

Sustainable Plant Protection Systems



Agroecology and Biotechnology of Stalk Rot Pathogens of Sorghum and Millet

Project KSU 210
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Summary

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 known to be a problem on maize in Egypt and there are published case of donkeys with leukoencephalomalacia. *Fusarium* species from section *Liseola*, with teleomorphs in the *Gibberella fujikuroi* species complex, are widely known from maize and sorghum in Egypt, but little detailed characterization has been made of these species. 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. We examined 353 *Fusarium* isolates within section *Liseola*, recovered from both maize and sorghum. Species among these isolates were identified with AFLP markers and sexual fertility testing. We recovered 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*), 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.

Objectives, Production and Utilization Constraints

Objectives

- Determine the presence of viable fungi and related mycotoxins in sorghum and millet grain.
- Use genetic and molecular traits to assessing genetic variability in populations of *Fusarium* from Egypt, Mali, Tanzania, Uganda, South Africa, and the United States.
- Provide pure cultures of fungi from our extensive collection to U.S. and LDC investigators to expedite diagnoses of fungal diseases of sorghum and millet.
- Conduct Scientific Writing and *Fusarium* identification training workshops.
- Prepare text for *The Fusarium Laboratory Manual*.

Constraints

Mycotoxin contamination limits the uses to which harvested grain can be put, and creates health risks for both humans and domestic animals. *Fusarium*-produced mycotoxins are among the most common mycotoxins found in cereal grains, yet have not been effectively evaluated 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.

Fusarium spp. associated with sorghum and millet does obvious damage as stalk rot, grain mold and pokkah boeng. All of these diseases can cause intermittently heavy losses in the United States and in developing countries. Breeding for resistance to *Fusarium*-associated diseases often is limited because resistant germplasm is either unavailable or has undesirable characters from which the resistance trait must be separated. The source of the pathogens in fields that have been fallow for some time has never been clearly defined.

Research Approach and Project Output

Research Methods

Recovery and culture of *Fusarium* isolates. *Fusarium* cultures were isolated from plant stalks of maize, and grain and forage sorghums. Also, *Fusarium* cultures were isolated from tops of sorghum plants that showed Pokka Boeng (twisted top) disease. Plant tissues were surface sterilized in 95% ethanol for two minutes and then planted on to semi-selective peptone PCNB medium (Nash and Snyder, 1962). The plates were incubated at 25 C for 4-7 days. *Fusarium* colonies emerged from the plant tissues were picked up and cultured on complete medium (Correll et al., 1987). Pure cultures of *Fusarium* were obtained by subculturing single spores separated by micromanipulator on 2% water agar medium. All cultures are maintained as spore suspensions in 15% glycerol at -70 C.

AFLP analyses and comparisons. Isolates for DNA extraction were cultured by inoculating approximately 1 ml of a spore suspension (typically 10^6 - 10^7 conidia) into 40 ml of liquid CM. Isolates were grown on a rotary shaker (150 rpm) for two days at room temperature (23-26°C) and harvested by filtration through milk filters. Mycelia were blotted dry between paper towels and the dried mycelia stored at -20°C until DNA extraction. DNA was extracted with a cetyltrimethylammonium bromide (CTAB) protocol as described and AFLPs generated by standard methods. *EcoRI* primers used in the final specific PCR amplifications were labeled with ^{33}P -ATP.

The presence or absence of polymorphic AFLP bands ranging in size from 100-800 bp in each gel was scored manually and the data recorded in a binary format. All polymorphic markers in this size range were scored, even those that were unique to a single individual. Bands appearing at the same mobility in different individuals were assumed to represent the same allele. Each band of differing mobility was treated as a single independent locus with two alleles (present or absent), and unresolved bands or missing data were scored as ambiguous.

Initially, a single AFLP primer pair was used to group isolates that could not be identified to a specific mating population on the basis of cross fertility. Results for each group were compared to profiles from isolates of previously identified *Fusarium* species. The resulting binary data set was analyzed with the UPGMA clustering option of PAUP (v 4.10b*) to suggest possible species associations for the unidentified isolates. UPGMA similarities were calculated based on these AFLP gels. Final UPGMA genetic distances within and between sets of species were calculated with the Dice coefficient and the CLUSTER option of SAS.

Sexual cross and data analysis. Standard tester strains representing *MAT-1* and *MAT-2* from each of the eight mating populations were used to determine mating type and female fertility of the field isolates collected from maize and sorghum. Crosses were made on carrot agar medium following standard procedures developed at KSU. Field isolates were used initially as males with the standard strain as the female parent. Once mating population and mating type were identified, the field isolate was used as a female parent to assess its female fertility. The cross was considered fertile when a cirrhous of ascospores was seen emerging from a mature perithecium.

Effective population number based on mating type ratios and relative frequency of female-fertile isolates of field isolates of different mating populations was calculated based on the equations derived previously for ascomycete fungi by Leslie and Klein. The average number of female sterile mutations per isolate was calculated by using the observed frequency of the hermaphrodites as the zero term in a Poisson distribution (Leslie and Klein, 1996).

PCR and DNA sequence and analysis. To determine the mating type of sterile *Fusarium* strains, we amplified portions of mating type idiomorph *MAT-1* or *MAT-2* with PCR-based assays. We used PCR primers Gfmat1a, Gfmat1b, Gfmat2c, and Gfmat2d to amplify ~200-bp *MAT-1* or ~800-bp *MAT-2* fragments as previously described. For the b-tubulin fragments, we used PCR primers T1, T2, and T3. PCR products of b-tubulin were purified with the Wizard DNA Clean Up kit. The purified DNA products were directly sequenced by using ABI Prism BigDye Terminator Ready Cycle Sequencing Kits. Sequencing reactions were run on an ABI Prism 3700 DNA Analyzer at the Kansas State University DNA sequencing facility.

DNA sequences were edited and aligned by using the Clustal X algorithm in the BioEdit program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Final alignments were optimized visually. Phylogenetic analyses of aligned DNA sequences were performed by using PAUP* version 4.0b10. The heuristic search option was used to infer maximum parsimony trees. Clade stability was assessed by 1000 bootstrap replications calculated from PAUP trees. Other

measures, including tree length, consistency index (CI), and retention index (RI) also were calculated with PAUP* 4.0b10.

Research Findings

Experimental rationale. *Fusarium* species are important pathogens of sorghum and millets, where they usually persist as endophytes until the end of the season when they can become serious pests causing stalk rot, head blight and grain mold. Most studies of *Fusarium* species done in Egypt used outdated taxonomy that placed virtually every isolate from either host in *Fusarium moniliforme*. This grouping is morphologically convenient but is inaccurate from a biological point of view with this previously single species now subdivided into 25+ species of varying frequency, distribution and economic importance. Our objectives in this study were to determine species diversity for isolates of *Fusarium* recovered from maize and sorghum, to estimate effective population number and thereby the relative frequency of sexual reproduction within these fungal populations, and to evaluate genetic diversity within these species using anonymous molecular markers (AFLPs).

Survey results. We evaluated 66 isolates from maize and 287 isolates from sorghum. For the isolates from maize, 47 were assigned to mating population A (MP-A), nine to mating population D (MP-D), two to mating population F (MP-F), and three to mating population G (MP-G). Five isolates were cross fertile with one another and are closely related to, but are distinct from *F. andiyazi* based on AFLPs and b-tubulin DNA sequences. These five isolates probably represent a previously unidentified *Fusarium* species. The general frequencies of these species is comparable to those seen in maize samples from other developed and underdeveloped countries in that MP-A is dominant, MP-D is present at a lower, but still significant, level, and MP-F is present, but not common.

Of the 287 isolates collected from sorghum, 148 isolates were assigned to MP-D, 75 to MP-F, 48 to MP-A, 3 to MP-G (*F. nygamai*) and 16 isolates to the putative new species. This distribution has several unusual features. First, *F. nygamai* has never before been reported from Egypt on any species. Second, MP-D (*F. proliferatum*) is the dominant species rather than MP-F (*F. thapsinum*). Most sorghum samples are dominated by MP-F, which produces relatively little fumonisins and results in a crop that is generally clean from the perspective of *Fusarium* mycotoxins present. The Egyptian samples were dominated by MP-D (> 50%) and also contained a significant proportion of MP-A, which also can produce high levels of fumonisins. Thus nearly 2/3 isolates of *Fusarium* from sorghum in this sample were capable of producing fumonisin mycotoxins. Even more interesting is the relative lack of MP-F, which produces little in the way of mycotoxins and dominates on sorghum crops grown in the United States and elsewhere in Africa. The complete absence of *F. andiyazi*, another species that produces little if any mycotoxins and is the dominant *Fusarium* species grown on sor-

ghum in South Africa, also was surprising. These results suggest that the *Fusarium* species colonizing sorghum may vary by location (or a correlated variable such as climate), a pattern that is not the same as that seen in maize, which is usually dominated by either MP-A (warmer/drier) or MP-E (cooler/wetter). They also suggest that fumonisin contamination of sorghum might be a problem in Egypt even though it is not a problem elsewhere. All of the species were found throughout the Nile River valley and there did not appear to be any gross difference in species distribution based on location within the country.

We have also isolated strains of the new species from grain and stalks of maize, sorghum and millet in other parts of Africa (Nigeria, Tanzania, and Uganda). The significance of this new species as either a plant pathogen or as a mycotoxin producer has yet to be determined, but it is clearly found in a pan-African pattern and is present on grains being used for human consumption.

Sexual cross fertility. Three MPs, MP-A, MP-D, and MP-F had sufficient numbers to be analyzed for effective population number. With respect to mating type, the distribution of the *MAT-1* and *MAT-2* alleles were not widely skewed, suggesting that female fertility rather than mating type was of primary importance in determining the relative frequency of sexual and asexual reproduction. Hermaphrodites were the most common in MP-A (71%) but were much less common in MP-D (23%) and MP-F (8%). An unusual feature for this sample was the presence of strains that could function as neither male nor female parents in a sexual cross (up to 9% of the MP-F population). These strains should represent asexual lines whose genetic composition changes only as a result of mutation.

