Sustainable Plant Protection Systems

Agroecology and Biotechnology of Stalk Rot Pathogens of Sorghum and Millet

Project KSU 210
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Summary

Fusarium is an intermittently severe cause of grain mold, stalk rot and pokkah boeng of sorghum. The taxonomy formerly applied to many sorghum and millet isolates, especially from Africa, often is inaccurate. We used genetic and molecular criteria to describe two additional species and to identify at least six additional new species. In Egypt a detailed population study found that F. proliferatum is common on sorghum, a crop where it usually is relatively rare (~5-10% of the population). This species is almost completely absent in West Africa, and is important because it can produce fumonisins. Most *Fusarium* species found on sorghum and millet produce little or no fumonisins, but often produce moniliformin, which is quite toxic to poultry. In a collaborative study with IITA of maize, sorghum and pearl millet grown side-by-side in farmer's fields in Nigeria, the maize was more heavily contaminated with aflatoxin and fumonisins than either the sorghum or the pearl millet. In Kansas, evidence for the evolution of putative new sorghum pathogen types was found through the analysis of strains recovered from a native tallgrass prairie. Zearalenone is a well-documented mycotoxin produced by several *Fusarium* species, and often diagnosed by thin-layer chromatography. The fungi that produce this toxin are not common in hot dry areas where sorghum usually is grown. We identified another compound, 8-O-methylbostrycoidin, that comigrates with zearalenone on TLC plates and is produced by many species recovered from sorghum. Thus, more sophisticated chemical technology should be used to screen sorghum for zearalenone to ensure that grain moving in international channels is not improperly labeled as contaminated. Biological control of *Fusarium* by four mycoviruses, which might confer hypovirulence, was investigated. These mycoviruses conferred no change in morphology, other than perhaps female sterility, suggesting that they will be of little value as biological control agents.

Objectives, Production and Utilization Constraints

Objectives

- Increase collection of *Fusarium* samples from sorghum and millet, and identify the species recovered and their mycotoxigenic potential.
- Develop genetic and molecular characters, e.g., mating type and Amplified Fragment Length Polymorphisms (AFLPS), for assessing genetic variation. Use these traits to analyze fungal populations from Egypt, Mali, Tanzania, India, Uganda, South Africa, and the United States.
- Provide pure cultures of fungi to others to expedite diagnoses of fungal diseases of sorghum and millet.
- Conduct Scientific Writing, Research Ethics, and *Fusarium* Laboratory workshops.
- Prepare text for *The Fusarium Laboratory Manual*.
- Edit Proceedings of 2000 Global Sorghum and Millet Pathology Conference.

Production and Utilization Constraints

- *Fusarium* spp. associated with sorghum and millet do obvious damage as stalk rot, grain mold and pokkah boeng, resulting in intermittently heavy losses in the United States and in developing countries. Breeding for resistance to *Fusarium*-associated diseases is difficult because the strains responsible for disease often are not accurately identified and used repeatedly in field challenges. Correct identification of these fungi is essential for the design of breeding and control measures. Without a thorough understanding of the pathogen's distribution, genetic diversity and population dynamics, effective control measures are difficult to design and resistant lines may have unexpectedly brief lives.
- Mycotoxin contamination limits the uses for harvested grain, and creates health risks for both humans and domesticated animals. *Fusarium*-produced mycotoxins are common in cereal grains, yet have been little studied in sorghum and millet. Since contamination often occurs on apparently sound grain, merely discarding obviously molded grain is not sufficient to avoid the mycotoxicity problems.
- Scientists in developing countries often are unfamiliar with the publication process for international journals and have little experience with contemporary research culture and laboratory practices. These problems may limit the international exposure that their work receives. The Scientific Writing, Research Integrity and Ethics, and *Fusarium* Laboratory workshops help address these needs through short-term training.

Research Findings and Project Output

Short-term training workshops. Three short-term training workshops are run through this project. *Fusarium* Laboratory workshops are taught annually by an international team of 5-7 instructors. These one-week workshops are approximately financially self-supporting from registration fees. Preparing for one of these workshops is a full-time job for two people for 3-4 months. The workshop is taught in odd years at Kansas State University and in even years at locations outside the United States (2002 - Sydney, Australia; 2004 - Pretoria, South Africa; 2006 - Bari, Italy; 2008 - Penang, Malaysia). To date, > 300 scientists have participated in one of these workshops.

Scientific Writing and Research Integrity and Ethics workshops are one day in length and require no special preparation. Costs are minimal, usually the cost of duplicating handouts and coffee breaks. These workshops are presented on request from host country scientists and include lectures and small teams working to solve sample problems. Since 2001, the Scientific Writing workshop has been taught in 12 countries with ~3000 participants. The Research Ethics workshop was developed in 2005 and has been presented in five countries for 384 participants.

Species identification and differentiation. Two new species were described, *Fusarium andiyazi*, and *Gibberella* sacchara. These species are part of the *Gibberella fujikuroi* species complex and used to be called *Fusarium moniliforme*, a name that has now been abandoned because of the confusion associated with its use. Both *F. andi-yazi* and *G. sacchara* can be distinguished on the basis of morphology, sexual cross fertility (or its lack), and differences in DNA sequences and AFLP markers. Additional species from Egypt and West Africa await description.

Egyptian *Fusarium* populations. Sorghum is the fourth most important cereal in Egypt (after maize, wheat and rice), and is the only one of these cereals that can be easily cultivated in the "new lands" or in very hot and arid Upper Egypt. *Fusarium* toxins are a problem on maize in Egypt and there are published cases of donkeys with leukoencephalomalacia. *Fusarium* species in the *G. fujikuroi* species complex, are widely known from maize and sorghum in Egypt, but little detailed characterization has been made. A common perception is that both crops have a common set of pathogens that cause stalk, ear and kernel rot and produce mycotoxins such as fumonisins and moniliformin. Three hundred fifty-three *Fusarium* isolates in the *G. fujikuroi* species complex recovered from both maize and sorghum were identified to species with AFLP markers and sexual crosses. Representatives of *G. fujikuroi* mating populations (MPs), MP-A (*F. verticillioides*, teleomorph *G. moniliformis*), MP-D (*F. proliferatum*, teleomorph *G. intermedia*), MP-F (*F. thapsinum*, teleomorph *G. thapsina*), and MP-G (*F. nygamai*, teleomorph *G. nygamai*), were recovered along with members of an undescribed biological species closely related to *F. andiyazi*. MP-A was the most frequently recovered MP from maize (71% of recovered isolates) and MP-D was the most frequently recovered MP from sorghum (52% of recovered isolates from sorghum). Female fertile isolates were most common within MP-A (71%) and much less common in MPs D and F. Our results suggest that sexual reproduction occurs more frequently within MP-A than within MP-D or MP-F. The relatively low female fertility within MP-D and MP-F may limit genetic exchange among individuals within these species relative to that possible in MP-A.

Mycotoxin production. We evaluated a number of *Fusarium* species for beauvericin, fusaproliferin, monili-formin, and fumonisins B1, B2 and B3 production (Table 1) of which moniliformin was synthesized most widely. Beauvericin was first identified for its antibiotic and insecticidal activities, but also is toxic to brine shrimp, and to human ematopoietic, epithelial, and fibroblastoid cells. Fusaproliferin is toxic to brine shrimp and human B-lymphocytes cell line IARC/LCL 171, and can induce teratogenic effects, e.g., cephalic dichotomy, macrocephaly and limb asymmetry, in chicken embryos. Moniliformin is extremely toxic to animals such as ducklings, rats, mice, chickens, and swine, and has been correlated with hepatitis in vervet monkeys and with a human heart condition, Keshan Disease, in China. Fumonisins B1, B2 and B3 are non-genotoxic carcinogens primarily produced by *F. verticillioides*. Dietary fumonisins are correlated with esophageal cancer in humans and can cause leukoencephalomalacia in horses, pulmonary edema in swine, and liver and kidney damage in rats. F. *concentricum* produced the most beauvericin (720 µg/g). *F. phyllophilum* produced the most moniliformin, 1,500 µg/g. *F. pseudonygamai* produced the most fusaproliferin (131 µg/g), and *F. phyllophilum* produced the most fumonisin B1 (2.5 µg/g). No culture produced fumonisin B3, and *F. bulbicola* produced none of the six mycotoxins evaluated. Several species can synthesize more than one toxin and synergistic interactions amongst these compounds need further investigation. Field samples of sorghum and millet grain from Mali contained significant levels of moniliformin.

