P3-ISU-2 WORKPLAN

Enhancing biological nitrogen fixation (BNF) of leguminous crops grown on degraded soils in Uganda, Rwanda, and Tanzania

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Project Problem Statement and Justification

Common beans are the most important legume crop in Uganda, Rwanda and Tanzania occupying a very large proportion of land devoted to legumes. For example, over 45% of the protein intake by Ugandans comes from beans providing 25% of dietary calories. Likewise, over 75% of rural households in Tanzania depend on beans for daily subsistence. Common bean is an important source of protein for low-income families in rural and urban areas providing about 38% of utilizable protein and 12-16% of daily caloric requirements. Improved bean production in Uganda, Rwanda, and Tanzania offer unique opportunities to address the deteriorating food security situation there and elsewhere in sub-Saharan Africa.

Loss of soil fertility is recognized as the most important constraint to food security in sub-Saharan Africa. Low levels of nitrogen and phosphorous are the primary fertility constraints. Because soils are increasingly becoming degraded, an affordable means of improving soil fertility and productivity of nitrogen-accumulating crops is critical. Properly nodulated legumes can leave up to 350 kg nitrogen per hectare in the soil, depending on effectiveness of the nitrogen fixation process, type of legume, length of time the legume is grown, soil nutrient levels and nitrogen already available. Because inoculum is much cheaper than inorganic fertilizer, use of inoculants can provide an affordable and sustainable way to improve production of nitrogen fixing legumes.

Numerous studies have shown the potential of improving legume productivity by enhancing nodulation through proper use of a biological inoculant. Yet field trials in sub-Saharan Africa have provided mixed results. Likely causes for variable response include poor quality control of inoculant formulation, failure to compete with local rhizobia, inhibition by indigenous microbial flora, or failure of the inoculant species to survive in low pH and/or droughty soils. Modern inoculant formulations designed to deliver a

synergistic suite of biological and chemical enhancements for biological nitrogen fixation under stressful soil conditions have been made available to our collaborative research project by Becker Underwood, Inc. Becker Underwood's *BioStacked*® inoculant technologies for legume crops consist of well stabilized *Rhizobium* bacteria, a biological fungicide, plant growth promoting rhizobacteria, and other biologically derived proprietary biostimulant technologies which promote plant growth and overall plant health. These stacked inoculants have been shown to decrease chemical fertilizer use in crop rotations, increase legume yields, suppress root diseases, and improve rhizosphere conditions for root growth. We anticipate they will be particularly effective under degraded soil conditions encountered on small-landholder farms in Uganda, Rwanda, and Tanzania.

To optimize BNF, it also is essential to identify germplasm with greatest capacity for this trait. Although common bean has the potential for BNF, it is reported to have the lowest percent N_2 derived from N fixation among legumes. Genetic variation for BNF has been reported within the primary gene pool, and lines with superior BNF have been identified. Superior BNF lines such as Puebla 152 and BAT 477 have been used as parents in crosses to generate populations for genetic studies and to examine selection and breeding for improved BNF. Few breeding lines with improved BNF, however, have been developed. The optimal selection environment for BNF is under low soil N since application of nitrogen fertilizer reduces N fixation capacity. Marker-assisted selection (MAS) under such conditions is highly sought after as a means to facilitate breeding for traits like BNF with low to moderate heritability.

Molecular mapping in combination with germplasm screening and MAS would be a powerful way to improve locally adapted germplasm for BNF in a host country. Recombinant inbred populations currently available are ideal for tagging and mapping genes that influence quantitative traits (QTLs). Few QTLs associated with BNF, however, have been identified to date, and those identified have not been validated. Identifying and validating QTL-conditioning enhanced BNF would be a major contribution to the scientific community, and represent a major step toward effective marker-assisted selection for BNF.

Our BNF-CRSP program objectives address the need to identify production systems that enhance BNF, develop germplasm that benefits most from symbiotic inoculation, and aggressively share this new information with small landholder farmers in sub-Saharan Africa whose health and wellbeing depend heavily on legume production.

Planned Project Activities

Objective 1: The *first strategic aim* is to improve BNF and seed yields of common beans significantly using superior seed inoculants such as Becker Underwood's BioStacked® inoculant through farmer-based experimentation and adoption of innovative production techniques.

Sub-Objective1a: *To evaluate effectiveness of biologically stacked inoculants on local and improved germplasm.*

We expect to:

1. Establish Field Trial 2 (MSU, WSU, NaCCRI, SUA, ISAR)

2. Quantify yield advantage of inoculation for second crops seasons (MSU, WSU, NaCCRI, SUA, ISAR)

Sub-Objective 1b: *To quantify genotype by environment interactions and constraints to enhancing BNF of inoculated plants.*
We expect ISU to:

3. Complete analysis of plant/soil/weather data completed (ISU, Makerere, NaCCRI, SUA, ISAR)
4. Identify unique responses to inoculant x genotype x environment (ISU, Makerere, MSU, WSU, NaCCRI, SUA, ISAR)
5. Extract and analyze soil DNA for indigenous rhizobia strains (WSU, Makerere, NaCCRI, SUA, ISAR)
6. Establish indigenous rhizobia levels and environmentally tolerant strains (MSU, WSU, NaCCRI, SUA, ISAR)

Collaborators: *Becker Underwood, Inc.* (BU) is an international developer of bio-agronomic and specialty products. The company is the leading global producer of inoculants, beneficial nematodes, and a wide range of agricultural and horticultural products. BU will produce the *Bio-stacked®* legume inoculants (see http://www.beckerunderwood.com/en/newsreleases/100104) for distribution to HC and US researchers in this CRSP project. BU has worked with numerous universities around the world and has implemented quality assurance programs and technical support to ensure proper formulation and field application.