The N_e values for the various MPs are MP-A (98%), MP-D (64%) and MP-F (29%). These values suggest that sexual recombination could rapidly generate new genotypes in MP-A, but would be unlikely to do so in MP-F. The relative proportion of MP-A strains participating in sexual reproduction is 3-6%, in MP-D it is up to 1.5%, and in MP-F it is between 0.5 and 1% of the total population. Compared with global averages for these species from various hosts, the values for MP-A are somewhat higher than that normally seen, those for MP-D are somewhat lower than those normally seen, and those for MP-F are similar to or slightly lower than those previously reported. MP-A populations could recombine with relative ease to generate new genotypes, while MP-D could do so only with difficulty and MP-F only with very great difficulty.

Variation with species. Variation within a species was evaluated by using AFLP markers (Table 1). These markers have been used to construct genetic maps and for a number of previous evaluations of variation in *Fusarium* spp. and are particularly appropriate for studies such as these in which the haploid nature of the target organism enables an easy and straightforward interpretation of the results obtained.

Table 1. AFLP variation in *Fusarium* species from the *Gibberella fujikuroi* species complex collected from maize and sorghum in Egypt.

Mating population (MP) (Biological species)	Maize	Sorghum	Total	Number of AFLP haplotypes
MP-A (<i>F. verticillioides</i>)	47	48	95	74
MP-D (<i>F. proliferatum</i>)	9	148	157	126
MP-F (<i>F. thapsinum</i>)	2	75	77	55
MP-G (<i>F. nygamai</i>)	3	0	3	3
New <i>Fusarium</i> spp.	5	15	20	7
Unidentified <i>Fusarium</i> sp.	0	1	1	1
Total	66	287	353	266

Within the *Fusarium* species, examined, most MP-A and MP-D strains had different AFLP fingerprints suggesting that these strains were not clones. In a few cases the same clone was recovered from both maize and sorghum plants growing in the same general area, but clones were not observed if the strains were recovered from geographically distant locations. MP-F is highly clonal in some locations, with as few as 10 types composing > 75% of the MP-F population from the United States. Thus the Egyptian populations appear to be relatively diverse genotypically, even though the N_e values suggest that a much more clonal structure should be expected, at least for MP-F. The presence of common clones in both the maize and the sorghum samples suggests that at least some exchange between pathogen populations is occurring. Such documented exchanges for the moment are unique to Egypt, but the mechanism by which the exchanges occur could be significant for other regions as well.

Networking Activities

Editorial and committee service (2004)

Editor of *Applied and Environmental Microbiology*

Member of the International Society for Plant Pathology, *Fusarium* Committee

Member of Senior Fulbright Scholar Review Panel (US – Australia – New Zealand)

Member of the MycoGlobe Steering Committee

Research Investigator Exchanges

Dr. Leslie made the following international scientific exchange visits (2004):

Australia – April 10-24
Belgium – October 21-23
China – May 9-16

Italy – June 4-9

Malaysia – April 24 – May 1

Nigeria/Benin – May 24 – June 3

Nigeria/Cameroon – October 9-21

South Africa – March 5-13, September 18 – October 2, November 2-23

South Korea – May 2-5

Seminar, Workshop & Invited Meeting Presentations

Participated in *Fusarium* Laboratory Workshop at FABI, University of Pretoria, South Africa from September 26-October 1; 40 participants and five instructors from 16 countries

15th International Plant Protection Congress, Beijing, People's Republic of China.

Science University of Malaysia, Penang, Malaysia.

Faculty of Agriculture & Life Sciences, Seoul National Univ., Seoul, South Korea.

School of Pharmacy, Peking University, Beijing, People's Republic of China.

International Institute for Tropical Agriculture, Cotonu, Benin.

African Crop Improvement Center, Univ. of KwaZulu-Natal, Pietermaritzburg, South Africa.

Dept. of Plant Science, University of the Free State, Bloemfontein, South Africa.

During 2004 *Fusarium* Cultures were Provided to:

Dr. Ranajit Bandyopadhyay, IITA, Ibadan, Nigeria

Drs. Robert L. Bowden, Larry E. Claflin, & Mitch Tuinstra, Kansas State University, Manhattan, Kansas.

Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, Kansas.

Dr. David Geiser, Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania.

Prof. Dr. Laszlo Hornok, Agricultural Biotechnology Center, Institute for Plant Sciences, Godollo, Hungary.

Prof. Dr. Yin-Won Lee, Department of Plant Pathology, Seoul National University, Su-Won, South Korea.

Dr. Antonio Logrieco, Institute of the Science of Food Production, CNR, Bari, Italy.

Prof. Dr. Walter F. O. Marasas, PROMEC, South African Medical Research Council, Tygerberg, South Africa.

Dr. J. Scott Smith, Department of Animal Sciences & Industry, Kansas State University, Manhattan, Kansas.

Dr. Brett Summerell, Royal Botanic Gardens-Sydney, Sydney, Australia.

Dr. Bettina Tudzynski, Westfaelische Wilhelms University, Muenster, Germany.

Drs. Mike Wingfield & Brenda Wingfield, Forestry & Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Dr. Baharuddin bin Salleh, School of Biological Sciences, Science University of Malaysia, Penang, Malaysia.

Dr. Kerry O'Donnell, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, Illinois.

Other Collaborating Scientists (Host Country)

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Drs. M. Flieger & S. Pazoutova, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic

Dr. Laszlo Hornok, Agricultural Biotechnology Center, Godollo, Hungary

Dr. Yin-Won Lee, Department of Plant Pathology, Seoul National University, Su-Won, South Korea

Drs. Antonio Logrieco, Antonio Moretti & Giuseppe Mulé, Institute of the Science of Food Production, CNR, Bari, Italy

Prof. Baharuddin Salleh, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

Dr. Brett A. Summerell, Royal Botanic Gardens, Sydney, Australia

Drs. Michael and Brenda Wingfield, FABI, University of Pretoria, Pretoria, South Africa

Other Collaborating Scientists (U.S.)

Dr. G. N. Odvody, Texas Agricultural Experiment Station, Corpus Christi, Texas

Publications and Presentations

Journal Articles

Heaton, L. A. & J. F. Leslie. 2004. Double-stranded RNAs associated with *Fusarium proliferatum* mitochondria. *Mycological Progress* **3**: 193-198.

Leslie, J. F., K. A. Zeller, A. Logrieco, G. Mulè, A. Moretti & A. Ritieni. 2004. Species diversity and toxin production by strains in the *Gibberella fujikuroi* species complex isolated from native prairie grasses in Kansas. *Applied and Environmental Microbiology* **70**: 2254-2262.

Leslie, J. F., K. A. Zeller, M. Wohler & B. A. Summerell. 2004. Interfertility of two mating populations in the *Gibberella fujikuroi* species complex. *European Journal of Plant Pathology* **110**: 611-618.

Saleh, A. A. & J. F. Leslie. 2004. *Cephalosporium maydis* is a distinct species in the *Gaeumannomyces-Harpophora* species complex. *Mycologia* **96**: 1317-1329.

Smith, J. S., J. Fotso, J. F. Leslie, X. Wu, D. van der Velde & R. A. Thakur. 2004. Characterization of bostrycoidin: an analytical analog of zearalenone. *Journal of Food Science* **69**: 227-232.

Summerell, B. A., and J. F. Leslie. 2004. Genetic diversity and population structure of plant pathogenic species in the genus *Fusarium*. In: *Plant Microbiology* (M. Gillings & A. Holmes, eds.), pp. 207-223. Bios, Oxford, United Kingdom. 290 pp.

Abstracts

Bowden, R. L., J. E. Jurgenson, J.-K. Lee, Y.-W. Lee, S. H. Hun, K. A. Zeller & J. F. Leslie. 2004. A second generation genetic map of *Gibberella zeae*. *Proceedings of the 15th International Plant Protection Congress*: 355.

Leslie, J. F. 2004. Genetics of *Gibberella zeae*. *Proceedings of the 15th International Plant Protection Congress*: 353.

Saleh, A. A., & J. F. Leslie. 2004. Biological species in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*) recovered from maize and sorghum in Egypt. *Proceedings of the 15th International Plant Protection Congress*: 369.

Low Input Ecologically Defined Management Strategies for Insect Pests on Sorghum

Project MSU 205
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Summary

With emphasis on research directed at establishing integrated pest management practices on sorghum, MSU-205 investigations involving both insect pests and diseases in large monoculture sorghum production systems were coordinated between entomologists and plant pathologists with government, university and producer organization participation. Unlike the low input, inexpensive pest control technology utilized on small subsistence farms, the commercial operations in Nicaragua and El Salvador can involve a much higher level of insect pest and disease management technology with greater costs to the farmer. Improved insect and disease pest management practices were compared with agricultural practices utilized by farmers in an on-farm demonstration-type project in Nicaragua. The improved technology system proved to be better when yields were compared. Insecticide and fungicide treatments were evaluated in different programs, with considerable benefits obtained with timed pesticide applications for control of insect pests and diseases, respectively. The All Disease and Insect Nursery (ADIN) included sorghums that showed levels of resistance to fall armyworm and several diseases, including gray leafspot, zonate spot, downy mildew, and anthracnose. Plant pathologists in El Salvador found several superior sorghum lines and varieties, as well as native maicillo criollos, with resistance to both insect pests and diseases. The

whitefly problem on sorghum in 2004 in El Salvador was not as severe as it was in 2003. In the United States the first year of a three-year sorghum-soybean crop rotation research program was completed in 2004 in Mississippi in which six crop systems were evaluated for effects on insect pests and diseases. In other studies, stink bug activities in sorghum were included in preliminary investigations; these involved identifying adult and nymph thresholds of crop damage, adult preferences for crops and non-crop vegetation and crop plant growth stages, evaluation of insecticides for stink bug control, and observations on seed diseases related to stink bug damage. Workshops on insect and disease identification and pest management with farmer participation were conducted in 2004 in Nicaragua and El Salvador. Research accomplishments have been and will be presented at professional meetings and published in scientific journals and agricultural extension-type publications.

Objectives, Production and Utilization Constraints

Nicaragua

- MSU 205 PI met with Central America collaborator scientists in INTA and UNA and NGO personnel in

ANPROSOR to develop collaborative sorghum production research plans.

- Collaborate with INTA and UNA scientists to investigate on-farm cultural and chemical methods for management of insect pests and diseases on sorghum.
- Evaluate sorghums for resistance to diseases and insect pests.
- Conduct IPM workshops for agricultural professionals and local sorghum producers.