Table 1. Production of the mycotoxins beauvericin (BEA), moniliformin (MON), fusaproliferin (FP), and fumonisins B_1, B_2 and B_3 (FB_1, FB_2 FB_3) by 15 new *Fusarium* species.

Fusarium species	Strain Number[1]	Mycotoxins BEA (μg/g)	MON (μg/g)	FP (μg/g)	FB_1 (μg/kg)	FB_2 (μg/kg)
F. acutatum	7544	6 ± 1	ND[2]	ND	147 ± 10	360± 23
F. begoniae	7542	ND	1000 ± 64	ND	66 ± 3	ND
F. brevicatenulatum	7531	ND	ND	ND	150 ± 7	ND
F. bulbicola	7534	ND	ND	ND	ND	ND
F. circinatum	7541	57 ± 2	ND	ND	ND	ND
F. concentricum	7540	720 ± 48	ND	ND	ND	ND
F. denticulatum	7538	ND	180 ± 7	ND	ND	ND
F. guttiforme	7539	72 ± 6	ND	85 ± 5	ND	ND
F. lactis	7532	ND	51 ± 3	ND	ND	ND
F. nisikadoi	7533	ND	0.6 ± 0.1	ND	ND	ND
F. phyllophilum	7543	ND	1500 ± 73	ND	2500 ± 100	T[3]
F. pseudoanthophilum	7530	2.2 ± 0.2	ND[b]	ND	ND	ND
F. pseudocircinatum	7536	ND	100 ± 16	12 ± 0.3	280 ± 3	360±30
F. pseudonygamai	7537	ND	53 ± 2	130 ± 2	ND	ND
F. ramigenum	7535	ND	46 ± 9	ND	ND	ND

[1]MRC: Medical Research Council of South Africa strain collection.
[2]ND - Not Detected.
[3]T-Trace.

Aflatoxins commonly contaminate cereals and may result in cancer, liver disease, immune suppression, retarded growth and development, and death depending on the amount and duration of toxin exposure. Maize is an introduced crop to Africa and there have been efforts for the last ~20 years to replace traditional cereal crops, e.g., sorghum and pearl millet, with maize. Maize was significantly more heavily colonized by aflatoxin-producing *Aspergillus* spp. than was either sorghum or millet and overall aflatoxin levels were correspondingly higher. Subsistence farmers in the African savannas consume locally grown maize 5.6 to 6.6 days a week. If the primary cereal were sorghum instead of maize, then the risk of aflatoxin-related problems is reduced 8-fold, and if it is pearl millet, then the risks are reduced 9-fold. Efforts to improve and maintain the traditional crops should be encouraged in areas marginal for the production of maize in Africa.

Evolution of *Fusarium* species in native grasslands. The origin of pathogenic *Fusarium* strains was exam-ined by evaluating native grasses from the Konza Prairie, a native tallgrass prairie that has never been plowed. Fifty-three of 241 *Fusarium* isolates recovered were potential sorghum pathogens. *Fusarium* proliferatum, a common sorghum pathogen that can cause the pokkah boeng disease, was the single most common species. In general, the species found in the prairie grasses paralleled those typically recovered from the maize or from sorghum crops grown in the adjacent area. The only species that we collected that has not been typically reported from either of these two crops was *G. konza*, and the only species commonly recovered from either maize or sorghum that we did not recover from the Konza Prairie was *F. andiyazi*. Toxin production by the Konza Prairie isolates was neither qualitatively nor quantitatively different from isolates of those same species from agricultural settings. Isolates of *F. proliferatum* produced as much or more fumonisins as did the isolates of F. verticillioides. One strain, X-10626, is of particular interest as its molecular markers are consistent with it being a hybrid between *F. fujikuroi* (usually a rice pathogen) and *F. proliferatum*. This strain could be part of a hybrid swarm between these two species that could help explain how these pathogens evolve and adapt to new agroecosystems. For example, such a hybrid could be the source of the capability of some strains of *F. proliferatum* to cause Pokkah boeng disease, since *F. fujikuroi* strains are capable of producing various plant growth promoters, most notably gibberellic acid. The number, pathogenicity and relatedness of such putative hybrid swarms remain important questions for further study and analysis in terms of sustainable and durable resistance to these ubiquitous fungal pathogens.

Zearalenone analog. Some strains produce a compound with identical migration to zearalenone on TLC plates. This compound eluted two min. before zearalenone on a C18-HPLC column. The analog also has an absorption peak at 500 nm that is not found for zearalenone. There was no evidence for zearalenone production by any of these cultures. The zearalenone analog had an elemental composition of C16H13O5N and a molecular weight of 299. It contained aromatic C–H, aliphatic C–H, C=O, CH2, C–OH, and C=CH2 based on IR, NMR, GC/MS, and ES-IMS analy-ses and was identified as 8-OMB. 8-OMB was first isolated from *Fusarium verticillioides* by Prof. Marasas's group in South Africa in 1979, as part of a search for the cause of equine leukoencephalomalacia. This group did not do any TLC analyses and failed to detect the similarity between 8-OMB and zearalenone under these simple analytical conditions. Strains of both *F. andiyazi*, which is common on sorghum, and *F. pseudonygamai,* which is common on millet, produced detectable levels of this compound. Thus, reports of zearalenone in sorghum and millet grain from hot, dry areas that are based on TLC analyses are likely to be false positives in which 8-OMB was present instead.

DsRNAs for biological control. Four *F. proliferatum* isolates contained one or more dsRNAs that might be useful as biological control agents. The dsRNAs from three strains are multipartite and mitochondrial-associated while the single dsRNA in the fourth is cytoplasmic. None of the dsRNAs alter morphological phenotypes or growth rates. None of the dsRNAs was sexually transmitted; the mitochondrial-associated dsRNAs are reliably transmitted vegetatively, but the single copy cytoplasmic dsRNA is not. These features are not conducive to the use of these dsRNAs as biological control agents for *Fusarium*.

Description of Methods of Work Used

Cultures. All cultures used are available through the KSU culture collection. Some isolates originated from other scientists but many are unique to the KSU collection. All strains were subcultured as single conidia before being accessioned in the KSU collection. Strains recovered from field samples are grown out on a medium semi-selective for *Fusarium*, and then subcultured. We have the necessary USDA-APHIS permits for working with *Fusarium* strains from around the world. Strains for morphological evaluation are grown on either Carnation Leaf Agar or Potato Dextrose Agar. Sexual crosses are made on carrot agar. Strains are maintained in long-term storage as spore suspensions in 15% glycerol frozen at -70°C.

Cultures for DNA extraction are grown in liquid Czapek's medium and DNA extracted with a CTAB protocol. AFLPs are labeled with 33P, resolved on sequencing gels, and scored manually following autoradiography. Each AFLP band is treated as a single independent locus with two alleles. Genetic similarities are calculated with the Dice coefficient. UPGMA clustering was carried out with the CLUSTER option of SAS (v 6.12). Mating type is identified following PCR amplification with degenerate primers or in crosses with strains of known mating type.

