INTRAVARIETAL VARIABILITY FOR BIOLOGICAL NITROGEN FIXATION IN SOUTHERNPEA (*Vigna unguiculata* (L.) Walp)

by

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ABSTRACT

Intravarietal Variability for Biological Nitrogen Fixation in Southernpea (Vigna unguiculata (L.) Walp)

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This investigation was conducted to determine whether intravarietal variability for nitrogen fixation in southerpea has a genetic basis. Two seed sources each of 'Brown Crowder' and 'Bush Purple Hull' were inoculated and grown in the greenhouse. Following acetylene-ethylene assay, plants representing low, medium, and high nitrogen fixation potential were selected from each of the four populations. Plants were propagated vegetatively and allowed to produce seed. The seed were then planted in a randomized block and assayed as before. 'Brown Crowder' had a greater nitrogen fixation potential than 'Bush Purple Hull' regardless of seed source. Sufficient variability for plant specific activity within varieties and sources was found to allow for the delineation of three phenotypic classes for first cycle selection. Although there was no significant difference in plant specific activity in the progeny, a positive trend was observed which may support a genetic basis for this variability.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The southernpea (*Vigna unguiculata* L. Walp) also known as cowpea or black-eyed pea is an important vegetable legume which is grown throughout the tropical areas of Africa, India, southeast Asia, and the coastal areas of Central and South America. Nigeria is thought to be the center of origin of the southernpea which has been cultivated for at least two thousand years (16). Southernpeas are also widely grown throughout the southern United States. In 1977, thirty thousand acres of southernpeas were planted in Texas. Fifteen percent were harvested for the fresh market and the remainder for dry peas (19).

The southernpea is a warm season annual herb that has a variety of growth habits including erect, semi-erect, trailing or climbing. The southernpea has pinnately trifoliate leaves and a deep taproot with profuse lateral roots. The flowers are borne on long peduncles and are white, yellow, or bluish-purple in color. The seed pods are borne in clusters above or scattered within the foliage. They are straight or curved and range from ten to thirty cm long. The colors of the mature pods include white, straw, green,red, and reddish-purple. Seeds are four to twelve mm long and may be black, blue,brown, buff, tan, red, maroon, gray, or white and mottled or speckled. Some types are eyeless, while in others the eye may be black, blue, brown, or red (2,16).

The southernpea is consummed in the form of immature and mature

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The style and format of this thesis follows that outlined by the *Journal of the American Society for Horticurtural Science*.

seeds and pods, young shoots and leaves, and mature leaves. In many areas the southernpea is grown for livestock forage. The protein content of the mature dried seed varies from twenty to forty percent but is deficient in the sulfur-containing amino acids methionine and cysteine. Southernpea is thus utilized pimarily as a protein source, supplementing starches such as cereals and cassava (7).

The southernpea, like other legumes, is able to biologically fix atmospheric nitrogen through its symbiosis with the Rhizobium bacteria. Through the biological fixation process, which is a complex interaction of host plant, bacteria. and the envionment, molecular nitrogen is reduced to ammonia. Ammonia is a plant-avaiable form of nitrogen and is used in the synthesis of proteins. Thus leguminous plants can grow on relatively poor soils without the addition of nitrogenous fertilizers. Therefore as a versatile legume with a high protein content, relative drought tolerance, minimal fertilizer requirements, and the ability to produce two or more crops a year in tropical regions, the southernpea has an excellent potential for use as an expanded source of protein for both humans and livestock (4).

Traditionally, plant breeders have concentrated on such easily recognizable traits as yield, seed color, and growth habit with no conscious selection for nitrogen fixation potential (12). Most nitrogen fixation research has dealt with the Rhizobium component of the symbiotic relationship. Recently however, researchers, studying southernpea and other leguminous species, have found intervarietal variability--that is, variability between varieties or genotypes--for nitrogen fixation potential (22, 23). However, many of these researchers have also observed what appears to be intravarietal variability--that is variability within varieties or genotypes (12,18). In several cases, this apparent intravarietal variability can be almost as great as the intervarietal variability (12). In order to more effectively develop varieties with enhanced nitrogen fixation potential, this observed intravarietal varability should be understood. Therefore the objective of this research was to determine whether the observed intravarietal variability for biological nitrogen fixation in southernpea has a genetic basis.

CHAPTER II

REVIEW OF LITERATURE

Intervarietal Variability

Intraspecific variability for nitrogen fixation potential has been reported in many species. In some species such as red clover (*Trifolium pratense*) (14) and alfalfa (*Medicago sativum*) (1) large extremes were found between host plant genotypes for susceptibility and resistance to nodulation. Non-nodulating varieties of soybean (*Glycine max*) (21) and field peas (*Pisum sativum*) (11) have been reported. Nodule number has been shown to be under host control in field pea (*Pisum sativum*) (8), subterranean clover (*Trifolium subterraneum*) (15), and white clover (*Trifolium repens*) (13). Lines of *Centrosema pubescens* that were selected for low or high nodulation were found to be stable in subsequent generations (3). Genotypes of broad bean (*Vicia faba*) were found to differ significantly in nodule number, nodule mass, and nodule size (6). Extensive variability between varieties of the common bean (*Phaseolus vulgaris*) for the ability to reduce acetylene has also been observed (20). These reports indicate that the host plant does have a significant effect on the symbiotic relationship and that it is possible to select for high-fixing varieties or genotypes.

Interspecific variability for biological nitrogen fixation was also observed in souternpea (*Vigna unguiculata* L.Walp). Zary *et al*. (23) screened one hundred different southernpea lines for nitrogen fixation potential. Plant specific activity ranged from 0.6 to 43.3 umoles

C_2H_4 /plant /hour. The nodule mass ranged from 0.1 to 3.0 grams and the nodule number ranged from 10 to 146 per plant. Five high-fixing genotypes and four low-fixing genotypes were then selected for a greenhouse verification study. Again, significant variability was found for all nitrogen fixation parameters measured. Plant specific activity ranged from 0.5 umoles for 'Bush Purple Hull' to 6.8 for 'Brown Crowder'. A field verification trial was also conducted with seven genotypes. Plant specific activity for 'Bush Purple Hull' was 36.2, while 'Brown Crowder' had a plant specific activity of 118.6 umoles C_2H_4 produced/plant/hour. The authors concluded that the observed variability is evidence for genetic control of the trait and that it may be possible to breed for increased nitogen fixation potential.

Zary and Miller (22) also conducted a study involving the influence of genotype on diurnal and seasonal patterns of nitrogen fixation in the southernpea. The diurnal patterns for 'Bush Purple Hull' and 'Brown Crowder' were similar, with two peaks of acetylene reduction at 1200 and 2400 hours. Zary and Miller also found that sampling for maximum rates of acetylene reduction should occur as the plants reach full flower.

Intravarietal Variability

Many workers have observed intravarietal variability for nitrogen fixation potential, but little has been published on this subject. Gibson (9) inoculated fifteen varieties of lucerne (Medicago sp.) with single Rhizobium strains. The seedlings were grown in an agar nutrient medium in test tube culture. In a preliminary study, considerable differences were found between bacterial strains and between varieties for dry weight. Significant differences in nodulation were found between the agronomically important varieties 'Hairy Peruvian', 'Hunter River', and 'DuPuits' and the Canadian varieties 'Rambler' and 'Rhizoma'. Also there was a wide range of the effectiveness of various Rhizobium strains for nodulation of the lucerne varieties. Gibson noted a high degree of intravarietal variability in many variety/bacterial strain treatments. He attributes this observation to genetic heterogeneity of the symbiosis-regulating factors in the host plants. Variability within a variety seemed to be greatest within varieties showing intermediate fixation potential levels. In many of these varieties, some individual plants had dry weights equivalent to the best individuals of very effective varieties, while others within this same variety has dry weights similar to uninoculated plants. This suggests the possibility of selection for higher nitrogen fixation potential within many varieties of lucerne.

Duhigg et al. (5) found that selection for acetylene reduction rates was effective in 'Mesilla' alfalfa (Medicago sativum). Individual plants were also evaluated for nodulation score, nodule color score, root score, dry weight of plant top, and percentage of Kjedahl nitrogen in the top growth. Fifteen plants with high and fifteen plants with low acetylene reduction rates were selected the 278 original plants. Plants within the high and low selections were selfed in all possible combinations. A considerable amount of variation for the parameters measured was found between the individual plants. The plants selected for high acetylene reduction had an average increase of 130 percent for acetylene reduction, 57 percent total nitrogen in the plant tops, and 100 percent increase in dry weight of the plant top when compared to the original population. Also, selections for low plant specific activity had readings below the average for all of the other parameters measured. The progeny of plants selected for high nitrogen fixation showed plant specific activities that were twice as high as those of progeny derived from the low selections. Dry weight of plant tops and total nitrogen of the plant tops showed similar variability between the two progeny groups. Acetylene reduction increased 82 percent in the progeny selected high plant specific activity from the rates of the original population. Almost 60 percent increases were found in dry weight of plant tops and total nitrogen of the tops from the original population. The progeny also showed higher nodulation than the original population. These results indicated that there was significant variability for nitrogen fixation parameters within the variety to select for enhanced nitrogen fixation.

Preliminary studies by Smittle and Brantley (18) indicated the presence of intravarietal variability in southernpea (*Vigna unguiculata* L. Walp), snapbean (*Phaseolus vulgaris*), and lima bean (*Phaseolus limensis*). Screening of southernpea varities 'Purple Hull Pinkeye', 'Mississippi Silver', and 'White Acre' revealed twenty to sixty-fold variation in acetylene reduction within a variety. However there was no report of an evaluation of the progeny for acetylene reduction. Even greater variability was observed within snapbean varieties (17).

CHAPTER III

MATERIALS AND METHODS

Specific studies included 1) a characterization of the original populations, 2) a mixed-strain inoculant study, and 3) a single-strain study.

Two southernpea varieties were used in the studies--'Brown Crowder' which has been indexed as a relatively high-fixing genotype and 'Bush Purple Hull' which is a relatively low-fixing genotype. Two seed sources of each variety, each originating from the same seed lot, were used. Seed source A was derived from plants that has been selfed in the greenhouse for seven generations, with the elimination of the possibility of outcrossing. Seed source B was derived from plants grown in the field for one generation with the possibility of outcrossing. 'El', an inoculant obtained from the Nitrgin Co., Milwaukee, Wis., consisting of five Rhizobium strains was used in the first two studies. The strain '32Hl' was used for the single-strain study.

Characterization of the Original Populations

On Feb. 11,1980, the seed were planted in one gallon black plastic pots containing a sterilized mix of sand and vermiculite (1:1 by volume). All seed was inoculated at planting by including approximately .25 gr of the 'El' inoculant (granular form) in the planting hole and covering with the sand-vermiculite growth medium.

The seedlings were thinned to one plant per pot. They were grown in a greenhouse with no artificial light. The temperature in the greenhouse ranged from 20°C to 30°C. The plants were watered with a nitrogen-free nutrient solution prepared with distilled water. At anthesis, the plants wer harvested and assayed for nitrogen fixation potential.