El Salvador

- Evaluate the efficacy of insecticide programs on sorghum varieties for control of stem borers and sorghum webworms.
- Evaluate sorghums for resistance to diseases and insect pests.
- Continue research relationships with entomologist at the University of El Salvador to collaboratively investigate the serious whitefly problem first encountered in El Salvador in 2003.
- Conduct workshop on insect pest identification and control measures.

United States

- Investigate the influence of sorghum-soybean crop rotation systems on insect pest and disease occurrence and population densities, and damage to the crops. Work collaboratively with plant pathologist in describing these relationships for plant diseases on both crops.
- Investigate the stink bug complex on sorghum, including species diversity and density, nymph and adult damage to the grain, developmental rates on crop seed and insecticide efficacy.
- Continue research and academic programs for two MSU-205 Ph.D. students.

Research Approach and Project Output

Nicaragua

Entomologists and plant pathologists at INTA and UNA worked together with ANPROSOR in conducting a number of integrated pest management investigations in sorghum on large and small farms in several areas of Nicaragua in 2004. Students from UNA participated in these investigations to gain experience in various aspects of agricultural crop production and specifically with insect and disease pest management strategies. Workshops in two country zones were conducted with farmers participating. Two workshops emphasized identification of insect and disease pests, and two workshops emphasized integrated pest management practices. Eighty sorghum producers attended the four workshops. A pest identification and integrated pest management practices document was developed by the scientists and given to each participant.

Four insect and disease pest management investigations were completed during the second crop growing season. In a study to determine the influence of insecticide (cypermethrine) to control fall armyworm larvae and fungicide (benomyl) to manage diseases on vegetative sorghum, chemical spray applications were made when insect infestation levels reached 20, 30, 40 or 50% and a fungicide spray was applied at 10, 20, 30 or 50% disease severity levels as the designated integrated chemical program spray treatments. The insect infestation did not reach the 50% level. Although diseases were not observed during the early vegetative plant growth stages of sorghum, several were obvious during mid- to late season. Diseases caused by *Ramulispora sorghi*, *Colletotrichum graminicola* and *Cercospora sorghi* were present and each reached 60% severity level during late season. The complex of diseases reached treatment levels up to 40%, but not the 50% severity level during the designated treatment period. In this chemical application program to manage insect and disease pests on sorghum in vegetative stages, highest yields were obtained when fall armyworm and diseases were managed with insecticide and fungicide, respectively. Lowest yield was obtained in the untreated plots. No significant differences were observed among chemical treatments suggesting that sorghum will tolerate fall armyworm infestations as high as 40% and disease infection level as high as 20% during early to mid-vegetative stages. These data indicate the benefit of a combination of insecticide and fungicide chemical spray applications to manage insect and diseases, respectively, on vegetative sorghum.

In a second on-farm investigation, the number of insecticide (cypermethrin) chemical spray applications (1, 2 or 3) and fungicide (benomyl) spray application at 10, 20 or 50% disease severity levels were treatment programs designated to manage foliage feeding lepidopterous caterpillars (mainly fall armyworm) and diseases, respectively, on vegetative sorghum in small plots during the second crop growing season. The first insecticide spray application was made when 40% of the plants were infested with fall armyworm larvae; the second spray three weeks after the first at 40% insect infestation. The treatment program including three insecticide sprays plus fungicide spray at 50% disease severity level was not initiated because the vegetative stages of the crop was too short due to drought, thus insect infestation and disease severity levels were not observed on vegetative plants. Chemical spray applications on the reproductive stages of sorghum were made to program treatments 1 and 2 (above) to uniformly manage seed head insects and diseases. Fall armyworm larval infestations were observed on very young sorghum plants and increased to highest levels during late vegetative stages. No apparent difference was observed between treatments with one or two insecticide spray applications to vegetative sorghum at treatment thresholds of 40% infested plants. Diseases of vegetative sorghum caused by *Colletotrichum graminicola*, *Ramulispora sorghi* and *Gloeocercospora sorghi* and diseases of reproductive stages caused by *Colletotrichum* and *Cercospora sorghi* were observed and reached severity lev-

els up to 20% of infected foliage during vegetative stages. Little apparent difference in disease severity level was observed between the two treatment programs (10 or 20% severity levels) and the untreated plots during vegetative plant growth stages. The diseases were observed at levels as high as 60% during the reproductive stages. The data suggests that one pyrethroid insecticide spray for fall armyworm control when plants are at 40% infestation level provided as good control of this pest as two insecticide applications (the second three weeks after the first) in an integrated pest management program when fungicide is applied at either 10 or 20% severity levels for disease control.

The disease caused by *Ramulispora sorghi* is not generally considered to be a significant constraint to sorghum production in Nicaragua, but this investigation revealed the vulnerability of the commercial variety Tortillero to the pathogen.

The second year of an on-farm study to compare improved insect and disease management practices with conventional sorghum production practices used by farmers was conducted. Production practices used by farmers included applications of cypermethrin insecticide, early and fall season weed control and fungicide (Benomyl or Python + pH plus) applied to the seed head. Improved practices included the biological insecticide Dipel, full season weed control and fungicide spray for disease control. Fall armyworm larvae were the principal defoliators during the vegetative stages and leaf-footed bugs during the seed development stages. The lowest cumulative infestation of fall armyworm larvae was recorded in the improved pest management treatments with the biological insecticide and the largest infestation in plots experiencing insect pest control practices with the chemical insecticide used by the farmers. Dipel was more effective than cypermethrin for control of this insect pest, but did not affect certain natural enemy (i.e., lady beetles) populations. The chemical insecticide cypermethrin was more effective than Dipel for control of leaf-footed bugs on seed heads. The plant diseases that were observed on vegetative plants were caused principally by *Macrophomina phaseolina* and *Fusarium proliferatum*. Calcium sulfate broth in the improved management treatments was more effective in disease management than either Benomyl or Python + pH plus used in the treatment representing practices used by the farmers. Diseases on the seed heads, mainly molds, were caused by *Fusarium thapsinum*, *Curvularia lunata* and *Aspergillus flavus* and severity of disease was lower in improved technology plots than in the plots utilizing pest management of insect pests and diseases than the conventional pest management practices employed by the farmers. The results obtained in this investigation indicate that the improved pest management technology in sorghum provided better management of insect pests and diseases than the conventional pest management practices used by the farmers.

Sorghum entries (50) in the All Disease and Insect Nursery were evaluated for resistance to insect pests and diseases during the second growing season. Insects evaluated were fall armyworm on vegetative stages and sorghum midge on the reproductive stage. Midge infestations were too low for meaningful analysis. The principal diseases included gray leaf spot caused by *Cercospora sorghi*, zonate spot caused by *Gloeocercospora sorghi*, downy mildew caused by *Peronosclerospora sorghi* and anthracnose caused by *Colletotrichum graminicola*. All sorghum entries were infested with fall armyworm larvae, with only three considered tolerant to this lepidopterous defoliator. Twenty-four lines were considered very susceptible to fall armyworm feeding damage. Six sorghum lines were considered tolerant to gray leaf spot, downy mildew and anthracnose, five lines tolerant to gray leaf spot and anthracnose, and 10 lines tolerant to anthracnose and downy mildew. Considering individual diseases, 32 lines were considered tolerant to anthracnose, 23 to downy mildew and 15 to gray leaf spot. Six of the seven sorghum lines with highest yield (greater than 1000 kg ha⁻¹) were considered tolerant to fall armyworm, but susceptible to one or more of the above diseases. This might suggest that damage by fall armyworm to sorghum during vegetative and reproductive stages should be limited to improve crop yield and that the above diseases should be further evaluated to determine influence on crop damage and yield.

El Salvador

A survey was conducted by CENTA scientists in 2004 to determine the relative importance of insect pests on sorghum as identified by sorghum farmers in five areas of El Salvador. White grubs were identified as the principal insect pest of concern in all five areas, with fall armyworm, sorghum webworm, stalk borers and sorghum midge as secondary pests. This information will be used in defining areas for establishing insect pest management studies.

Based on information obtained in the insect pest survey, two studies were conducted on a farm located where the specific pests of interest have been a problem in sorghum production. In one test, insecticide (Lorsban) applications were made at different dates to determine efficacy of the chemical pesticide on stalk borers (*Diatraea* sp.). Insecticide was applied 15 days after planting and in a second treatment 25 days after planting. These treatments were designed to protect the plants during vegetative stages. When the two insecticide treatments were compared with the untreated control, the two insecticide treatments had less damage than the control and higher yields, and although the treatment at 15 days post plant had less damage than the treatment at 25 days post plant, no yield differences was observed between these two treatments. This data suggests that a single timed insecticide spray application during early vegetative stages of sorghum can be as effective as two spray applications on vegetative sorghum for

managing stalk borers on sorghum, thus reducing the cost of chemical management of this pest on sorghum.

A test was conducted to evaluate the level of sorghum webworm (*Nola sorghiella*) control obtained when Lorsban insecticide was applied at the milk stage of sorghum seed. A second treatment included Lorsban spray application at the milk stage and a second spray application 16 days after the first application. These treatments were compared with the untreated control. The insecticide treatments and the untreated control did not differ in levels of seed damage or yield. Because this insect can be responsible for considerable damage to sorghum grain at times when infestations are very large on the panicles, this type of study should be conducted again to determine appropriate insect pest management tactics.

In recent years, the evaluations of sorghum materials in the All Disease and Insect Nursery (ADIN) in El Salvador resulted in the selection of sorghums with tolerance/resistance to certain diseases. More recently, insect damage has been evaluated on sorghums in the ADIN to complement the disease evaluations. In 2004, selected sorghum lines and varieties, and native sorghums (maicillos) were evaluated at two locations for both disease and insect resistance. Information was obtained on agronomic characteristics, incidence and severity of diseases and insect damage. Ten superior lines were selected from the 2003 ADIN based on tolerance to diseases and insects, size and color of the grain, length of the panicle, aspects of plant growth, and yield for further evaluation in the 2004 ADIN. Three lines, Sureno, D2CA4624 and 96CD635, were selected from the 2004 ADIN based on the above characteristics and will be planted in 2005. Five native macillos criollos were evaluated for disease and insect resistance at two locations in 2004. One maicillos, Indio Macartus, showed resistance to diseases, whereas two macillos, Indio Macartus and Indio, showed lower levels of resistance to disease and insect pests. The rust caused by *Puccinia sorghi* appeared to be the most important disease of sorghum in El Salvador.

