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July 8, 1993

Charles Sloger
Agriculture Advisor, Office of Agriculture
Bureau for Research and Development
Agency for International Development
Washington, D.C. 20523

RE: PSTC #8.052, Grant DPE-5542-G-SS-8015-00

Dear Dr. Sloger:

At long last here is the final report for this project. Please review it with the thought that I can make any additions or corrections you feel appropriate. I have contacted Dr. Ahmad and await more information regarding technology transfer and future work in Jamaica. However, I have decided not to delay sending this any longer. If you feel more information is needed please let me know as I may have more by then.

Thank you again for your patience on this project.

Sincerely,

Fred D. McElroy, PhD
President

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SELECTION OF COMPATIBLE VA MYCORRHIZAL FUNGI, RHIZOBIA AND INOCULATION METHODS TO INCREASE COWPEA YIELDS

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FINAL REPORT

Covering Period: June 1988 - January 1992

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

Selection of Compatible VA mycorrhizal Fungi, Rhizobia and Inoculation Methods to Increase Cowpea Yields

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I. RESEARCH OBJECTIVES

A. Overall Aim and Specific Objectives

The overall aim of this research was to increase the effectiveness of *Rhizobium* and vesicular-arbuscular mycorrhizal (VAM) fungi in improving the growth and yield of cowpea (*Vigna unguiculata*) in low fertility farming systems. The tripartite legume-*Rhizobium*-VAM fungus system is a highly interactive and interdependent mutualism which functions well under low to moderate soil fertility conditions. If one or more components of the system are not compatible with the others, the plan will not achieve its maximum yield potential. In Jamaica, cowpea varieties and rhizobia nodulate poorly with no apparent relationship to any soil physical or chemical parameter. These soils are also high in their phosphate-fixing capacity. Both nodulation and dinitrogen fixation are highly dependent on an adequate supply of phosphate. VAM fungi are well known for their ability to improve plant phosphate levels and increase nodulation and dinitrogen fixation. There has been no consideration of the VAM fungi with regard to the introduction of cowpea varieties or rhizobia. In fact, there has been no research conducted on VAM fungi in Jamaica. Recent evidence suggests that for a given legume, it may be possible to select combinations of VAM fungi and rhizobia which are more beneficial to the plant than others. This research addressed a selection process which maximized the effectiveness of these beneficial microorganisms for improving cowpea yield. Specific objectives were as follows:

1. Select VAM fungi and rhizobia for their ability to colonize cowpea roots effectively in non-sterile, phosphate-fixing soils.

2. Develop improved methods and criteria for the co-selection of compatible highly effective VAM fungi and rhizobia for cowpea.

3. Define conditions for the successful establishment of effective and compatible VAM fungi and cowpea rhizobia under field conditions.

4. Provide training for the establishment of a VA mycorrhiza research program in Jamaica.

B. Relevance to Development

The proposed research is strongly aligned with the Program in Science and Technology Cooperation (PSTC) high priority Research Modules #2 (Plant Biotechnology) and #6 (Diversity of Biological Resources). Data obtained from this work will directly affect considerations for AID/SCI research priorities and objectives relative to legumes. Projects such as NifTAL will especially benefit from our findings.

As a less developed country (LDC), Jamaica will benefit from the development of a VA

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mycorrhiza research program which will be useful for improving the growth of many crops (not just legumes) in their P-fixing, low fertility agricultural soils. Specific problems of poor cowpea nodulation and low success in establishment of introduced rhizobia will be addressed. Our work is especially timely since *Rhizobium* strain selection and inoculant production are now under development in Jamaica.

The research has a high degree of relevance to LDC development in that both VAM fungi and rhizobia are able to improve the growth of legumes in low fertility soils. It should be stressed here that methodologies developed in our research are applicable to all legume crops, thus the work has a much wider significance than only cowpea in Jamaica. Another point relevant to LDC development is the fact that VAM fungi, which colonize the roots of many different crops, are an important component in promoting sustainable agriculture. VAM fungi improve plant P uptake, soil structure, and reduce plant drought stress. These factors, combined with the enhancement of *Rhizobium* effectiveness, contribute to the maintenance of soil organic matter and soil fertility, which then benefits subsequent crops. VAM fungus and *Rhizobium* biotechnology development can be accomplished at relatively low cost and produce very large benefits in crop productivity. These are important considerations for adoption and utilization by LDCs. With proper care to prevent the buildup of plant pathogens in the VAM fungus culturing, we envision no adverse side effects from our research.

C. Innovative Aspects

Currently, VAM fungi and rhizobia are selected for their effectiveness primarily under sterilized soil conditions and without regard for any weakly synergistic or competitive interactions these organisms can have with each other. Competition for carbon between these endophytes can be detrimental to the host by reducing the N_2 fixation potential of the rhizobia and the P uptake abilities of the VAM fungus. Our innovative approach was to establish criteria for the co-selection of rhizobia and VAM fungi which form a compatible, mutualistic response within the host. The research combined recent information on inoculation methodologies for both microorganisms which will enhance their establishment and competitive abilities with less effective native soil populations. The research was not a mere adaptation of existing technology, but a new multi-organism approach to biotechnology development for low-fertility farming systems.

D. Peninsu-Lab's Support of the Project

Peninsu-Lab (PL), the recipient of this grant award, provided the base of employment for Dr. R. Ames while he worked on this project. In doing so, PL provided the administrative and accounting framework to administer this project, and Dr. Ames' salary for the project. The support facilities of PL also provided for the space for experimentation, isolation and culture of the microorganisms, preparation of samples for nutrient analysis, facilities for training of Jamaican researchers, and computers and software for data collection, evaluation,

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and report preparation.

II. RESEARCH STUDIES

The research studies conducted under this project were divided between laboratory work at PL in the U.S., and laboratory and field work in Jamaica. The results of the experiments are presented below under those headings. The U.S. research was designed to gather background information for application to research studies to be carried out in Jamaica, therefore this report of the results of the U.S. studies is somewhat abbreviated since it is reported in greater detail as a part of the Jamaica studies.

A. U.S.

1. Impact of Banding Rhizobia and VAM -Soil Type 1

a. Materials and Methods

The purpose of this experiment was to determine if banding VAM fungus inoculum (mixed with selected rhizobia) below cowpea seed would result in a competitive advantage of the introduced organisms over that of the indigenous soil population. The experiment used a non-sterile soil which contained indigenous rhizobia and VAM fungi.

The soil used in this experiment was a loamy sand, Ph 5.8, 2.0 ug N g^{-1}, 7.8 ug P g^{-1}, and 47 ug K g^{-1}. This soil contained an indigenous *Rhizobium* population which was serologically distinct from the inoculated strains. It also contained a native VAM fungus (*Glomus* spp.) which differed from the other two *Glomus* species used in this experiment.

Inoculum of *Glomus macrocarpum* or *G. mosseae* and rhizobia, TAL 173 or IRC 256 were placed in a band 3-5 cm below cowpea seed in each 2 liter pot. This was a 2x2 factorial, completely randomized design conducted in a plant growth chamber for a period of 66 days. There was one plant per pot. One nutrient application had to be made early in the experiment due to poor growth of the control (non-inoculated) treatments.

b. Results

The root systems of all plants became heavily colonized by VAM fungi and nodulated by *Rhizobium*. The indigenous rhizobia were not detected from nodules of plants inoculated with strains TAL 173 or ICR 256. Plant growth response resulted from the strain of *Rhizobium* present rather than the species of VAM fungus. There was no significant interaction between VAM fungi and rhizobias. Fewer and smaller pods and a larger number of nodules were obtained with indigenous compared to the introduced rhizobia.

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c. Conclusions

1. In a non-sterile soil containing an indigenous population of rhizobia and VAM fungi, it is possible to establish introduced isolates of these microorganisms by mixing them together and banding them 3-5 cm below cowpea seed.

