PD-ABB-916
G. URIAS

A RESEARCH PROJECT

Submitted to

United States Agency for
International Development

For Research On

Factors Affecting Symbiotic Nitrogen
Fixation and yield of Cowpeas in Central
Alabama and Jamaica

by

TUSKEGEE AGRICULTURAL EXPERIMENT STATION
(A Division of the Carver Research Foundation)

TUSKEGEE INSTITUTE
Tuskegee Institute, AL 36088

Submitted by:

James R. Allen
Name
Principal Investigator

Name
Co or Assoc. Investigator

Name
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Name
Dean of School

Dr. Bobby R. Phills
Director
Tuskegee Agricultural Experiment Station

TITLE: FACTORS AFFECTING SYMBIOTIC NITROGEN AND YIELD OF COWPEAS IN CENTRAL ALABAMA AND JAMAICA

OBJECTIVES:

1. To determine the effect of Mn, Zn and P on the growth of cowpeas at different pH levels under central Alabama and the Jamaica climatic conditions.
2. To determine the effect of P x Mn and P x Zn interaction on the growth, yield and nitrogen fixation of cowpeas.

PROCEDURE

A. Objective I

Cowpea cultivars will be planted in plots 4.6 x 4.2 m treated with Mn and Zn at rates of 0 (residual soil conc.), 5, 10 and 20 kg/ha.

Each micronutrient rate will be applied at pH levels 5.0 and 6.5. Before Mn, Zn and phosphorous (P) treatments are applied, the soil will be tested to determine residual Mn, Zn and P levels. Control plots will be untreated (no applied Mn, Zn or P). At Tuskegee Institute the soil will be a Norfolk sandy loam of medium fertility. For each level of applied Mn or Zn, two levels of P will be applied - the one recommended by soil test and double that rate. Before micronutrients or P treatments are applied, lime or sulfur will be added to the soil to lower or raise the pH to

desired levels. After application of Mn, Zn and P, Rhizobium inoculated cowpea seeds will be sown in the plots at a rate of 36 kg/ha in rows one m wide and 6.2 m long. There will be four rows per plot. The experimental design will be a split-plot with four replications per treatment. Main plots will be cowpea cultivars and pH levels, and micronutrients and P as subplots. For each pH level there will be eight treatment combinations of Mn and P or Zn and P. These treatments combinations are as follow:

1. Control A - no applied micronutrient or P (only residual soil concentration will be present)
2. Recommended amount of P + 5 kg Mn or Zn
3. Recommended amount of P + 10 kg Mn or Zn
4. Recommended amount of P + 20 kg Mn or Zn
5. Double the recommended rate of P + 5 kg Mn or Zn
6. Double the recommended rate of P + 10 kg Mn or Zn
7. Double the recommended rate of P + 20 kg Mn or Zn
8. The recommended rate of P and no applied Mn or Zn

Nitrogen and potassium will be applied to all plots according to soil test recommendation.

At the Tuskegee Institute site two cowpea cultivars will be used; 'Mississippi Silver' and 'Pinkeye Purple Hull.' At the Jamaican site two of the commonly grown domestic cultivars will be used. The soil in Jamaica will

be the highly weathered lateritic type which is inherently low in P. The experimental design and lay-out will be exactly the same at the Jamaican site as at Tuskegee.

After flowering 10 plants will be randomly harvested from each plot, dried at 70°C for 24 hours and dry matter determined. Each dried sample will then be divided into root and above ground sections and analyzed for Mn, P and Zn content. Mn and Zn content will be determined by atomic spectrophotometric analysis and P by colorimetry. During the growing period, plants will also be monitored for nutrient deficiency or toxicity symptoms. Soil of each plot will be sampled and analyzed for Mn, Zn and P content after treatment but before plant emergence.