Approaches and Methods: In Rwanda, Tanzania, and Uganda multiple sites will be used to evaluate popular cultivars of both determinate bush and indeterminate vine growth habit types for response to different rhizobia-inoculum treatments. Site selection will be defined by where beans are already grown and consumed, and will encompass the range of soil types and weather conditions documented at each site (1c). Four cultivars will be chosen representing different market types, evolutionary origin, in addition to the different plant types. For example in Tanzania popular cultivars (genotypes) representing the major speckled purple-Kablanketi (Type III, Andean), yellow-Njano (Type I, Andean), Red Kidney (Type I, Andean), and Carioca (Type II, MA) market types would be tested. Adapted non-nodulating genotype(s) (~BAT477, DOR364 from CIAT) will be useful for this and subsequent BNF trials as checks. Rhizobia inoculum treatments will include *Bio-stacked®,* other commercially available inoculants (e.g. Bio-N-Fix), and no inoculum. The *Bio-stacked®* inoculum from Becker-Underwood, Inc. is formulated for enhanced BNF under stressful soil conditions (see product note from Becker Underwood, Inc.). A RCBD with four replications, and moderately large plot size will be used (4 to 6 rows wide by 5 to 7 m length), Established research station sites will be used initially and expanded to on farm and community co-op trials using select genotypes which exhibit greatest BNF response. It is envisioned by year 2 that HC Extension personnel, NGO, or other business partners will be identified to help develop and implement strategies for technology dissemination to numerous farmers (Strategic Aim 3). A low N treatment will be targeted the first few years and expanded to include low and high N in subsequent years as HC and US project participants gain training and experience with experimental protocols and procedures.

Standard agronomic practices will be employed in the controlled location studies (Opio et al 2001). Incidence and severity of disease and pest damage will be recorded to determine their indirect impact on N-fixation, plant performance and response to inoculant treatments. Agronomic data collected for each treatment includes: soil analysis, final plant stand (pl/m), seed yield (kg/ha), disease and insect pest ratings (mid-season for leaves), days to physiological maturity, pods per plant, seed quality (color, % not mature, % mottled, and economic return on investment in the inoculant technology. The latter will be assessed by careful record keeping of agronomic input costs and grain sales. Thermometers, rain gauges, and soil moisture sensors will be positioned on site for recording local weather conditions. Plant N (multiple subsamples per plot), seed N (multiple subsamples per plot), biomass, and seed yield at harvest maturity on a plot basis will be used to measure BNF response of the different genotypes and treatments. These measurements are the most affordable in terms of cost and labor and correlate well with seasonal BNF. Select genotypes or treatments with large or interesting BNF responses, could be further characterized by evaluation of root biomass, nodulation number and mass, isotope assays, or post crop response. Data collection will be coordinated by HC scientists and students.

Objective 2: The *second strategic aim* is to examine the inheritance of genetic and environmental variation in BNF in common bean, and to identify molecular markers associated with QTL conditioning for enhanced BNF.

Sub-Objective 2a: *To identify parental materials for inheritance studies of BNF.*
We expect to:

7. Screen germplasm for BNF in low soil N +/- inoculants in HC field trials (MSU, WSU, NaCCRI, SUA, ISAR)
8. Initiate greenhouse screening trials on selected lines for BNF response (ISU, WSU)
9. Initiate Greenhouse BNF screening on selected lines (WSU)
10. Test parental lines for BNF in the field: US and HC sites (ISU, MSU, WSU, NaCCRI, SUA, ISAR)

Sub-Objective 2b: *To phenotype existing mapping populations for BNF response, populate with molecular markers, and conduct QTL analysis.*
We expect to:

11. Test selected populations for BNF in the field: US and HC sites (ISU, MSU, WSU, NaCCRI, SUA, ISAR)
12. Establish correlative response of BNF in field and GH trials (ISU, MSU, WSU, Makerere, NaCCRI, SUA, ISAR)
13. Establish nodule rhizobia occupancy established on selected lines (WSU)
14. Advance selected RILs to F2 (ISU, WSU, NaCCRI, SUA, ISAR)

Collaborators: Host country field managers at NaCRRI, ISAR, and SUA and experiment station managers at ISU, MSU, and WSU in the US.

Approaches and Methods: We will collect and increase seed of representative commercial market types and advanced breeding lines from host countries Rwanda, Uganda, Tanzania and the US; lines known to differ for BNF (BAT 477, Pueblo 152, CAL 143, RIZ lines, etc.); super-nodulating and non-nodulating; and select parents of existing mapping populations; in total about 50 materials. These materials will be tested for BNF response under low N conditions in the field (single locations in Rwanda, Tanzania, and Uganda) and greenhouse (US-WSU). The materials will be split into groups of 30 genotypes each. The plan is to test half the lines in Rwanda and the other half in Tanzania in Year 1, and vice versa in Year 2. The plots will be smaller (single row, 3 m length) and with fewer reps (2 to 3).

The materials will also be tested in the greenhouse in the US (WSU and ISU). Single plants will be sown in 1 liter pots containing 50% sand/potting soil mixture, N-deficient fertilizer solution, and arranged in RCBD with 5 replications, and at least two treatments – non-inoculated and inoculated (with mixture of rhizobia strains). The materials will be similarly grouped (20 to 30 materials each group) for the GH experiments conducted over a period of two years. BNF response will be measured by plant N (multiple subsamples per plot), seed N (multiple subsamples per plot), biomass, and seed yield at harvest maturity for field studies. For greenhouse studies, plant biomass on shoot/root basis, nodulation score, and plant N concentration at 12 wks after planting will be used to measure BNF response.

Crosses will be conducted between parents with contrasting BNF response (low vs. high) to initiate generation of genetic mapping populations (recombinant inbred line – RIL populations). It takes three years to obtain mapping populations and increase seed for F5 or later derived RILs for replicated multi-site testing. Four populations will be developed (two by ARS-Prosser and two by ARS-East Lansing) consisting of approximately 150 lines each. Efforts will be made to cross high X low parents that are adapted for each country (Rwanda, Tanzania, Uganda, and US). Basic agronomic information will be collected, e.g. biomass at flowering, biomass at harvest, shoot N at harvest, seed yield, seed N, HI and NHI. From these RIL populations we expect to obtain advanced breeding lines with good BNF, good agronomic performance, and identify acceptable HC market types.