Mycotoxin production, isolation and extraction. Strains were cultured on cracked corn or maize grits for 2-4 weeks. Analytical procedures depended on the lab doing the analysis (Marasas - South Africa or Smith - KSU) with the results confirmed by comparison with authentic standards and spiked samples. Moniliformin is found in an aqueous extract and fusaproliferin and beauvericin in a methyl chloride extract. The amount of toxin present was quantified by HPLC. Fumonisins are recovered in an ethyl acetate extract and measured, after derivatization if necessary, by HPLC or LC-MS. The zearalenone analog was purified by TLC and HPLC, and structural analyses made of the HPLC-purified compound with IR (Infrared spectrometry), GC/MS (Gas Chromatography/Mass Spectrome-try), NMR (Nuclear Magnetic Resonance), and ESIMS (Electrospray Ionization Mass Spectrometry).

Networking Activities

Editorial and Committee Service

- Editor, Proceedings 3rd Global Conference on Sorghum and Millet Diseases
- Editor, Applied and Environmental Microbiology (2000-2006)
- International Society for Plant Pathology, *Fusarium* Committee (2000-2007)
- Mycoglobe Steering Committee (2003-2007)
- Senior Fulbright Scholar Review Panel (U.S./Australia/New Zealand 2003-06; chair 2005-06)

Research Investigator Exchange

Australia – November 4-11, 2000; October 10-14, 2001; January 26 – August 20, 2002; January 20 – February 1, 2003; April 10-24, 2004; August 11-26, 2006. Belgium – October 21-23, 2004. Benin – June 1-3, 2004. Burkina Faso – October 9-12, 2003. Cameroun – October 19-21, 2004. China – May 9-16, 2004; April 21-30, 2007. Egypt – April 27 - May 8, October 14-23, 2000; April 25 – May 4, 2001; July 30 – August 3, 2002; September 12-18, 2003. Ethiopia – November 9-23, 2002. Ghana – September 7-12, 2001; October 12-15, 2003; September 9-15, 2005. Hungary – November 29 – December 3, 2000. India – September 28 – October 6, 2001. Italy – September 18-26, 2003; June 4-9, 2004; May 27 – June 10, September 24-30, 2006. Kenya – November 24-25, 2002. Malaysia – November 12-18, 2000; October 7-9, 2001; January 19-25, 2002; February 1-4, 2003; April 24 – May 1, 2004; August 26 – September 2, 2006. Mozambique – October 28-31, 2001. The Netherlands – December 17-19, 2000; September 21-24, 2006. Nigeria – April 25 – May 4, October 15-25, 2003; May 24-31, October 9-18, 2004; November 22-27, 2006. South Africa – December 4-16, 2000; November 1-20, 2001; September 22 – October 11, 2002; October 31 – November 21, 2003; March 5-13, September 18 – October 2, November 2-23, 2004; October 20 – November 4, 2006. South Korea – October 14-18, 2001; February 4-9, 2003; May 2-5, 2004; September 3-6, 2006; May 1-5, 2007. Uganda – October 9-14, 2000.

Seminar, Workshop & Invited Meeting Presentations (International Locations Only)

Australia: 8th International Mycology Congress, Cairns; AustralAsian Plant Pathology Society, Mudgee; CSIRO Publishing, Melbourne; CSIRO Plant sciences, Canberra; Flinders University, Adelaide; Royal Botanic Gardens, Sydney; University of Adelaide, Adelaide; University of Melbourne, Melbourne; University of Queensland, Brisbane; University of Sydney, Sydney. Benin: International Institute of Tropical Agriculture, Cotonou. China: 15th International Plant Protection Congress, Beijing; Dalian Nationalities University, Dalian; Peking University, Beijing; Shenyang Agricultural University, Shenyang. Egypt: Egyptian National Agricultural Library, Dokki; Plant Pathology Research Institute, ARC, Giza. Ghana: Savanna Research Institute, Tamale. Hungary: Agricultural Biotechnology Center, Godollo. India: ICRISAT, Patancheru. Italy: Institute for the Science of Food Production, Bari. Malaysia: Universiti Sains Malaysia, Penang. Mexico: International Workshop on Sorghum and Millets Pa-thology, Guanjuato. Nigeria: International Institute for Tropical Agriculture, Ibadan. South Africa: Agricultural Research Council, Potchefstroom; Medical Research Council, Tygerberg; Stellenbosch University, Stellenbosch; University of the Free State, Bloemfontein; University of KwaZulu-Natal, Pietermaritzburg; University of Northwest, Potchefstroom; University of Pretoria, Pretoria. South Korea: Seoul National University, Seoul & Su-Won. Uganda: Makerere University, Kampala.

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Publications and Presentations (2006 and 2007)

Leslie, J. F. & B. A. Summerell. 2006. The *Fusarium* Laboratory Manual. Blackwell Professional Publishing, Ames, Iowa. 385 pp.

Leslie, J. F., R. Bandyopadhyay & A. Visconti, eds. 2007. Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI, Kew, UK.

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Hornok, L., C. Waalwijk & J. F. Leslie. 2007. Genetic factors affecting sexual reproduction in toxigenic *Fusarium* species. (accepted).

Bandyopadhyay, R., M. Kumar & J. F. Leslie. 2007. Relative severity of aflatoxin contamination of cereal crops in West Africa. (accepted).

Leslie, J. F., L. L. Anderson, R. L. Bowden & Y.-W. Lee. 2007. Inter- and intra-specific genetic variation in *Fusarium*. (accepted).

Reynoso, M. M., S. N. Chulze, K. A. Zeller, A. M. Torres & J. F. Leslie. 2007. Genetic structure of *Gibberella moniliformis* populations isolated from maize in Argentina. (accepted).

Low Input Ecologically Defined Management Strategies for Insect Pests on Sorghum

Project MSU 205
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Summary

MSU-205 sorghum plant protection research and institution building activities that had been centered in Honduras for over 20 years was de-emphasized in 2001 and expanded into Nicaragua and El Salvador, with emphasis on pest management in improved cropping systems on large agricultural farms on the Pacific coastal plain, representing agricultural systems unlike the low input, subsistence farming systems in Honduras. Activities on large farms can involve a much higher level of insect and disease pest management technology with greater cost to the farmer. Initial investigations in Nicaragua and El Salvador included studies on insect biology, behavior, ecology and population dynamics of the principal insect pests. In 2003 the MSU-205 project assumed responsibility of sorghum plant pathology research activities as part of the INTSORMIL plant protection program in this ecogeographic zone. MSU 205 collaborated in research activities with the Instituto Nicaraguense de Technologia (INTA), The Universidad Nacionial Agraria (UNA), the Nicaraguan National Sorghum Producers Association (ANPOSOR) in Nicaragua, and the University of El Salvador and the Centro de Technologio de Agricola (CENTA) in El Salvador. Information from collaborative participation in research involving entomologists and plant pathologists in university, government and producer organizations was used in developing cultural, biological and chemical control tactics for implementation in insect and disease management programs for specific pests or a complex of pests. Complementary research was conducted on insect pests and diseases in the United States. Professional agricultural workshops have increased agricultural capabilities of professionals in this region of Central America. Collaborative research with scientists in host countries and host country graduate student education has been fruitful in developing greater research capacity and furthering institution building activities in the respective host countries. Popular sorghum crop management articles have been published for farmer utilization in the application of recommended crop protection technology, sorghum research papers have been presented at meetings, and scientific papers on sorghum pest management have been published in professional journals.

Objectives, Production and Utilization Constraints

Nicaragua/El Salvador

- Determine principal insect pests and diseases on sorghum, identify damage and severity levels due to specific pest situations, and develop safe, implementable and economically acceptable pest management programs.
- Study biology, ecology, seasonal occurrence and population dynamics of insect pests and host plant relationships of plant disease pathogens with sorghum plants in different cropping systems.
- Tactics for management of insect pests and diseases are evaluated and include planting date, crop variety (host plant resistance), and pesticide efficacy.
- Investigate quality of stored sorghum grain and determine the levels aflatoxin associated with storage facilities.