When cowpea plants in these plots reach the 10% bloom stage, one plant from each plot will be dug up and intact roots removed by cutting at the base of the stem. Roots will then be washed to remove adhering soil and then each intact root will be placed in sealed septum bottles for nitrogen fixation determination, using the acetylene reduction technique. The top portion of the plant will be dried at 70°C for 24 hours, dry weight determined and then divided into stem and leaf samples. These samples will then be analyzed for Mn, Zn and P as mentioned above. Atomic spectrophotometric analysis will be done exclusively at

Tuskegee Institute, while colorimetric analysis will be performed both in Jamaica and Tuskegee. To relate micronutrient and P treatments to yield and rate of nitrogen fixation, data will be analyzed statistically using the AOV method. To further determine the micronutrient and P levels that are contributing most to the variation in yield and rate of nitrogen fixation, stepwise regression analysis will be done. This analysis will automatically eliminate variables that do not contribute to variation in yield or rate of nitrogen fixation from the regression equation. In so doing, a clear picture of micronutrient and P treatments that enhance or adversely affect cowpea production can be deduced. An overview of the relationship among variables will be accomplished by simple correlation matrix derived from simple correlation analysis.

OBJECTIVE II

This objective will also be achieved from the plots established for Objective I. After colorimetric analysis of plant tissue for P and atomic spectrophotometric analysis for Mn and Zn, data will be statistically analyzed as described for Objective I. Significant Mn x P and Zn x P (as determined by LSD test of significance) effects on nutrient uptake, yield and rate of nitrogen fixation will be presented graphically to show whether the presence of Zn or

Mn increased or decreased the uptake of P and also to show the extent to which this increase or decrease affected yield and rate of nitrogen fixation. Since no atomic spectrophotometric analysis will be done in Jamaica, efforts will be made to ship dried ground samples to Tuskegee where they will be analyzed for Mn and Zn. The Jamaican soil may contain enough free Al at acid pH levels to possibly cause Al toxicity problems in cowpeas. Soil samples will be taken from micronutrient treated plots at pH 5.0 and analyzed for free Al. This data will be statistically analyzed for possible Al x Mn or Al x Zn effect on the growth (dry matter yield) and rate of nitrogen fixation. In addition, top growth and nitrogen fixation rate will be directly related to soil Al content and at the same time compared to Mn and Zn treatments to see if growth and nitrogen fixation responses could possibly be caused by the Al and not the Zn or Mn.

JUSTIFICATION

1. Cowpeas are nutritious and are consumed by individuals in all of the social and economic classes in developing countries such as Jamaica. Thus, it is becoming a major source of protein in diets of people in the Caribbean basin. In

Jamaica, cowpeas are one of the three most important seed legume crops. However, work on this crop is mostly confined to cultivar and fertilizer trials and pesticide effects while the factors affecting symbiotic nitrogen fixation has been virtually ignored.

2. Cowpeas have a relatively high level of drought resistance, thrive well on soils of low to medium fertility and acid pH levels, conditions which may limit growth in central Alabama and Jamaica.

3. Nitrogen fertilizer is expensive in developing countries and thus severely limits crop productivity. However, this nitrogen fertilizer problem could be partially solved by incorporating more cowpeas in the cropping system and more effectively use the nitrogen produced through symbiotic N_2-fixation.

4. Phosphorus is needed for the growth of legumes. However, under acid conditions, its availability may be reduced by being tied up with micronutrients, i.e., Mn and Zn. Also, at acid pH levels these micronutients can be toxic and prevent root development.

LITERATURE REVIEW

Limited availability and increase in the cost of nitrogen fertilizer have severely hurt the productive capabilities of small or limited resource farmers in both the United States and developing countries. Presently, a principal means of increasing crop production is by increasing the application of nitrogen fertilizer. However, in legume production, the only time when nitrogen application has proven to be beneficial is when used as a starter fertilizer and in small amounts (3). Studies have shown that generally the rate of nitrogen fixation will decrease with an increase in the rate of nitrogen fertilizer application (9, 21).