Given the three year time frame necessary to generate new mapping populations, existing mapping populations with promise for mapping QTL conditioning BNF response will be tested in HC and US. Two existing mapping populations will be phenotyped for BNF response (EP=Eagle/Pueblo 158, 78 F8 RILs, 357 markers; RC=Rojo/CAL 143, 147 F5 RILs, no markers). Seed of the RILs will be increased (January-May, Year 1, EP by ARS, East Lansing and RC by ARS-Prosser). The parents for EP, RC, and a few other bi-parental populations will be tested in the GH to confirm divergent phenotypic response for BNF (January – Year 1, WSU). Given divergent response for the parents the EP population will be tested at two sites (ARS-Prosser and -East Lansing) under low N, using 1-2 row plots and 3-4 reps as determined by seed availability, using a RCBD design (summer Year 1).

Objective 3: The *third strategic aim* is to improve the productivity, profitability, and sustainability of agricultural systems on degraded soils through effective dissemination of new information and technologies to small-landholder farmers.

Sub-objective 3a: *To improve farmer awareness of inoculation technologies*
We expect to:

15. Conduct farmer KPA evaluations on benefits of inoculation and BNF (VEDCO)
16. Train Extensionists at HC institutions in benefits of BNF and inoculant use (VEDCO, NaCCRI, SUA, ISAR)
17. Establish format for field demonstrations at HC research stations (ISU, MSU, WSU, VEDCO, Makerere, NaCCRI, SUA, ISAR)
18. Conduct field days in each HC to present research results (VEDCO, NaCCRI, SUA, ISAR)

Sub-objective 3b: *To conduct on-farm demonstrations comparing inoculant strategies*
We expect to:

19. Identify farmer cooperators for on-farm trials (VEDCO, NaCCRI, SUA, ISAR)
20. Train farmer cooperators on proper methods for conducting on-farm trials (VEDCO, NaCCRI, SUA, ISAR)
21. Initiate on-farm trials initiated with selected farmer cooperators (WSU, MSU, VEDCO, NaCCRI, SUA, ISAR)

Sub-objective 3c: *To strengthen farmers' collective capabilities to purchase inoculants and incorporate them into a profitable and sustainable system for small landholders,*
We expect to:

22. Create training materials to disseminate through PELUM farmer network (ISU, WSU, MSU, Makerere, VEDCO, NaCCRI, SUA, ISAR)
23. Conduct advocacy meetings with farmer groups and agribusiness interests (VEDCO)

Collaborators: PELUM is an a network of 207 civil society organizations in Eastern, Central and Southern Africa working towards poverty eradication, food security, and sustainable community development (see http://www.pelumrd.org/).

Approaches and Methods: Ultimately our outreach activities will include training field staff on the use and potential benefits of inoculation technology, selecting farmers to participate in on-farm trials, sensitizing farmers and farmer groups about inoculant technology, identify local bean varieties to include in the field trials, training farmers on proper methods for conducting on-farm trials, data management, economic returns, and supporting data collection for site characterization. This will be completed through on-farm demonstrations, mass media, field schools, and local forums that the PELUM network has established in the region.

Our approach in Year 2 and 3 will be to disseminate information about the application of inoculant technologies directly to small landholder farmers through our partner connections in PELUM. PELUM's work focuses on enhancing farmers' livelihoods through sustainable agriculture, seed and food security. PELUM has active networks in 10 countries: Botswana, Kenya Lesotho, Malawi, Rwanda, South Africa, Tanzania, Uganda, Zambia and Zimbabwe. As a network their strength lies in efficient and effective collaboration and communication.

Objective 4: "I*ncrease the capacity, effectiveness and sustainability of agriculture research institutions which serve the bean and cowpea sectors in developing countries"*

Capacity building in terms of degree training includes formal education for seven (7) MS level graduate students and five (5) undergraduate students from host countries. Two graduate students will be trained in the Soil Science Department at Makerere University under the direction of Dr. Mateete Bekunda, Professor of Soil Science. Two graduate students will be trained at Sokoine University of Agriculture under the direction of Dr. Susan Mchimbi, Associate Professor of Plant Breeding and Genetics. One HC graduate student will be trained at Washington State University under the co-direction of Dr. Lynn Carpenter-Boggs, Assistant Professor of Soil Microbiology and Biochemistry, and Dr. Phillip Miklas, Legume Research Geneticist with USDA-ARS. One HC graduate student will be trained at Iowa State University under the direction of the program PI, Dr. Mark Westgate, Professor of Crop Production and Physiology. And one HC graduate student will be trained at Michigan State University under the co-direction of Dr. Jim Kelly, Professor of Crop Breeding and Genetics, and Dr. Karen Cichy, Research Geneticist with USDA-ARS.

It is expected that HC students training in the US will spend some time conducting practical field work in their home country. The student enrolled at Michigan State University in Crop and Soil Sciences, for example, will conduct in depth studies on promising lines identified in Rwanda field trials and will develop linkage maps of a recombinant inbred line population to conduct QTL analysis of BNF capacity. Once the student has completed his or her coursework at MSU, he or she will spend an estimated 4 months in Rwanda gathering data on BNF variability in the RIL population. The student will then return to MSU to complete degree requirements. It also is expected that HC students trained at US institutions will return to their home countries to engage in research in their chosen field.

Capacity building in terms of non-degree training includes formal internships for five (5) undergraduate students and training of HC laboratory technicians, field agronomists and extension staff on use and agricultural benefits of seed inoculants. In the first year, three undergraduate students will be assigned to the three field sites in Rwanda to assist in germplasm evaluation. These students will be supervised by Dr. Augustine Musoni, and interact directly with US PIs during their visits to the field sites. Two undergraduate interns will be assigned to work with VEDCO staff on information dissemination in Year 2 and 3.

An additional short-term training activity is planned for SUA microbiologist Mr. Hamisi Tindwa in the microbiology lab of Dr. Lynne Carpenter-Boggs at Washington State University. The intent of this training activity is for Mr. Tindwa to learn about modern molecular and biochemical methods to identify and quantify soil microflora.

Training/Capacity Building Workplan Format (January 1 to September 28, 2010)
This program includes formal training for seven MSc students, five undergraduate interns, and one visiting scientist to Washington State University. Five graduate students and three undergraduate interns will be identified in during the first funding period. Two graduate students and two interns will begin training in year 2.