2. An introduced strain of *Rhizobium* may dominate nodule production over that obtained by an indigenous population.

3. Different species of VAM fungi introduced into a non-sterile soil may co-exist within cowpea roots colonized by native VAM fungi.

4. The strain of *Rhizobium* present appears to have a greater influence on cowpea growth than the species of VAM fungus.

2. Impact of Banding Rhizobia and VAM -Soil Type 2

This experiment was a repeat of Experiment 1 using a different soil type. The soil used in this experiment was a sandy loam, Ph 6.5, 19 ug N g^{-1}, 13.1 ug P g^{-1}, and 131 ug K g^{-1}, and contained VAM fungi but not cowpea rhizobia.

Rhizobium isolate TAL 169 was mixed into the soil of all treatments since an indigenous strain was not present. Inoculum of either *Glomus macrocarpum* (350 spores/40g) of *Glomus mosseae* (360 spores/40g) was applied as a band approximately 3 cm below the seed. A non-inoculated treatment was also established to examine effects of the indigenous VAM fungus on cowpea growth. One mL of *Rhizobium* isolates TAL 173, ICR 256 or sterile YMA broth was applied over each of the three seeds per pot. After one week, the plants were thinned to one plant per pot. There were three replications for each of the nine treatments: three VAM fungi, three rhizobia. Plants were grown for 61 days. Water was applied from below by filling the saucers that held each pod. No nutrients were applied.

b. Results

Plant growth was twice that obtained in Experiment 1 due to the higher nutrient content of the soil.Excellent mycorrhiza formation and nodulation by all three introduced rhizobia was obtained. No interactions occurred between VAM fungi and strains of cowpea rhizobia. TAL 173 significantly increased nodule number and leaf nitrogen while shoot dry weight was significantly increased by TAL 169.

c. Conclusions

Both experiments 1 and 2 indicated that the strain of *Rhizobium* has a greater influence over plant growth and nutrient content than soil type or species of VAM fungus. In addition, both

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experiments demonstrated that there is no interaction between the species of VAM fungi and strains of *Rhizobium* used. The introduced mycorrhizal fungi and rhizobia are able to colonize the roots of cowpea grown in non-sterilized soils with indigenous strains of these microorganisms already present.

3. Impact of Pre-establishment of Rhizobia and VAM Fungi on Subsequent Nodulation of More Aggressive Rhizobia.

a. Materials and Methods

This study was in response to the belief that aggressive but ineffective strains of *Rhizobium* in Jamaica rapidly colonize cowpea roots after seed germination and prevent efficient nodulation by the effective, introduced strains. Three strains of *Rhizobium*, TAL 169, TAL 658, and IRC 256 were grown in yeast extract-mannitol broth and 3.0 ml, 1.0 ml per each of 3 seeds/pot, were inoculated at 1.8×10^9, 1.2×10^9, and 7.1×10^6 CFU mL^{-1}, respectively. The rhizobia were applied alone or with 60g of *Glomus mosseae* or 75g of *Glomus macrocarpum* inoculum, which contained 228 and 320 spores of each of the respective fungi. There were also treatments with the VAM fungi alone. Sterilized soil (500 cm^3) which is the same as that used in Experiment 1, was used to establish cowpea plants pre-inoculated with the above organisms or left uninoculated. The plants were grown for 18 days, after which they were transplanted to the non-sterilized soil in 1.5 L pots which had been inoculated with TAL 173. Using extra plants, pre-colonization by the appropriate VAM fungi and rhizobia was confirmed. The plants were then grown for an additional 5 weeks. There were 3 replications per treatment.

b. Results and Conclusions

The results of this experiment indicated that pre-establishment of either the VAM fungi of rhizobia had no effect on subsequent nodulation by indigenous rhizobia. These results were confirmed by field trials conducted in Jamaica (see below).

B. JAMAICA

1. Co-selection of Compatible Rhizobia and VAM

a. Materials and Methods

The separate sterilized (autoclaved) and non-sterilized soil experiments were conducted in Jamaica. The soil for both experiments was collected on campus at the University of the West Indies, Kingston, Jamaica, from the Caribbean Agricultural Research and Development Institute's experimental farm. The black loam soil contained 2 ug g-1 NO3-N, 28 ug g^{-1} P and had a pH of 6.3. For each treatment replicate, plastic bags 25 x 13 x 10 cm. were filled with air-dried soil.

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Rhizobium strains JRC14, JRC 19, and JRC 29 were isolated from Jamaican soils and TAL169 was obtained from NifTAL, Hawaii, USA. TAL169 and JRC19 were the most effective strains selected from sterilized soil tests while JRC14 and JRC29 were selected from non-sterilized soil testing. The three VAM fungi, *G. pallidum, G. aggregatum and S. microcarpa*, were isolated from Jamaican soils and increased in potted plant culture with corn (*Zea mays*) in a sterilized sandy soil. The undiluted mycorrhiza inoculum produced 59.5, 54.5 and 58.3% root colonization in cowpeas from the *G. pallidum, G. aggregatum*, and *S. microcarpa* culture material, respectively, in a 3-week inoculum standardization test. The mycorrhizal fungus inocula consisted of spores, hyphae, root fragments, and soil from the corn pot cultures. Twenty-five grams of each of the VAM fungal inocula, containing approximately 1000 spores, were placed 2-3 cm below the seed in each VAM fungus treatment. Each bag of soil was then seeded with two surface-sterilized cowpea seeds, cv. Laura B., which were later thinned to one plant per bag. The rhizobia were grown in yeast-mannitol broth and 1.0 ml. containing 1 x 10^8 cells, of the appropriate inoculum was applied over each seed and covered with soil. The plants were grown for 59 days in a greenhouse with average day and night temperatures of 29°C and 22°C, respectively. Water was applied as needed and alternated with a dilute nutrient solution. Care was taken to prevent cross-contamination of the microorganisms.

The non-sterilized soil study consisted of a randomized factorial design of five rhizobia (JRC14, JRC19, JRC29, TAL169, and the indigenous strain) and four VAM fungi (*G. pallidum, G. aggregatum, S. microcarpa*, and the native VAM fungi). There were five replicates per treatment. The sterilized (autoclaved) soil experiment was conducted similarly, except the indigenous rhizobia and native VAM fungi were killed by autoclaving and these treatments were not inoculated. There were four replicates per treatment in the sterilized soil study.

At harvest, the shoot was severed at the soil surface, oven-dried (70°C), and weighed. The shoot material was ground and a subsample was digested (Thomas et. al. 1967) and analyzed for P (Murphy and Riley 1962) and N (Nessler test, Vanselow 1940). Nodules were removed from the roots, counted, then air-dried and weighed.

Ten nodules from each treatment were tested to confirm colonization by the introduced rhizobia, using antibiotic resistance profiles (McLaughlin and Ahmad 1984) or a fluorescent antibody assay. The roots were washed, cut into segments of 2-3 cm, and subsampled for VAM fungal colonization. The percentage mycorrhizal fungus colonization of the roots was determined using a line-intercept method (March 1971) after clearing and staining to reveal the VAM fungi (Phillips and Hayman 1970). The data were analyzed by one-way analysis of variance. Significant differences between treatment means was determined by a t-test.

b. Results

Sterilized Soil Study: Antibiotic and fluorescent antibody assays

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of the nodules showed that those sampled were colonized by only the introduced rhizobia. No cross-contamination was detected and no nodulation occurred in the non-inoculated controls.

JRC14 and JRC19 produced significantly greater numbers and weights of nodules than the rhizobia (Table 1, See Appendix under title of this study). In plants without the rhizobial inoculant, the percentage mycorrhizal colonization, shoot dry weight, and shoot N and P content were significantly ($P<0.01$) reduced compared to the *Rhizobium*-inoculated treatments. Although mycorrhizal development was significantly greater in the JRC29 versus TAL 169 treatments, there were no differences between *Rhizobium* strains in shoot dry weight and N and P content.