Harvest and Assay Procedures

Harvesting occurred between 1100 and 1300 hours, which has been determined by diurnal studies to be the peak of nitrogen fixation. The plants were decapitated at the cotyledonary node. The fresh weight of each plant was determined and two basal cuttings were obtained from each individual plant. The roots of the plant were removed from the planting medium and placed in a 500 CC glass canning jar fitted with a rubber septum. Twenty-five CC of acetylene (generated by adding calcium carbide to water) was injected into each jar. After incubation for sixty minutes at 25°C, a 15 CC sample of the gas was removed and injected into a 10 CC evacuated Vacutainer. The roots were removed from the jar and placed in individually labelled plastic bags for freezer storage. At a later date nodule number, nodule weight, and root weight were determined. A .5 CC sample of the gas from the Vacutainer was injected into a flame-ionization gas chromatograph with a Poropak N column to determine the amount of C_2H_4 produced during the incubation period. The nitrogenase activity was expressed both as plant specific activity (umoles C_2H_4 produced/plant/hour) and nodule specific activity (umoles C_2H_4 produced/ gr nodule/hour).

Selection and Growth of Cuttings

The basal cuttings were dipped in 'Rootone F' and placed in sand in a mist system. When the relative plant specific activity of the individual plants was determined, those cuttings representing the high and low extremes and also the midrange of nitrogen fixation potential were selected. These cuttings were then transplanted into two-gallon black plastic pots containg sand, peat, and vermiculite (1:1:1 by volume). The plants were fertilized with a water-soluble 20-20-20 fertilizer. The plants were allowed to grow to maturity, and the seed were collected from individual plants. This seed formed the first selection generation, which was used in the second and third studies.

First Selection Generation with Mixed-strain Inoculant

On Sept. 9, 1980, seed from the individual plants of the original population was planted in a randomized block with five replications and treated as before using the mixed-strain inoculant. The plants were harvested and assayed as before. Analysis of variance was used to determine if the first selection generation behaved as their parent for nitrogen fixation potential.

First Selection Generation with Single-strain Inoculant

A third study, initiated Jan. 14, 1981, was conducted to eliminate the effects of possible strain interactions and also to study the effects of varying acetylene concentrations on plant specific activity and nodule specific activity. In this study seed from the cuttings of the original study was again used. It was treated as that for the mixed-strain study with two exceptions. The plants were inoculated with a single strain of *Rhizobium* ('32H1'). Also two levels of acetylene--25 and 50 (five and ten precent of the total volume of the incubation jar, respectively) were injected into the jars. Analysis of variance was again used to evaluate the results.

CHAPTER IV

RESULTS AND DISCUSSION

Characterization of the Original Populations

The original populations were characterized in the initial study. The frequency distribution for plant specific activity in the original population of 'Brown Crowder' from seed source B is shown in Figure 1. There was substantial variability, ranging from 2 to 36 umoles C_2H_4 produced/plant/hour, which is similar to that observed by Smittle (18). In addition, the distribution appears to be bimodal with peaks at 14 and 24 umoles. The frequency distribution of the 'Brown Crowder' original population from seed source A is illustrated in Figure 2. Again, wide variability for plant specific activity was found, ranging from 6 to 42 umoles C_2H_4 produced. However, in this case the distribution appeared to be concentrated at 16 umoles. This indicated that selfing with the elimination of outcrossing may tend to reduce nitrogen fixation potential. Similar results were obtained with 'Bush Purple Hull'.

The means of the three selection classes defined on the basis of plant specific activity are given for each variety in Table 1. The low class for 'Bush Purple Hull' from seed source A had an average plant specific activity of 5.7, while the high class had an average of 18.5. The low and high classes of 'Bush Purple Hull' from seed source B had an average plant specific activity of 8.3 and 23.9 respectively. Similar ranges were observed in the 'Brown Crowder' populations. It is evident

Figure 1 FREQUENCY DISTRIBUTION OF 'BROWN CROWDER' ORIGINAL POPULATION FROM SEED SOURCE B

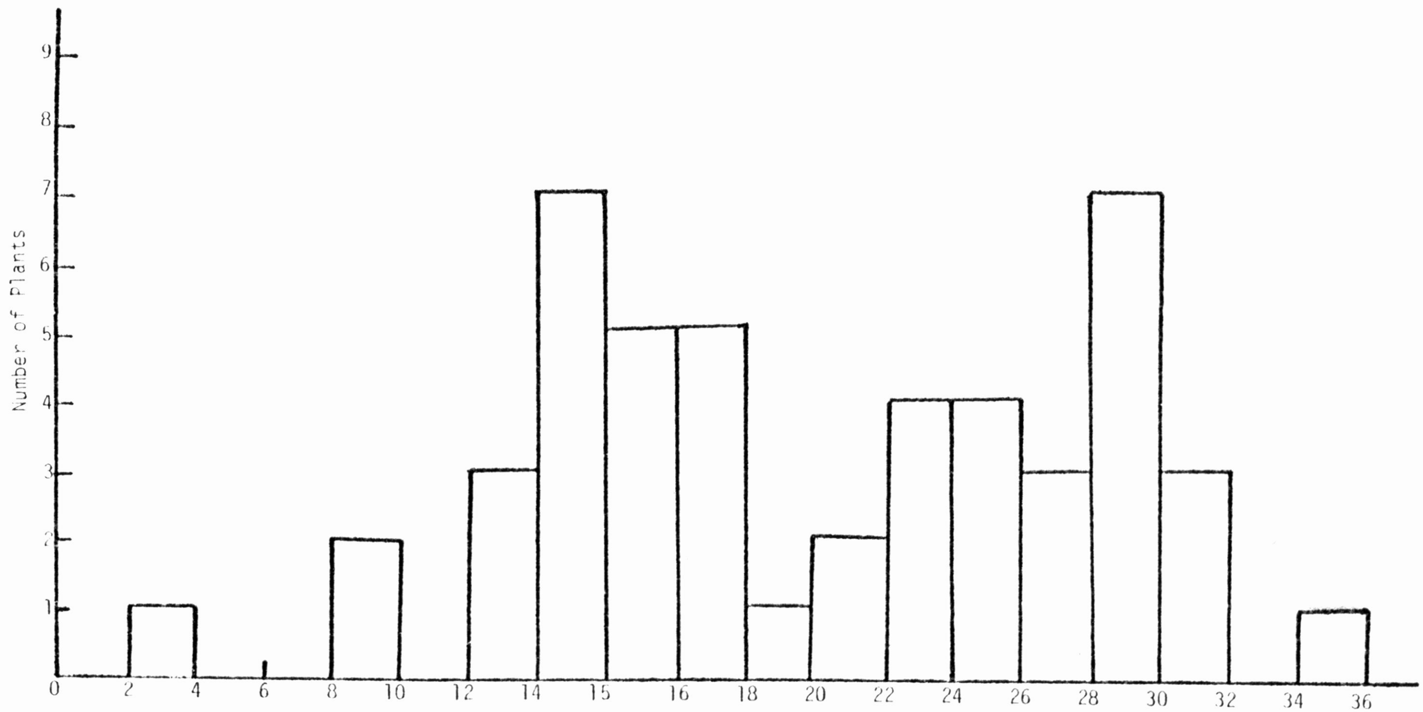


Figure 2 FREQUENCY DISTRIBUTION OF 'BROWN CROWDER' ORIGINAL POPULATION FROM SEED SOURCE A

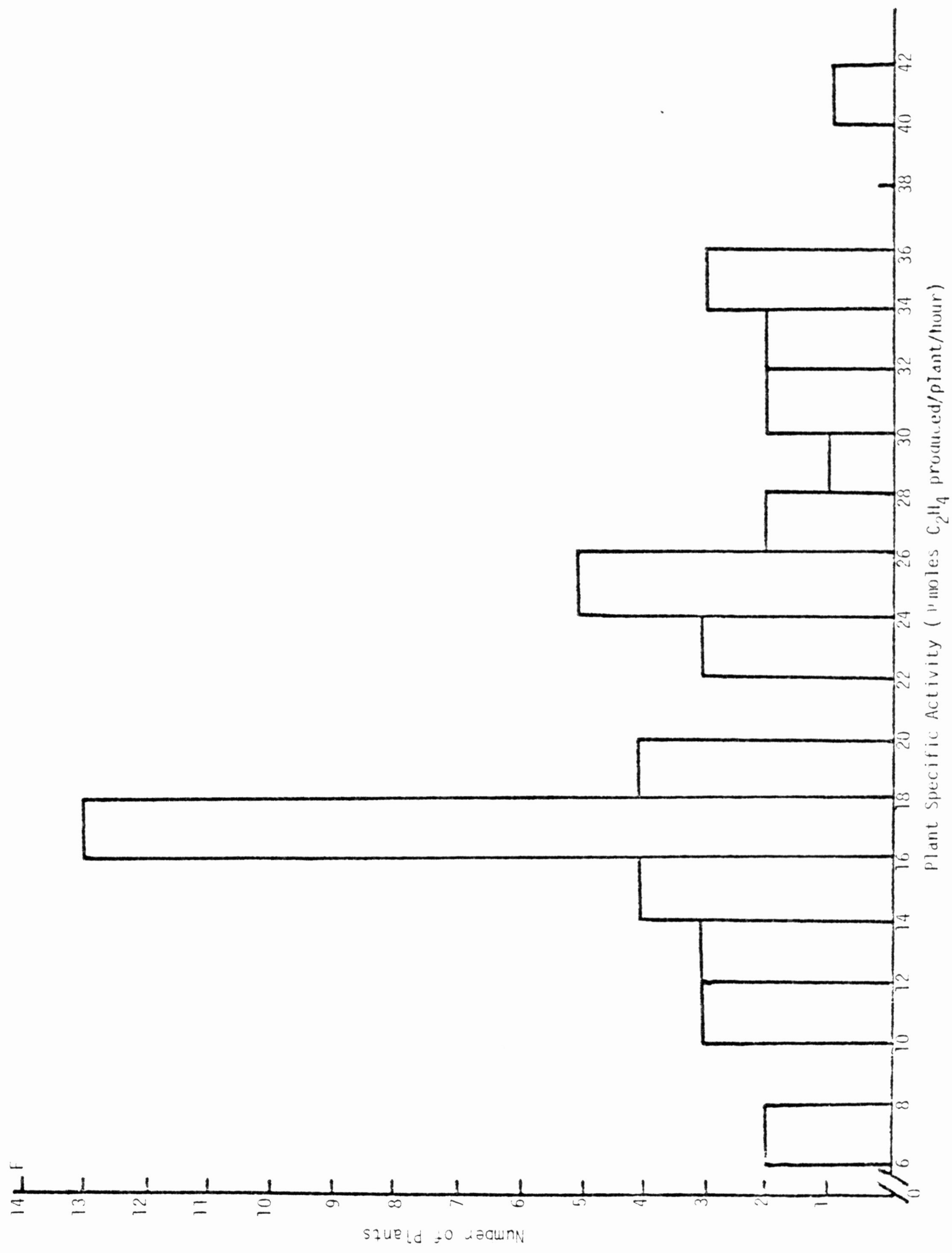


Table 1 MEAN PLANT SPECIFIC ACTIVITY AND CONFIDENCE LIMITS FOR THREE SELECTION CLASSES AS INFLUENCED BY VARIETY AND SOURCE

	VARIETY			
	'Bush Purple Hull'		'Brown Crowder'	
Source	A	B	A	B
Low	5.7 ± 6.5[z]	8.3 ± 3.8	9.9 ± 4.6	12.5 ± 2.5
Medium	10.0 ± 1.9	13.8 ± 2.11	18.2 ± 5.2	22.5 ± 4.3
High	18.5 ± 6.4	23.9 ± 6.5	27.3 ± 7.0	32.5 ± 3.4

[z] Plant Specific Activity = μ moles C_2H_4 produced/plant/hour

that ther were significant differences between low and high selected populations regardless of seed source or variety. The averages of the low, medium, and high selections for seed source B of 'Bush Purple Hull' were 8.3, 13.8, and 23.9, respectively, while those of seed source A were 5.7, 10.0, and 18.5. Asimilar trend was observed in 'Brown Crowder', indicating again that regardless of variety, the exclusion of outcrossing tended to reduce nitrogen fixation potential. Also it is obvious when examining the results of a single seed source source that 'Brown Crowder' was a relatively high-fixing genotype when compared to 'Bush Purple Hull' (23).