Whiteflies, encountered for the first time on rice, sorghum and corn in 2003 by scientists at the University of El Salvador, were again infesting these crops in 2004, although populations were lower than in 2003. Of particular research interest in 2004 was whether the whiteflies would survive during the dry season (November-April) in El Salvador. Whiteflies were collected in low numbers in late February, 2004, indicating the potential for these pests to survive the harsh hot, dry period. The insects infested rice during mid-year, then moved to corn and sorghum during the second growing season (early-September). Crops were sampled in a number of areas in El Salvador to determine the range of this pest in the country. A number of areas not sampled in 2003 were determined to be infested with whiteflies in 2004. Whiteflies were decimated on rice with insecticide sprays but survived on wild host plants until corn and sorghum were planted. A grass, *Elasine indica*, contributed to the ability of the whiteflies to survive between first and second growing seasons.

Other grass species may be important in the ecology of this pest in this region of Central America. Natural enemies, many identified in 2003, were at low levels in 2004, thus contributed little to the low population levels during the year.

United States

Sorghum-soybean rotation has been reported to improve yields of the two crops over continuous cropping of individual monocrop systems. Insect pests and diseases are limiting factors in the production of these crops. Studies were conducted in 2004 and will continue through the 2006 growing season in Mississippi to evaluate sorghum-soybean rotation systems for reducing pest pressure and increasing yields of these crops. Six sorghum-soybean planting, systems were initially established in small replicated plots in 2004, namely, 1) continuous sorghum, 2) continuous soybean, 3) sorghum-soybean-sorghum rotation, 4) soybean-sorghum-soybean rotation, 5) sorghum-soybean-soybean rotation, and 6. soybean-sorghum-sorghum rotation. Insect pests were sampled at 10 day intervals and disease incidence was evaluated monthly; samples for nematodes were made in May and September. Although three-cornered alfalfa hopper and bean leaf beetle adults and velvetbean caterpillar larvae were prevalent on soybeans, and corn earworm and sorghum webworm larvae and sorghum midge adults were numerous on sorghum, the populations of the pests were below economic thresholds on the respective crops. Zonate spot was the most prevalent of the diseases on sorghum. Frogeye leaf spot and stem canker were at low to moderate levels on soybeans. Spiral nematode was at greater numbers on soybean than on sorghum, but generally considered a minor pest on both crops. Root knot nematode was at low levels on both crops. No yield differences were recorded among treatment plots for each crop in the 2004 study, as might be expected because the crops were in the first plantings in each system.

Aflatoxins occur in sorghum and can be damaging to animals feeding on the crop. The feed industry requires that seed must contain less than 20 ppb aflatoxin, the legal limit. The relationship between insect pests and levels of mycotoxin forming fungi in sorghum-soybean rotational cropping systems was initiated in 2004 and will continue in 2005 and 2006. Aflatoxins in sorghum seed in the first year of the rotational system were determined using the Vicam Afla Test. Sorghum samples from test plots contained less than 20 ppb.

Stink bugs frequently become a concern to sorghum producers in the southeastern United States. Several species, including the southern green stink bug and green stink bug, infest the sorghum panicles and feed on the seed. Sorghum seed weight, quality and yield are reduced. Studies were initiated in 2004 and will continue in 2005 and 2006 in Mississippi to obtain information on the stink bug complex on sorghum. Both the southern green and green stink bugs were observed in large numbers on sorghum and soybeans during mid-and late season in North Mississippi. The southern green stink bug was

the more prevalent of the two species. The green stink bug declined in numbers during this period, whereas the southern green stink bug continued to develop to even larger populations on sorghum before the onset of cold weather. Preliminary tests were conducted utilizing southern green stink bug adults or nymphs. Tests included: 1.) levels of adult or nymph infestations responsible for economic plant damage (economic injury level) and yield reduction, 2.) adult stink bug host plant preference for sorghum, corn, soybean or cotton, 3.) adult stink bug preference for sorghum in different plant growth stages, 4.) developmental rate of stink bugs on sorghum, corn or soybean seed, 5.) evaluation of insecticides for control of stink bugs on sorghum panicles, and 6.) observations on seed disease related to stink bug damage. As these studies were preliminary, experimental procedures will be refined and the studies will be repeated in 2005.

Networking Activities

The sorghum crop production and IPM workshop organized by INTSORMIL (MSU 205 and KSU 211), UNA, INTA, and ANPROSOR in Nicaragua in 2002 served as the stimulus for further development of similar scientific meetings and workshops involving scientists, farm organization personnel, and sorghum producers in Nicaragua and El Salvador in 2003 and 2004. Two workshops were conducted in Nicaragua and one in El Salvador in 2004 and additional meetings and workshops are planned for 2005. They included farmer participation in reporting pest problems and crop production methods, as well as research needs, and aspects of integrated insect pest and plant disease management. The workshops were successful because of detail coordination by scientists and administrators at UNA, INTA and ANPROSOR in Nicaragua and CENTA in El Salvador.

Networking with ANPROSOR in Nicaragua provides opportunities to conduct on-farm integrated insect pest and disease management research with cooperation from many farmers associated with this National Sorghum Producers Association. Extension of research activities on the whitefly problem with scientists at the University of El Salvador proved to be very important in understanding this new pest situation in sorghum in this region of Central America.

Popular articles and departmental reports on sorghum pests published during the past two years provide information for farmers to use in managing insect pests and diseases on sorghum to improve yield. Publications are distributed by INTA and UNA in Nicaragua and CENTA in El Salvador into farm communities with assistance from local agricultural professionals.

Publications and Presentations

Books, Book Chapters and Proceedings

Serrano, Cervantes L., R. Guzman de Serrano, A.E. Moran, C.A. Borja Melara, M. Azahar Barrera, J.L. Mayen Rafael, A. De Garcia Andrade, J.A. Trujillo, M. Hernandez and H.N. Pitre. 2004. Population behavior of the whitefly, *Aleurocybotus occidius*, in rice and sorghum in 2004 at Nueva Concepcion, Chalatenango, El Salvador, C.A. Proc. 9th IPM International Congress. Nov. 3-5, 2004. San Salvador, El Salvador.

Dissertations and Theses

Zeledon, J.J. 2004. Methods of infestation, damage and economic injury level for fall armyworm, *Spodoptera frugiperda* (J.E. Smith), in Mississippi grain sorghum. PhD dissertation. Mississippi State University, Mississippi State, MS. 73pp.

Presentations

Serrano, Cervantes L. et. al. 2004. Population behavior of the whitefly, *Aleurocybotus occidius*, in rice and sorghum in 2004 at Nueva Concepcion, Chalatenango, El Salvador, C.A. Ninth IPM Int'l. Congress. San Salvador, El Salvador. Nov. 3-5, 2004.

Pichardo, Sergio T., Richard E. Baird and Henry N. Pitre. 2004. Occurrence of insect pests and diseases in sorghum (*Sorghum bicolor*) (Linn.) Moench and soybean (*Glycine max* (L.) Merr. rotations in Mississippi. Miss. Assoc. Plant Pathologists and Nematologists Ann. Meeting. Feb. 22-24. Stoneville, MS

***Striga* Biotechnology Development and Technology Transfer**

**Project PRF 213
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Summary

Witchweeds (*Striga* spp.) are obligate parasitic weeds of significant economic importance. Control methods available to date have been costly and beyond the means of farmers in developing countries. While combining several control measures may be necessary for eradication of *Striga*, crop losses to *Striga* can be effectively minimized through host-plant resistance. Our goal is to exploit the unique life cycle and parasitic traits of *Striga* especially the chemical signals required for germination, differentiation, and establishment.

In this report, we summarize our activities on integrated *Striga* management in Ethiopia during 2002 and 2003 crop seasons. A bigger document highlighting project activities 2001-2004 is currently under preparation. On-farm evaluation of an ISM package was conducted in four *Striga* endemic regions of Ethiopia. The package included a *Striga* resistant sorghum cultivar, soil moisture conservation, and fertilization to synergize reduction of *Striga* infestation and increased sorghum yield. A secondary objective was to promote the use of newly released *Striga* resistant sorghum cultivars and to assist in the establishment of a community based seed multiplication program. Farmers in all four regions were positive on the power of the ISM package in reducing parasitic infestation and increasing yield. Data collected clearly showed that mean *Striga* count from the ISM package was ten to fifteen times lower while grain yield of sorghum was two to three times higher than plots planted to local sorghum cultivars.

Objectives, Production and Utilization Constraints

The overall objectives of our research are to further our

understanding of 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. is 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 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, using different 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 (both *ex ante* and *ex post*) of 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 Approach and Project Output

Research Methods

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 chemical signals produced by 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 these 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. In the last four years, 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 is currently in progress 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 plan to develop and test economically feasible and practicable integrated *Striga* control packages for testing on farmers' fields in selected countries in Africa. 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.

Research Findings

Integrated Striga Management (ISM) on Sorghum in Eastern Africa

Striga parasitizes its host plant by attaching to the root, penetrating the vascular tissue and becoming a sink for water, nutrients and photosynthates. Successful parasitism results from a succession of signals released by the host plants and required for *Striga* to germinate, attach to the host roots, and penetrate the host vascular tissue. Several control measures have been suggested to reduce *Striga* infestation but taken individually, none present complete and durable control. Hand weeding practiced yearly, and even with several weeding dur-

ing a cropping season, does not provide a durable control of *Striga*. Hand weeding is also labor intensive competing for a scarce resource at critical parts of the crop season. Chemical inputs such as herbicides, fumigants or germination stimulants that lead to suicidal germination of *Striga* are rarely considered as viable options since these inputs are often unaffordable for subsistence farmers. Crop rotation, catch or trap cropping, as well as mixed-cropping are also not practicable in areas where population pressure has put greater demand on availability of arable land. Use of resistant crop cultivars, perhaps is the only practicable and economically feasible *Striga* control measure.