No VAM fungus colonization was detected in the uninoculated controls in the sterilized soil (Table 2). The number of nodules was significantly ($P<0.001$) increased by inoculation with S. microcarpa and G. pallidum but not by G. aggregatum. Nodule dry weight, shoot dry weight, percentage VAM fungus colonization, and shoot P content were all significantly ($P<0.001$) increased with the VAM fungus inoculations. Shoot N was not affected.

Poor plant growth and a low nutrient content were observed in cowpeas without *Rhizobium* or VAM fungi (Table 3). JRC 14 and JRC 19 increased nodule numbers more than JRC29 and TAL169 only when mycorrhizal fungi were absent, or in the presence of *G. aggregatum*, which was the least effective VAM fungus. Nodule dry weight differences occurred only in the absence of mycorrhizal fungi. The *Rhizobium* isolate had a significant effect on mycorrhiza (% VAM) development only in the presence of *G. palladium*.

VAM fungus - *Rhizobium* interactions significantly affected the cowpea shoot dry weight (Table 3). The optimum shoot weight was obtained with *G. pallidum* and JRC19 and JRC29. *G. aggregatum* increased the shoot weight when co-inoculated with JRC14 and JRC19. No shoot weight differences occurred between the four rhizobia in the presence of *S. microcarpa*.

The shoot N content was not significantly ($P> 0.05$) affected by VAM fungus - *Rhizobium* interactions. The shoot P content was significantly improved in the VAM fungus + *Rhizobium* treatments compared with the single inoculation treatments (Table 3).

Non-sterilized Soil Study: Antibiotic resistance and fluorescent antibody assays confirmed the presence of the introduced rhizobia in each appropriate treatment. Although no cross-contamination with the introduced rhizobia was seen, we did not measure nodule occupancy by the indigenous rhizobia because no antibiotic resistance profiles had been developed for them.

Inoculation with the introduced strains of *Rhizobium* significantly increased the shoot dry weight, percentage VAM fungus colonization, and shoot N content compared with the

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indigenous strain(s) (Table 4). JRC14 and JRC29 significantly ($P<0.01$) improved nodule dry weight, shoot dry weight and shoot P content over that of JRC19 and TAL169.

The shoot dry weight, percent VAM fungus colonization, and shoot P content was significantly greater for the introduced than indigenous VAM fungi (Table 5). Nodule number and shoot N content were not affected by the introduced VAM fungi. The largest shoot P content was obtained by the *G. pallidum* and *S. microcarpa* treatments.

The interaction of JRC14 and JRC29 with the three introduced VAM fungi resulted in significantly ($P<0.05$) higher shoot dry weight and percent VAM fungus root colonization compared with the other rhizobia paired with the same VAM fungi (Table 6), JRC14 and JRC29 significantly improved shoot P when paired with *S. microcarpa* and nodule dry weight and shoot P when paired with *G. pallidum*.

2. Field Trials with Co-Selected Rhizobia and VAM

a. Materials and Methods

In a greenhouse study, we tested the compatibility of three native strains of VAM fungi (two *Glomus* species and one *Sclerocystis* species) with four cowpea rhizobia strains (three native strains, JRC14, JRC 19, and JRC29, and one NifTAL strain, TAL169). Two VAM fungi (*Glomus pallidum* and *Sclerocystis microcarpa*) and two rhizobial strains (JRC14 and JRC29) showed the most effective and compatible microbial pairing in sterilized and nonsterilized soils and enhanced the growth of cowpea under greenhouse conditions. (Ames et. al. 1991). However, the effect of inoculation of cowpea with such compatible pairings of VAM fungi and cowpea rhizobia under field conditions was not determined. In this work, we have examined the response of cowpea to inoculation with each of the compatible pairings of VAM fungi (*Glomus* sp. and *Sclerocystis* sp.) and rhizobia strains (JRC14 and JRC298) under field conditions in two soil types (black sandy clay loan and red bauxitic silt loam) at two locations in Jamaica.

The VAM fungi (*Glomus pallidum* and *Sclerocystis microcarpa*) were isolated from Jamaican soils and were multiplied in potted plant culture under greenhouse conditions, using maize (*Zea mays*) as host plant in a sterilized sandy soil (Ames et. al. 1991). After 10 weeks the plants were harvested, and the roots were chopped and mixed with the potting medium and stored at 5°C until used. The cowpea rhizobial strains JRC14 and JRC29 were isolated from Jamaican soils and grown as described previously (Ahmad et. al. 1981b).

The first field inoculation experiment was conducted at the agricultural experimental field, University of West Indies, Kingston, Jamaica. The soil type was black sandy clay loan (pH 7.0). The available phosphorus was 14.7 ug.g soil $^{-1}$. No basic fertilizer was applied. Cowpea seeds (cv. Laura B) were surface sterilized (Ahmad et. al. 1981a) and planted as two seeds in each hole. The seeds were inoculated with VAM fungal inoculum (1000 spores

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in 50 mL potting medium placed 2 cm below seed level) an 1 mL of rhizobial culture (10^8 cells.mL^{-1}). The control treatment received the same amount of heat-killed inoculum. After 7 days the plants were thinned to one plant per hole. The treatments included plants each inoculated with JRC14, JRC29, *G. pallidum*, or *S. microcarpa* alone or with each combination of rhizobia and VAM fungi. Each plot consisted of nine rows, 7.5 m long with 1 m space between rows. Each row contained nine treatments, having 1 m space between plants. A randomized complete block design with nine replicates was used.

The second field experiment was carried out at a commercial farm at Trafalgar, St. Ann, in red bauxitic silt loam soil (pH 6.1). The available phosphorus was 9.7 ug.g soil $^{-1}$, and the available nitrogen was 1.8 ug.g soil $^{-1}$. Basic fertilizers (5 kg N, 5 kg P_2O_5, and 5 kg. K_2O per hectare, respectively) were applied before planting. The treatments, inoculation method, and planting were similar to those described in Experiment 1.

A third experiment was done at the same site (Trafalgar farm, St. Ann) employing a "prior establishment" method for rhizobia (Kosslak et. al. 1983) and VAM inoculation (Islam and Ayanaba 1981). Soil was collected from the surface (15-20 cm) of the site, autoclaved on two consecutive days, each time for lh (Aarons and Ahmad 1987), and filled into 400-mL disposable plastic cups (8 x 12 cm). Cowpea seeds were surface sterilized (Ahmad et. al 1981a) and planted into plastic cups containing autoclaved soil. Each seed was inoculated with VAM fungi and rhizobia alone and in combination, as described in Experiment 1. The cups were kept in the greenhouse for 20 days for the establishment of inoculum and then the plants were transplanted to the field. The treatments, replicates, and spacing were the same as that of Experiment 1.

Cowpea plants were harvested 90 days after planting (DAP). The number of pods and pod dry weight were recorded. The leaf and shoot material were oven-dried and ground, and subsamples were digested (Thomas et. al. 1967) and analyzed for total P (Murphy and Riley 1962) and N content (Vanselow 1940). The roots were washed, cleaned, and stained (Phillips and Hayman 1970), and the percentage of VAM infection was determined by a line intersect method (March 1971). Nodules were collected and washed, and rhizobia were isolated and identified by their intrinsic antibiotic resistance (IAR) patterns according to Uddin et. al. (1984). The data were analyzed by two-way ANOVA.

B. Results

Cowpea inoculated with the VAM fungus and *Rhizobium* strain pairing produced significantly more pods and produced higher N and P content than either single inoculations or uninoculated control in black sandy clay loam soils (Table 1, See Appendix under title of this study) ($p < 0.05$). However, there was no significant difference within the treatments of VAM fungus (*G. pallidum* and *S. microcarpa*) or of *Rhizobium* (JRC14 and JRC29) when used alone. Poor yield (pod number and pod dry weight) and low nutrient content were observed in the uninoculated control (Table 1). The VAM fungal infection in the

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uninoculated control was 34%, which suggests that a considerable VAM fungal population is present in these soils. When VAM fungus was paired with rhizobia, mycorrhizal infection and nodule formation were significantly increased over that of the control. The percentage nodulation by *Rhizobium* strain JRC14 was higher than that of JRC29, suggesting that JRC14 may be more competitive than JRC29.