Degree Training:
Seven host country M.Sc. graduate students
First and Other Given Names: TBD
Last Name
Citizenship: Uganda
Gender:
Training Institution: Iowa State University
Supervising CRSP PI: Westgate
Degree Program for training: M.S.
Program Areas or Discipline: Plant Physiology
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training:
Thesis Title/Research Area: TBD
Start Date: Fall 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed):pending
Type of CRSP Support (full, partial or indirect) for training activity: full

First and Other Given Names: TBD
Last Name:
Citizenship: Uganda
Gender:
Training Institution: Makerere University
Supervising CRSP PI: Bekunda
Degree Program for training: M.S.
Program Areas or Discipline: Soil Science
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training: Makerere University
Thesis Title/Research Area: TBD
Start Date: Summer 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed): pending
Type of CRSP Support (full, partial or indirect) for training activity: full

First and Other Given Names: TBD
Last Name:
Citizenship: Uganda
Gender:
Training Institution: Makerere University
Supervising CRSP PI: Bekunda
Degree Program for training: M.S.
Program Areas or Discipline: Soil Science
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training: Makerere University
Thesis Title/Research Area: TBD

Start Date: Summer 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed): pending
Type of CRSP Support (full, partial or indirect) for training activity: full

First and Other Given Names: TBD
Last Name:
Citizenship: Rwanda
Gender:
Training Institution: Michigan State University
Supervising CRSP PI: Kelley, Cichy
Degree Program for training: M.S.
Program Areas or Discipline: Plant Breding/Genetics
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training: ISAR
Thesis Title/Research Area: TBD
Start Date: 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed): pending
Type of CRSP Support (full, partial or indirect) for training activity: full

First and Other Given Names: TBD
Last Name:
Citizenship: Tanzania/Rwanda/Uganda
Gender:
Training Institution: Washington State University
Supervising CRSP PI: Carpenter-Boggs, Miklas
Degree Program for training: M.S.
Program Areas or Discipline: Soil Microbiology/Biochemistry
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training: Sokoine University Agriculture
Thesis Title/Research Area: TBD
Start Date: Fall 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed): pending
Type of CRSP Support (full, partial or indirect) for training activity: full

First and Other Given Names: TBD
Last Name:
Citizenship: Tanzania
Gender:
Training Institution: Sokoine University Agriculture
Supervising CRSP PI: Mchimbi, Tindwa
Degree Program for training: M.S.
Program Areas or Discipline: Breeding and Genetics
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training: Sokoine University Agriculture
Thesis Title/Research Area: TBD
Start Date: Summer 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed): pending
Type of CRSP Support (full, partial or indirect) for training activity: full

First and Other Given Names: TBD
Last Name:
Citizenship: Tanzania
Gender:
Training Institution: Sokoine University Agriculture

Supervising CRSP PI: Mchimbi, Tindwa
Degree Program for training: M.S.
Program Areas or Discipline: Breeding and Genetics
If enrolled at a US university, will Trainee be a "Participant Trainee" as defined by USAID? YES
Host Country Institution to Benefit from Training: Sokoine University Agriculture
Thesis Title/Research Area: TBD
Start Date: Summer 2010
Projected Completion Date: Summer 2012
Training status (Active, completed, pending, discontinued or delayed): pending
Type of CRSP Support (full, partial or indirect) for training activity: full

Short-term Training:

Two undergraduate internships at VEDCO
Type of training: Undergraduate Internship on inoculant technologies, management and benefits
Description of training activity: Participation in field operations
Location: Varied, depending on staff and farmer group locations
Duration: 8 weeks
When will it occur? Summer 2011
Participants/Beneficiaries of Training Activity: Extension staff, farmer groups
Anticipated numbers of Beneficiaries (male and female): Up to 22,000 VEDCO farmers
PI/Collaborator responsible for this training activity: VEDCO, Musoke
List other funding sources that will be sought (if any):
Training justification: Adaptation of new technology requires user understanding of appropriate use management, and pitfalls.

Three undergraduate internships at ISAR
Type of training: Undergraduate Internship on inoculant technologies, management and benefits
Description of training activity: Participation in field operations
Location: Varied, depending on staff and farmer group locations
Duration: 8 weeks
When will it occur? Summer 2011
Participants/Beneficiaries of Training Activity: Extension staff, farmer groups
Anticipated numbers of Beneficiaries (male and female): 1000 farmers
PI/Collaborator responsible for this training activity: ISAR, Musoni
Training justification: Adaptation of new technology requires user understanding of appropriate use, management, and pitfalls.
Type of training: Staff and farmer education on inoculant technologies, management and benefits
Description of training activity: Formal lecture and open discussion
Location: Varied, depending on staff and farmer group locations
Duration: 4 hours
When will it occur? Spring-summer 2010
Participants/Beneficiaries of Training Activity: Extension staff, farmer groups
Anticipated numbers of Beneficiaries (male and female): 50 male, 50 female
PI/Collaborator responsible for this training activity: VEDCO/ISAR/SUA, Musoke, Nchimba, Musoni
Training justification: Adaptation of new technology requires user understanding of appropriate use, management, and pitfalls.
Type of training: Staff and farmer education on inoculant technologies, management and benefits
Description of training activity: Formal lecture and open discussion
Location: Varied, depending on staff and farmer group locations
Duration: 4 hours
When will it occur? Spring-summer 2010
Participants/Beneficiaries of Training Activity: Extension staff, farmer groups
Anticipated numbers of Beneficiaries (male and female): 50 male, 50 female
PI/Collaborator responsible for this training activity: Westgate, Musoke
Training justification: Adaptation of new technology requires user understanding of appropriate use, management, and pitfalls.

Contribution of Project to Target USAID Performance Indicators
Graduate and undergraduate training is central to this project. Supporting advanced education for HC students with world-class scientist and training field technicians will contribute directly to HC capacity building.

Training of farmers and farmer groups on technologies to improve bean productivity will contribute to income and food security of small landholder farmers.

Improved on-farm productivity will enhance marketing opportunities for farmer associations.

Advancing inoculant technology for legumes will promote agricultural enterprise associated with inoculant production and sales.