Variability similar to that for plant specific activity was found in the other nitrogen fixation parameters measured (Table 2). For example, nodule number had a range of 5 to 57 nodules per plant in the 'Bush Purple Hull' population derived from seed source A. Nearly ten-fold differences were observed in nodule weight within seed sources of the two varieties. Approximately three-fold differences were found in both root and top fresh weight.

Propagation of cuttings from the original study was very effective with a 99 percent success rate.

First Selection Generation with Mixed-strain Inoculant

In the summary of analysis of variance, the significant main effects and interactions for top fresh weight, plant specific activity, and nodule specific activity are given (Table 3). For fresh weight the source x variety interaction was significant at at least the one percent level. The main effects of source and variety were highly significant for both plant

Table 2 RANGES IN FIVE NITROGEN FIXATION PARAMETERS OBSERVED IN THE ORIGINAL POPULATIONS OF TWO SOUTHERNPEA VARIETIES FROM TWO SEED SOURCES

VARIETY	'Brown Crowder'		'Bush Purple Hull'	
SEED SOURCE	A	B	A	B
Plant Specific Activity[z]	6.05-40.54	2.42-37.27	0.60-28.50	3.89-29.04
Nodule Number	4-37	6-37	5-57	4-45
Nodule Weight[y]	0.11-1.04	.25-.92	0.02-1.10	0.15-.876
Root Weight[y]	3.02-10.37	2.78-9.22	3.27-10.00	3.06-8.58
Top Weight[y]	6.22-22.36	5.15-15.32	6.62-17.60	4.02-14.04

[z] Plant Specific Activity = μ moles C_2H_4/plant/hour

[y] g/plant

Table 3 SIGNIFICANT MAIN EFFECTS AND INTERACTIONS FOR FRESH WEIGHT, PLANT SPECIFIC ACTIVITY AND NODULE SPECIFIC ACTIVITY REVEALED BY ANALYSIS OF VARIANCE

	Fresh Weight of Plant Top[z]	Plant Specific Activity[y]	Nodule Specific Activity[x]
Source	***	***	***
Variety	***	***	**
Source X Variety	**		
HML[w]			
Source X HML[w]			
Variety X HML[w]			
Source X Variety X HML[w]			

*	.01 - .05	Probability of a greater F value
**	.001 - .01	Probability of a greater F value
***	.0001 - .001	Probability of a greater F value

[z] g/plant

[y] μ moles C_2H_4/plant/hour

[x] μ moles C_2H_4/g nodule fresh weight/hour

[w] high, medium, and low selection classes

and nodule specific activity. No significant interactions were detected for these two parameters. Table 4 summarizes the significant main effects and interactions for nodule number and nodule and root weights. The interaction of seed source x variety was highly significant for nodule number. A third order interaction, seed source x variety x selection class, was observed for nodulation weight. The interaction seed source x variety was significant at at least the five percent level for root weight.

Figure 3 depicts the interaction seed source x variety for top fresh weight. 'Brown Crowder' plants with a fresh weight of 20 and 23 g/plant were significantly greater than 'Bush Purple Hull' with fresh weights of 13 and 19 g/plant regardless of seed source. Also plants of seed source A had a significantly lower fresh weight regardless of variety than did plants from seed source B. These data indicated that 'Brown Crowder' has a higher nitrogen fixation potential than 'Bush Purple Hull', and that the elimination of outcrossing tends to reduce nitrogen fixation potential and general vigor.

Two main effects--variety and seed source--were significant for plant specific activity. Figure 4 indicated that 'Bush Purple Hull' with a plant specific activity of 14 umoles C_2H_4 produced/plant/hour was significantly lower for this parameter than 'Brown Crowder' at 22 umoles. This data again supports results obtained by Zary et al. (23). Figure 5 shows the effect of seed source on plant specific activity. Plants of seed source A, which had an average plant specific activity of 16.5 regardless of variety. However, plants of seed source B, derived from field-grown seed, had an average plant specific activity of almost 20 umoles. Thus, the relative average plant specific activities of the two varieties and seed sources does appear to be genetically controlled.

Table 4 SIGNIFICANT MAIN EFFECTS AND INTERACTIONS FOR NODULE NUMBER, NODULE WEIGHT AND ROOT WEIGHT REVEALED BY ANALYSIS OF VARIANCE

	Nodule Number	Nodule Weight[z]	Root Weight[z]
Source	***	***	*
Variety	***	***	***
Source X Variety	***		*
HML[y]			
Source X HML[y]			
Variety X HML[y]			
Source X Variety X HML[y]		*	

*	.01 - .05	Probability of a greater F value
**	.001 - .01	Probability of a greater F value
***	.0001 - .001	Probability of a greater F value