JRC14 and JRC29 when paired with VAM fungus significantly ($P < 0.05$) increased the pod yield, nutrient content, mycorrhizal infection, and nodule number of cowpea over either single inoculations or uninoculated control in a bauxitic silt loam soil (Table 2). The percentage nodulation by both strains of rhizobia was higher in red bauxitic silt loam soil than in black sandy clay loam soil (Tables 1 and 2). In red bauxitic silt loam soil the native population of cowpea rhizobia is generally low (Aarons and Ahmad 1987), which may be one of the possible reasons for increased nodule numbers by introduced rhizobia.

A third field experiment was done in red bauxitic silt loam soils using the prior establishment method of inoculation (Table 3). As in the second experiment, an improvement of growth responses was observed when cowpea plants were inoculated with the VAM fungus - *Rhizobium* pairing. Using the method, VAM fungus or *Rhizobium* as a single inoculum significantly increased pod number and pod dry weight over control ($P < 0.05$).

Various workers have shown that the inoculation of legumes with VAM fungi improves growth and yield (Islam and Ayanaba 1981; Ganry et. al. 1982). Whether the yield of a given legume can be increased by dual inoculation with compatible pairings of VAM fungus and *Rhizobium* is not well documented. Whereas Badr El-Din and Moawad (1988) indicated that dual inoculation with VAM fungi and rhizobia enhanced the growth of legumes, Bagyaraj et. al. (1979) and Dela Cruz et. al. (1988) have shown that there is no significant difference in yield of legumes when inoculated with *Rhizobium* and VAM fungus as compared with *Rhizobium* alone. However, Pocovsky et. al (1986) have claimed that by changing *Bradyrhizobium* strain mycorrhizal plants have shown improvement in the yield of soybean. None of the above workers co-selected the compatible pairing of VAM fungus - rhizobia prior to their use as dual inocula.

Dual inoculations increased pod dry weight 25 and 16% over single inoculations of VAM fungi and rhizobia, respectively in both soil types. On an average, JRC14 and JRC29 each paired with *G. pallidum* increased the pod dry weight 45.6 and 39%, respectively, over the uninoculated control in the three experiments. Similarly, JRC14 and JRC29 each paired with *G. pallidum* increased the pod dry weight in the three experiments. Similarly, JRC14 and JRC29 each paired with *S. microcarpa* increased the pod dry weight 46 and 39.6%, respectively. Generally, JRC14 either paired with *G. pallidum* or *S. microcarpa* showed better performance than JRC29, indicating that JRC14 is not only effective and compatible but also a competitive strain.

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To our knowledge this is the first report on the field testing of co-selected strains of rhizobia and VAM fungi on cowpea. We have shown that the growth of cowpea in a given soil type can be maximized by inoculation with compatible *Rhizobium* - VAM fungus pairing.

3. Greenhouse Evaluation of VAM Influence on Competitive Ability of *Bradyrhizobium* spp.

a. Materials and methods

The VAM fungus *Glomus pallidum* was isolated in Jamaica and multiplied in a sterile soil: sand mixture using corn (*Zea mays*) as the host plant (Ames et. al. 1991). The corn plants were grown for 3 months and then the roots were chopped and stored at 4°C until used for inoculation in soil. The *Bradyrhizobium* spp. strains JRC14 and JRC29 were isolated from cowpeas in Jamaica (Aarons and Ahmad 1987; McLaughlin and Ahmad 1984). *Bradyrhizobium* spp. strain IRC256 was isolated from cowpeas in West Africa, Nigeria (Eaglesham et. al 1982). The intrinsic antibiotic resistance pattern of rhizobial strains was examined according to McLaughlin and Ahmad (1984). IRC256 was resistant to ampicillin (20 ug ml^{-1}) and sensitive to streptomycin (5 ug ml^{-1}) and kanamycin (5 ug ml^{-1}). JRC29 was resistant to streptomycin (50 ug ml^{-1}), rifampicin (100 ug ml^{-1}), and ampicillin (100 ug ml^{-1}) but sensitive to kanamycin (20 ug ml^{-1}). JRC 14 was resistant to streptomycin (10 ug ml^{-1}), but sensitive to rifampicin (5 ug ml^{-1}) and ampicillin (5 ug ml^{-1}). All rhizobial cultures were grown in yeast extract mannitol broth according to Ahmad et. al. (1981a).

Cowpea cv. Laura B was grown under greenhouse conditions in plastic pouches (15x18x28 cm) with perforated bottoms, containing non-sterilized garden soil. The soil type was a sandy clay loan (pH 7.0), with available P of 14.7 ug g^{-1}. Cowpea seeds were surface-sterilized (Ahmad et. al. 1981b) and inoculated with *Bradyrhizobium* spp. strains (10^8 cells ml^{-1}) according to Eaglesham et. al. (1982). The enumeration of rhizobia in culture was done with a Petroff-Hausser chamber. When necessary, *Bradyrhizobium* sp. cultures were diluted with sterilized yeast extract mannitol broth to give the desired cell density (10^8 cells ml^{-1}). Before inoculation the appropriate combination of *Bradyrhizobium* spp. cultures was mixed in a 1:1 ratio. The VAM fungus was inoculated as described previously (Ames et. al 1991). A total of 12 treatments with four replicates in a random complete block design was used. At 35 days after planting the plants were harvested, nodules were picked off, separated as dark and pink nodules, and counted. The nodules were surface-sterilized (Ahmad et. al 1981a), crushed aseptically, and streaked directly on yeast extract mannitol agar plates supplemented with appropriate concentrations of antibiotics (Uddin et. al 1987). The plates were incubated at 30°C for 7-10 days and observed for growth. Each test included the corresponding parent strain as control. The native *Bradyrhizobium* spp. were sensitive to antibiotics and did not grow on any of the antibiotic plates. None of the native *Bradyrhizobium* spp. strains produced dark brown nodules on the cowpea.

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B. Results

In tripartite symbiosis between legume, *Rhizobium* sp. and VAM fungi, establishment of a VAM fungus onto the root system can alter rhizospheric microbial populations (Ames et. al. 1984; Meyer and Linderman 1986), which might affect the competitive interaction between introduced and native rhizobia for nodulation sites. Table 1 summarizes the data on the effect of VAM fungus on the competition among cowpea *Bradyrhizobium* spp. strains in non-sterilized soils. Dual inoculations of the cowpea by VAM fungus + *Bradyrhizobium* spp. strains increased the total nodule formation significantly (P = 0.05) over the respective *Bradyrhizobium* spp. treatments and the uninoculated control. When JRC14 was used as a single inoculation, the nodule occupancy was 76.2%, which significantly increased to 83.2% in the presence of *Glomus pallidum*. Similar significant increases in nodule occupancy were observed for JRC29 (from 59.2 to 71.2%) and for IRC256 (from 46.1 to 52.5%) with dual inoculations of VAM fungus and *Bradyrhizobium* spp. (Table 1, See Appendix under title of this study). In summary, all three strains (JRC14, JRC 29, IRC 256) showed increased nodule occupancy in the presence of VAM fungus (Table 1).

The usefulness of a dark brown nodule strain (IRC256) in competition studies was suggested in soils where native *Bradyrhizobium* spp. do not produce dark nodules in cowpeas (Eaglesham et. al. 1982; Uddin et al. 1987). When a mixed inoculation of JRC14 and IRC256 was used, JRC14 occupied 51.7% and IRC256 occupied 25.8% of total nodules (Table 1). It is clear that JRC14 is more competitive than IRC256. The addition of a VAM fungus (JRC14+IRC256+*Glomus pallidum*) significantly enhanced (P=0.05) the nodule occupancy of JRC14 (increased from 51.7 to 63.6%) and made JRC14 highly competitive. Whereas in the case of JRC29+IRC256 mixed inoculations, IRC256 was more competitive (43.4%) than JRC29 (33.5%). However, with the VAM fungus (JRC29+IRC256+*Glomus pallidum*), the nodule occupancy of IRC256 increased from 43.4 to 47.3%, though the increase was not significant, but nodule occupancy by the dark brown nodule strain IRC256 was dominant in all the replicates.