Considerable progress has been made in identifying good sources of host plant resistance in several crops although the degree of resistance is variable across crops. With the use of genetic resistance, a host plant is grown that lacks the capacity to produce signals required for *Striga* to successfully complete its life cycle. Resistant crop cultivars could also produce inhibitors leading to failed parasitism, a potentially effective control since it both limits damage in the current crop and avoids the build up of *Striga* seed population in the soil. Breeding for durable resistance to *Striga* in sorghum as has been a focus in our group at Purdue University where several improved *Striga* resistant sorghum cultivars have been developed and released for wide cultivation.

A more enhanced control of *Striga* can be achieved by combining two or more individual control approaches synergistically. Yet, there has been limited on-farm research under-

taken to evaluate the benefits of integrating multiple control options against *Striga*. Even with highly resistant crop cultivars, a more enhanced *Striga* control and increased crop productivity can be achieved by synergistic combination of one or more agronomic practices with host plant resistance. Because damage caused by *Striga* is more severe on host crops that are already under abiotic stress caused by soil fertility depletion and moisture shortage, we proposed an Integrated *Striga* Management (ISM) project through our INTSORMIL/Purdue University collaborative program in Eastern Africa. The project targets several countries where *Striga* pressure is severe. The ISM Pilot Project started in 2001 in Ethiopia, and expanded more recently in Eritrea and Tanzania.

The goal of the ISM program is to reduce the ravages of *Striga* on sorghum production where the parasitic weed is endemic. Specific objectives were: 1) to promote a technology package that integrates host plant resistance, soil fertility enhancement, and water conservation measures for control of *Striga* and enhanced crop yield; 2) to establish a functional seed program by promoting seed production as a commercial entity and by developing seed growers' organizations; 3) to increase profitability for farmers involved in the ISM project by promoting new markets and products for sustainable use of their technology package.

Progress of work in Ethiopia is reported in here. The ISM Pilot Project was carried out in four *Striga* endemic regions of Ethiopia: Oromia, Amhara, Tigray, and Southern (Fig. 1). The implementation of the ISM program was coordinated by

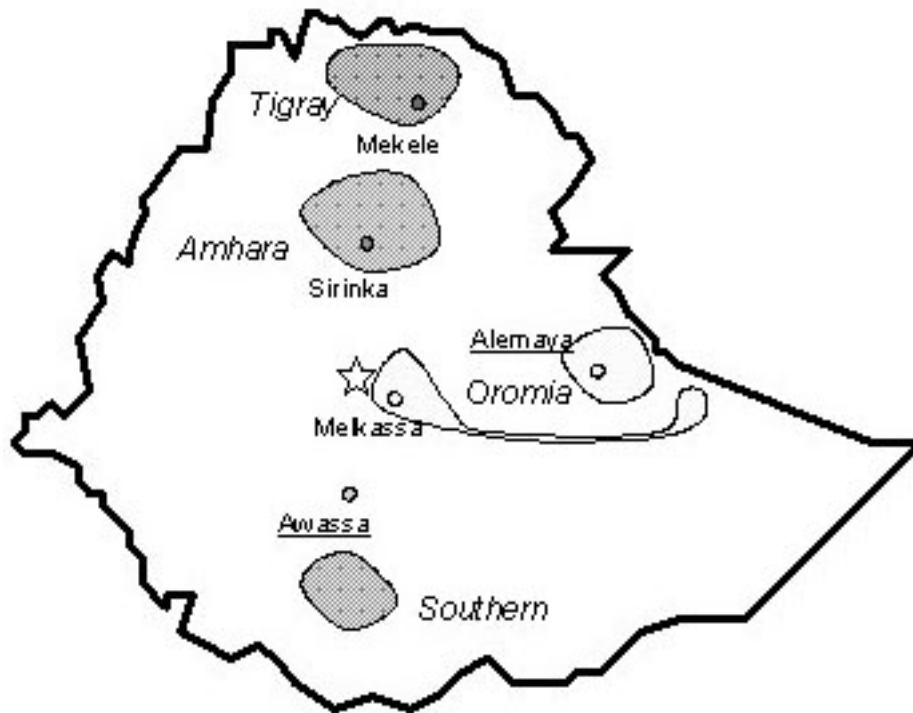


Figure 1. ISM pilot project conducted in four regions of Ethiopia: Tigray, Amhara, Oromia and Southern region (in italic), in 2002 and 2003.

the Ethiopian Agricultural Research Organization (EARO) and in collaboration with Regional Research Centers, higher learning institutions, and regional Bureaus of Agriculture (BoA). Coordination of activities and overall management was provided by Melkassa Agricultural Research Center (MARC) and Alemaya University (AU) for Oromia region; by Sirinka Agricultural Research Center (SARC) for Amhara; Tigray Agricultural Research Institute (TARI) for Tigray, and by Awassa Research Center (ARC) for the Southern region.

Project activities were conducted on farmers' fields that were primarily selected based on their history of heavy *Striga* infestation. The project was designed for one-half hectare plot sizes, though farms of various sizes were included based on land availability. Three sets of activities were carried out: 1) demonstration of the ISM package of host plant resistance, fertilization, and tied ridges; 2) popularization of *Striga* resistant varieties without providing fertilizer inputs; 3) seed production of *Striga* resistant sorghum varieties under as optimal a condition available to the farmer. Farmers were selected and grouped for each of the three activities based on their keenness to participate, apparent knowledge base, and land type and degree of infestation by *Striga* and wild sorghums.

Of eight *Striga* resistant sorghum cultivars released by Purdue University/INTSORMIL in 1995, two selections (P9401 and P9403) were officially recommended for wide cultivation under the local names of "Gubiye" (P9401) and "Abshir" (P9403). Good quality seed of 'Gubiye' and 'Abshir' were produced in large quantities both at Purdue University and Melkassa Agricultural Research Center during the previous crop season and were distributed to farmers selected to participate in the ISM project. Nitrogen fertilizer in the form of urea and diammonium phosphate (DAP) were purchased from the local market and provided to selected participants. Tied ridgers were fabricated in the local industrial area in Nazret from a design provided by the Melkassa Research Station.

The primary technology focus was to demonstrate the benefits of combining host plant resistance with improved agronomic practices of fertility restoration and water conservation. Farmers with fields heavily infested with *Striga* and easily accessible for others to visit were selected to demonstrate the ISM package. These farmers were required to also have fields cultivated with the local landraces and under local practices nearby as control plots. For this activity, farmers were provided with *Striga* resistant sorghum seed, fertilizers (DAP and urea), and with tied ridgers as a strategy for water conservation unless other local practices were available. When needed, farmers were also shown how to row plant the seed, apply fertilizer, and construct and maintain tied-ridges.

Farmers whose fields were heavily infested with *Striga* but not in easy reach were given free seed only so they could test the improved sorghum varieties for local adaptation and

their efficacy on *Striga* control as a popularization of the genetic component of the package. Fertilizer and tied ridgers were not provided to these farmers. Seed of either 'Gubiye' or 'Abshir' were distributed based on potential fit to the environment from prior testing.

The third activity of the ISM pilot project was intended to promote organized seed production and to train local farmers as seed growers. For this activity, farms were selected on the basis of soil type, soil fertility, and isolation from other sorghum fields and wild relatives of the crop. In this case, when available, farm lands free of *Striga* were chosen. If *Striga* was found in the field, special care was taken during post harvest seed processing activities to minimize contamination. Farmers involved as seed growers were advised to adopt use of inputs and improved agronomic practices. Inputs were provided free to seed producers and the use of improved water conservation practices highly recommended. Seed producers were inspected at different times during the season and the quality of their seed crop assessed. Seed crops deemed of acceptable quality were bought back by the project from farmers at an agreed upon premium over the going market price.

In both the 2002 and 2003 crop seasons, the ISM Pilot Project was undertaken in four administrative regions (Fig. 1) and focused on the three primary activities of popularization, demonstration, and seed production. For each of the three primary activities, seed of *Striga* resistant sorghums were provided free to farmers involved in the project. In 2002, about 3.6 tons of seed were distributed to 472 farmers and 6 tons were provided to 1340 farmers in 2003. Of these 6 tons of seeds, half were obtained from on farm seed multiplication activity conducted by participating farmers the previous year. The balance of seed was produced on research farms supervised by project coordinators. Farmers participating in the ISM demonstration and seed multiplication activities also received 13.8 tons of fertilizers in 2002 and 12.6 tons in 2003. Ninety tied ridgers were fabricated and distributed to participating farmers the first year and an additional 12 tied-ridgers were provided during the second year. Seed producers were assured of a premium price for their produce provided that the seed met inspection and quality standards outlined by the project personnel.

Though plots involved in the popularization activities were not monitored and performance data were not recorded by project personnel, oral reports indicated that the results were encouraging in all four regions. Farmers were impressed not only by the high level of resistance to *Striga* manifested by these cultivars, but also by the early maturity, drought tolerance, as well as the good grain quality of the *Striga* resistant varieties. During the two crop seasons, 1337 farmers grew the *Striga* resistant sorghum cultivars with seed provided by project personnel. The number of farmers who received seed of *Striga* resistant cultivars during the second year through informal exchange with neighboring farmers who grew these cultivars the previous year is not known.

Demonstration

During the first year, 145 farmers received the three components (seed, fertilizer, and ridgers) to participate in the demonstration of the ISM package promoted by the project. In the second year, 135 farmers were involved in the demonstration activity. Data on *Striga* count and grain yield recorded on the demonstration plots showed the value of the package in *Striga* control and increased productivity. The results of the demonstration activity were consistent across farms and years. Plots that received the ISM package showed drastically reduced *Striga* count with significant and concomitant increase in grain yield. Grain yields as high as 5.5 tons were recorded in some demonstration plots that received the package. In contrast, many of the local sorghum landraces grown with no input and only the local practice of hand weeding failed totally with no measurable yields recorded.

Seed Production

Emphasis here was in promoting the concept of seed as different from grain. Farmers were instructed in treating seed production differently from grain production, a lesson often difficult for traditional farmers to grasp. Traditional community-based seed production efforts often fail for this reason. Farmers do not like removing outcrosses by rogueing and meeting isolation requirement is often seen as waste of resources. During the first year of the pilot project, 83 farmers were involved in the seed multiplication activity. Of these, 46 successfully produced a total of 26.5 tons of seeds. Grow-out tests showed acceptable levels of contamination. Out of this amount, about eight tons were reported to have been purchased by NGOs, the BoA and the pilot project itself. These seed was then redistributed to 925 farmers including the 477 farmers who received the seed from the project in 2003. It has been reported that a significant quantity of seed produced by farmers who participated in the 2002 seed multiplication activity have been informally distributed to neighbors to further the popularization and diffusion of these cultivars. The exact quantity of seed that has been exchanged informally is not known, however. In the second year, 112 farmers were involved as seed growers.