- Conduct IPM workshops for agricultural professionals and local sorghum producers.
- Prepare extension-type publications on sorghum crop protection from insect pests and diseases for distribution into farm communities, and scientific papers for professional journals.
- Attend professional meetings and present scientific papers.

United States

- Investigate stink bug complex on sorghum, including species diversity and density in date-of-planting systems, economic thresholds, and ecological relationships with crop and non-crop host plants.
- Investigate the influence of sorghum-soybean crop rotation systems on insect pests and diseases, and on crop yield.
- Coordinate entomology and plant pathology research activities with collaborative scientists in Nicaragua and El Salvador, and participate in preparation of plant protection literature for publication and distribution.
- Supervise academic and research activities of MSU 205 M.S. and Ph.D. students.

Research Findings and Project Output

Nicaragua

The principal insect pest constraints to sorghum production on the coastal plain of Nicaragua are fall armyworm, sorghum midge, chinch bugs and stalk borers. Sorghum midge research resulted in extension articles and scientific journal publications on pest occurrence and ecology, host plant relationships and pest management tactics that will assist farmers in management of this pest on sorghum. A sorghum plant protection workshop was conducted in Nicaragua in 2002 by the INTSORMIL principal investigators of MSU 205 and KSU 211 with collaboration by scientists at INTA, UNA and AMPROSOR. Technical presentations included entomology and plant pathology pest management principles, pest management tactics and strategies, defining integrated pest management programs, and specific insect and disease agent pest constraints to sorghum production in the region.

Insect pest and disease management investigations were conducted on large farms during a three year period (2004-2005) including chemical insecticides and fungicides. Chemicals were applied when pests reached potentially damaging infestations or infection levels for respective insect pests and diseases. Highest yields were obtained when fall armyworm (the most damaging insect pest) and a complex of diseases were managed with insecticides and fungicides, respectively. Information suggests that sorghum can sustain armyworm infestations as high as 40% and disease infection level as high as 20%. In a follow-up study comparing improved sorghum pest management practices with conventional crop protection practices, farmers using conventional practices had significantly reduced sorghum yields than those using improved technology (timely application of pest control chemicals at effective rates and at pest economic threshold levels).

Sorghums were evaluated on farms each year to determine reaction to specific insect pests and diseases. Principal diseases included gray leaf spot, zonate spot, downy mildew, and anthracnose. Sorghum lines were identified with resistance to the plant pathogens responsible for these diseases. Low levels of plant resistance to full armyworm and sorghum midge were observed in foliage and panicles, respectively, of some sorghum lines. These lines were included in sorghum breeding programs to develop hybrids with resistance to insect pests and diseases with high yield potential.

Phytosanitary condition of sorghum in commercial grain storage facilities and potential association of aflatoxin in stored grain were investigated in 2005-2006. Insects commonly associated with the seed included grain beetles and rice weevil. Six Aspergillus fungi, two which are commonly associated with aflatoxins, were identified from samples of seed from storage facilities. Less than 20 ppb of toxin was recorded from the seed with aflatoxin; 20 ppb toxin is the highest level of aflatoxin that is acceptable.

Two sorghum crop protection workshops were conducted with scientists from UNA, INTA and AMPROSOR participating. These workshops emphasized crop production and crop protection for use by extension specialists and farmers. The collaborating scientists attended Cooperative Meetings to Improve Crop and Animal Production in Central America (PCCMCA) annually and presented research papers on aspects of sorghum crop production and crop protection.

El Salvador

Insects of greatest interest as constraints to sorghum production in El Salvador include a complex of soil inhabiting insects (mostly white grubs), defoliators (particularly fall armyworm), and stalk borers (neotropical cornstalk borer). At CENTA, insecticides were evaluated for efficacy against fall armyworm: chiten inhibitor growth regulators providing best results, with less control by the chemical insecticide Lorsban; nuclear polyhedrosis virus, fungi and the botanical Neem providing inadequate control. In studies to determine insecticide efficacy when the chemical is applied at specific plant growth stages, at various rates and volumes of water, and in one of more applications, fall armyworm infestations were lowest with each increase in rate and volume of water and with each additional spray application during each vegetative plant growth stage. The data indicates that in this cropping system in Central America, insecticides should be used with complete knowledge of the stage of sorghum development at the time of fall armyworm infestation and potential for this pest to cause irreversible feeding damage to the developing plant. In some situations sorghum plants damaged by defoliator pests during early vegetative plant growth stages can compensate for this damage during later stages of plant development. In economic threshold studies with fall armyworm during 2001-2002, whorl stage sorghum infested with this defoliator at levels ranging from 0 to 80% had similar yields at harvest. In other insecticide studies, chemical sprays applied for control of stalkborers had some effect on damage caused by this pest, and sorghum was infested with fewer stalk borers than corn in sorghum corn intercropped systems. Insecticide sprays did influence stalk borer infestations as a well timed application during early vegetative plant growth stage when borer moths are laying eggs can be as effective as two spray applications for managing this pest on sorghum, thus reducing the cost of chemicals in protecting the plants.

Each year sorghums have been evaluated for resistance to plant diseases and insects in the All Disease and Insect Nursery

(ADIN) in El Salvador. Lines were selected to be carried forward to future ADIN trials. These lines were selected for tolerance to diseases and insects, size and color of the grain, length of the panicle, aspects of plant growth and yield. Sixteen sorghum lines appeared to have some levels of resistance to certain plant diseases and insects; none of the lines had resistance to stem borers.

Whiteflies were first encountered on sorghum, rice and corn in Nicaragua in 2003 by scientists at the University of El Salvador. The damage caused by this pest on these crops is lethal to young plants and later infestations reduce yield significantly. Working with scientists at the University of El Salvador, aspects of the ecology and population dynamics of this whitefly have been elucidated, as has the identity of naturally occurring beneficial insects that play a role in the dynamics of whiteflies. Populations of this pest have declined in these crop production systems since 2003. Sorghum lines have been evaluated for whitefly resistance, with some lines having some levels of tolerance. The reason(s) for the whitefly population to have become an economic problem on these crops in El Salvador is not clear. However, the explosion of this pest in these systems possibly can be related to the extended and misuse of toxic chemicals in the system, particularly on rice, with associated harmful effects on the biological organisms (predators and parasites) that previously held the whitefly population at levels that did not cause damage to these susceptible crops.

Several extension-type publications related to specific plant diseases on sorghum and the whitefly problem in El Salvador have been published by the scientists at CENTA and the University of El Salvador with INTSORMIL MSU 205 participation. Professional meetings were attended and research papers presented.

United States

The economic threshold for caterpillar pests on whorl stage sorghum was investigated in 2001 and 2002 in Mississippi. Optimum procedures were determined for infesting plants with various numbers of larvae at different times during the day to improve artificial infestation methods for scientific studies. This research is required to elucidate pest infestation levels needed to warrant the practical use of insecticides. The generally recommended economic threshold of one larvae per plant was confirmed for whorl stage 2 (5 leaves) sorghum. This threshold level was determined to be too low for subsequent whorl stage plants.

The influence of sorghum-soybean rotational cropping systems on insect pest populations and incidence of plant diseases was investigated during 2003 through 2006 crop growing seasons. This crop rotation system improves yields of the two crops over continuous cropping of individual monocrop systems and is practiced to some extent in both the United States and some areas in Central America. Research plots were too small to obtain critical information on the very mobile insect pests that move throughout the study areas. All species remained below economic threshold levels throughout the study. The most prevalent disease on sorghum was zonate spot, whereas gray leaf spot was prevalent on soybeans. Gray leaf spot, brown spot and frogeye leaf spot fungal pathogens contributed to reduced yields in continuous sorghum and continuous soybean compared with soybean-sorghum rotations or sorghum-soybean rotations.