[z] g/plant

[y] high, medium, and low selection classes

Figure 3 EFFECT OF SEED SOURCE X VARIETY ON PLANT FRESH WEIGHT

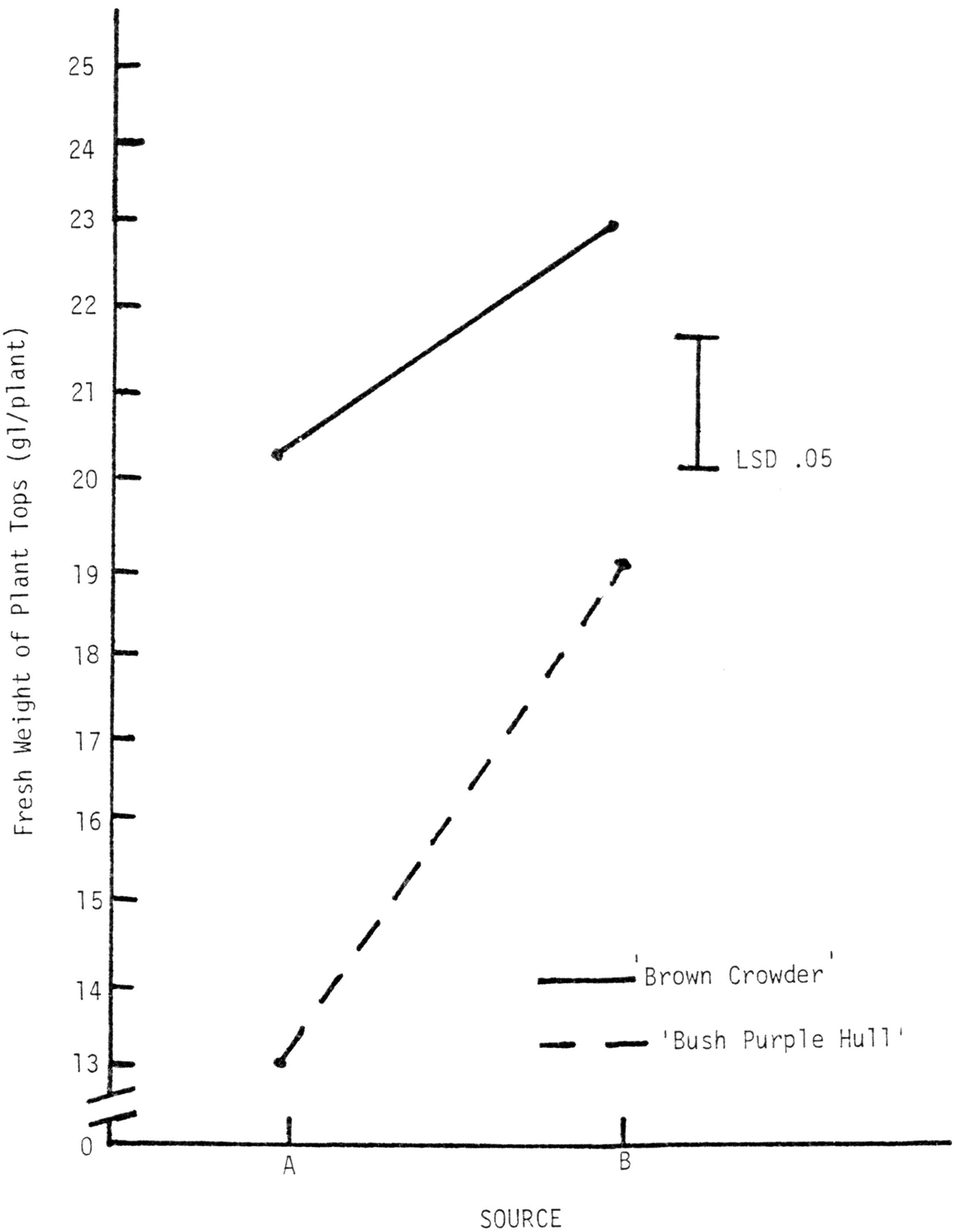


Figure 4 EFFECT OF VARIETY ON PLANT SPECIFIC ACTIVITY

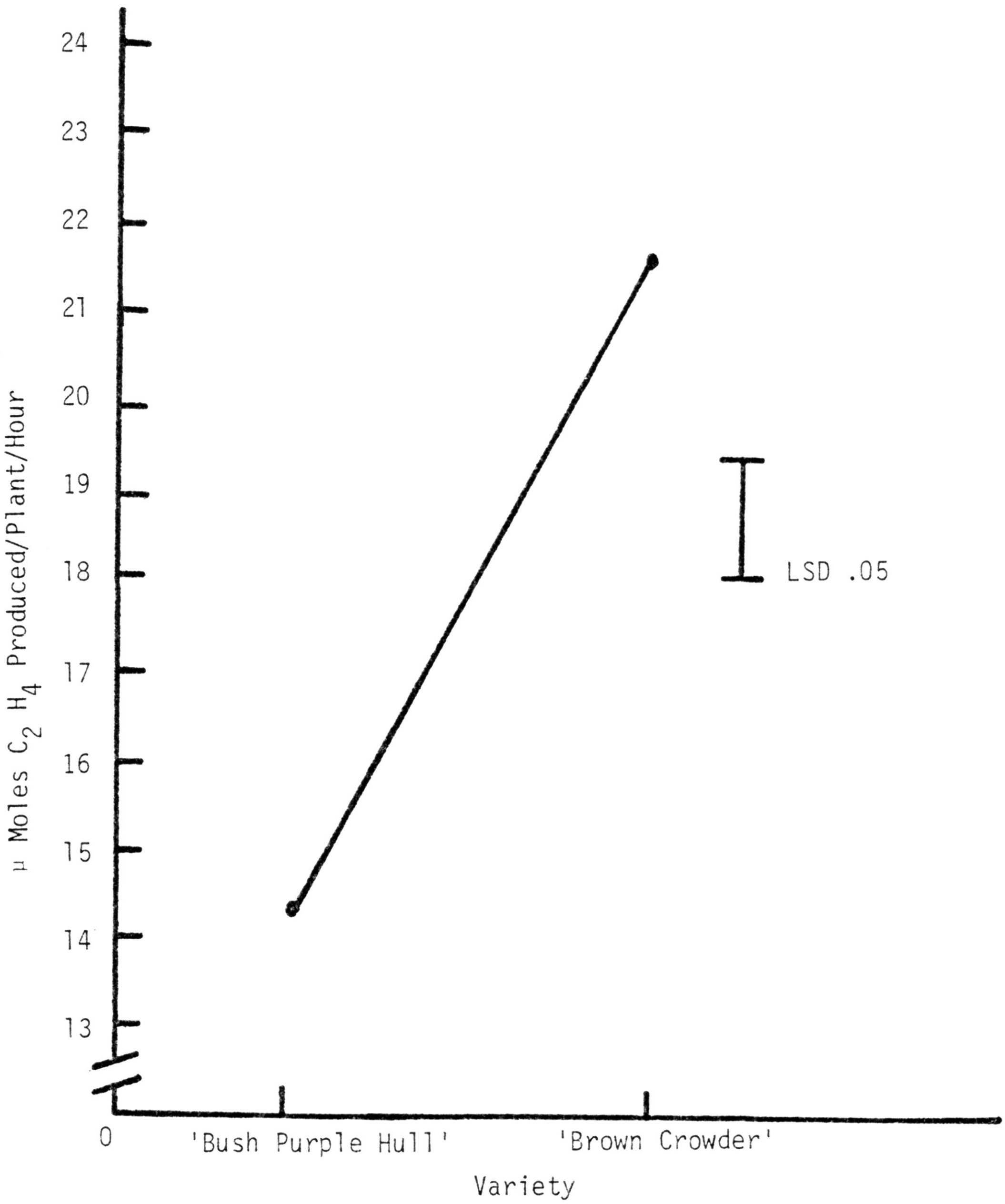
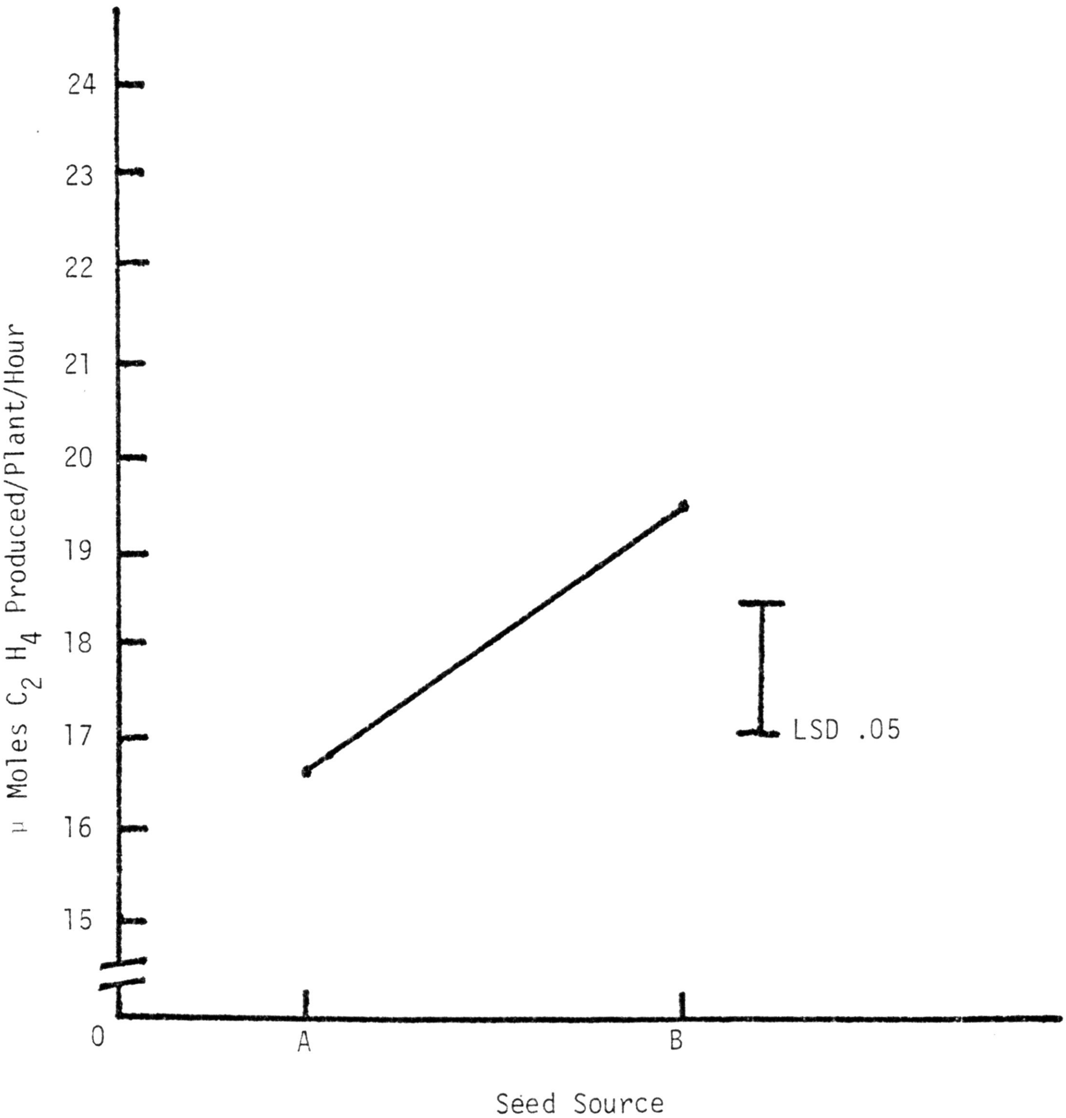


Figure 5 EFFECT OF SEED SOURCE ON PLANT SPECIFIC ACTIVITY



The effect of selection class on plant specific activity is shown in Figure 6. Although the differences within varities varieties are not statistically significant, a general trend was observed in the progeny of the three selection classes. The progeny of plants selected for high plant specific activity showed a higher plant specific activity themselves than those progeny of the medium and low selection classes. Perhaps the selection criteria of the original populations were not sufficiently stringent--that is a much smaller proportion of the original populations should have been selected for each class.

Nodule specific activity was also affected by the main effects of variety and seed source as is shown in Figures 7 and 8 respectively. 'Bush Purple Hull' had a nodule specific activity of 16.5 umoles of C_2H_4 produced/g nodule/hour, while 'Brown Crowder' had a significantly higher nodule specific activity at 19 umoles. Plants from seed source A had a nodule specific activity of 15, whereas plants of seed source B had a much greater nodule specific activity of 21 umoles. The results for nodule specific activity as with those for plant specific activity indicated that 'Brown Crowder' has a substantially higher fixation potential than does 'Bush Purple Hull', and that continued selfing with no outcrossing reduces fixation potential in the population.

Significant interactions were found for nodule number, nodule weight, and root weight. Figure 9 illustrates the interaction of seed source x variety on nodule number. 'Bush Purple Hull' had a higher nodule number than did 'Brown Crowder' regardless of seed source. Within 'Bush Purple Hull' plants from seed source A had a significantly lower nodule number, with 32 nodules per plant than did plants from seed source B with an average of 58. However, there was no significant difference between seed