Habte and Barrion (1984) showed that a soil fungus inhibited the growth of *Bradyrhizobium* spp. populations under laboratory conditions, but had little or no effect on the nodulating ability of the rhizobial strains. Kosslak and Bohlool (1985) reported no alteration in competitiveness between *Bradyrhizobium japonicum* strains (USDA 110 and USDA 123) in the presence of some antagonistic actinomycetes in soybean rhizosphere. However, *Glomus* macrocarpum was shown to increase nodule activity in VAM-treated cowpea plants (Ames and Bethlenfalvay 1987). In a previous study we have shown that nodule formation by *Bradyrhizobium* spp. strains on cowpea plants was increased by VAM inoculations. We have also reported a significant increase in mycorrhizal infections in cowpea roots when inoculated with JRC14+*Glomus pallidum* or JRC29+*Glomus pallidum* (Thiagarajan et al. 1992). Our earlier results indicated that this VAM fungus can enhance the competitive ability of introduced *Bradyrhizobium* spp. strains for nodulation, which led in the present competition study. The results from the present study suggest this VAM fungus enhances

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the competitive ability of introduced *Bradyrhizobium* spp. strains. To our knowledge this is the first report on the influence of a VAM fungus on competition by cowpea rhizobia in non-sterilized soils where a dark brown nodule-producing *Bradyrhizobium* sp. strain was used.

4. VAM Fungi Isolated in Jamaica

a. Materials and Methods

Root and rhizospheric soil samples of various plant species grown in various parts of Jamaica were collected. Samples were dried under cool air, and stored at 4°C until used for spore extraction. Rhizospheric soil samples (50g) were thoroughly mixed, and the spores were separated by wet sieving and decanting method described by Gerdeman and Nicolson (Gerdemann & Nicolson, 1963), followed by sucrose centrifugation (Smith & Skipper, 1979). The VAM fungal spores were carefully collected under dissection microscope (Wild Heerburg, Switzerland) and mounted in polyvinyl alcohol lactophenol mixture for observation. The species of VAM fungi were identified by spore color, size, wall layers, wall ornamentation, attached mycelium and various other morphological characters, by following the Manual for Identification of VA-Mycorrhizal fungi (Schenck & Perez, 1990). The spores were photographed under Zeiss microscope (Zeiss MC 63, West Germany). A collection of specimens on polyvinyl alcohol lactophenol slides has been placed at the Biotechnology Centre, University of the West Indies, Kingston, Jamaica.

b. Results

The spores isolated from the rhizospheric soil samples from various plant species were grouped into 9 different species representing four different genera. These genera include *Glomus* (five species), *Sclerocystis* (two species) *Scutellospora* (two species). The species representing *Glomus* were *Glomus aggregatum*, *Glomus pallidum*, *Glomus tenue*, *Glomus diaphanum* and *Glomus hoi*. *Sclerocystis* were represented by *Sclerocystis microcarpa* and *Sclerocystis taiwanensis*. *Scutellospora* was represented by *Scutellospsavannicola* and *Scutellispora corolloides*.

Spores with slight morphological differences were found, but they fell within these four genera. The representative species are as described below.

***Glomus aggregatum* Schenk & Smith:** Chlamydospores globose to subglobose, found in aggregates of 4-6 spores, spore diameter 75-90 when globose or 65 x 95um when subglobose, light yellow to light brown, spore walls yellow brown 2 um to 2.5 um thickness consisting of two wall layers easily separable with slight pressure. Outer wall is thicker than inner wall, subtending hyphae 5-6 um diameter. Isolated from rhizospheric soil of sugar cane (*Saccharum officinarum*) from Clarendon, which is the sugarcane belt of the Island. Also isolated from the rhizosphere of corn (*Zea mays*) in St. Catherine.

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Glomus diaphanum **Morton & Walker:** Spores found singly or in small cluster of 8-15 spores in soil, globose to ellipsoidal and hyaline in color with one large oil globule or many small oil globules. If globose (50)-75-(100)um diameter or 35-75 x 60-120um when ellipsoidal. Collected from aluminum rich soils at a bauxitic mine wasteland under some wild grasses, in St. Ann.

Glomus hoi **Berch & Trappe:** Spores found singly in soil, globose to subglobose, sometimes ellipsoidal 60-100 x 50-90um, light brown, wall two layered easily distinguishable and separable. Outer layer with fractures and sloughs 4-7um thick. Subtending hyphae cylindrical and becoming wider towards the spore, 8-12um diameter. Isolated from rhizosphere of coffee plants (*Coffea arabica*) from the Blue Mountains.

Glomus pallidum **Hall:** Spores formed in loose clusters, each cluster may consist of 100-300 spores. Spores globose to sub-globose 35-75 x 30-65 um diameter, wall 1-5um thick becomes laminated with aging. Subtending hyphae 4-8um diameter, with pore open when the spore is young closed when spores matured. Spores and hyphae become laminated with aging. Isolated from sugar cane (*Saccharum officinarum*) rhizosphere.

Glomus tenue **(Greenall) Hall:** Spores 10-15 um diameter when matured, yellowish brown to dark brown, generally globose. Walls dark brown, 1.8 to 2.2 um thick. Isolated under the rhizosphere of various plant species particularly from various grasses. This seems to be an ineffective VAM fungus routinely found around University of the West Indies, Mona campus.

Sclerocystis microcarpa **Iqbal & Burshra:** Sporocarps light to dark brown, 350-450um diameter, globose, spores tightly packed in radial layers around a central plexus of hyphae. Chlamydospores 100-120 x 45-65 um, clavate or cylindric clavate with a minute pore connecting the subtending hyphae. Collected under fern rhizosphere, Fern-gully, St. Ann.

Sclerocystis taiwanensis **Wu & Chen:** Sporocarps brown to reddish brown, Globose 240-300 um diameter. Chlamydospores arranged radially in a tightly packed manner around a central plexus of hyphae. Chlamydospore clavate, triangular or sometimes irregular with septum at the pointed base. Spore walls laminate, thicker at the base,yellow-brown. Isolated fern rhizosphere, Fern-gully, St. Ann.

Scutellospora corolloides **(Trappe, Gerdemann & Ho) Walker & Saunders:** Azygospores formed singly in soil, subglobose 330-380 x 300 x 350 um diameter, dark brown when matured. Spore wall continuous, 8-12 um thick, outer wall ornamented with irregularly chapped warts and ridges 2-5 um tall radiating outside with a small suspensor-like cell. Isolated from a sandy clay loamy soil under bermuda grass (*Cynadon dactylon*).

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***Scutellospora savannicola* (Herrer & Ferrera) Walker & Sanders:** Azygospores formed singly in soil, hyaline to white, glittering, ellipsoid to irregular, 100-200 x 190 - 280 um. Wall consists of many layers, outer wall not smooth. Subtending hyphae hyaline, not long, spores normally found near the roots of guinea grass (*Panicum maximuon*) the University campus.

Most of the collected isolates were purified and multiplied on corn plants. Out of these 9 species of VAM fungi isolated, *Glomus pallidum, Glomus aggregatum and Sclerocystis microcarpa* were found to be effective for cowpea (Ames, et. al. 1991) and some other crops (Unpublished data). *Glomus pallidum* and *Sclerocystis microcarpa* were very effective for cowpea even under field conditions along with selected *Rhizobium* strains (Thiagarajan et. al., 1992). This effectiveness could be due to the production of higher number of spores in a cluster in *G. pallidum* or many spores in a sporocarp as in *S. microcarpa* and also because of the high rate of viability (unpublished data). Now we have *S. microcarpa, G. pallidum* and *G. aggregatum* as pure inoculum multiplied on corn roots. Similar reports on VAM flora from various geographical, or regional areas has been presented. Most of them might be already described species with slight morphological differences, but could be new to that region or country (Blaszkowski, 1989, Blaszkowski, 1990, McGee, 1986).