Training and outreach turned out to be an important aspect of the ISM pilot project program. Project staff at MARC trained about 100 individuals from the Amhara, Oromia and Southern regions. Trainees included Subject Matter Specialists (SMS), Development Agents (DA) from the Bureaus of Agriculture and selected farmers. Regional research centers in Tigray (TARI) and Amhara (SARC) also conducted additional training for their respective staff and selected farmers. In addition, SARC provided college students from Mersa Agricultural and Technical Vocational College with lectures on the biology and control of *Striga*. DAs from different localities of the Southern and Oromia regions participated in a workshop on agronomic practices, sorghum seed production, and *Striga* biology and control. Furthermore, workshops were

also organized at the end of each of the two years to gauge progress and provide a mid-term review of the ISM pilot project with participants from each of the four regions.

Fields days were also organized each year and at each of the regions to extend the technology to other farmers, to create awareness among policy makers, and to demonstrate the benefits of control of *Striga* through the ISM technology package. In 2003, MARC organized two field days involving farmers and DAs from Oromia region in western Hararghe, and in east Hararghe. Two field days were also organized in 2002 and repeated at the end of the 2003 cropping season at Sirinka. Several field days were also organized by TARI at several locations in the region.

Voluntary involvement of NGOs in the ISM program served as a good indication of the success the diffusion of the ISM technology package. During the 2002 crop season, two NGOs; (Kobo Giranna Valley Development Program (KGVDP) and Hope Ethiopia) purchased seed of *Striga* resistant sorghum cultivars produced by the research farm at SARC for distribution to collaborating farmers in the Amhara region. In the Oromia region the Red Cross purchased 1.6 tons of seed from Alemaya University to redistribute to farmers in the Fedis area. Catholic Relief Services purchased an additional 2 tons of *Striga* resistant sorghum seed from farmers involved in the seed multiplication activity in Eastern Hararghe. All of the seed was presumably distributed to farmers in the same region.

Interest in the use of *Striga* resistant sorghum cultivars and the use of the integrated *Striga* management (ISM) package of cultivars, fertilizers, and water conservation practice has increased considerably in Ethiopia. The broad-based involvement and participation of farmers, development agents, research scientists, and government officials has facilitated this success. Popularization of *Striga* resistant sorghum was conducted widely across four regions of Ethiopia. Feedback from farmers and BoA agents were all very positive, recognizing the *Striga* resistance, drought tolerance, and excellent grain quality of the two cultivars ('Gubiye' and 'Abshir') in wide distribution in the country. The demonstration of the ISM technology package showed very dramatic results in *Striga* control as well as significant enhancement of sorghum grain yield. The rationale of the ISM package of improved cultivars, fertilization, and water conservation was greatly appreciated. Increased diffusion of seed of the improved cultivars as well as acceptance of the ISM package were greatly assisted by the planned field days and workshops held at various sites during the project period. In light of the success of the efforts undertaken and recommendation of participants at regional centers, in June 2003 the government of Ethiopia declared the ISM package to be widely practiced as a national campaign in *Striga* endemic areas of the country.

Lack of a functional seed multiplication effort for sorghum in Ethiopia had hampered further diffusion of improved

varieties in spite of the great demand in many areas. The ISM program has demonstrated that good quality seed could be produced by farmers selected to participate as seed growers for multiplying *Striga* resistant sorghum varieties. However the sustainable development of a viable seed multiplication enterprise requires the creation of proper incentives and formulation of a structure and entity that needs to be put in place. Furthermore, a functional seed enterprise depends on farmers' ability to pay for inputs, which in turn depends on the profitability of the practice in the farm community. We are currently working towards developing a cooperative-based community seed enterprise with necessary market outlets for both seed and grain.

Networking Activities

Workshop and Program Reviews

We have been involved in a number of engagements in our *Striga* research and development this past year. Two major programs have been underway in Ethiopia and Eritrea directed at the promotion of an integrated *Striga* management using a mix of technologies, including *Striga* resistant sorghum cultivars, nitrogen fertilization, as well as tied-ridges as a water conservation measure. In Ethiopia, over one thousand demonstration plots have been planted in four regions of the country with very exciting and promising results. Plots planted to the IPM technology yielded consistently higher and up to four times the yield of the untreated farmer-managed plots. Project efforts in Eritrea were similar, but scaled down in number with only one hundred demonstration plots this first year. The second, but very important, objective of the project in both countries focuses on promoting a functional seed multiplication efforts based on sale of good quality seed for a premium price. Keen farmers were identified, trained, and encouraged to engage in seed business. While early results of the quality of seed from these organized multiplication efforts have been good, it is too early to judge if the concept of seed as a business entity has taken hold yet.

A training workshop was held in Ethiopia to kick off the demonstration and seed multiplication activities. In addition, presentations on the *Striga* biotechnology research were made both at the First Sorghum and Millet Improvement Workshop in Nazret, Ethiopia and at the 2002 INTSORMIL PI Conference in Addis Ababa, Ethiopia.

Research Investigator Exchange

We have had individuals from India, Ethiopia, Uganda, Burkina Faso, Eritrea, Mali, Niger, and Kenya visit our *Striga* research facility at Purdue University. In addition, Dr. Hamidou Traore, a Fulbright fellow spent a year at Purdue conducting *Striga* research in our facility. His work focused on identification of sorghum lines with multiple mechanisms of *Striga* resistance.

Germplasm Exchange

Seed of *Striga* resistant sorghum lines have been filled on a request basis. In addition, an International *Striga* Resistant Sorghum Nursery has been organized and distributed to a number of African national programs, who have agreed to collaborate on free will. This past year, the nursery has been sent to Ethiopia, Kenya, Eritrea, Niger, Mali, and Tanzania.

Publications

Refereed Papers

Rich, P.J., C. Grenier and G. Ejeta. 2004. *Striga* resistance in the wild relatives of sorghum. *Crop Sci.* 44:2221-2229.

Conference Proceedings

Kapran, I., C. Grenier, and G. Ejeta. 2004. Introgression of genes for *Striga* resistance into African landraces of sorghum. McKnight Foundation Collaborative Research Program, Workshop on Millet and Sorghum-based Systems in West Africa. Niamey, Niger.

Toure, A., B. Dembele, M. Kayentao, and G. Ejeta. 2004. Genetic improvement of *Striga* resistance in sorghum. McKnight Foundation Collaborative Research Program, Workshop on Millet and Sorghum-based Systems in West Africa. Niamey, Niger.

Grenier, C., A. Deressa, Z. Gutema, G. Gebeyehu, H. Shewayrga, M. Mekuria, A. Belay, T. Tadess, N. Mengistu, O. Oumer, A. Adugna, B. Tsegaw, and G. Ejeta. 2004. Integrated *Striga* Management (ISM) in East Africa. McKnight Foundation Collaborative Research Program, Workshop on Millet and Sorghum-based Systems in West Africa. Niamey, Niger.

Invited Presentations

Ejeta, G. 2004. Understanding key developmental processes in parasitic weeds, a keynote address at the Weed Science Society of America Annual Meetings, June 21-24, Durban, South Africa.

Sustainable Management of Insect Pests

Project WTU 200

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Summary

The PI traveled to Botswana, Mozambique, and South Africa in March to review collaborative research to manage insects and develop IPM approaches for sorghum and discuss Ph.D. programs for prospective students. Texas sorghum lines were resistant, but most Botswana sorghums were damaged by sugarcane aphids at Botswana College of Agriculture. Sorghum sprayed with extracts from local plants were less damaged by aphids, sorghum midge, panicle bugs, and grain mold than the nontreated check in Mali. Stored sorghum grain treated with local plants was less damaged by lesser grain borer in Mali. Six sorghums were resistant to sorghum midge in Niger. Survival and damage by millet head miner and yield of pearl millets were evaluated in Niger. A M.S. student who graduated and returned to Mozambique in August finished evaluating resistance of stored sorghum and found weight losses of 0.8–46.8% at 105 days after infestation with maize weevils. Light and scanning electron microscopy was used to compare seed coats of susceptible versus resistant sorghums, which were twice as thick. Plant differentials were used to determine greenbugs from Kansas and Texas were biotype I. A total of 2,493 sorghums developed by TAM 223 and two commercial seed companies were evaluated for resistance to greenbug biotypes E and I. A M.S. student graduated in August after finishing assessing tritrophic effects and finding greenbugs from resistant sorghum negatively affected lady beetle eggs. A Malian M.S. student began assessing effects of photoperiod on greenbug biotypes. An Ethiopian began Ph.D. studies in fall 2004. A Malian who came to West Texas A&M University to learn English began a M.S. program in 2005.

Research results were presented at entomology, extension, farmer, and sorghum meetings. The PI advised extension personnel and the National Grain Sorghum Producers on management of insects.

Objectives, Production and Utilization Constraints

Objectives

Southern Africa

Support scientists from Botswana, Mozambique, and South Africa with research to evaluate resistance and develop IPM strategies for such sorghum insect pests as sugarcane aphid, stalk borers, termites, and storage beetles. Educate graduate students in entomology and IPM.

West Africa

Support scientists from Mali and Niger with research to develop and transfer strategies, especially non-chemical methods, to manage insect pests and improve yield and income from sorghum and pearl millet. Educate graduate students in entomology and IPM.

United States

Study biology, ecology, and population dynamics of insects so effective management strategies and longer-lasting

plant resistance can be developed. Use standard and molecular techniques to identify biotypes of greenbugs from the field. Assess effects of resistant sorghum on lady beetles feeding on greenbugs from the sorghum. Evaluate sorghum grain for resistance to storage insect pests and use microscopy to relate grain characteristics with resistance. Collaborate with breeders, commercial seed companies, and molecular biologists to develop sorghums for greater yield potential and resistance to major insect pests. Supervise graduate student research and education in entomology and IPM. Advise extension and commodity organizations on managing insects of sorghum. Participate in professional meetings to transfer insect pest management information.