The stinkbug complex on sorghum was investigated with emphasis on stink bug development and behavior, and ecological relationships between the most prevalent stink bug (southern green stink bug) and its host and non-crop host plants in date-of-planting crop production systems. Studies also included economic thresholds on sorghum at three stages of plant development, namely milk stage, soft-dough stage and hard-dough stage. Developmental time from nymph to adult and percent mortality was 28 days (10%) on sorghum, 29 days (14%) on soybeans, and 28 days (23%) on corn. In host plant preference studies this stink bug preferred soybeans over corn, sorghum or cotton. The adults showed no feeding preference for sorghum in prebloom, bloom or milk, hard-dough or mature seed stages. Four or five adults or 20 nymphs per panicle during the milk stage to maturity of grain development increased the number of punctures per seed and reduced seed weight and seed germination compared with panicles infested during the milk to maturity stages, but densities of 4 or 5 adults per panicle reduced seed germination when confined on panicles during hard-dough to maturity stages of seed development. This stink bug information is useful in developing effective insect pest management programs on sorghum during the reproductive plant growth stages.

Two Ph.D. students will complete their respective graduate academic and research programs and graduate from Mississippi State University in May, 2007. One will return to Nicaragua as plant pathologist at UNA and the other to El Salvador as entomologist in CENTA to become involved in teaching and agricultural research programs.

The MSU 205 principal investigator traveled to Central America in each year of the project reporting period to coordinate multidisciplinary research programs and work with collaborator scientists in developing extension-type crop protection articles for publication and distribution into agricultural communities and scientific papers for publication in respective agricultural discipline journals. Professional meetings were attended by the principal investigator of MSU 205.

Networking Activities

Networking with ANPROSOR in Nicaragua provided opportunities to conduct on-farm integrated insect pest and disease management research with cooperation from many farmers associated with this National Sorghum Producers Association. Research activities on whiteflies on multiple crops in collaboration with scientists at the University of El Salvador proved to be very important in understanding this new devastating pest situation on sorghum, rice and corn in this region of Central America.

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Striga Biotechnology Development and Technology Transfer

Project PRF 213
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Summary

The parasitic weed *Striga*, particularly *S. asiatica* and *S. hermonthica*, remain major biotic constraints to cereal (sorghum, millet, maize) production in Africa. Remaining largely uncontrolled, *Striga* is responsible for keeping crop productivity in many of the *Striga* endemic regions of Africa at or below subsistence level. We have made significant progress in our *Striga* research at INTSORMIL focused on genetic control of the parasite specifically in sorghum. Our approach is based on developing a better understanding of the biology of interaction between the parasite and the hosts it attacks. We hypothesized that host plant genetic resistance to *Striga* spp. can be described in terms of specific points in the parasitic life cycle that require signal exchange between the host and parasite.

Using this approach, we have identified various characters that may improve *Striga* resistance in sorghum. We are able to import *Striga* seed and grow it in the laboratory by special permit from USDA-APHIS-PPQ. Thanks in large part to the stable and continued funding provided by INTSORMIL/USAID, we maintain the only operational parasitic weed containment facility in the U.S. for *Striga* research. Using this facility, we have developed and refined laboratory techniques that allow us to observe interactions between *Striga* and sorghum from weed seed germination through attachment, establishment of vascular connections and early growth on the host root system. We have utilized the extensive network of collaboration made possible through the INTSORMIL CRSP to test and verify our laboratory findings with field performance in *Striga* prone areas and introduce germplasm to African breeders in the earliest stages of development. By combining our work and that of other scientists working on root parasitic plants, we have revised and refined our understanding of the biology of host-parasite interactions.

Objectives, Production and Utilization Constraints

The overall objectives of our research have been to elucidate the biological interactions between *Striga* and its hosts, and to devise control strategies based on host resistance. In addressing our goal of developing sorghum cultivars that are resistant to *Striga*, we emphasize the vital roles of the multiple signals exchanged between the parasite and its hosts, which coordinate their life cycles. To develop control strategies based on host-plant resistance, we employ integrated biotechnological approaches combining biochemistry, tissue culture, plant genetics and breeding, and molecular biology.

Striga spp. are economically important parasites of sorghum, millets and other cereals in tropical Africa and Asia. Yield losses of sorghum due to *Striga* infestation, coupled with poor soil fertility, low rainfall, and lack of production inputs, all contribute to survival difficulties for subsistence farmers. Eradication of *Striga* has been difficult due to the unique adaptation of *Striga* to its environment and the complexity of the host-parasite relationship. Suggested control measures, including mechanical or chemical weeding, soil fumigation, nitrogen fertilization, have been costly and beyond the means of poor subsistence farmers. Host plant resistance is probably the most feasible and potentially durable method for the control of *Striga*. Host resistance involves both physiological and physical mechanisms. Our goal is to unravel host resistance by reducing it to components based on the signals exchanged and disrupt their interactions at each stage of the *Striga* life cycle. The specific objectives of our collaborative research project are as follows:

- To develop effective assays for resistance-conferring traits and screen breeding materials assembled in our *Striga* research program for these traits.

- To elucidate basic mechanisms for *Striga* resistance in crop plants.
- To combine genes for different mechanisms of resistance, through traditional breeding assisted by biotechnological approaches, into elite widely adapted cultivars.
- To test, demonstrate, and distribute (in cooperation with various public, private, and NGOs) elite *Striga* resistant cultivars to farmers and farm communities in *Striga* endemic areas.
- To develop integrated *Striga* control strategies, with our LDC partners, to achieve a more effective control than is presently available.
- To assess the adaptation and use of these control strategies, in cooperation with collaborating agricultural economists.
- To train LDC collaborators in research methods, breeding approaches, and use of integrated *Striga* control methods and approaches.

Research Findings and Project Output

We have maintained the Purdue University Parasitic Weed Containment Facility since its establishment in 2000. The facility is the only place in the US where *Striga* can be grown. It is annually inspected by USDA-APHIS-PPQ. Here we maintain our collection of S. hermonthica from several East and West African countries and the Carolina strain of S. asiatica. In addition to research, the facility is used to train African scientists in basic biological research on *Striga*.

Striga resistant sorghums developed at Purdue/INTSORMIL have been extensively tested and released for wide cultivation in a number of African countries including Niger, Ethiopia, Tanzania, Eritrea, and Eritrea.

We have developed laboratory methods that allow detailed observation on specific mechanisms of *Striga* resistance in sorghum. With these bioassays, we have been able to identify new and novel variants of sorghum with resistance to *Striga*. These include low germination stimulant production, low haustorial initiation capacity, and hypersensitive and incompatible responses to infection. Some of these *Striga*-resistance traits have come from wild sorghums.

We have effectively introgressed these traits into more productive sorghum varieties and shared this improved germplasm with collaborating breeding programs in Africa. Some have been officially released and distributed being widely grown on *Striga* prone farms under various local names (“Gubiye”, “Abshir” and “Brhan” in Ethiopia; “Hakika”, “Wahi” in Tanzania).

With supplemental funding from USAID/OFDA, we introduced an “Integrated *Striga* Management” (ISM) program for control of *Striga*. We first undertook this as an emergency relief effort in areas of Ethiopia and Eritrea that have been plagued by the ravages of drought, *Striga*, and subsequently famine. This project was a success as it demonstrated relief to the *Striga* problem through the combined use of *Striga* resistant sorghums, moisture conservation practice, and improved soil fertility through the use of inorganic or organic fertilizers. Yields were increased multiple-fold in many places. In Ethiopia alone, an estimated 100,000 families have benefited from the ISM technology.