The VAM fungi plays an important role in the uptake and regulation by the host plants (Dueck et. al., 1986, Meggc & Angle, 1990). Hence, since most Jamaican soils are aluminum rich bouxitic type, these isolates of VAM fungi could play a role in the rehabilitation of the bouxitic waste lands.

III. IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

The overall aim of this research was to increase the effectiveness of *Rhizobium* and vesicular-arbuscular mycorrhizal (VAM) fungi in improving the growth and yield of cowpea (*Vigna unguiculata*) in low fertility farming systems. This program has been highly successful in accomplishing that goal. Optimization of cowpea yield in Jamaica soils was attained by utilization of appropriate pairs of co-selected strains of VAM fungus-*Rhizobium* combinations. It was also demonstrated that in the presence of VAM fungus, introduced *Bradyrhizobium* spp. strains become more competitive than native rhizobia. These findings are of significance not only to the production of legumes in Jamaica and other countries, but they also constitute the first report of these phenomena. The methodologies have also been established to expand this approach to other crop systems both in Jamaica and other countries. These research findings have already resulted in expansion of this research by scientists in Jamaica.

As a result of the training and the research results of this project the scientists in Jamaica now have the capabilities to utilize this technology, not only to continue expansion of this research, but also to apply this knowledge for the increased production of cowpea and other similar crops. The research will be facilitated by the microscope, fertility analysis equipment,

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and the shade house supplied under this grant. The discovery of new beneficial microorganisms, and the methodologies to manipulate them to optimize plant production will result in a long term benefit to the industry in Jamaica and other LDCs.

In the U.S., plans are being developed to utilize this technology to enhance production of chickpea by combining it with biological disease control agents (BDCAs) to optimize a sustainable yield. This non-chemical approach, utilizing naturally occurring beneficial microorganisms, will not only reduce disease and enhance crop production without the addition of chemical fertilizers, but will also be an environmentally safe approach. The grant-supplied equipment at PL has already been utilized to begin research to develop a system to combine BDCAs and mycorrhiza. PL also plans to continue to work with USAID to develop projects along these lines of incorporating BDCAs into the Rhizobia/VAM fungal complex. There is also the possibility of continued interaction between PL and Jamaica to work toward solution of other agricultural constraints in Jamaica.

IV. PROJECT ACTIVITIES/OUTPUTS

A. Meetings

November, 1989. R.N. Ames attended the workshop on Biocontrol in the Caribbean sponsored by the USAID Mission in Jamaica, Caribbean Agricultural Research and Development Institute (CARDI), and CICP.

July, 1990. M. H. Ahmad attended the BOSTID/PSTC BNF Networking Meeting.

B. Training

R. N. Ames conducted training sessions throughout the course of this project, either in Jamaica or at Peninsu-Lab. He made visits to Jamaica on 2/7-17/89, 7/16-22/89, 3/8-15/90, and 7/18-20/90 to carry out training of researchers and technicians on methods of isolation, identification, and culture of VAM fungi. W. McLaughlin and V. Morgan visited Peninsu-Lab on 4/5-19/89 to receive training in the methodologies for collecting data from cowpea plants inoculated with VAM fungi and *Rhizobium*. Training was also provided for the use of the fluorescent antibody technique to identify cowpea rhizobia.

C. Publications

Thiagarajan, T.R., Ames, R.N., Ahmad, M.H. 1992. Response of cowpea (*Vigna unguiculata* (L) Walp) to inoculation with co-selected VAM fungi and *Rhizobium* strains in field trials. Can J. Microbiol. 38:573-576.

Ames, R.N., Thiagarajan, T.R., Ahmad, M.H. & McLaughlin, W. 1991. Co-selection of compatible rhizobia and VA mycorrhizal fungi for cowpea in sterilized and non-sterile soil.

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Biol. Fertil. Soil, 12:112-116.

Thiagarajan, T. R. and M. H. Ahmad. 1993. Influence of a vesicular-arbuscular mycorrhizal fungus on the competitive ability of *Bradyrhizobium* spp. for nodulation of cowpea *Vigna unguiculata* (L.) Walp in non-sterilized soil. Biol Fertil Soils 15: (in press).

V. PROJECT PRODUCTIVITY

As stated earlier the ultimate aim of this research, to increase the effectiveness of *Rhizobium* and VAM fungi in improving the growth and yield of cowpea in low fertility farming systems, has certainly been met under this program. As has been shown under the research results, the four Specific Objectives outlined under IA of this report have been more than adequately met.

VI. FUTURE WORK

Some of the proposed future work has been discussed under section III above. Once this project has been successfully brought to conclusion, more specific plans for future work along these lines will be developed. Dr. F. McElroy has already met with several project leaders in Washington D.C. to discuss the possibilities of combined work on plant disease biocontrol, Rhizobia and mycorrhiza for LDCs.

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Tables 1, 2, and 3: Co-selection of compatible rhizobia and VAM.

Table 1. Effects of different isolates of *Rhizobium* on cowpea growth, nodulation, and mycorrhizal development in a sterilized soil

Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
None	0	0	2.80b	63.9c	16.4b	1.21b
JRC14	87.6a	218.0a	4.65a	70.7ab	23.9a	2.14a
JRC19	89.1a	221.1a	4.90a	70.9ab	23.5a	2.14a
JRC29	73.9b	201.4b	4.74a	72.4a	23.3a	2.12a
TAL169	77.3b	196.8b	4.59a	69.3b	23.2a	2.09a

Numbers within columns are means of 16 observations. Values within columns not sharing the same letter are significantly different ($P \leq 0.05$) based on a one-way analysis of variance and *t*-test. VAM, vesicular-arbuscular mycorrhizae

Table 2. Effects of different species of vesicular-arbuscular mycorrhizal fungi on cowpea growth, nodulation, and mycorrhizal development in a sterilized soil

Fungus	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
None	73.1b	179.3b	3.36b	0	20.8a	1.45b
G. pallidum	87.5a	227.5a	4.73a	69.3a	23.4a	2.11a
G. aggregatum	77.9b	212.9a	4.54a	68.6a	21.6a	2.07a
S. microcarpa	90.3a	219.3a	4.71a	70.2a	22.3a	2.14a

Means of 20 observations. *G.*, *Glomus*; *S.*, *Sclerocystis*. For other explanations, see footnotes to Table 1

Table 3. Interactions among three vesicular-arbuscular mycorrhizal fungi and four isolates of *Rhizobium* in effects on cowpea growth, nodulation, and mycorrhizal development in a sterilized soil

Fungus	Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
None	None	0	0	1.90c	0	14.3b	0.95c
	JRC14	84.3a	195.7a	3.42b	0	22.9a	1.65ab
	JRC19	78.0a	209.6a	3.90a	0	22.2a	1.70a
	JRC29	61.5b	167.1b	3.78a	0	22.2a	1.45b
	TAL169	68.8b	144.8c	3.81a	0	22.6a	1.50ab
G. pallidum	None	0	0	3.11c	64.6e	16.0b	1.35c
	JRC14	90.5a	230.8a	4.94b	69.5c	25.7a	2.20ab
	JRC19	95.3a	231.5a	5.50a	72.1b	25.9a	2.38ab
	JRC29	81.7a	229.0a	5.54a	74.6a	25.5a	2.60a
	TAL169	81.0a	218.9a	4.79b	67.0d	24.3a	2.15b
G. aggregatum	None	0	0	3.02c	63.3b	17.4b	1.20b
	JRC14	83.0a	217.3a	5.06a	70.7a	24.4a	2.30a
	JRC19	90.0a	219.2a	5.07a	69.4a	22.6a	2.20a
	JRC29	66.0b	199.0a	4.74b	70.5a	21.3a	2.30a
	TAL169	72.5b	216.0a	4.81b	69.0a	22.5a	2.33a
S. microcarpa	None	0	0	3.16b	63.7b	18.0b	1.33b
	JRC14	92.8a	228.2a	5.19a	71.7a	22.7a	2.43a
	JRC19	93.3a	224.2a	5.12a	71.2a	23.3a	2.30a
	JRC29	88.5a	217.3a	5.11a	72.7a	24.6a	2.25a
	TAL169	86.8a	207.4a	4.96a	71.8a	23.2a	2.40a

Means of four observations. Statistical comparisons were only made between rhizobia within each VAM fungus. For other explanations, see footnotes to Table 1

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Tables 4, 5, and 6: Co-selection of compatible rhizobia and VAM.