While nitrogen fertilizer application does not always improve legume production, indications are that when used in a crop rotation system the amount of nitrogen that had to be applied to succeeding crops was significantly reduced (2, 10). Cowpeas have been shown to fix as much as 84 kg/ha nitrogen per year (4). In addition to being tremendously important as a source of nitrogen for further crop production, a legume such as cowpeas can be of vital importance in alleviating protein deficiency in the human diet and livestock since it has been proven to be highly nutritious (10). Pope et al (15) showed that steers fed

5.7 kg cowpeas/steer had an average daily gain of 4.7 kg. This compared to those that gained 4.8 kg while being fed an equal amount of protein in the form of cotton seed cake. It was also noted that the cowpea cost 76% less than the cotton seed cake (15).

A problem in legume production is that often the role of micronutrients in plant growth is ignored. However, some studies have shown that when present in optimum amounts some of these micronutrients can potentially enhance the growth of legumes (6, 8, 17). Sallee and Smith (16) in their research on blackeye beans showed that this legume did not respond to nitrogen, phosphorus and potassium but did to Zn.

Kapur and co-workers (6) examined the influences of Zn on nitrogen fixation in soybeans and discovered that application of 5-10 ppm of Zn sulfate to a Zn-deficient silt loam of pH 7.1, significantly increased yield and nitrogen fixation . Often, however, the problem of zinc nutrition is one brought about by toxicity or deficiency in acid soils (1, 2, 19, 20). Smith _et al_ (18) increased nodulation of Bur and Dutch clovers by adding lime and at the same time noted that nodulation decreased with toxic levels of Zn. Another micronutrient that could possibly be beneficial in legume production is Mn. Studies on tropical legumes showed that Mn is capable of enhancing Rhizobium-legume

relationships (4). While research on cowpeas is limited, several studies on soybeans have supported the idea that Mn could enhance legume production (11, 12, 13, 14, 17, 19, 20).

Cultural practices in cowpea production usually call for relatively high rates of phosphorus application. However, there is evidence to indicate that phosphorus could possibly tie-up some of these essential micronutrients. Greenhouse studies by Martens et al (6) showed that when phosphorus was added to Zn-deficient soil, Zn deficiency was shown in tomato plants. Khan et al (6) showed that chlorotic leaves of pinto beans grown in a Zn-deficient soil had higher P and P/Zn levels than green healthy plants.

CURRENT RESEARCH

At Tuskegee Institute, research is being conducted to determine the effects of B, Mn and Zn on the rate of nitrogen fixation and yield of southernpeas (cowpeas) at various soil pH levels. Field studies have shown that at pH 6.0 both seed yield and rate of nitrogen fixation was highest with 2.5 kg/ha B. Seed yield and rate of nitrogen fixation were also enhanced by the addition of Mn and Zn but at a rate of 10 kg/ha. Studies are also underway in which the cowpeas are being intercropped with corn. This study includes the collection of data on the effect of phosphorus

and nitrogen on yield and rate of nitrogen fixation within the intercropping systems.

These studies are closely linked to the one proposed since preliminary data already obtained will be used as a basis for designing field studies to be done by the Jamaican counterparts.

FACILITIES AND EQUIPMENT

A. Tuskegee Institute

The research will be conducted at the Tuskegee Institute Experiment Station and Laboratories in the Department of Agricultural Sciences and Carver Research Foundation. Gas chromatograph and colorimeters are available in the Department of Agricultural Sciences and an atomic spectrophotometer at Carver Laboratories. Ovens and pH meters are also available in the Department of Agricultural Sciences for biomass and pH determinations. In addition, land and field equipment are available on the research farm for field plot establishment.

B. The Botany Department, U.W.I.

(1) Greenhouses for pot experiments

(2) Land for plot trials

(3) GLC for nitrogen fixation analysis
(Acetylene reduction assay)

(4) Laminar flow and incubators for insolation and growth of strains of Rhizobia

(5) Autoclaves

(6) A jeep for traveling

(7) Laboratory space for the project

RESEARCH TIME TABLE

This project is expected to be completed in two years. It will be initiated in October, 1983 and end in October, 1985. The project will proceed as follows:

(a) Fiscal year 1983-84, field research studies will be initiated in November, 1983 in Jamaica and by May, 1984 at Tuskegee Institute.