Target Outputs
New knowledge on bean germplasm x inoculant x environment interactions to inform ongoing variety development programs in the U.S. and host countries about specific improvements in BNF needed to realize enhanced yield, nutritional value, and marketability of dry beans and other pulses.

Seven graduate students and (at least) five undergraduate students trained in agricultural research and extension.

Methods and conditions for profitable use of superior legume inoculants determined.

Engagement of USAID Field Mission(s)
Work in this project is closely aligned with USAID's goals of increasing agricultural production, enhancing the sustainable use of natural resources, reduce threats to biodiversity, and improve food security. USAID assistance seeks to increase and diversify commercial agricultural production and increase Uganda's competitiveness in local and international markets. This project will contribute to USAID's mission of strengthening producer organizations by working with individual farmers and farmer groups. In particular, the CRSP project explores the benefits of modern agricultural (micro-biological) technology to increase agricultural productivity and income to small landholder farmers.

Outcomes of this CRSP program directly support the USAID Rwanda Mission program for economic growth and expanded opportunities in rural areas, increase household incomes, employment, and corresponding rural financial services for targeted communities. The central Mission goal of increasing agricultural productivity is promoted by developing sustainable production practices to increase legume yields through training and access to modern agricultural inputs. Knowledge and experiences gained through VEDCO's dissemination activities in Uganda provide an excellent model for disseminating information to farmer groups in rural communities in Rwanda.

The major objectives of the USAID Mission in Tanzania is to stabilize population growth, prevent the spread of HIV/AIDS, arrest environmental degradation and promote democracy, human rights and broad-based national and regional economic growth. CRSP activities Tanzania will contribute to USAID's mission of strengthening producer organizations by working with individual farmers and farmer groups. Through our participatory approach, this program will disseminate new knowledge about sustainable agricultural technologies and build capacity of farmer groups and associations. This program also contributes directly to the US Presidential Initiative to End Hunger In Africa, which is designed to help Africa countries reduce hunger in half by 2015.

Networking Activities with Stakeholders

We anticipate our direct interaction with these programs will expand the impact of current CRSP-funded variety development programs in the US. Dr. Phil Miklas has ongoing research activities with the bean breeding program at the Sokoine University of Agriculture. This connection will provide direct linkage between US and Tanzanian scientists using molecular genetics tools to select for improved bean germplasm.

Prof. Jim Kelly at Michigan State University has ongoing germplasm development projects with colleagues at the Institit des Sciences Agronomiques du Rwanda/ISAR in Rwanda. Our research team has ongoing collaboration with bean breeders at the Rwanda through PABRA (CIAT and ECABREN) in the area of exchange of germplasm, esp. snap beans, climbing beans and root rot resistant bean lines.

Dr. Michael Ugen and colleagues at NaCRRI in Uganda work in collaboration with CIAT and ECABREN (East and Central Africa Bean Research Network) under PABRA (Pan African Bean Research Alliance) for germplasm exchange, sharing equipment and research results, trainings, support to monitoring tours, exchange of scientists, backstopping national research programs (breeding, pathology, participatory monitoring and evaluation and seed system), supervision of students, co-designing 5-year collaborative research programs.

Through VEDCOs leadership in the PELUM network, we will work with farmers groups and associations and agribusiness concerns in Rwanda, Tanzania, Uganda, and Kenya using participatory methods to understand local livelihoods, agronomic practices, their previous and current linkages with various types of institutions and service providers (governmental and non-governmental), private sector traders, transporters, their livelihood aspirations, assets, capabilities, and strategies. Involving local leadership is a key component of this approach to mobilization of farmers and local agricultural concerns.

CSRL uses 'Learning Forums' regularly to interact with various institutions and service providers (governmental and non-governmental), private sector traders, agricultural processors and distributors etc., to gain and maintain appropriately broad perspectives on key issues in production, the value chain, benefit from their special expertise, and build new collaborative relationships for high levels of success.

Leveraging of CRSP Resources

US Institutions have committed $154,236 in 'in-kind' dollars towards the successful completion of the projects outlined in this proposal. Our industrial partner, Becker Underwood, Inc. (BU) is contributing about 43% of this amount. This level of commitment from an industry partner is significant and clearly indicative of the potential for leveraging additional industry funds to expand the program. Through its collaboration with the Lutheran World Relief, Becker Underwood is currently supporting the expansion of Inoculant Technology in Burkina Faso, Niger, Tanzania, Kenya, and Mali. This activity involves local seed companies and is designed to minimize dependence on inorganic N fertilizer. While a formal commitment of funds from the CSRL program is not possible, many of the management, development, and research activities conducted by the Center with our partners in Sub Saharan Africa Uganda support the research and development activities outlined in this proposal.

TMAC EVALUATIONS AND RECOMMENDATIONS PLUS PI RESPONSES

Phase III Project: PIII-ISU-2, *Enhancing biological nitrogen fixation (BNF) of leguminous crops grown on degraded soils in Uganda, Rwanda, and Tanzania*

Lead U.S. PI: Mark E. Westgate, Iowa State University

TMAC Comments Regarding the FY11 Workplan

1. The TMAC views this as an important project for the Pulse CRSP, with the potential to have substantial impact.

2. **Objectives 1 and 3:** Perspectives of testing the inocula from Becker Underwood under objective 1 is exciting and is commended as a potentially important innovation. However, this same technology appears to be slated for dissemination already in the work plan for 2011 under objective 3. While interaction with farmers on the technology should in fact take place, true dissemination should occur only with validated technologies. This is an issue of how the technology is presented, and how the interaction with farmers evolves. Objective 1 should lead to objective 3 almost seamlessly, but farmers should understand in this process that they are participating in experimentation, and should not be led to think that the technology is already validated.

3. **Objectives 2 and 4:** Concerns are expressed about the research methods and approaches of objective 2. Since much of this work will be carried out as student theses, these concerns are relevant for student research under objective 4. Factors that affect BNF have not been fully considered.

- Crop phenology and plant habit will affect BNF, and just a few days difference in flowering or maturity can have significant effects on total N fixed. Genotypes must be classed by phenology and growth habit groups for comparison within groups.