Production Constraints

Southern Africa

Stalk borers; sugarcane aphid, *Melanaphis sacchari*; panicle bugs; termites; and sorghum midge, *Stenodiplosis sorghicola*; infest and reduce yields of sorghum. Sorghum midges can destroy 100% of kernels. Stalk borers bore into the plant and kill the central shoot or break the peduncle. Bugs and associated infection by pathogens reduce yield and quality of grain. Beetles consume and contaminate stored grain.

West Africa

Panicle bugs, sorghum midge, stalk borers, and beetles in stored grain are the most damaging insects of sorghum in West Africa. The worst insects of pearl millet are millet head miner, *Heliocheilus albipunctella*, that reduces grain yield and quality, and *Coniesta ignefusalis* stalk borer.

United States

Major insect pests are greenbug, *Schizaphis graminum*; sorghum midge, panicle bugs and caterpillars, and beetles and moths in stored grain. Monoculture of sorghum disrupts the ecosystem, increases severity of pests, and results in increased production costs and less yield. Insecticides prevent damage and yield loss, but overuse results in increased production costs, disruption of the ecosystem, outbreaks of secondary arthropods, resurgence of the targeted pest, and environmental contamination. Biology, insect-plant interactions, amount of damage, and economic and ecological costs of insecticides need to be understood to manage insects. Biological and cultural management tactics such as use of resistant plants are needed to prevent damage by insect pests.

Research Approach and Project Output

This project emphasizes collaborative research and education. The IPM approach is used to develop strategies to manage insect pests economically, ecologically, and environmentally. 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, resistant varieties, and insecticide when necessary. Information and technology from research is transferred to farmers, extension, and others.

Southern Africa

From March 10-21, 2005, the PI traveled to Botswana, Mozambique, and South Africa to view current and plan collaborative research and discuss Ph.D. programs for prospective students. Mr. Chitio assessed amount of damage storage insects caused in fields of sorghum planted at three dates at Nampula Research Station in Mozambique. In South Africa, Drs. du Plessis and Peterson were assisted with evaluating sorghums for resistance to sugarcane aphid. Dr. van den Berg and students at North West University, South Africa, surveyed 25 sorghum fields each week from boot until kernel maturity and found 35 species of panicle bugs that differed in abundance over time.

In collaborative research at Botswana College of Agriculture, D. C. Munthali, M. Obopile, and M. Kelatlhilwe evaluated for resistance to sugarcane aphid seven sorghums common in Botswana (BSH 1, Mahube, Mmabaitse, Marupantsi, Macia, Phofu and Segalane) and Texas-bred 04CA 10391, 04L 217, and 04L 295 lines. Each genotype was planted in a 7-m row in each of two cages 5.2 m long, 3.0 m wide, and 2.5 m tall. A completely randomized design was used to allocate genotypes to rows. Seeds were planted with 30 cm between plants and 50 cm between rows. Plants 4 weeks after germination in one cage were artificially infested with sugarcane aphids. The aphids were left until grain filling. Plants in the second cage were not infested. Abundance and damage by sugarcane aphids was assessed on 10 randomly selected plants in each row. Damage was scored 1-5, where 1 = 0-20, to 5 = 81-100% leaves dried or drying per plant. Damage scores were similar and significantly less (1.1-2.1) on noninfested than infested plants of all genotypes except Mmabaitse and 04AC 10391 (Table 1). Infested Mahube, Phofu, Macia, Segalane, and Marupantsi were significantly more damaged than other genotypes. Infested 04L 217 and 04L 295 were significantly least damaged (1.1 score). Aphids caused 4.5, 3.3, 3.0, 3.0, and 2.2 fold more damage on infested than noninfested Mahube, Phofu, Segalane, Marupantsi, and Macia, but there were no differences in damage between noninfested and infested 04L 217 or 04L 295.

West Africa

Madani Telly finished English training and began a M.S. program at West Texas A&M University. Tiecoura Traore from Mali started the second year of a M.S. program.

Dr. Yaro Diariso assessed efficacy of local neem and *Calotropis procera* plants against aphids, sorghum midge, bugs,

Table 1. Resistance of southern African and Texas sorghums to sugarcane aphid in Botswana.

Sorghum genotype	Mean damage score (\pm SE) on sorghum plants not infested or infested by sugarcane aphids		Overall variety or line damage score
	Non-infested plants	Infested plants	
Macia	2.1 \pm 0.10 b-e	4.6 \pm 0.16 a	3.4 f
Phofu	1.5 \pm 0.27 c-e	4.9 \pm 0.10 a	3.2 f
Mahube	1.1 \pm 0.10 e	5.0 \pm 0.00 a	3.0 f
Segaolane	1.6 \pm 0.22 c-e	4.5 \pm 0.22 a	3.0 f
Marupantsi	1.4 \pm 0.22 de	4.2 \pm 0.47 a	2.8 f
Mmabaitse	2.0 \pm 0.26 b-e	2.4 \pm 0.16 bc	2.2 g
BSH1	1.1 \pm 0.10 e	2.4 \pm 0.22 bc	1.8 gh
04CA 10391	1.4 \pm 0.16 de	2.8 \pm 0.33 b	2.1 g
04L 295	1.5 \pm 0.17 c-e	1.1 \pm 0.10 e	1.3 h
04L 217	1.3 \pm 0.15 de	1.1 \pm 0.10 e	1.2 h
Overall treatment score	1.5 \pm 0.10	3.3 \pm 1.04	2.4

CV = 0.242

Means followed by the same letter in a column are not significantly different (Tukey, $P < 0.05$).**Table 2. Damage by sorghum midge, panicle bugs, and grain mold and kernel weight of S34 sorghum treated with extracts from local plants.**

Treatments	Damage score by sorghum midge on 10 panicles	Damage score by bugs on 10 panicles	Rate of grain mold on 10 panicles	Weight (g) of grain from center rows
Non-sprayed check	3.1 a	4.0 a	2.2 ab	433.3 a
Neem seed jelly (200 g/l)	3.1 ab	3.2 b	2.2 ab	566.3 c
Neem seed jelly (250 g/l)	2.2 bc	3.0 b	2.1 ab	600.3 c
<i>Calotropis procera</i> juice (10 l/ha)	2.4 a-c	4.1 a	2.5 a	466.7 b
Dursban (5.3 ml/l)	1.5 cd	2.0 c	1.9 c	666.7 cd
Panicles protected by bag	1.0 d	1.0 d	1.0 c	700.0 d
CV	0.3673	0.1416	0.0677	
Probability	0.048	0.00	0.00	
Significance	S	HS	HS	HS

Means followed by the same letter in a column are not significantly different (Duncan range test, $P < 0.05$).

and grain mold on bug-susceptible S34 sorghum in Mali. A Fisher block design with six treatments and three replications was used. Plant juice was filtered and sprayed on seedlings, at the end of flowering, and at hard dough. Insects were counted a day before and week after treatment. After treatment, fewer aphids infested seedlings, especially treated with neem or Dursban. Three times more aphids were in plots before as after treatment with *C. procera*. At hard-dough, numbers of bugs were less than half after plots were sprayed with Dursban or plant extract. Fewest bugs (5) were in plots sprayed with Dursban, followed by 250 (7) or 200 g/l of neem seed jelly (<10). No bugs were on panicles protected by bags. Damage to panicles protected by bags or Dursban did not differ from each other but differed from panicles sprayed with plant extract (Table 2). Scores of damage by sorghum midge, bugs, and grain mold were 3.1, 4.0, and 2.2 on check plants and 1.5, 2.2, and 1.9 on panicles sprayed with Dursban. Panicles sprayed with greater doses of neem seed jelly (200 or 250 g/l) had less mold than panicles sprayed with lesser doses. Weight of protected grain was greater than that of the check or panicles sprayed with *C. procera*. Bags or Dursban better protected panicles from insects and mold than did plant extracts, which were better than the check.

Dr. Yaro Diarisso treated four replications of Malisor 92-1 grain in cotton-cloth bags with powder from *Calotropis procera* or *Cassia nigricans* plants. Percentage of grain damaged by lesser grain borer, *Rhyzopertha dominica*, was assessed each month for 6 months by counting and weighing numbers of damaged and nondamaged grains per treatment and replication. Damage by lesser grain borer started the third month and increased over time (Table 3). Nontreated grain was most damaged. Plant powders were more effective at greater doses. Lesser grain borer destroyed 0.4 and 2.3% of grain the third month to 4.3 and 5.1% the sixth month after treatment with 6 and 3 g, respectively, of plant powder per kilogram. Loss of nontreated grain was 3.4-7.1%. Efficacy of powder decreased and insects increased over time.

Hame Abdou Kadi Kadi and collaborators from INRAN, INTSORMIL, and ICRISAT evaluated 38 single-seed descent sorghum lines and improved varieties and 33 varieties from ICRISAT for resistance to sorghum midge at the Regional Agricultural Research Center at Maradi, Niger. At 25-50% anthesis, spikelets at the top and immature spikelets at the bottom of a panicle were removed so flowering spikelets in the middle were exposed to sorghum midge. A wire frame covered with net 20 cm wide and 40 cm long was placed

Table 3. Sorghum grain loss to lesser grain borer after treatment with powder of *Calotropis procera* or *Cassia nigricans* at Sotuba, Mali, in 2004.

Grain treatment	% loss at months after treatment			
	3 months	4 months	5 months	6 months
<i>Calotropis procera</i> powder (6 g/kg grain)	0.3 c	1.3 c	2.3 c	4.4 b
<i>Calotropis procera</i> powder (3 g/kg grain)	2.2 b	3.2 b	4.4 b	5.0 b
<i>Cassia nigricans</i> powder (6 g/kg grain)	0.5 c	1.7 c	2.7 c	4.2 b
<i>Cassia nigricans</i> powder (3 g/kg grain)	2.4 b	3.3 b	4.5 b	5.1 b
Nontreated check	3.4 a	4.7 a	5.7 a	7.1 a
CV	0.2619	0.1706	0.1274	0.1392
PPDS	0.7641	0.7595	0.7641	1.137
Significance	HS	HS	HS	HS

Means followed by the same letter in a column are not significantly different (Duncan's range test, $P < 0.05$).

Table 4. Mean (\pm SE) percentages of millet head miner stages developed, damage score, and yield for infested pearl millet at Maradi, Niger, in 2004.

