation in symbiotic nitrogenase activity as measured by acetylene reduction was not altered by changes in root morphology during root tuberization. This aspect of nitrogenase activity was no different than that of the normal rooted legume Vigna unguiculata (L.) Walp. The ratio of maximum to minimum activity varied from 1.4:1 to 1.9:1 under field conditions. Diurnal changes in nitrogenase were shown to result from fluctuations in the environment, and not from an endogenous rhythm. Prolonged darkness of 174 hours reduced nitrogenase specific activity of tuberous-rooted plants to 40% of the original dark period level. This was not direct evidence that the tuberous root supports nitrogen fixation because other investigators have observed similar results with normal rooted legumes.

Storage organ accumulation was shown to follow the phasic pattern of partitioning. In the field, root tuberization and seed development were equal, competitive sinks for carbon but the reproductive structures competed more strongly for nitrogen than did the tuberous roots. Thus inflorescences were not able to exploit the positional advantage for fixed carbon and the tuberous root was not able to exploit its advantage for assimilation of translocated nitrogen.

Deflowering P. erosus increased root tuberization and total root nitrogen and could be practiced on a field scale since the inflorescences rise well above the unstaked leaf canopy. The marketable yield of tuberous roots after 15 weeks of growth was increased 30% by periodic flower removal (24.8 and 18.7 metric tons/ha for deflowered and control treatments respectively).

CHAPTER VII

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APPENDIX I

Productivity of Root Crops in Hawaii

Crop	yield [1] (lbs/ac. x 10^3)	average time to harvest [2] (days)	average price ($/lb.)	gross productivity ($/ac./day)
Taro	16.7	405	0.127	5.24
Sweet potato	12.5	150	0.220	18.33
Lotus root	5.0	210	0.841	20.02
Irish potato	12.5	120	0.198	20.63
Carrot	12.5	95	0.173	22.76
Daikon	21.6	65	0.122	40.54
Dasheen	27.0	300	0.478	43.02
Burdock	19.8	160	0.396	49.00
Ginger	25.9	300	0.658	56.81
Onion	23.1	125	0.356	65.79
Radish	11.5	35	0.236	77.54

[1] per acre yield and per pound value from Statistics of Hawaiian Agriculture. 1977. Hawaii Agricultural Reporting Service.

[2] time to harvest from Planting Guide for Vegetables in Hawaii. Hawaii Cooperative Extension Service.

APPENDIX 2

Effects of Rhizobium Effectiveness on the Components of Yield and Nitrogen Content of Pachyrhizus erosus 1/

Rank	NiFTAL isolate	Original host	Dry Matter 2/ (g.) Root	(g.) Shoot	(g.) Total	Relative effectiveness (% of max.)	Proportion of dry matter in tuberous root (%)	Nitrogen concentration (%) root	(%) shoot	Nitrogen Accumulation (mg) root	(mg) shoot	(mg) total	Relative effectiveness (% of max)	Proportion of Nitrogen in tuberous root (%)
1	734	Crotalaria juncea	6.37	2.46	8.83	100	64	1.60	4.09	100.6	100.8	201.4	100	50
2	727	Calopogonium caeruleum	5.71	2.43	8.14	92	70							
3	1000	Arachis hypogaea	4.84	1.83	6.67	76	73							
4	657	Pachyrhizus erosus	3.98	2.36	6.34	72	63	1.84	4.57	73.0	106.5	179.5	89	41
5	651	Calopogonium mucunoides	4.01	2.25	6.26	71	64	1.91	4.30	76.5	97.1	173.6	86	44
6	744	Dolichos sp.	3.01	2.62	5.63	64	53							
7	22	Phaseolus lunatus	3.59	1.77	5.31	60	68							
8	648	Psophocarpus tetragonolobus	2.86	2.11	4.97	56	58							
9	731	Canavalia sp.	2.64	2.25	4.89	55	54							
10	861	Indigofera hirsuta	3.10	1.74	4.84	55	64	1.07	3.87	40.9	67.4	108.3	54	38
11	1102	Lupinus sp.	2.52	1.28	3.80	43	66	0.67	2.83	16.8	35.9	52.7	26	32
12	848	Pithocellobium jiringa	2.32	1.23	3.55	40	65							
13	794	Erythrina indica	1.47	1.13	2.60	29	57							
14	169	Vicna unguiculata	1.36	0.92	2.28	26	60	1.31	4.17	19.5	39.0	58.5	29	33
15	187	Sphenostylis stenocarpa	1.26	0.54	1.80	20	70							
16	819	Clitoria laurifolia	1.21	0.57	1.78	20	68							
17	309	Dolichos africanus	1.12	0.57	1.69	19	66	1.01	3.37	11.4	19.6	31.0	15	37
18	82	Leucaena leucocephala	.96	0.57	1.53	17	63							
19	43	Voandzeia subterranea	0.96	0.55	1.51	17	64							
20	742	Desmodium heterophyllum	1.00	0.46	1.46	17	68	0.28	1.35	2.9	6.3	9.1	5	32
21	182	Phaseolus vulgaris	0.99	0.47	1.46	17	68							
22	849	Pithocellobium jiringa	.71	.51	1.22	14	58							
23	656	Pachyrhizus erosus	.67	.50	1.17	13	57	1.35	3.29	8.9	16.2	25.1	12	35
Controls														
+N	70 ppm N as KNO_3		7.47	3.32	10.79	122	69	0.60	2.66	44.5	87.6	132.1	66	34
-N	Uninoculated		1.00	0.46	1.46	17	68	0.52	1.33	5.17	6.17	11.3	6	46
								0.33	0.35	22.48	17.61			
Bayes LSD(.05)			1.23	0.50										

1/ Two plants per 1.1 liter Leonard Jar, harvested after 60 days.
2/ Two seeds; 0.42 g, 4.12% N, 17.3 mg. N

APPENDIX 3

Dry matter and nitrogen accumulation for *V. unguiculata* and *P. erosus* after eight weeks of growth in the field

component	per plant		
	V. unguiculata	*P. erosus*	t test
Total plant (g)	29.7	18.4	*
nodule (g)	0.29	0.13	*
root (g)	0.98	2.53	**
shoot (g)	28.4	15.8	*
% N in shoot	3.00	3.65	*
Total shoot nitrogen (mg)	842	591	*

*, ** indicate the .05 and .01 confidence levels, respectively

APPENDIX 4

Nodule mass and specific nitrogenase activity of field grown *P. erosus* over time

weeks since emergence	nodule mass per plant (g)	Nitrogenase specific activity (μmoles ethylene·g nodules^{-1}·hr^{-1})
3	0.101	116.8
4	0.256	112.1
5	0.253	108.3
6	0.396	124.4
7	0.454	137.4
8	0.519	nd
12	0.281	42.8
Bayes LSD (.05)	0.134	22.5

APPENDIX 5

Effect of ethylene incubation* on dry matter production of *Pachyrhizus erosus* using tuberous roots as propagules+

Plant part	Dry weight from tuberous roots	
	Incubated	Unincubated
	g	
Shoot	0.600	0.096
Root	0.578	0.280
Total	1.18	0.376

ANOVA	df	F
Treatments	1	26.5**

+ 6.4 ppm ethylene for 30 min.

** significant at the 99% level