Description of Methods of Work Used

Field evaluation of crops for *Striga* resistance has been slow and difficult, with only modest success. Our research addresses the *Striga* problem as a series of interactions between the parasite and its hosts, with potential for intervention. We recognize that successful *Striga* parasitism is dependent upon a series of gene products from its host.

The working hypothesis is that an intricate relationship between the parasite and its hosts has evolved exchange of signals and interruption of one or more of these signals results in failed parasitism, leading to possible development of a control strategy. Our general approach has been to assemble suitable germplasm populations for potential sources of resistance, develop simple laboratory assays for screening this germplasm, establish correspondence of our laboratory assay with field performance, establish mode of inheritance of putative resistance traits, and transfer gene sources into elite adapted cultivars using a variety of biotechnological means. Whenever possible, the methods developed will be simple and rapid, in order to facilitate screening large numbers of entries.

We place major emphasis on developing control strategies primarily based on host-plant resistance. To this end, we have in place a very comprehensive *Striga* resistance breeding program in sorghum. Over the last several years, we have generated and selected diverse and outstanding breeding progenies that combine *Striga* resistance with excellent agronomic and grain quality characteristics. All previously known sources of resistance have been inter-crossed with elite broadly adapted improved lines. Almost all resistant sources ever recorded have been assembled and catalogued. We undoubtedly have the largest, most elite and diverse *Striga* resistance germplasm pool, unmatched by any program anywhere in the world. However, while all resistance sources have been introgressed to elite and most readily usable backgrounds, the only mechanism of resistance we have fully exploited has been the low production of germination signal. We have not had the ability to screen for other mechanisms of resistance in the infection chain or the host-parasite interaction cycle. Since 2001, we have placed significant emphasis on developing additional effective methods for screening host plants for *Striga* resistance at stages in the parasitic life cycle beyond germination, including low production of haustorial initiation signal, failure to penetrate, hypersensitive reaction, incompatibility, or general cessation of growth after penetration. Work continues on refining these assays and integrating them into our plant breeding procedures for effective transfer of genes of *Striga* resistance into new and elite sorghum cultivars.

The wealth of germplasm already developed in this program also needs to be shared by collaborating national programs in *Striga* endemic areas of Africa. To this end, we have organized international nurseries for distribution of our germplasm on a wider scale. This has served as an effective way to network our *Striga* research with NARS that have not been actively collaborating with INTSORMIL. As we combine and confirm multiple mechanisms of resistance in selected genotypes, the efficiency and durability of these resistance mechanisms can be better understood through such a wide testing scheme.

Furthermore, in cooperation with weed scientists and agronomists in various NARS, we have developed and tested economically feasible and practicable integrated *Striga* control packages in farmers' fields in selected African countries. While most INTSORMIL projects have been directed as bilateral collaborative ventures focusing on individual NARS, this *Striga* project is handled as a regional or more "global" program, because of the commonality of the *Striga* problem and because no other agency has the mandate or is better suited to do the job.

Networking Activities

We have held several programs and workshops in target countries, promoting the ISM package (described in the Research Findings and Project Output section) as well as training in seed multiplication and laboratory methods for *Striga* biotechnology research.

In November, 2006 we hosted a gathering of international scientists in the fields of botany, plant biology, molecular biology, plant physiology, biochemistry, plant breeding, weed science, biological control and agronomy as well as economics in Addis Ababa, Ethiopia in a conference dealing with integrating new technologies for *Striga* control. The six day conference included a discussion of future funding opportunities and plans to cooperate in a multidisciplined approach to the *Striga* problem.

In addition to the graduate students trained with the support of INTSORMIL funding, we have had visitors from India, Ethiopia, Uganda, Burkina Faso, Eritrea, Mali, Niger, and Kenya visit our *Striga* research facility at Purdue University. Some have received extensive training in *Striga* biotechnology in our laboratory.

We have also exchanged germplasm with our African collaborators. They have sent us some of their breeding materials for laboratory characterization and in turn field test ours. Our various international *Striga* resistant sorghum nurseries have been organized and distributed to a number of African national programs, who have agreed to collaborate on free will basis. We have had nursery trials in Ethiopia, Kenya, Eritrea, Niger, Mali, and Tanzania. Seed of agronomically improved *Striga* resistant sorghum lines have been filled on a request basis.

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Sustainable Management of Insect Pests

Project WTU 200
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Summary

Collaborative research and educational activities in integrated pest management of insect pests of sorghum and pearl millet in the field and storage were done with entomologists, plant breeders, and others from government agricultural research stations and universities in nine African countries and the United States. Biological and cultural control strategies, especially resistant cultivars, were developed and evaluated. Amplified fragment length polymorphism was used to locate molecular differences among biotypes of greenbug, and the biology of greenbug biotypes was determined on wild and cultivated hosts and in relation to temperature, photoperiod, soil fertility, and soil moisture. The information was used to more accurately evaluate resistance to greenbug biotypes of 3,434 sorghum genotypes developed by sorghum breeders with the Texas Agricultural Experiment Station and commercial seed companies. Resistance to maize weevils of different genotypes of stored sorghum grain and possible causes of resistance (grain size, hardness, moisture, protein, pericarp morphology) were determined. Stored grain retained as much as 99.2% of original weight after infestation by maize weevils. Light and scanning electron microscopies showed the pericarp of the most-resistant sorghum was twice as thick as that of the most-susceptible sorghum. Hundreds of sorghum and pearl millet genotypes were evaluated for resistance and grain yield and quality against aphids, panicle bugs, sorghum midge, stalk borers, shoot fly, storage insects, termites, millet head miner, and grain mold in the field and storage in Botswana, Mali, Mozambique, Niger, and South Africa. Research was published in 62 articles and presented 111 times at farmer, extension, and professional meetings in the U.S. and at many meetings in Africa. Since 2002, one Ph.D. student from Ethiopia and nine M.S. students from Mali, Mozambique, India, and the U.S. were educated at West Texas A&M University. The students returned to research and increase institutional capacity at agricultural research centers in their countries or are earning Ph.D. degrees from other universities.

Objectives, Production and Utilization Constraints

Objectives

Africa. Support scientists from Botswana, Mali, Mozambique, Niger, South Africa, and other countries to increase yield and income through research to evaluate resistance and develop IPM strategies for managing panicle-infesting bugs; sorghum midge, *Stenodiplosis sorghicola*; sugarcane aphid, *Melanaphis sacchari*; stalk borers, and storage insects in sorghum and millet head miner, *Heliocheilus albipunctella,* in pearl millet. Educate students in IPM and entomology. United States. Study biology, ecology, and population dynamics of insects so effective management can be developed. Evaluate sorghum grain for resistance to storage insects. Collaborate with breeders, commercial seed companies, and molecular biologists to develop sorghums with greater yield potential and resistance to insects. Educate students in IPM and entomology. Advise extension and commodity organizations on managing sorghum insects. Participate in meetings to transfer insect pest management information.

Production Constraints

Africa. Panicle bugs, sorghum midge, stalk borers, sugarcane aphid, and beetles in storage are the most damaging insects that reduce yield and quality of sorghum in Africa. The worst insects of pearl millet are millet head miner and *Coniesta ignefusalis* stalk borer.

United States. Major insect pests are greenbug, Schizaphis graminum; sorghum midge; panicle bugs; and caterpillars in the field and beetles and moths in stored grain. Biology, insect-plant interactions, amount of damage, and economic and ecological costs of control need to be understood. Biological and cultural strategies such as use of plant resistance are needed to prevent damage.

Research Findings, Project Output and Description of Methods of Work Used

This project emphasized collaborative research and education. The IPM approach was used to develop strategies to manage insects. The insect must be identified; its biology, ecology, and population dynamics understood; abundance determined in relation to crop damage and yield loss; and control tactics used, especially conservation of natural enemies, cultural controls including resistant varieties, and insecticide when necessary. Information and technology from research was transferred to farmers, extension personnel, researchers, and others.