Figure 6 EFFECT OF SELECTION CLASS ON PLANT SPECIFIC ACTIVITY

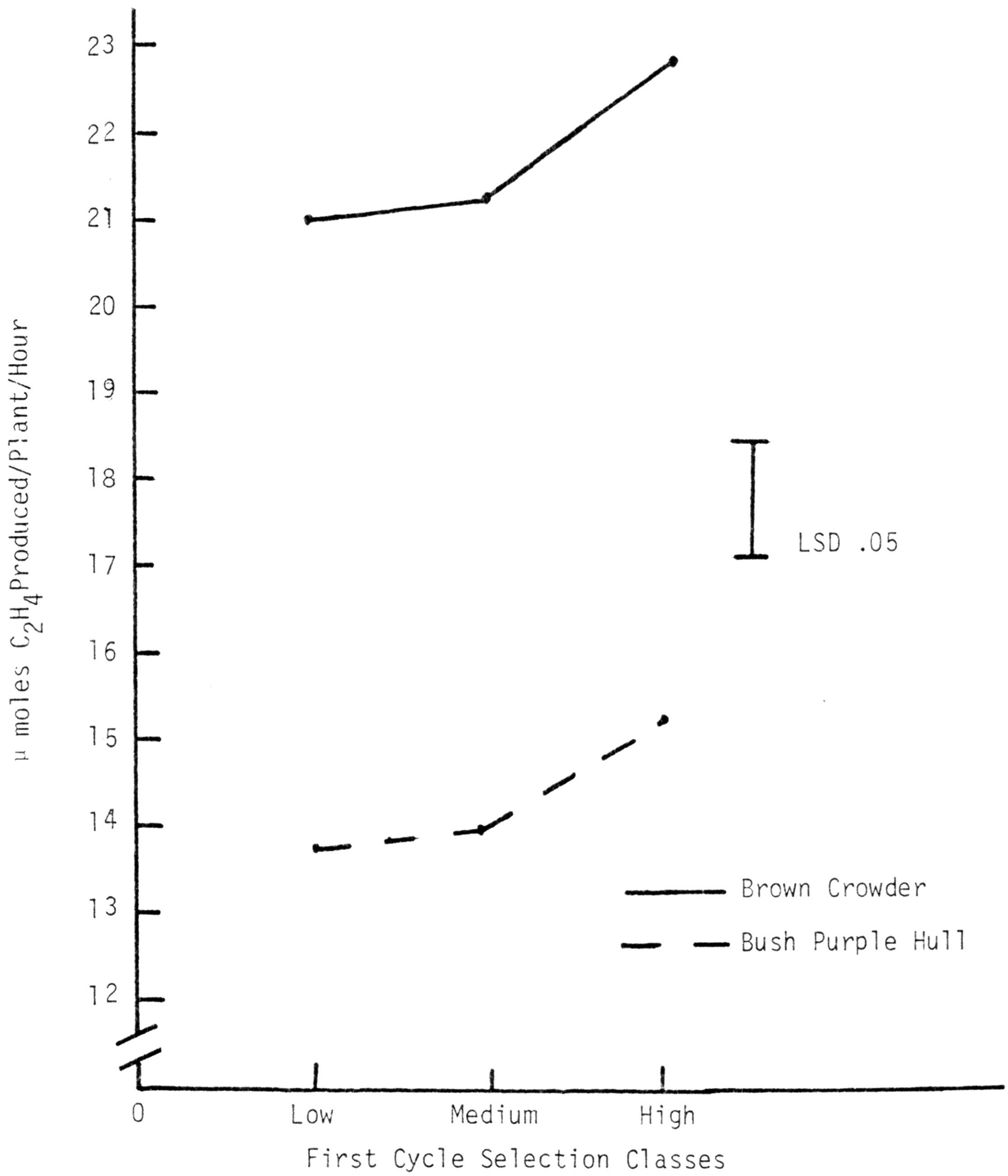


Figure 7 EFFECT OF VARIETY ON NODULE SPECIFIC ACTIVITY

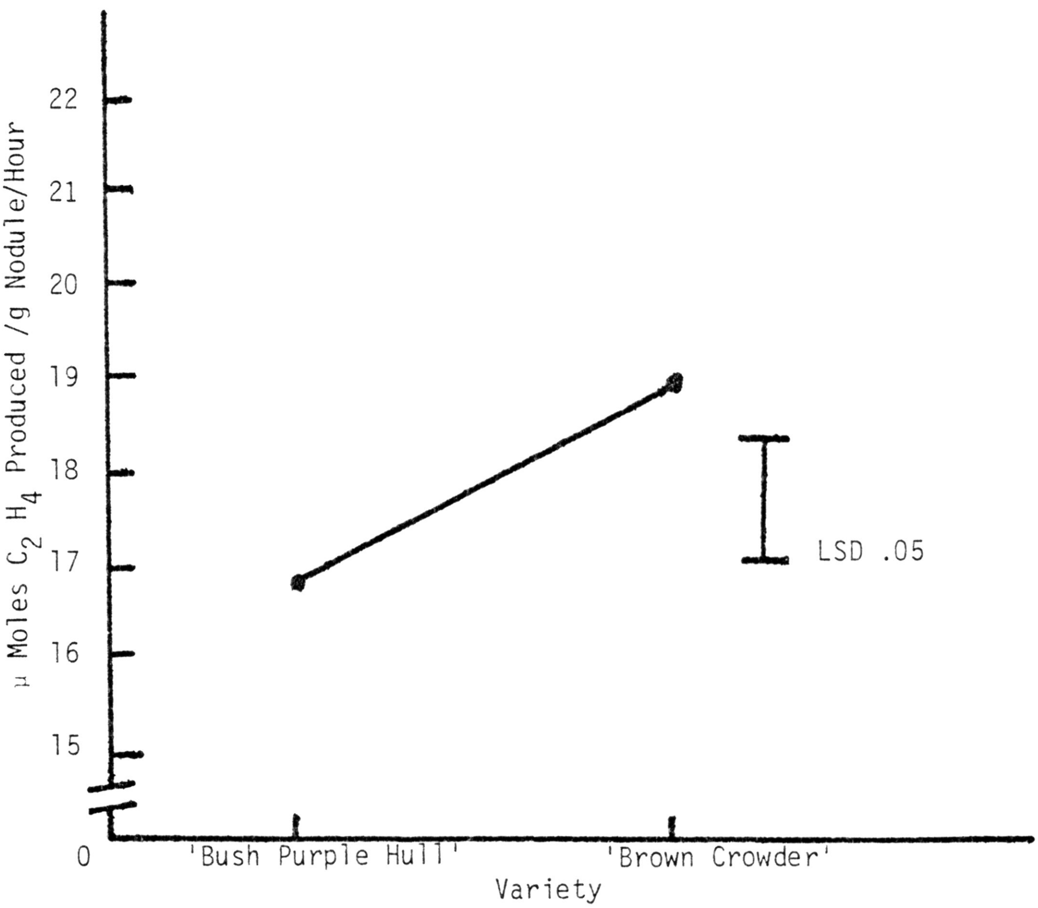


Figure 8 EFFECT OF SEED SOURCE ON NODULE SPECIFIC ACTIVITY

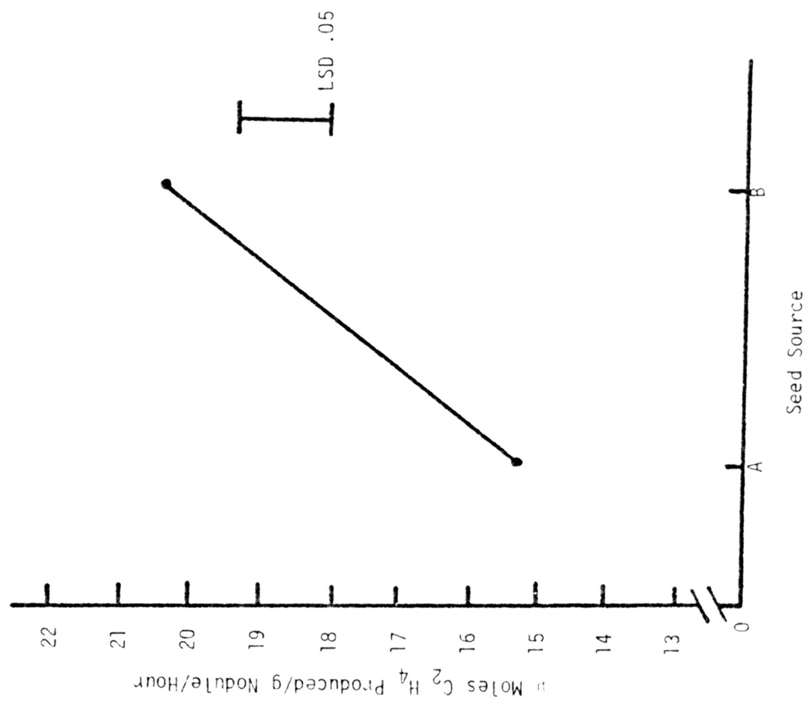
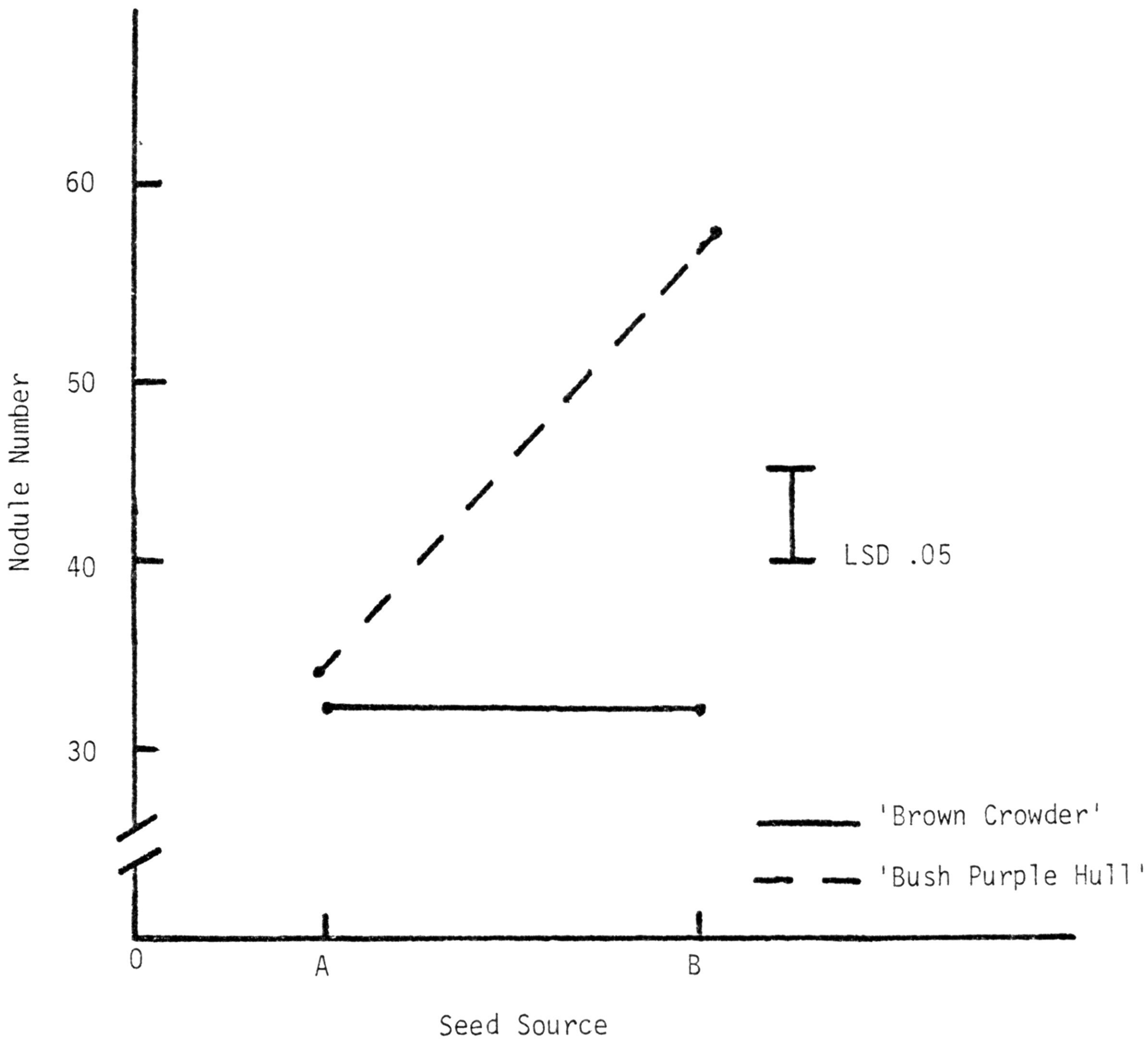


Figure 9 EFFECT OF SEED SOURCE X VARIETY ON NODULE NUMBER



sources for the 'Brown Crowder' variety. The third order interaction of seed source x variety x selection class for nodule weight is depicted in Figure 10. In all cases, 'Brown Crowder' plants had a higher total nodule weight per plant than did 'Bush Purple Hull' regardless of variety or selection class. Within each variety, seed source A plants had a higher nodule weight than plants of seed source B. Also, nodule weight follows the same general trend for selection class--progeny of plants selected for high plant specific activity had a higher nodule weight than did progeny of plants selected for low plant specific activity. The results of the interaction of seed source x variety for root weight are shown in Figure 11. 'Brown Crowder' plants had a higher root weight than did 'Bush Purple Hull' plants regardless of seed source. Plants of 'Bush Purple Hull' from seed source A had a significantly lower root weight than those of seed source B. However, there was no signifcant difference between seed sources within the 'Brown Crowder' variety. Thus, 'Brown Crowder' plants had a higher nodule weight and root weight, but 'Bush Purple Hull' had a larger number of nodules per plant. This indicated that 'Bush Purple Hull' plants had a large number of relatively small and inefficient nodules.