- Evaluating tropical germplasm at higher latitudes in Washington and Iowa will be affected by photoperiod response of the germplasm if done under long summer days. Germplasm has varying degrees of sensitivity and genotypes will not respond uniformly, creating even greater differences in phenology. This would require doing these studies in fall and winter months when light intensity will be a factor. Is this contemplated?

- Similarly, developing RILs at high latitudes will take longer if one or the other parent is photoperiod sensitive.

- No mention is made of field technique. Historically progress in BNF research has been limited or facilitated by having good low-N field sites, especially with sandy soil. Field sites must be systematically evaluated and selected with partners for

their adequacy for BNF research. On station sites might not be adequate or typical of production areas. Protocols are needed that take into account how to deal with differences in habit and phenology. Addressing these methodological details is critical for the development of student theses.

- No mention is made of working with climbers, although these are genotypes that naturally fix more and that can contribute more N to the system than they extract. It is likely that climbers would be included by national partners (especially in ISAR-Rwanda) among their genotypes for testing, but the program in Uganda also has interest in climbers. Some explicit plan should be made for climbers. Evaluating climbers would require separate trials. Inoculum could be supplied to the bean program in Ecuador to test with elite climbers, with no additional involvement or expense to the ISU-2 project.

- The currently available RIL populations may have limitations. Puebla 152 is tried-and-true BNF source, but the Eagle/Puebla 152 population was developed for root rot resistance, and Eagle is a snap bean. Snap beans are selected for slow seed development, so N-use within the plant may not be typical of dry grain types. These two parents are growth habits I and III, segregation of which will influence BNF. BAT 477 is another reliable BNF source and a RIL population and map are available.

- While the best parental types are identified and RILs are developed, 3 or more years will have passed, followed by 1 or 2 years of phenotyping including field work to obtain reliable data over sites and seasons. By that time molecular techniques will have advanced even more, and more knowledge of BNF genes could be available. At that time QTL analysis may or may not be the best method. The emphasis in the short term should be on developing several populations (more than the 4 that are cited) from promising parental materials that can be advanced in generations (perhaps as bulks to reduce expenses) while the most interesting parents and traits are identified. Pure lines can be derived when the most promising parents are confirmed. The most appropriate molecular method can be chosen at that time.

- A large USDA-funded CAP project involving sequencing and phenotyping of beans is housed in North Dakota State University. Information from this project that could be relevant for mapping activities could eventually be forthcoming. This should be monitored for possible useful interaction as research progresses.

4. **Partnering:** The project would be strengthened by cross-project interaction with the project UPR-1 (Development, testing, and dissemination of genetically improved bean cultivars for Central America, the Caribbean, and Angola) and PSU-1 (Improving bean production in drought prone, low fertility soils of Africa and Latin America – an integrated approach).

- Significant synergies would be derived from interaction among the three projects that have a component of BNF or soil fertility and roots.
- The research group in the UPR-1 project has the following strengths:
    - A tried-and-true low N site
    - Experience in breeding for BNF and excellent germplasm for low N that could participate as candidates for RIL parents
    - Expertise in microbiology in tropical soils, including experience in testing Becker Underwood strains
    - Experience and a mandate for working with tropical germplasm and adapting it for temperate regions (TARS)
    - Experience in receiving populations from partners in the temperate region for winter nurseries to expedite advance of generations.
    - Seed of the RIL population including BAT 477 as a parent
- The PSU-1 project deals with
    - Root structure, which could affect nodulation
    - Acquisition of P which is a major limitation on BNF
- Additionally, the N2Africa project of CIAT-TSBF is carrying out very similar activities as in objective 1, and the two projects overlap in Rwanda. Communication should be established with Kenton Dashiell K.Dashiell@cgiar.org and activities should be coordinated.
- The TMAC appreciates that coordination of the project will be a special challenge that requires significant time on behalf of the PI

TMAC Recommendations and PI Responses:

1. A workshop should be organized among the participants in the ISU-2, UPR-1 and PSU-1 projects to explore integration and synergies. The PI should work with the MO to determine a budget if additional funds are needed for this. Topics should include:
    - Interchange of germplasm
    - The planting of trials in Puerto Rico or possibly other sites where BNF research is ongoing
    - Possible use of winter nurseries

    Response: This is an excellent recommendation, which we fully endorse. Preliminary discussions with the PIs for UPR-1 and PSU-1 have already taken place. [Jonathan Lynch, Jim Beaver, Consulo Estevez, Soareo Xerinda] Lynch, Beaver, and Westgate plan to meet at the American Society of Agronomy meetings in Long Beach, CA early in November to plan the timing, focus, and participation in this workshop. The lead PI for ISU-2 (Westgate) will take the lead in planning this activity. We will work with the CRSP-MO to coordinate funding as plans develop.

In addition, The PI (Westgate) has been in contact with Ken Giller at Wageningen, Lead PI of the BMCF- funded N2Africa project to identify areas of synergy and complementation. Musoni Augustine, the HC PI of the Pulses CRSP BNF, is a key collaborator of N2Africa project of CIAT-TSBF in Rwanda. He is the assistant PI of similar "Soil Health Climbing beans Cropping Systems" in ISAR funded by the Alliance for a Green Revolution in Africa, AGRA. He will ensure experiences and synergies are drawn from the 3 projects in Rwanda. The projects will present opportunity to study different inoculants from different sources as well as offer a broader range of bean germplasm.

2. Consideration of climbers should be included in some national partner work plans, as a fully viable object of research.

   Response: We recognize climbing beans are popular in southwestern Uganda and Rwanda. As such, we fully intended to include climbing beans in our experiments as well. It has been reported they may be better at fixing N than bush beans and there is usually more leaf production with climbing bean production. In Uganda, the NaCRRI and Mbarara locations will evaluate bush beans, while the Kachwekano site will be specifically for climbers. Due to nature of terrain in the area where the climbers will be tried, the plot size may be reduced compared to NaCRRI and Mbarara sites. In Rwanda, some of the recently released climbers: MAC 9, MAC 49, MAC 44, RWV 2070, Gasilida, Mamesa or Nyiramata will be included in the inoculation trials according to the agro-ecological sites of adaptation. The CRSP BNF project will forge linkages and collaboration with a Wageningen-CIAT led N2Africa and another AGRA supported Soil Health project that are specifically looking at climbers and their interaction with Inoculant * P* Organic Manure.