West Africa. Dr. Yaro Diarisso determined percentages of grain damage and loss by lesser grain borer, *Rhizopertha dominica*, and other storage insects from April-September 2006 in Mali. Acar and 97-SB-F5 were least damaged (0.95 and 0.5%). Most damaged (5.3%) was 04-CZ-F5P. Grain damage and loss of Acar, Malisor84-7, 04-CZ-F5P, and 97-SB-F5DT was less in metal than cloth, plastic, or polyethylene containers at Sotuba, except Darrelken was less damaged in a plastic than metal container.

Dr. Yaro Diarisso interviewed 10 farmers (seven males and three females) in each of 19 villages (four, 10, and five in Koulikoro, Ségou, and Sikasso regions) in 2007 for their perceptions of pest control on millet and sorghum. Of 190 farmers, 131 controlled pests in the field (55 used insecticide), while 36 used no control (Table 1).

Sixty-eight farmers (35.8%) used local plants, especially bénéfin (*Hyptis armigera*), neem (*Azadarachta indica*), and tamarin (*Tamarindus indica*), to control storage pests in granaries in Mali (Table 2). Some farmers believed local plants were effective while others said they could not afford to use chemicals to control pests of stored grain.

Southern Africa. The PI traveled to Botswana, Mozambique, and South Africa from 6-19 March 2007 to review and discuss research with entomologists and plant breeders. Mr. Chitio planted four replications of ATX635, Macia, Malisor84-17, Sima, and Sureño sorghum on 14 January 2005 and February 2006 and 2007 at Nampula Research Station in Mozambique. Maize weevils started to appear in the field on 15 April 2005 when the grain was at the hard-dough stage. Abundance of maize weevils peaked three weeks later. Sureño and Macia were least infested by maize weevils in the field. Yields were 2.3, 2.1, 1.7, 1.1, and 0.4 tons per hectare for Sureño, Sima, Macia, Malisor84-17, and ATx635. Although Sima was most infested, it yielded well at 2.1 tons per hectare. In 2006, no maize weevils were found in sorghum where legumes had been planted the previous year.

From 2004-2006, Dr. van den Berg and students enclosed whole panicles in plastic bags and removed them from fields and used a D-Vac to vacuum panicles at 26 sites in four provinces of South Africa. A total of 23,798 (14,590 adults and 9,208 nymphs) of 43 species of herbivorous Hemiptera was collected. This is compared to 57 species of panicle-feeding Hemiptera in the world and 42 species on sorghum in Africa. Fewer than eight individuals

Table 1. Control methods for pests in fields in the Koulikoro, Ségou, and Sikasso regions of Mali in 2007

Control method	Number of farmers using by region			
	Koulikoro	Ségou	Sikasso	Total
None	10	14	12	36
Insecticide	8	37	10	55
Apron at planting		18		18
Watcher	3	9	5	17
Rhonier leaves (Borassus flabellifer var aethiopum)	5	5	2	12
Chasing birds and monkeys	4	3	2	9
Seed treatment at planting	2	3	1	6
Burning cotton residue in the field, use of fertilizer	1	1	1	3
Crop rotation		2		2
Bamboo leaves (Oxythenantera abyssinica) and baobab fruit (Adamsonia digitata)		1	1	2
Early harvesting			2	2
Burning of blister beetles in the field	1	1		2
Burning of blister beetles in the field and chasing birds		1		1
Neem jelly + soap		1		1
Neem juice			1	1
Total	**34**	**96**	**37**	**167**

Table 2. Use of local plants to control storage insects in Koulikoro, Ségou and Sikasso regions of Mali

Plant name	Scientific name	Plant part used	Number of farmers using by region Koulikoro	Ségou	Sikasso	Total
Bénéfin	*Hyptis armigera*		3	20	4	27
Neem	*Azadarachta indica*	All parts		2	9	11
Tamarin	*Tamarindus indica*	Leaves	8			8
N'Tiribara	*Cochlospernum tinctorium*	Leaves	1	1		2
N'Djiro plante à serpent	*Securidata longepedonculata*	Leaves		2		2
Mougoudro		Leaves		2		2
Dimokotoli	*Cassia nigricans*	Leaves	1			
Samacara	*Swarzia madagascariensis*	Fruit	1			1
Caïcedrat	*Khaya senegalinsis*	Bark		1		1
N'Gonan	*Sclerocarya birrea*	Leaves		1		1
Piment	*Capsicum*	Fruit		1		1
Bere		Leaves		1		1
Combretum	*Guiera senegalinsis*	Leaves				
N'Binikan		Leaves				
Ash			10			10
Gasoline			1			1
Total			**25**	**31**	**13**	**68**

Dr. Yaro Diarisso found most farmers threshed and stored sorghum and millet grain in Koulikoro and Ségou regions of Mali; in Sikasso, most farmers stored the entire sorghum panicle (13.9%) or millet spike (14.5%). Clay and cement (38.3%), clay on rock (18.5%), and clay alone (14.4%) were most often used for granaries. Different kinds of granaries were used in Koulikoro and Ségou regions than in the Sikasso region.

per 100 panicles were found of most species and are not considered pests of sorghum in South Africa, but many are pests of sorghum in West Africa, North and South America, and India. Twelve to 30 bugs per 100 panicles were found of Nezara viridula, two *Eurystylus* spp., *Campylomma* sp., one *Mirid* sp., and *Nysius natalensis*. Five species of Eurystylus were most abundant at 67.9 per 100 panicles. Bugs were present from flowering until grain hardening. Damage by bugs resulted in more kernels having rotten germ. Resistance to bugs differed among sorghum varieties.

United States. Temperature affects abundance of corn leaf aphid, *Rhopalosiphum maidis*, eaten by lady beetles (family Coccinellidae) that stay to eat greenbugs and other insect pests of sorghum and other crops. M.S. student Shivakumar Bheemappa used 80 individual corn leaf aphids in clip cages on Tx399 x RTx430 sorghum at 10, 15, 20, 25, 30, and 35°C in an incubator. The aphid in the clip cage was discarded after it produced an aphid that was retained. The pre-reproductive periods per aphid were 39.9 and 4.4 days at 10 and 30°C. The reproductive periods were 31.3 and 12.2 days at 15 and 30°C. Fecundity was greatest (54.1 nymphs per aphid) at 20°C. No nymphs were produced at 35°C. Each aphid at 10 and 35°C lived 66.3 and 5.7 days. (Table 3)

Madani Telly, an M.S. student from Mali, identified insects found on mature kernels of sorghum in the field and evaluated genotypes of stored sorghum grain for resistance to maize weevil. Dead maize weevils per gram of sorghum grain at 42 days after infestation in vials ranged from 0.02 for Sureño or ICSR-939 to 0.12 for Tx7078. Total numbers of weevils were 0.22 on Tx7078 and 0.92 on 87EON366*90EON328 at 42 days after infestation. Sureño was most resistant, while ICSR-939 and 87EON366*90EON328 were least resistant.