First Selection Generation with Single-strain Inoculant

Ranges in fixation parameters for the third study involving a single-strain inoculant and varying acetylene levels is given in Table 5. Approximately three-fold difference were found within varieties and seed sources for plant and nodule specific activity. Nodule number ranged as high as 20 to 70 nodules per plant with seed source B of 'Bush Purple

Figure 10 THE EFFECT OF SEED SOURCE X VARIETY X SELECTION ON NODULE WEIGHT

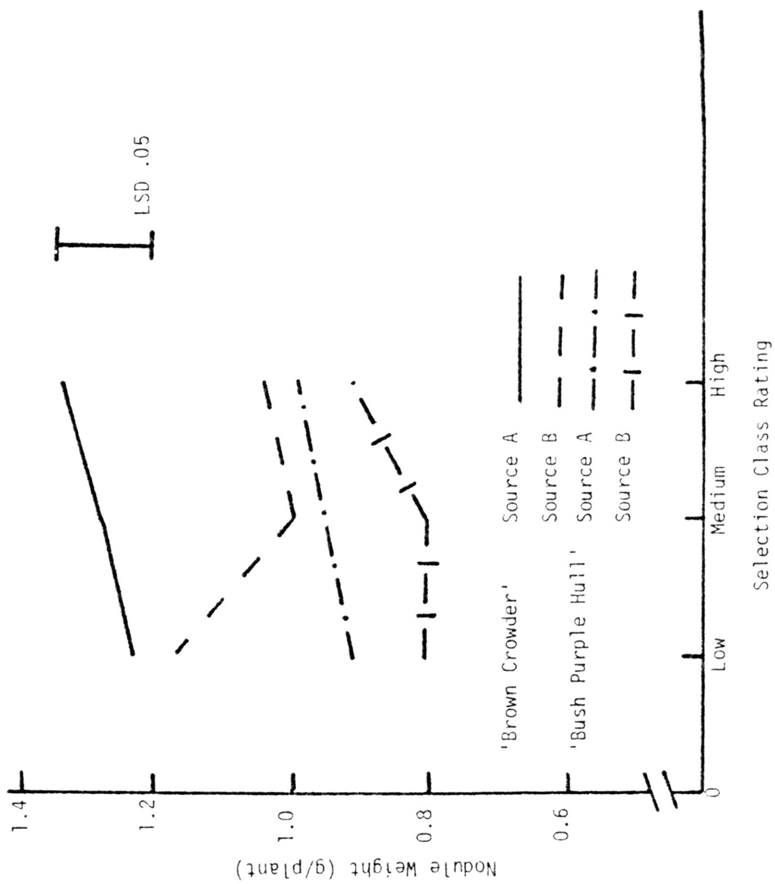


Figure 11 EFFECT OF SEED SOURCE X VARIETY ON ROOT FRESH WEIGHT

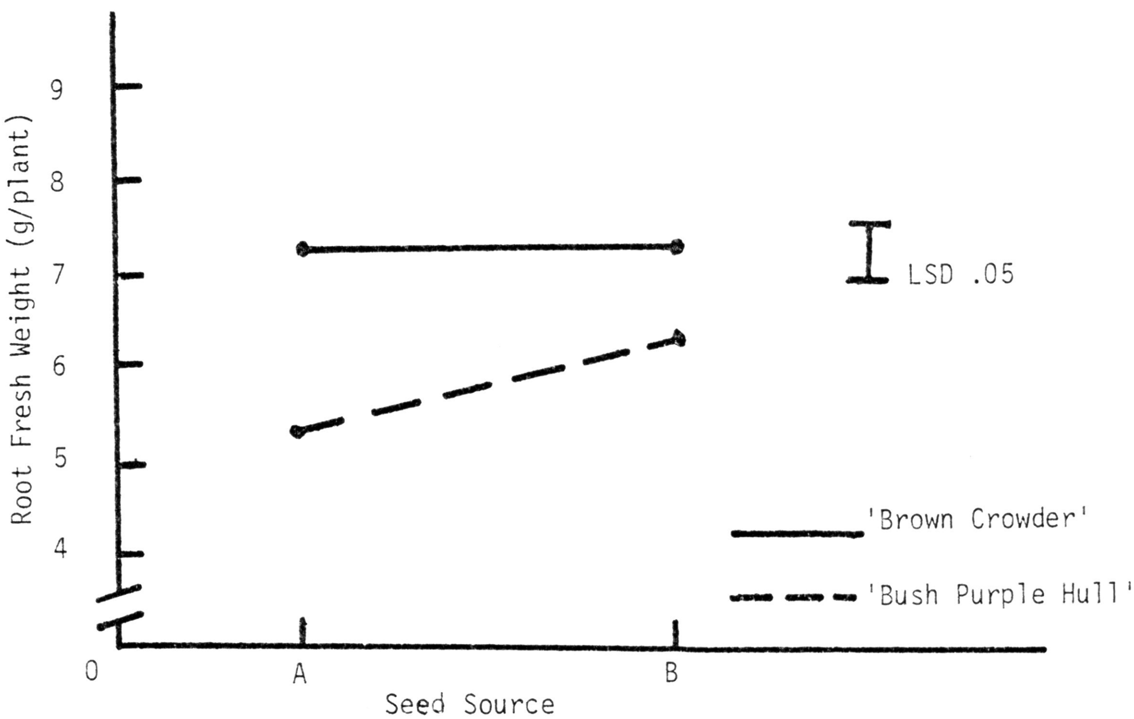


Table 5 RANGES IN SIX NITROGEN FIXATION PARAMETERS OBSERVED IN TWO SOUTHERNPEA VARIETIES FROM TWO SEED SOURCES INOCULATED WITH A SINGLE <u>RHIZOBIUM</u> STRAIN

VARIETY	'Brown Crowder'		'Bush Purple Hull'	
SEED SOURCE	A	B	A	B
Plant Specific Activity[z]	3.42-17.34	3.38-14.88	3.59-9.06	4.31-11.20
Nodule Specific Activity[y]	17.50-45.88	13.05-40-31	12.19-31.49	13.99-25.75
Nodule Number	12-31	11-48	18-60	20-70
Nodule Weight[x]	.308-.525	.115-.549	.221-.519	.268-.540
Root Weight[x]	1.95-5.41	1.77-5.19	1.56-4.37	1.49-4.73
Top Weight[x]	4.07-13.93	4.88-13.78	5.36-11.00	7.19-12.36

[z] Plant Specific Activity = μmoles C_2H_4/plant/hour

[y] Nodule Specific Activity = μmoles C_2H_4/g nodule/hour

[x] g/plant

Hull'. Approximately two-fold differences were observed in nodule weight per plant. Also two to three-fold differences were found in root and top weight.

Analysis of variance of the nitrogen fixation parameters that had significant main effects and interactions are summarized in Table 6. For both plant specific activity and nodule specific activity, the main effects of variety and acetylene levels were significant. Variety was also highly significant for both nodule number and the fresh top weight. No significant interactions were observed. Also there were no significant main effects or interactions for root or nodule weight in this study.

Both variety and acetylene levels had an effect on plant specfic activity. The plant specific activity of 'Bush Purple Hull' at 6.7 umoles C_2H_4 produced/plant/hour is significantly less than that of 'Brown Crowder' at 9.8 umoles (Figure 12). The effect of acetylene level on plant specific activity is shown in Figure 13. When the roots were incubated with 25 CC of acetylene, the average plant specific activity was 7.3 umoles C_2H_4 regardless of variety. However, when the plants were incubated with 50 CC of acetylene, the plant specifc activity was 9.0 umoles. Although the amount of acetylene was significant, suggesting that the nitrogenase enzyme was not saturated with the acetylene substrate (10), the results indicated that a five percent concentration by volume is sufficient to determine relative differences in nitrogen fixation potential in southernpea.

Nodule specific activty also serves to demonstrate differences in varieties and acetylene concentrations. The effect of variety on nodule specific activity when a single-strain inoculant is used is represented in

Table 6 SIGNIFICANT MAIN EFFECTS AND INTERACTIONS FOR NITROGEN FIXATION PARAMETERS OBSERVED WITH SINGLE STRAIN INOCULANT[z]

	Plant Specific Activity[y]	Nodule Specific Activity[x]	Nodule Number	Fresh Weight of Plant Top[w]
SOURCE				
VARIETY	***	***	***	***
SOURCE x VARIETY				
ACET[v]	**	**		
VARIETY x ACET				
SOURCE x VARIETY x ACET				

* .01 - .05 Probability of a greater F value

** .001 - .01 Probability of a greater F value

*** .0001 - .001 Probability of a greater F value

[z] No significant main effects or interactions observed for nodule or root weight

[y] Plant Specific Activity = μmoles C_2H_4/plant/hour

[x] Nodule Specific Activity - μmoles C_2H_4/plant/hour

[w] g/plant

[v] 25 or 50 cc. C_2H_2 injected at incubation

Figure 12 EFFECT OF VARIETY ON PLANT SPECIFIC ACTIVITY (Single-Strain Inoculant)