(b) The first report will be written by November, 1984.

(c) Fiscal 1984-85, field and/or greenhouse studies will be repeated. Laboratory and statistical analyses will be conducted.

(d) Final report will be written between the period December, 1985 - January, 1986.

PERSONNEL SUPPORT

1. James R. Allen, Professor
   Department of Plant and Soil Science
   Tuskegee Institute, AL 36088
   Present Research Involvement:
   USDA/CSRS: The effect of micronutrients on yield of southernpeas. Grant # AL.X-3-.'.(A-03(2).

2. Dr. P. V. Devi Prasad
   Senior Lecturer
   Department of Botany
   University of the West Indies
   Mona, Kingston, Jamaica West Indies

3. Dr. G. H. Sidrak, Head
   Department of Botany
   University of the West Indies
   Mona, Kingston, Jamaica West Indies

INSTITUTIONAL UNITS INVOLVED

(a) Department of Agricultural Sciences
Provides laboratory for plant and soil analysis.

(b) Tuskegee Institute Agricultural Experiment Station provides land for field plot research - Equipment for land preparation - transportation for transplanting plant and soil samples to and from the farm.

(c) Carver Research Laboratories
Will make an atomic absorption spectrophotometer available for Mn and Zn analysis.

(d) Department of Botany, University of the West Indies Establish greenhouse and field studies - collect and analyze field and greenhouse data.

IMPACT

A. The Jamaican counterpart feels that a study of this nature will do the following:

(1) Will reduce the problem of importation of pulses thus saving valuable hard currency.

(2) More indigenous proteninaceous food will be available.

(3) If production could be increased significantly, these could be an item for export within the Caricom Region to earn hard currency.

(4) Better yields might induce more farmers to cultivate the suitable (but at present idle) land.

LITERATURE CITED

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U.S. DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION

PROPOSAL BUDGET

OMB-0531-0009

ORGANIZATION AND ADDRESS
Tuskegee Institute
Department of Agricultural Sciences

PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S)
James R. Allen

DURATION PROPOSED: Months: 12

S&E USE ONLY: Months: _____

	S&E FUNDED WORK MONTHS Calendar	Academic	Summer	FUNDS REQUESTED BY PROPOSER	FUNDS APPROVED BY S&E (If different)
A. Salaries and Wages					
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)					
b. ___ Senior Associates	2,000			$ 2,000	$
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorate	4,000			4,000	
b. ___ Other Professionals					
c. ___ Graduate Students				1,500	
d. ___ Pre-Baccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop, and Other					
Total Salaries and Wages					
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits *(A plus B)*					
D. Nonexpendable Equipment *(Attach supporting data. List items and dollar amounts for each item.)*					
E. Materials and Supplies				2,300	
F. Travel					
1. Domestic *(Including Canada)*				200	
2. Foreign *(List destination and amount for each trip.)*				3,300	
G. Publication Costs/Page Charges				150	
H. Computer (ADPE) Costs					
I. All Other Direct Costs *(Attach supporting data. List items and dollar amounts.* Details of subcontracts, including work statements and budget, should be explained in full in proposal.)				2,300	
J. Total Direct Costs *(C through I)*					
K. Indirect Costs *(Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)*				2,063	
L. Total Direct and Indirect Costs *(J plus K)*					
M. Less Residual Funds *(If applicable)*					
N. TOTAL AMOUNT of this REQUEST *(L minus M)*				$ 17,813	$
O. COST SHARING	$				

NOTE: Signatures required only for Revised Budget

This is Revision No. ►

NAME AND TITLE (type or print)	SIGNATURE	DATE
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR		
AUTHORIZED ORGANIZATIONAL REPRESENTATIVE		

Form S&E-55 (Aug 81)

USDA-S&E

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Budget Explanation

Request for 1983 -1984

a. Research Associate

This individual will be at the University of the West Indies as requested by Dr. Sidrak.