   We also have begun the process of collecting and multiplying other unique germplasm for our experiments. We received seeds of BAT 477, Puebla 152 and DOR 364 from CIAT, which we are multiplying this season to be included in the coming season experimentation. We would gladly test the BAT477 RIL population if the seed and marker data set were available to us. It is agreed that the Eagle/P152 RIL population has serious issues, which will render it less useful for BNF studies. Routine crosses and populations will be generated for breeding improved BNF into African materials and will be available for analyses using new methods as they become available. We plan to closely monitor and make use of the molecular tools generated by the BeanCAP at North Dakota State University.

3. Technical details regarding the expressed concerns have to be resolved and consigned to protocols, especially for the benefit of students and the defense of theses plus the achievement of technical objectives.

   Response: Detailed field plans were not elaborated in the proposal due to space constraints, but these have been developed to meet established scientific standards for evaluating field trials. In summary, the sites to be used have been described both in terms of physical and chemical properties and those with very low N have been selected. The CRSP BNF will as much as possible use BILFA (Bean Improvement

for Low Soil Fertility) established sites in Rwanda, Uganda and in Tanzania (for example - NaCRRI, Mbarara, and Kachwekano ZARDIs). For many years of collaborative research on Bean Improvement for Low Soil Fertility in Africa (BILFA) with CIAT-Pan Africa Bean Research Alliance, hotspot sites for low soil N and P were characterized and were established in Rwanda, Uganda and Tanzania for the routine screening of bean germplasm that were tolerant to these deficiencies. The same sites have been zeroed on for conducting the inoculation trials and demonstrations. In case of Rwanda, the specific ones were planted with corn to enhance the mining of soil N further.

Three to five of these sites were sampled for physical and chemical characterization out of which the best sites with low N are being used. Three locally adapted varieties are being tested with or without additional P with 3 rhizobia sources (MAK, USA, UON). These treatments are replicated 3 times; standard plot size is 3m x 5m.

A similar approach will also be carried out for on-farm trials. We anticipate variations even within fields especially on-farm where smaller fields are used for varied crops. But we hope to minimize this by increased sampling and more extensive field characterization before planting. On-farm trials will start next season.

Additional attention will be paid to phenological characteristics of climbing varieties. The interaction of climbing bean varieties with full indeterminate growth habit (Type III and IV) with the inoculants will be studied along-side the indeterminate bush types with respect to their phenological/morphological differences. Unlike the bush beans, climbers have staggered growth and development and at certain stages, flowering, pod formation and filling as well as maturation stages occur simultaneously. The twining, heavy branching, multiple lateral inflorescence system and enhanced pod-load of the climbing beans could have a significant implication on the N-fixation and utilization capacity that need to be investigated. These same traits are positively correlated with the high yield potential of 3 to 5 tons per hectare (in Rwanda), which represents a 3-fold increase over the yield of determinate bush beans. Because of the economic returns and the need for their staking, farmers usually integrate fast growing N-fixing tree species like *Leucaena, Calliandra,* and *Sesbania* in climbing bean cropping systems with added potential BNF beneficial effects. The impact of this integration will be considered during on-farm evaluations of inoculant technologies.

3. PIs from this Pulse CRSP project should attend the next Bean Improvement Cooperative meeting in November 2011

   Response: Attending this meeting will be a priority for the ISU-2 program. As many of the team members as possible will be encouraged to attend. We will work with the CRSP-MO to ensure funding is available for the HC team members to attend.

Dry Grain Pulses CRSP
Research, Training and Outreach Workplans
(October 1, 20010 -- September 30, 2011)

FY 2011 PERFORMANCE INDICATORS
for Foreign Assistance Framework and the Initiative to End Hunger in Africa (IEHA)

Project Title: Enhancing biological nitrogen fixation (BNF) of leguminous crops grown on degraded soils in Uganda, Rwanda, and Tanzania

Lead U.S. PI and University: Mark E. Westgate, Iowa State University
Host Country(s): Rwanda, Tanzania, Uganda

Output Indicators	2011 Target (October 1, 2010-Sept 30, 2011)	2011 Actual (October 1, 2010-Sept 30, 2011)
Degree Training: Number of individuals enrolled in degree training		
Number of women	4	
Number of men	3	
Short-term Training: Number of individuals who received short-term training		
Number of women	50	
Number of men	50	
Technologies and Policies		
Number of technologies and management practices under research	1	
Number of technologies and management practices under field testing	1	
Number of technologies and management practices made available for transfer	1	
Number of policy studies undertaken		
Beneficiaries:		
Number of rural households benefiting directly	1000	
Number of agricultural firms/enterprises benefiting	6	
Number of producer and/or community-based organizations receiving technical assistance	50	
Number of women organizations receiving technical assistance	3	
Number of HC partner organizations/institutions benefiting	6	
Developmental outcomes:		
Number of additional hectares under improved technologies or management practices	1000	

Dry Grain Pulses CRSP : SECOND PERIOD

Enhancing biological nitrogen fixation of leguminous crops grown on degraded soils in Uganda, Rwanda and Tanzania