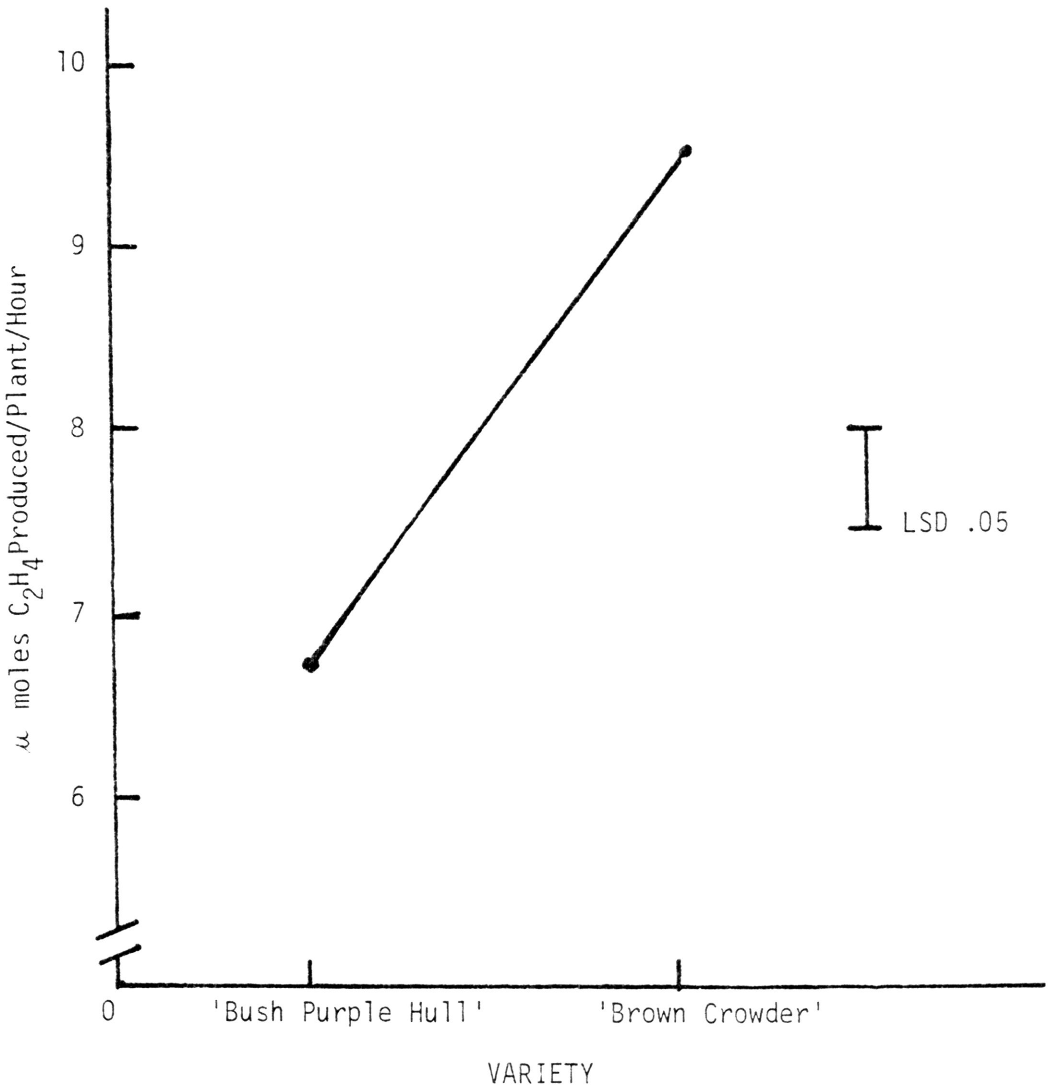


Figure 13 EFFECT OF ACETYLENE AMOUNT ON PLANT SPECIFIC ACTIVITY (Single-Strain Inoculant)

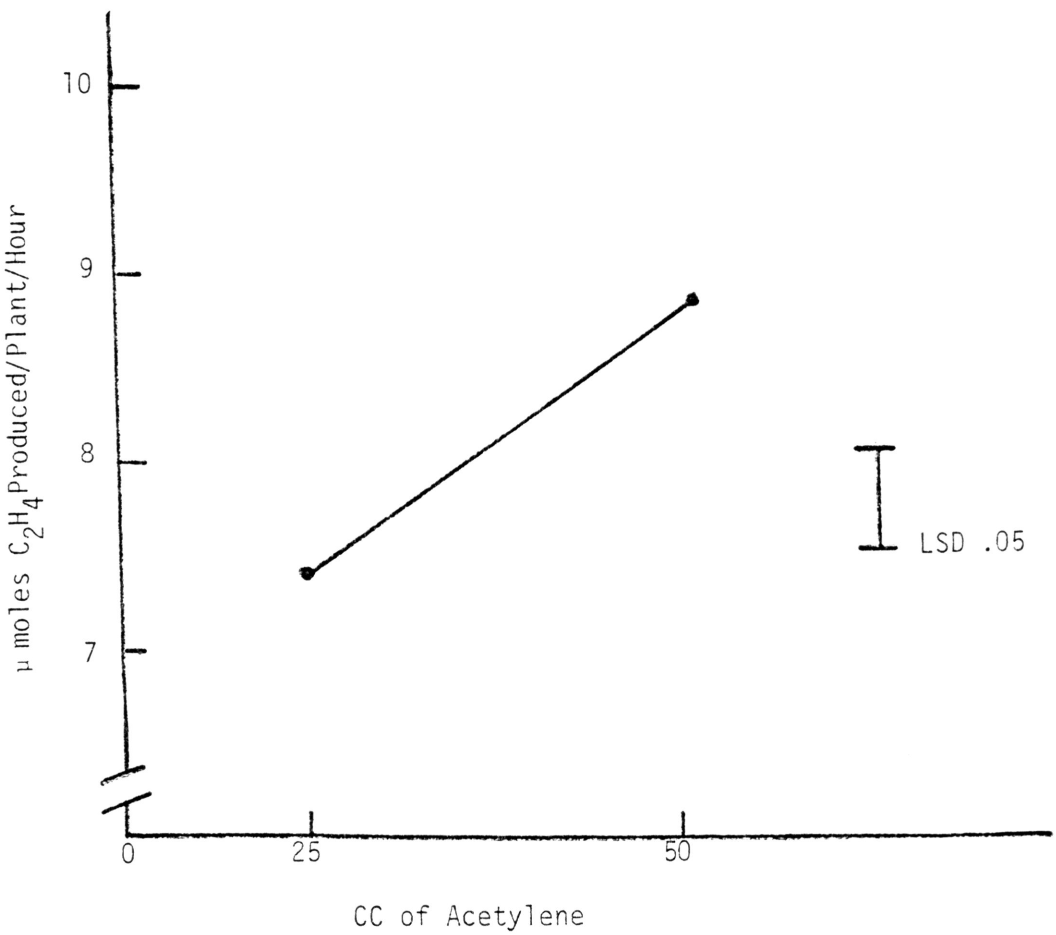


Figure 14. 'Bush Purple Hull' plants has a nodule specific activity of 19 umoles C_2H_4 produced/g nodule/hour. 'Brown Crowder' plants had a significantly higher nodule specific activity at 25 umoles, again supporting the conclusion that 'Brown Crowder' is a relatively high-fixing genotype. The acetylene concentration results shown in Figure 15 indicated that pl ants incubated with 25 CC of acetylene had anodule specific activity of 20.2 umoles, while plants incubated with 50 CC of acetylene produced 24 umoles /g nodule. This again indicated that the amount of acetylene used in the incubation process is a significant factor in the reduction assay, unless only relative differences are needed.

Variety also affected nodule number and fresh weight when the plants were inoculated with a single-strain inoculant. Figure 16 shows the effect of variety on nodule number. Plants of 'Bush Purple Hull' had significantly more nodules per plant than did 'Brown Crowder' plants. Thus, the single-strain inoculant and mixed-strain inoculant affected the nodule number of the two varieties in a similar manner. The single-strain and mixed-strain inoculants also affected the top fresh weight of the two varieties in a similar manner, as is shown in Figure 17. 'Bush Purple Hull' at 9 g/plant was significantly lower than 'Brown Crowder' at 11 g/ plant. Again 'Brown Crowder' was shown to be the larger of the two varieties.

Figure 14 EFFECT OF VARIETY ON NODULE SPECIFIC ACTIVITY (Single-Strain Inoculant)

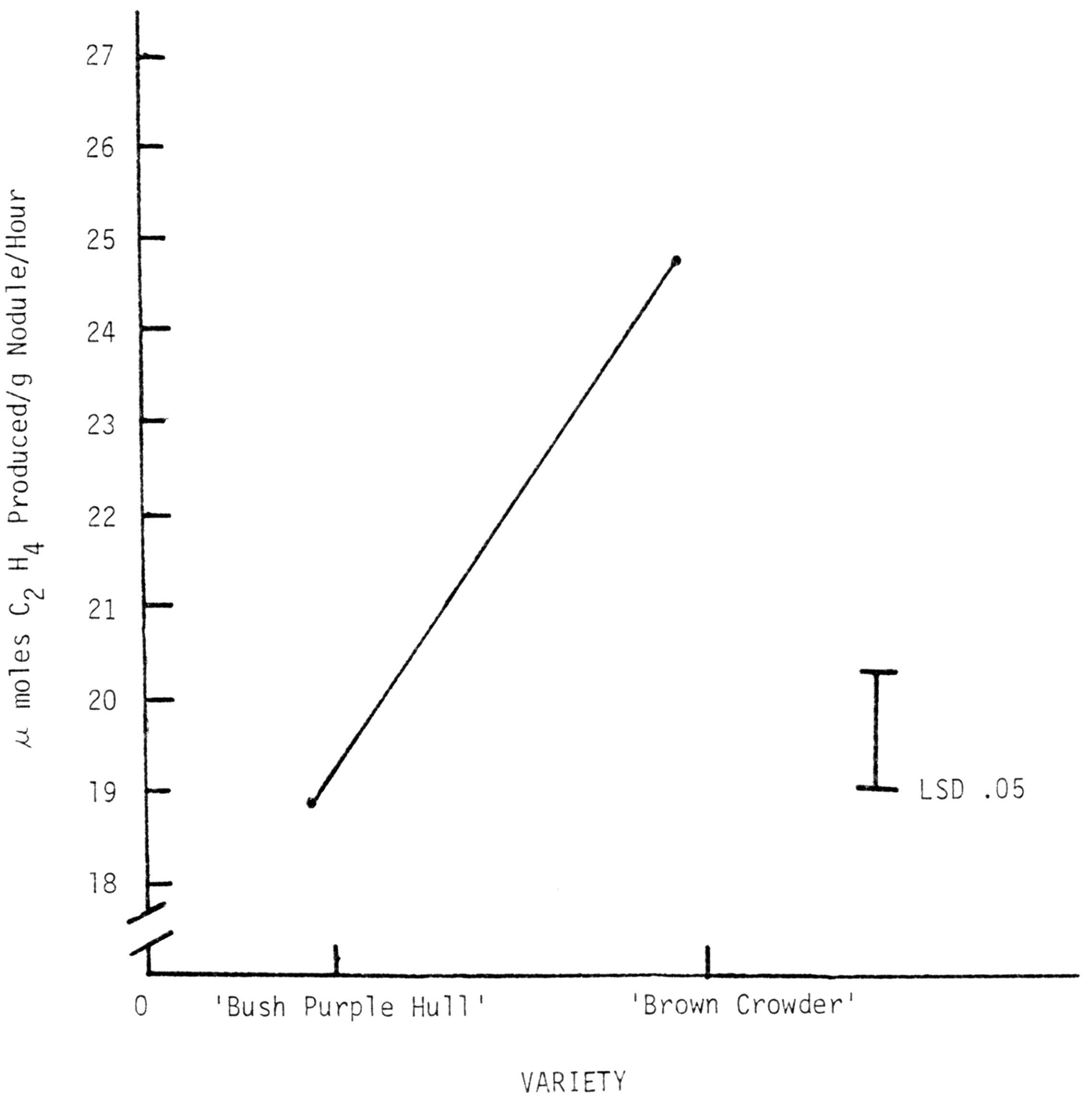


Figure 15 EFFECT OF ACETYLENE AMOUNT ON NODULE SPECIFIC ACTIVITY (Single-Strain Inoculant)