Table 4. Effects of different isolates of *Rhizobium* on cowpea growth, nodulation, and mycorrhizal development in a non-sterilized soil

Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
Indigenous	101.0a	230.8ab	4.65c	56.8c	20.9b	1.61b
JRC14	106.4a	248.0a	5.49a	70.5a	23.9a	2.09a
JRC19	103.6a	226.0b	4.95b	62.8b	22.8a	1.79b
JRC29	112.2a	246.6a	5.45a	69.5a	23.5a	2.08a
TAL169	105.5a	220.9b	4.91b	61.7b	22.9a	1.78b

Means of 20 observations. For other explanations, see footnotes to Table 1

Table 5. Effects of different species of vesicular-arbuscular mycorrhizal fungi on cowpea growth, nodulation, and mycorrhizal development in a non-sterilized soil

Fungus	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
Indigenous	101.4a	225.2b	4.71b	56.3b	21.8a	1.53c
G. pallidum	109.3a	248.3a	5.25a	67.8a	23.4a	2.05a
G. aggregatum	105.2a	230.8b	5.20a	66.8a	23.2a	1.88b
S. microcarpa	107.0a	235.7ab	5.20a	66.0a	22.7a	2.04a

Means of 25 observations. For other explanations, see footnotes to Table 1

Table 6. Interactions among three vesicular-arbuscular mycorrhizal fungi, four rhizobia, and indigenous microflora on cowpea growth, nodulation, and mycorrhizal development in a non-sterilized soil

Fungus	Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
Indigenous	Indigenous	99.0a	221.7a	4.27b	51.9b	19.4a	1.28b
	JRC14	102.2a	228.3a	4.81a	59.0a	23.9a	1.52a
	JRC19	101.4a	225.4a	4.75a	56.7a	22.2a	1.58a
	JRC29	102.4a	224.8a	5.04a	58.2a	20.9a	1.60a
	TAL169	102.0a	225.6a	4.68a	55.7ab	22.6a	1.54a
G. pallidum	Indigenous	105.0a	226.9b	4.73b	57.9d	22.1bc	1.74b
	JRC14	112.4a	269.4a	5.88a	76.4a	25.1ab	2.28a
	JRC19	103.2a	223.6b	5.04b	67.2c	22.5abc	1.88b
	JRC29	118.4a	291.0a	5.67a	75.1a	25.5a	2.42a
	TAL169	107.6a	226.1b	4.90b	62.5b	21.8c	1.92b
G. aggregatum	Indigenous	97.0a	222.9a	4.82b	58.1c	21.5a	1.58b
	JRC14	105.8a	251.5a	5.65a	73.8a	22.9a	2.08a
	JRC19	105.2a	226.4a	4.96b	63.8b	24.3a	1.84ab
	JRC29	113.2a	240.7a	5.57a	73.5a	24.2a	2.04a
	TAL169	104.6a	207.7a	5.00b	65.0b	23.1a	1.86a
S. microcarpa	Indigenous	103.0a	251.8a	4.77b	59.3c	20.5a	1.84b
	JRC14	105.2a	242.6a	5.63a	72.8a	23.5a	2.48a
	JRC19	104.4a	228.4a	5.04b	63.5b	22.1a	1.86b
	JRC29	114.8a	229.8a	5.52a	71.1a	23.6a	2.24a
	TAL169	107.8a	223.3a	5.04b	63.5b	23.9a	1.78b

Means of five observations. Statistical comparisons were only made between rhizobia within each VAM fungus. For other explanations, see footnotes to Table 1

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Tables 1, 2, and 3: Field trials with co-selected rhizobia and VAM

TABLE 1. Effect of dual inoculation of VAM fungi and rhizobia on growth, nodulation and mycorrhizal development of cowpea (*V. unguiculata*) in a sandy clay loam soil

Inoculant		Pod (no.)	Pod dry wt. (g)	Nodules formed (%)*	VAM fungi (%)	Shoot P (mg/g)	Shoot N (mg/g)
VAM fungus	*Rhizobium*						
None	None	19*f*	17.4*c*	0	34*d*	1.6*e*	17.0*d*
	JRC14	26*cd*	21.4*b*	53.3	48*c*	1.9*d*	21.4*b*
	JRC29	25*de*	20.5*b*	37.5	46*c*	1.7*e*	20.3*c*
G. pallidum	None	22*ef*	19.3*bc*	0	56*b*	2.1*c*	19.8*c*
	JRC14	33*a*	25.0*a*	54.3	62*a*	2.5*a*	23.5*a*
	JRC29	30*ab*	24.6*a*	43.8	63*a*	2.4*ab*	22.0*ab*
S. microcarpa	None	22*ef*	18.8*bc*	0	54*b*	1.9*d*	19.7*c*
	JRC14	33*a*	24.6*a*	59.4	60*a*	2.4*ab*	23.0*a*
	JRC29	29*bc*	23.9*a*	46.7	61*a*	2.3*b*	21.8*ab*

NOTE: All values within columns are the means of nine replicates. Numbers followed by the same letters do not significantly differ at $P = 0.05$.

*Percentage of nodules formed by the inoculated rhizobia (no statistical comparison was made).

TABLE 2. Effect of dual inoculation of VAM fungi and rhizobia on growth, nodulation and mycorrhizal development of cowpea (*V. unguiculata*) in a bauxitic silt loam soil

Inoculant		Pod (no.)	Pod dry wt. (g)	Nodules formed (%)*	VAM fungi (%)	Shoot P (mg/g)	Shoot N (mg/g)
VAM fungus	*Rhizobium*						
None	None	24*e*	32.2*c*	0	23*g*	1.4*d*	14.1*e*
	JRC14	36*cd*	38.0*b*	59.4	36*e*	1.7*c*	18.8*c*
	JRC29	36*cd*	36.7*b*	53.3	32*f*	1.6*c*	18.1*c*
G. pallidum	None	31*de*	35.1*c*	0	50*c*	1.9*b*	16.5*d*
	JRC14	45*a*	46.8*a*	70.0	59*a*	2.1*a*	22.6*a*
	JRC29	42*ab*	42.2*ab*	60.0	55*b*	2.1*a*	20.9*b*
S. microcarpa	None	32*de*	35.9*c*	0	46*d*	1.9*b*	16.8*d*
	JRC14	42*ab*	45.7*a*	68.5	54*b*	2.1*a*	22.2*a*
	JRC29	43*ab*	44.3*a*	63.3	58*a*	2.0*ab*	21.6*ab*

NOTE: All values within columns are the means of nine replicates. Numbers followed by the same letters do not significantly differ at $P = 0.05$.

*Percentage of nodules formed by the inoculated rhizobia (no statistical comparison was made).