Networking Activities

The PI, graduate students, and collaborators attended and gave three presentations at the 55th Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists, Corpus Christi, Texas, 19-22 February 2007; nine presentations at the joint conference of the National Sorghum Producers and Southern Seed Association, Santa Ana Pueblo, New Mexico, 14-16 January 2007; three posters at the 54th Annual Meeting of the Entomological Society of America, Indianapolis, Indiana, 10-13 December 2006; oral presentations at the 18th Annual Texas Plant

Table 3. Effect of different constant temperatures on corn leaf aphids on sorghum

Temperature (°C)	Pre-reproductive period (days)	Reproductive period (days)	Fecundity (nymphs)	Longevity (days)
10	36.9 ± 1.98 a	29.6 ± 2.41 a	12.5 ± 1.75 d	66.3 ± 5.10 a
15	14.4 ± 0.29 b	31.3 ± 1.42 a	46.8 ± 2.41 ab	63.4 ±2.08 a
20	7.8 ± 0.13 d	19.5 ± 0.67 b	54.1 ± 2.06 a	41.9 ± 0.99 b
25	5.2 ± 0.15 e	18.3 ± 0.72 b	38.3 ± 2.02 bc	32.7 ± 0.89 c
30	4.4 ± 0.10 e	12.2 ± 0.57 c	28.9 ± 1.65 c	18.5 ± 0.67 d
35	9.8 ± 2.46 c	0.0 ± 0.00 d	0.0 ± 0.00 d	5.7 ± 0.68 e

Means followed by the same lowercase letter are not significantly different (LSD, $P = 0.05$).

Protection Conference, 5-6 December 2006 and at the Entomology Science Conference, 24-26 October 2006, College Station, Texas; and presented an invited seminar on management of insect pests in the US and Africa for the Department of Agricultural Sciences, West Texas A&M University, Canyon, 27 September 2006. From 6-19 March 2007, the PI traveled to Botswana, Mozambique, and South Africa to discuss and view collaborative entomology research. The PI advised extension, National Sorghum Producers, and commercial seed companies on management of sorghum insect pests in the U.S. Four hundred one sorghum lines developed for resistance to biotype I greenbug were evaluated for Milo Genetics. Reference books, supplies, and/or funding were provided to Mr. Abdou Kadi Kadi in Niger, Mr. Chitio in Mozambique, Dr. Yaro Diarisso in Mali, and Dr. Munthali in Botswana.

Publications and Presentations

Journal Articles

Peterson, G.C., K. Schaefer and B.B. Pendleton. 2007. Registration of Tx2962 through Tx2978 biotype E and I greenbug-resistant sorghum germplasm lines. Crop Science 47:453-455.

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Book

Cronholm, G., A. Knutson, R. Parker and B. Pendleton. 2007. Managing Insect and Mite Pests of Texas Sorghum. Texas Agricultural Extension Service Bulletin B-1220, College Station, TX.

Proceedings

Bheemappa, S., B.B. Pendleton and G.J. Michels, Jr. 2007. Corn leaf aphid fecundity and longevity at different constant temperatures on sorghum. P. 35. Proceedings of the 55th Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists, 19-22 February 2007, Corpus Christi, TX.

Bheemappa, S., B.B. Pendleton and G.J. Michels, Jr. 2007. Development of corn leaf aphid, Rhopalosiphum maidis (Fitch) (Hemiptera: Aphididae) at different temperatures on sorghum. Joint conference of the National Sorghum Producers and Southern Seed Association, 14-16 January 2007, Santa Ana Pueblo, NM.

Bowling, R.A., B.B. Pendleton and G.J. Michels, Jr. 2007. Managing spider mites and resistance in maize and sorghum. Joint conference of the National Sorghum Producers and Southern Seed Association, 14-16 January 2007, Santa Ana Pueblo, NM.

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Chitio, F., B.B. Pendleton and M.W. Pendleton. 2007. Resistance of sorghum grain to maize weevil. Joint conference of the National Sorghum Producers and Southern Seed Association, 14-16 January 2007, Santa Ana Pueblo, NM.

Damte, T. and B.B. Pendleton. 2007. Texas sorghum producers' perception of sorghum midge (*Diptera*: *Cecidomyiidae*). P. 36. Proceedings of the 55th Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists, 19-22 February 2007, Corpus Christi, TX.

Damte Belete, T. and B.B. Pendleton. 2007. Texas sorghum producers' perceptions of sorghum midge (*Diptera*: *Cecidomyiidae*). Joint conference of the National Sorghum Producers and Southern Seed Association, 14-16 January 2007, Santa Ana Pueblo, NM.

Damte Belete, T., B.B. Pendleton and L.K. Almas. 2007. Economic benefit of using a resistant sorghum hybrid to manage sorghum midge (*Diptera*: *Cecidomyiidae*). Joint conference of the National Sorghum Producers and Southern Seed Association, 14-16 January 2007, Santa Ana Pueblo, NM.

Pendleton, B.B. 2007. Insect pests of sorghum. Joint conference of the National Sorghum Producers and Southern Seed Association, 14-16 January 2007, Santa Ana Pueblo, NM.

Telly, M. and B. Pendleton. 2007. Resistance of stored sorghum to maize weevil (*Coleoptera*: *Curculionidae*). Pp. 40-41. Proceedings of the 55th Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists, 19-22 February 2007, Corpus Christi, TX.

Thesis

Bheemappa, S. 2007. Effect of Temperature on Growth and Development of Corn Leaf Aphid (*Hemiptera*: *Aphididae*) on Sorghum. M.S. thesis, West Texas A&M University, Canyon, TX.

Presentations

55th Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists, Corpus Christi, TX, 19-22 February 2007 – S. Bheemappa, B. Pendleton and J. Michels, *Development of corn leaf aphid, Rhopalosiphum maidis (Fitch) (Hemiptera: Aphididae)*, at different temperatures on sorghum; T. Damte and B. Pendleton, *Texas sorghum producer's perception of sorghum midge (Diptera: Cecidomyiidae)*; M. Telly and B. Pendleton, *Resistance of stored sorghum to maize weevil (Coleoptera: Curculionidae)*.

Joint conference of the National Sorghum Producers and Southern Seed Association, Santa Ana Pueblo, NM, 14-16 January 2007 – T. Damte Belete and B.B. Pendleton. *Texas sorghum producers' perceptions of sorghum midge (Diptera: Cecidomyiidae)*; T. Damte Belete, B.B. Pendleton and L.K. Almas, *Economic benefit of using a resistant sorghum hybrid to manage sorghum midge (Diptera: Cecidomyiidae)*; S. Bheemappa, B.B. Pendleton and G.J. Michels, Jr., *Corn leaf aphid fecundity and longevity at different constant temperatures on sorghum;* S. Bheemappa, B.B. Pendleton and G.J. Michels, Jr., *Development of corn leaf aphid, Rhopalosiphum maidis (Fitch) (Hemiptera: Aphididae) at different temperatures on sorghum*; R.A. Bowling, B. B. Pendleton and G.J. Michels, Jr., *Managing spider mites and resistance in maize and sorghum*; R.A. Bowling, B.B. Pendleton, G.J. Michels, Jr. and R. Bowling, *Alternatives to organophosphates and carbamates for managing aphids in wheat and sorghum*; F. Chitio, B.B. Pendleton and M.W. Pendleton, *Resistance of sorghum grain to maize weevil*; B.B. Pendleton, *Insect pests of sorghum.*

54th Annual Meeting of the Entomological Society of America, Indianapolis, IN, 10-13 December 2006 – R. Bowling, B.B. Pendleton and G.J. Michels, *Miticide evaluations in corn in the northwest Texas Panhandle*; T.D. Belete and B.B. Pendleton, *Texas sorghum producers' perceptions of sorghum midge (Diptera: Cecidomyiidae);* S. Bheemappa, B.B. Pendleton and G.J. Michels, *Development of corn leaf aphid, Rhopalosiphum maidis Fitch (Hemiptera: Aphididae), at different temperatures on sorghum*.

18th Annual Texas Plant Protection Conference, College Station, TX, 5-6 December 2006 – B.B. Pendleton, *Managing aphids in sorghum production.*

Entomology Science Conference, College Station, TX, 24-26 October 2006 – S. Bheemappa, B. Pendleton and J. Michels, *Effect of temperature on corn leaf aphid on sorghum*.

Department of Agricultural Sciences, West Texas A&M University, Canyon, 27 September 2006 - B. Pendleton, *Management of insect pests in the U.S. and Africa.*