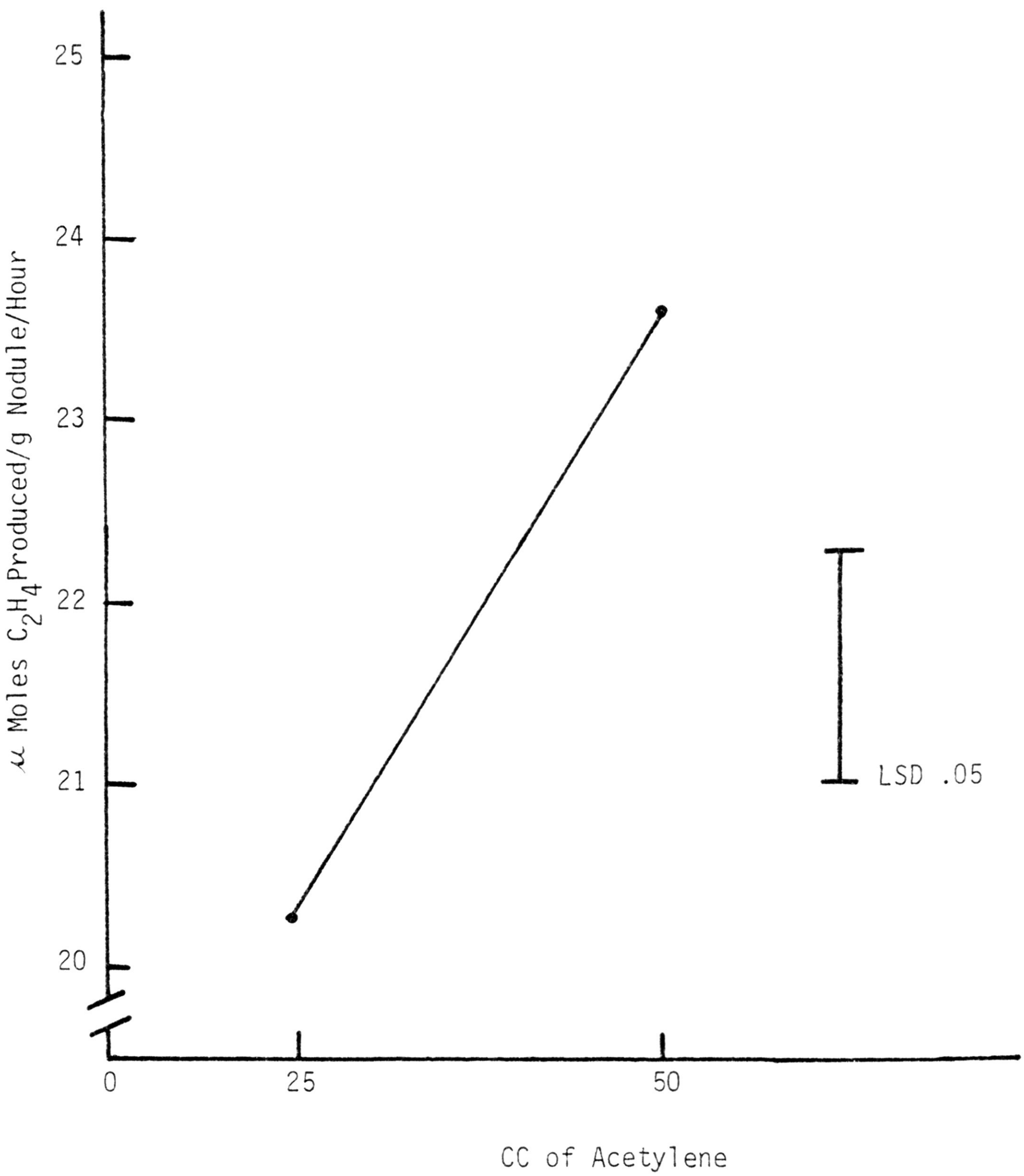


Figure 16 EFFECT OF VARIETY ON NODULE NUMBER (Single-Strain Inoculant)

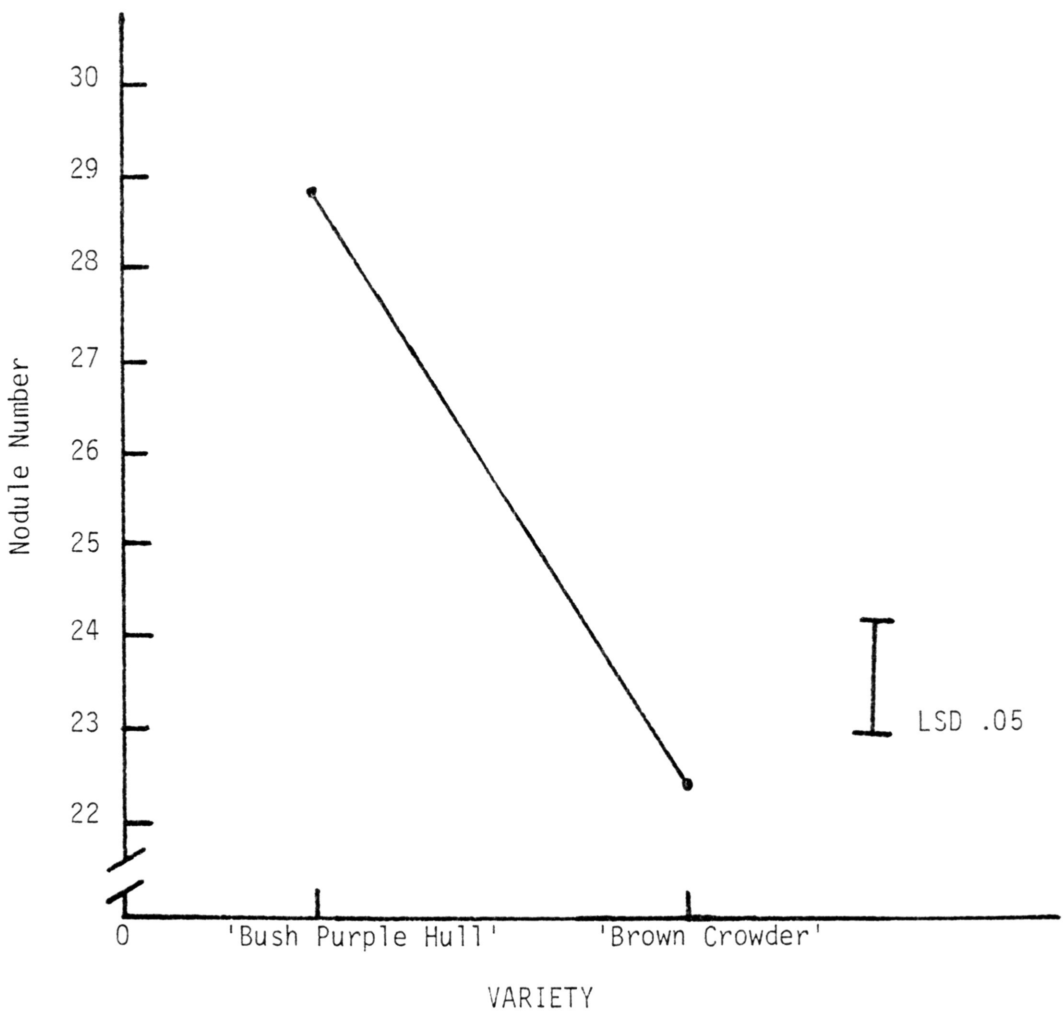
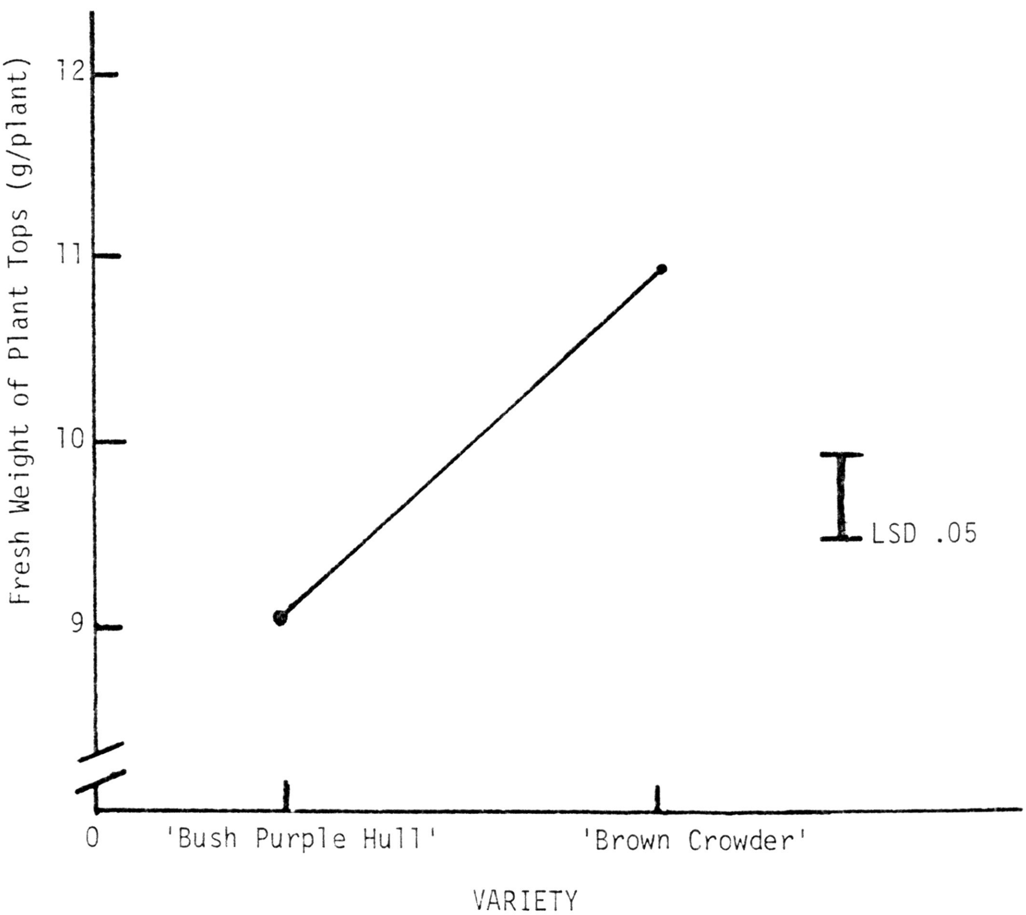


Figure 17 EFFECT OF VARIETY ON PLANT FRESH WEIGHT (Single-Strain Inoculant)



CHAPTER V

CONCLUSIONS

At least four conclusions can be drawn from this research:

1) 'Brown Crowder' in both the original populations and those resulting from first cycle selections had greater nitogen fixation potential than 'Bush Purple Hull' regardless of seed source.

2) Plants grown from seed derived from field-grown plants were shown to have a higher nitrogen fixation potential than plants grown from seed derived with the elimination of outcrossing regardless of variety.

3) Sufficient variability for plant specific activity within varieties and sources was found to allow for the delineation of three phenotypic classes for first cycle selection. However, there was no difference in the performance of the progeny, but a trend was observed which may support a genetic basis for intravarietal variability.

4) Phenotypic variability for nitrogen faxiton potential within varieties may be the result of heterozygosity for the genes

controlling this trait. Therefore, selfing the original population while excluding outcrossing may be required before selecting for enhanced nitrogen fixation.

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