10/01/10 - 09/30/11

	U.S. Institution	U.S. for Host Country	HC or U.S. Institution (1)	HC or U.S. Institution (2)	HC or U.S. Institution (3)	HC or U.S. Institution (4)	HC or U.S. Institution (5)	HC or U.S. Institution (6)	HC or U.S. Institution (7)	HC or U.S. Institution (8)	HC or U.S. Institution (9)
Institution Name	**ISU**		**MSU/Kelly**	**ARS/Cichy**	**WSU/Boggs**	**ARS/Miklas**	**Makerere U**	**VEDCO**	**NaCCRI**	**ISAR, Rwanda**	**Sokoine Univ**
a. Personnel Cost											
Salaries	$0.00	$19,615.00	$17,232.00	$0.00	$18,128.00	$18,592.00	$7,200.00	$8,400.00	$6,000.00	$9,000.00	$15,600.00
Fringe Benefit	$0.00	$2,589.00	$1,854.00	$0.00	$5,741.00	$2,454.00	$0.00	$0.00	$0.00	$0.00	$0.00
b. Travel	$8,650.00	$2,700.00	$7,200.00	$4,200.00	$3,900.00	$7,950.00	$7,450.00	$3,860.00	$13,145.00	$2,465.00	$6,515.00
c. Equipment ($5000 Plus)	$0.00		$0.00	$0.00	$0.00	$0.00	$0.00	$6,500.00	$0.00	$0.00	$0.00
d. Supplies	$6,200.00	$0.00	$2,000.00	$5,000.00	$5,000.00	$5,000.00	$2,250.00	$9,050.00	$3,300.00	$3,000.00	$3,000.00
e. Training											
Degree	$0.00	$4,582.00	$9,438.00	$0.00	$0.00	$10,134.00	$1,500.00	$0.00	$0.00	$0.00	$3,600.00
Non-Degree	$0.00		$0.00	$0.00	$0.00	$0.00	$0.00	$1,200.00	$0.00	$3,600.00	$0.00
f. Other	$1,350.00		$600.00	$0.00	$1,400.00	$800.00	$1,300.00	$1,800.00	$1,500.00	$1,300.00	$550.00
g. Total Direct Cost	**$16,200.00**	**$29,486.00**	**$38,324.00**	**$9,200.00**	**$34,169.00**	**$44,930.00**	**$19,700.00**	**$30,810.00**	**$23,945.00**	**$19,365.00**	**$29,265.00**
h. Indirect Cost	$10,687.00		$15,021.00	$1,022.00	$17,016.00	$4,992.00	$1,970.00	$4,622.00	$2,395.00	$0.00	$0.00
i. Indirect Cost on Subcontracts (First $25000)	$16,066.00										
j. Total Indirect Cost	$26,753.00	$0.00	$15,021.00	$1,022.00	$17,016.00	$4,992.00	$1,970.00	$4,622.00	$2,395.00	$0.00	$0.00
Total	**$42,953.00**	**$29,486.00**	**$53,345.00**	**$10,222.00**	**$51,185.00**	**$49,922.00**	**$21,670.00**	**$35,432.00**	**$26,340.00**	**$19,365.00**	**$29,265.00**
Grand Total	$369,185.00										

	Amount	Percentage
Total direct cost budgeted for U.S. institution(s)	**$142,823.00**	**48.35%**
Total direct cost budgeted for H.C institution(s)	**$152,571.00**	**51.65%**

Cost Share	U.S. Institution	U.S. for Host Country	HC or U.S. Institution (1)	HC or U.S. Institution (2)	HC or U.S. Institution (3)	HC or U.S. Institution (4)	HC or U.S. Institution (5)	HC or U.S. Institution (6)	HC or U.S. Institution (7)	HC or U.S. Institution (8)	HC or U.S. Institution (9)	**Total**
In-kind	$43,890.00		$6,184.00	$0.00	$3,507.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$53,581.00
Cash												$ -
Total	$ 43,890.00	$ -	$ 6,184.00	$ -	$ 3,507.00	$ -	$ -	$ -	$ -	$ -	$ -	$ 53,581.00
Attribution to Capacity Building												
Percentage of effort	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Amount corresponding to effort	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00	$0.00

Enhancing biological nitrogen fixation of leguminous crops grown on degraded soils in Uganda, Rwanda and Tanzania

SEMI-ANNUAL INDICATORS OF PROGRESS BY INSTITUTIONS AND TIME PERIOD

Project Title: *Enhancing biological nitrogen fixation (BNF) of leguminous crops grown on degraded soils in Uganda, Rwanda, and Tanzania*

	Abbreviated name of institutions															
	ISU		MSU		WSU		VEDCO		NaCCRI		SUA		Makerere		ISAR	
Identify Benchmark Indicators by Objectives	4/1/11	9/30/11	4/1/11	9/30/11	4/1/11	9/30/11	4/1/11	9/30/11	4/1/11	9/30/11	4/1/11	9/30/11	4/1/11	9/30/11	4/1/11	9/30/11
Objective 1																
Field Trial 2 established			x		x				x		x				x	
Quantified yield advantage of inoculation for two crops seasons				x		x				x		x				x
Analysis of plant/soil/weather data completed		x								x		x		x		x
Unique responses to inoculant x genotype x environment identified		x		x		x				x		x		x		x
Soil DNA extracted and analyzed for indigenous rhizobia strains						x				x		x		x		x
Established indigenous rhizobia levels and environmentally tolerant strains						x				x		x				x
Objective 2																
Screened germplasm for BNF in low soil N +/- inoculants in HC field trials			x		x				x		x				x	
Greenhouse screening trials initiated on selected lines for BNF response	x				x											
Parental lines tested for BNF in the field (US and HC sites)	x		x		x				x		x				x	
Selected populations tested for BNF in the field (US and HC sites)		x		x		x				x		x				x
Correlative response of BNF in field and GH trials established		x		x		x				x		x		x		x
Nodule rhizobia occupancy established on selected lines						x										
Selected RILs advanced to F2				x		x				x		x				x
Objective 3																
Trained Extensionists at HC institutions in benefits of BNF and inoculant use								x		x		x		x		x
Conducted farmer KPA evaluations on benefits of inoculation and BNF							x									
Established format for field demonstrations at HC research stations		x		x		x		x		x		x		x		x
Conducted field days in each HC to present research results								x		x		x		x		x
Identified farmer cooperators for on-farm trials							x		x		x				x	
Trained farmer cooperators on proper methods for conducting on-farm trials							x		x		x				x	
Initiated on-farm trials with selected farmer cooperators				x		x		x		x		x				x
Created training materials to disseminate through PELUM farmer network		x		x		x		x		x		x		x		x
Conducted advocacy meetings with farmer groups and agribusiness interests								x								
Objective 4																
Graduate students identified	x		x		x						x		x			
Graduate research programs initiated	x		x		x						x		x			
Undergraduate student interns identified							x								x	
Undergraduate student projects intiated							x								x	
Visiting scientist activites completed																

Name of the PI responsible for reporting on benchmarks	Westgate	Cichy	Miklas	Musoke	Ugen	Mchimbi	Bekunda	Musoni
Signature/Initials:								
Date:								