b. Travel

1. Request for: the principal investigator to visit the University of the West Indies to initiate the project.

2. The Jamaican counterpart (Dr. Sidrak) to visit Tuskegee Institute for consultation.

3. Dr. Sidrak for Travel Expenses from the the field to the laboratory at the Mona Campus of the University of the West Indies.

c. Expendable Supplies

Two thousand dollars ($2,000) is requested by Dr. Sidrak of the University of the West Indies and the rest will be for supplies at Tuskegee Institute.

d. Other

Two thousand three hundred dollars ($2,300) is requested by Dr. Sidrak and his group to operate field equipment for field plot layout and maintenance.

U.S. DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION

PROPOSAL BUDGET

OMB-0531-0009

ORGANIZATION AND ADDRESS	DURATION PROPOSED	S&E USE ONLY
Tuskegee Institute Department of Agricultural Sciences	Months: 12	Months: _____
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) James R. Allen	FUNDS REQUESTED BY PROPOSER	FUNDS APPROVED BY S&E (If different)

	S&E FUNDED WORK MONTHS Calendar	Academic	Summer	FUNDS REQUESTED BY PROPOSER	FUNDS APPROVED BY S&E (If different)
A. Salaries and Wages					
1. No. of Senior Personnel					
a. ___ (Co)-PI(s)/PD(s)	2,000			$2,000	$
b. ___ Senior Associates					
2. No. of Other Personnel (Non-Faculty)					
a. ___ Research Associates-Postdoctorate	4,200			4,200	
b. ___ Other Professionals					
c. ___ Graduate Students				1,500	
d. ___ Pre-Baccalaureate Students					
e. ___ Secretarial-Clerical					
f. ___ Technical, Shop, and Other					
Total Salaries and Wages ►					
B. Fringe Benefits (If charged as Direct Costs)					
C. Total Salaries, Wages, and Fringe Benefits *(A plus B)* ►					
D. Nonexpendable Equipment *(Attach supporting data. List items and dollar amounts for each item.)*					
E. Materials and Supplies				2,346	
F. Travel					
1. Domestic *(Including Canada)*				200	
2. Foreign *(List destination and amount for each trip.)*				3,300	
G. Publication Costs/Page Charges				150	
H. Computer (ADPE) Costs					
I. All Other Direct Costs *(Attach supporting data. List items and dollar amounts.* Details of subcontracts, including work statements and budget, should be explained in full in proposal.)				1,500	
J. Total Direct Costs *(C through I)* ►					
K. Indirect Costs *(Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)*				1,991	
L. Total Direct and Indirect Costs *(J plus K)* ►					
M. Less Residual Funds *(If applicable)* ►					
N. TOTAL AMOUNT of this REQUEST *(L minus M)* ►				$17,187	$
O. COST SHARING ►	$				

NOTE: Signatures required only for Revised Budget — *This is Revision No.* ►

NAME AND TITLE (type or print)	SIGNATURE	DATE
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR		
AUTHORIZED ORGANIZATIONAL REPRESENTATIVE		

Form S&E-55 (Aug 81) USDA-S&E

Request for 1984 - 1985

a. Research Associate

Four thousand two hundred dollars ($4,200) is requested for salary for this individual who will be at the University of the West Indies.

b. Travel

1. Requested for: the principal investigator to evaluate the progress of the research at the University of the West Indies.

2. Dr. Sidrak to pay a return visit to Tuskegee for consultation.

3. The Jamaican counterpart also requests additional travel to and from field plots to laboratories at the University campus and to purchase materials. The rest will be used for research related travel at Tuskegee Institute.

c. Expendable Supplies

1. Two thousand dollars ($2,000) is requested by Dr. Sidrak of the University of the West Indies and the rest will be for supplies at Tuskegee Institute.

d. Other

One thousand five hundred dollars ($1,500) is requested by Dr. Sidrak and his group to operate field equipment for field plot layout and manintenance.