TABLE 3. Effect of dual inoculation of VAM fungi and rhizobia on growth, nodulation and mycorrhizal development of cowpea (*V. unguiculata*) in a bauxitic silt loam soil by using a prior establishment method

Inoculant		Pod (no.)	Pod dry wt. (g)	Nodules formed (%)*	VAM fungi (%)	Shoot P (mg/g)	Shoot N (mg/g)
VAM fungus	*Rhizobium*						
None	None	26*d*	27.3*c*	0	21*f*	1.3*d*	15.4*d*
	JRC14	36*bc*	36.1*ab*	68.8	26*e*	1.6*c*	18.9*b*
	JRC29	37*bc*	36.9*ab*	66.7	29*e*	1.7*bc*	19.7*b*
G. pallidum	None	34*bc*	32.7*b*	0	57*cd*	1.8*bc*	17.3*c*
	JRC14	43*a*	40.3*a*	73.3	64*a*	2.3*a*	22.7*a*
	JRC29	44*a*	39.6*a*	71.9	63*ab*	2.2*a*	21.9*a*
S. microcarpa	None	33*bc*	33.9*b*	0	54*d*	1.9*b*	17.2*c*
	JRC14	45*a*	42.4*a*	71.0	66*a*	2.4*a*	22.8*a*
	JRC29	42*ab*	39.3*a*	70.0	60*bc*	2.2*a*	21.7*a*

NOTE: Values within columns are the means of nine replicates. Numbers followed by the same letters do not significantly differ at $P = 0.05$.

*Percentage of nodules formed by the inoculated rhizobia (no statistical comparison was made).

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Table 1: Greenhouse evaluation of VAM influence on competitive ability of *Bradyrhizobium* spp.

Table 1. Effect of a vesicular-arbuscular mycorrhizal fungus (*Glomus pallidum*) on the competitive ability of *Bradyrhizobium* spp. strains on the cowpea (*Vigna unguiculata*) in soil containing native rhizobia

Treatments	Total no. of nodules per plant	Fraction of nodules		No. of pink nodules formed with inoculum	No. of pink nodules formed by native rhizobia
		Dark	Pink		
Uninoculated control	56.3c	0	56.3 ± 3.1 (100)cd	(0)	56.3 ± 3.1 (100)a
G. pallidum	61.0bc	0	61.0 ± 2.4 (100)cd	(0)	61.0 ± 2.4 (100)a
JRC14	74.8ab	0	74.8 ± 3.1 (100)ab	57.0 ± 2.4 (76.2)b	17.8 ± 1.5 (23.8)de
JRC29	71.0abc	0	71.0 ± 4.7 (100)b	42.0 ± 3.6 (59.2)d	29.0 ± 2.1 (40.8)c
IRC256	70.5abc	32.5 ± 2.9 (46.1)b	38.0 ± 1.7 (53.9)e	0	38.0 ± 1.7 (53.9)b
JRC14 + *G. pallidum*	80.5a	0	80.5 ± 5.2 (100)a	67.0 ± 1.5 (83.2)a	13.5 ± 2.2 (16.8)e
JRC29 + *G. pallidum*	74.5ab	0	74.5 ± 5.0 (100)ab	53.0 ± 4.3 (71.2)bc	21.5 ± 3.4 (28.9)d
IRC256 + *G. pallidum*	79.0a	41.5 ± 2.0 (52.5)a	37.5 ± 1.1 (47.5)e	0	37.5 ± 1.1 (47.5)b
JRC14 + IRC256	71.0abc	18.3 ± 1.9 (25.8)c	52.7 ± 1.8 (74.2)d	36.7 ± 0.8 (51.7)d	16.0 ± 2.4 (22.5)de
JRC29 + IRC256	72.5abc	31.5 ± 1.7 (43.4)b	41.0 ± 2.2 (56.6)e	24.3 ± 4.6 (33.5)e	16.7 ± 2.2 (23.1)de
JRC14 + IRC256 + *G. pallidum*	80.3a	18.0 ± 2.4 (22.4)c	62.3 ± 3.2 (77.6)c	50.3 ± 1.2 (62.6)c	12.0 ± 1.7 (15.0)e
JRC29 + IRC256 + *G. pallidum*	75.0ab	35.5 ± 1.5 (47.3)b	39.5 ± 4.2 (52.7)e	22.5 ± 1.8 (30.0)e	17.0 ± 3.2 (22.7)de

Values are means of four replicates ± SE. Nodule numbers followed by same letters are not significantly different at $P = 0.05$, by Duncan's multiple range test. Numbers in parenthesis indicate percentages of total nodules

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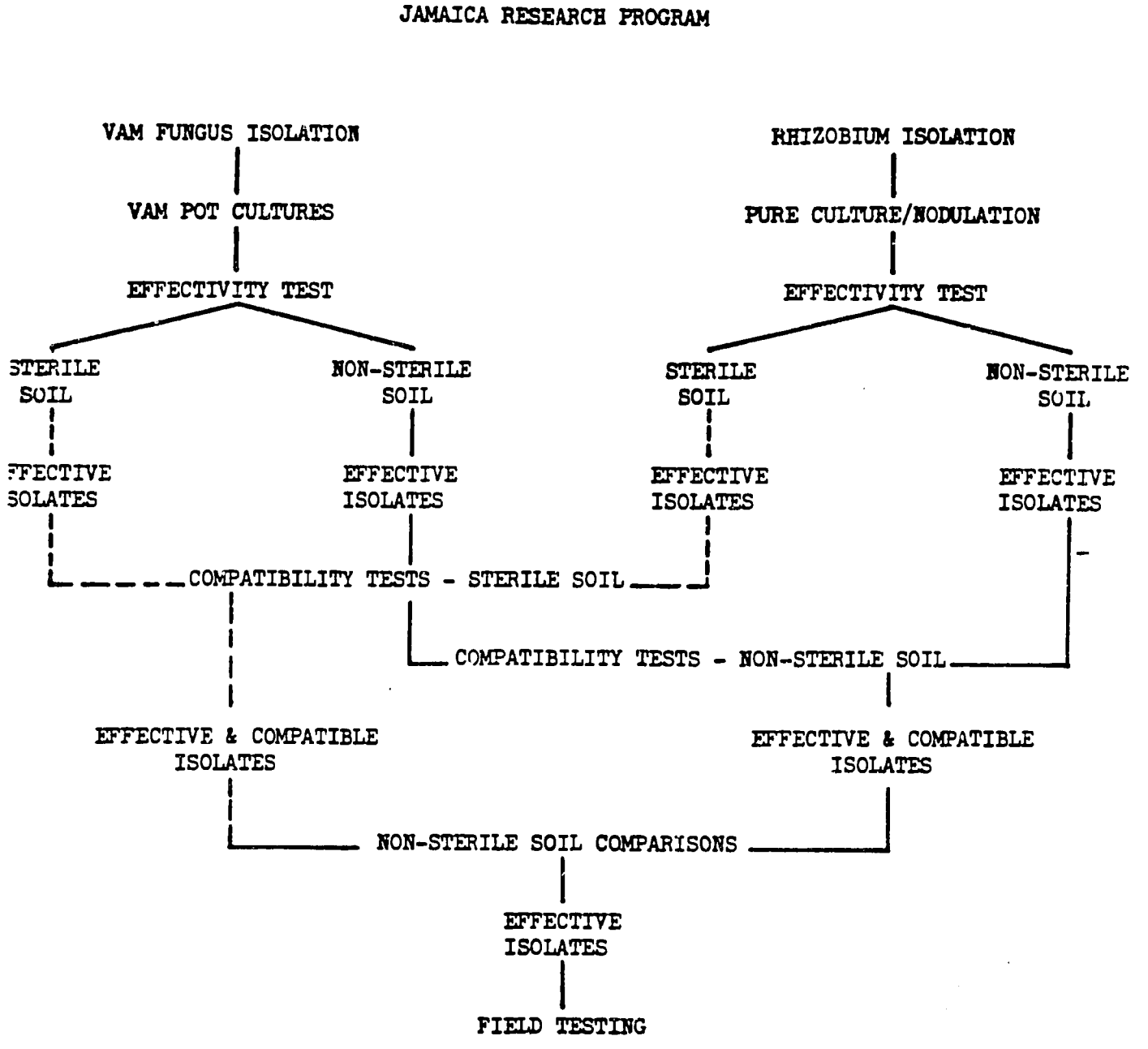

FIG. 1. Flow chart of research program for Jamaica.

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