Pearl millet variety	% neonate larvae	% medium larvae	% large larvae	% pupae	Damage score	Yield (kg ha ⁻¹)
1A x TMK	14.4 \pm 10.4 a-d	1.3 \pm 0.7 bc	1.3 \pm 0.7 b	1.3 \pm 0.7 ab	2.0 \pm 0.4 ab	1133.4 \pm 34.3 a
ICMV IS 99001	13.8 \pm 8.2 a-d	2.5 \pm 2.5 a-c	0.6 \pm 0.6 b	0.6 \pm 0.6 b	2.3 \pm 0.8 ab	1083.4 \pm 34.4 a
HKP-GMS	28.5 \pm 11.2 ab	11.4 \pm 7.1 a	4.9 \pm 4.9 ab	1.1 \pm 1.1 b	2.1 \pm 0.6 ab	1065.1 \pm 33.2 a
TMK	21.3 \pm 8.0 a-d	2.5 \pm 2.5 a-c	0.0 \pm 0.0 b	0.0 \pm 0.0 b	1.0 \pm 0.0 b	1021.7 \pm 13.9 a
1A x KBH	9.9 \pm 7.7 a-d	1.7 \pm 1.7 a-c	0.9 \pm 0.9 b	1.3 \pm 0.9 b	1.3 \pm 0.3 b	1000.1 \pm 27.3 a
ICMH 2003	26.3 \pm 9.4 ab	0.0 \pm 0.0 c	0.0 \pm 0.0 b	0.0 \pm 0.0 b	1.3 \pm 0.3 b	991.7 \pm 42.9 b
ICMH 2104	18.2 \pm 7.7 a-d	11.0 \pm 6.6 ab	2.9 \pm 2.2 ab	2.9 \pm 2.2 ab	3.3 \pm 0.9 a	950.1 \pm 85.4 b
ICMV IS 92326	7.5 \pm 7.5 b-d	1.9 \pm 1.9 a-c	0.6 \pm 0.6 b	0.6 \pm 0.6 ab	1.5 \pm 0.5 b	900.1 \pm 96.2 b
ZATIB	23.8 \pm 8.5 a-c	3.2 \pm 3.2 a-c	1.8 \pm 1.8 ab	2.3 \pm 2.3 ab	1.4 \pm 0.4 b	850.0 \pm 29.4 b
¼ HK B-78	31.4 \pm 9.2 a	3.8 \pm 2.4 a-c	8.3 \pm 8.3 ab	0.4 \pm 0.4 b	1.8 \pm 0.8 ab	825.0 \pm 25.7 b
SOSAT-C 88	8.6 \pm 3.5 ab	8.5 \pm 4.8 a-c	11.9 \pm 8.9 a	3.5 \pm 2.4 ab	2.5 \pm 0.9 ab	776.7 \pm 59.5 b
ICMV IS 90311	3.4 \pm 3.8 cd	2.5 \pm 2.5 a-c	1.3 \pm 1.3 b	1.3 \pm 1.3 ab	1.5 \pm 0.5 b	741.7 \pm 26.8 b
ANKOUTESS	8.2 \pm 3.6 b-d	8.2 \pm 3.6 a-c	4.8 \pm 2.9 ab	4.8 \pm 2.9 a	2.0 \pm 0.7 ab	733.4 \pm 22.8 b
KBH	0.0 \pm 0.0 d	0.0 \pm 0.0 c	0.0 \pm 0.0 b	0.0 \pm 0.0 b	1.0 \pm 0.0 b	628.4 \pm 83.9 c
Mean	16.8	4.2	2.8	1.4	1.8	907.2
LSD	21.9	9.9	10.6	4.1	1.6	70.6
CV	58.9	67.8	24.7	25.7	64.4	54.2

Means followed by the same letter in a column are not significantly different (Student T-test, $P < 0.05$).

around a panicle. Twenty female sorghum midges collected between 0600 and 0900 were placed in each cage on two consecutive days. Five to 10 panicles of each genotype were infested. Cages were removed 15 days later and damage was scored 1-9, where 1 = <10, to 9 = >81% damaged spikelets. Panicles from the middle row were rated for damage and harvested, and panicle and grain weights determined. Sorghum midges were more abundance and caused more damage to sorghum planted on the first date. Yield losses of 99 SSD F9-1, 99 SSD F9-5, 99 SSD F9-6, 99 SSD F9-19, 99 SSD F9-27, and IRAT 204 were 60.0-70.5%. Yield losses of 99 F9-31, ICSV 745, and 99 F9-37 were 7.4, 9.3, and 10.6%. Yields were 7.6, 12.2, 14.2, and 15.2% less for ICSV 745, 99 F9-17, 99 F9-35, and 99 F9-29 planted on the second date. For ICRISAT sorghum, the greatest damage score was 7.0 and yield loss 75.6% for ICSV 93071. Damage was 2.5 for Mota Maradi, IRAT 204, and ICSV 90001, with yield losses of 33.5, 54.2, and 57.6%. Yield losses were 2.1, 6.4, and 13.5% for ICSV 90013, ICSV 90011, and 99 SSD F9-35, while damage

was 1.0. DJ 6514, ICSV 197, ICSV 745, ICSV 93077, 99 SSD F9-35, and ICSV 90011-13 were resistant to sorghum midge, and DJ 6514 and ICSV 90011 were very infested but yielded only 20-30% less.

Hame Abdou Kadi Kadi and collaborators evaluated improved pearl millet varieties and ICRISAT-Niamey millet genotypes at the Regional Agricultural Research Center at Maradi, Niger. The experiment was a completely randomized block with four replications. Each 15-m² sub-plot had five rows 5 m long, with 1 m between rows and 1 m between hills. Spikes were covered at boot. A cage 70-90 cm long x 30 cm diameter from a wire frame covered by cotton mesh was placed over a spike at 1/3 exsertion (5-10 cm). A sticker with 40 millet head miner eggs from a farmer's field was pinned to a spike 2-3 days later. Four spikes of each genotype per replication were infested. Five days after infestation, spikes were checked for eggs. Survival of millet head miner life stages (different sized larvae, pre-pupae, and pupae) was monitored,

and damage was scored 1-9. At maturity, spikes were cut and damage was assessed. Most (31.4%) and no millet head miner eggs on $\frac{3}{4}$ HK B-78 and KBH hatched (Table 4). Most medium larvae (11.4 and 11.0) were on HKP-GMS and ICMH 2104. Most large larvae (11.9%) developed on SOSAT-C88. No medium or large larvae developed on ICMH 2003 or KBH. Most pupae (4.8%) were on ANKOUTESS. No pupae developed on TMK, ICMH 2003, or KBH. Damage to ICMH 2104 was 3.3. Damage to TMK and KBH was 1.0. 1A x TMK, ICMV IS 99001, HKP-GMS, TMK, and 1A x KBH yielded most (1,133-1,0001 kg ha⁻¹). KBH yielded least (628 kg ha⁻¹). Hybrids ICMH 2003 and ICMH 2104, with 1.3 and 3.3 damage and 991.7 and 950.1 kg ha⁻¹ yields, had tolerance to millet head miner. Improved varieties ICMV IS 99001, HKP-GMS, and TMK had some tolerance. Flowering of checks ZATIB and $\frac{3}{4}$ HK B-78 escaped millet head miner.

United States

Five hundred twelve and 1,625 sorghums were evaluated for resistance to greenbug biotypes I and E for TAM223. Two hundred twenty-one and 135 sorghum lines developed by Pioneer Hi-Bred International, Inc. and Milo Genetics were evaluated for resistance to biotype I.

Standard sorghum and wheat differentials were used to determine that greenbugs from Barton, Ellis, Kiowa, McPherson, Pratt, and Rush Counties, Kansas, were biotype I. Greenbugs from Archer, Baylor, Clay, Hale, Hardeman, Hidalgo, Jack, Moore, Randall, Swisher, Wichita, and Young Counties, Texas, were biotype I. In collaboration with Dr. Zhu-Salzman, molecular markers that differentiate greenbug biotypes are being used to identify and verify the biotypes of greenbugs collected from Kansas and Texas.

Master's student Tiecoura Traore began assessing effects of photoperiod on pre-reproductive period, fecundity, and longevity of greenbug biotypes E and I to understand differences between biotypes and determine best conditions to use when evaluating sorghums for resistance.

Master's student Murali Ayyanath finished assessing effects of biotype I greenbugs from resistant PI550607 versus susceptible RTx430 sorghum on the life cycle of convergent lady beetles at 23 and 30°C and photoperiod of 14:10 light:dark hours. Lady beetle larvae and adults consumed similar numbers of greenbugs from resistant or susceptible sorghum. Larvae ate 1.7 and adults ate 2.0-3.1 times more greenbugs at 23 than 30°C. Each adult ate 17,124 and 16,325 greenbugs from resistant and susceptible sorghum at 23°C. Almost 8.5 times more eggs were produced per lady beetle fed greenbugs from susceptible sorghum at 23°C (2,893) than by lady beetles fed greenbugs from resistant sorghum at 30°C (342). Numbers of eggs were greatest from lady beetles fed greenbugs from susceptible sorghum. At 23°C, 91.0% hatched of 2,893 eggs by lady beetles fed greenbugs from susceptible

sorghum. Only 21.8-39.4% of eggs hatched from other pairs of beetles paired as adults in four ways based on the sorghum source of greenbugs from which the lady beetles had been fed. Greenbugs from resistant sorghum negatively affected the number and viability of eggs produced by convergent lady beetles.

Master's student Fernando Chitio finished evaluating resistance of 20 genotypes of stored sorghum grain to maize weevil, *Sitophilus zeamais*, graduated, and returned to Mozambique. Five newly emerged maize weevils were put with 5.0 g of grain in each of 10 vials for 105 days. Twenty sorghums were used. Each day, each grain in the 10 vials of one kind of sorghum was evaluated for damage on a scale of 1-5, numbers of live and dead weevil adults were counted, and grain in each vial was weighed. Fewest maize weevils emerged from Sima, Macia, and Sureño - 1.7, 2.8, and 3.1 per gram of grain. Most weevils emerged from CE151 (14.2 per gram). Weight loss of grain of Sureño, Sima, Macia, and Malisor-84-7-167 was 0.8-6.6% at 105 days after infestation. Weight loss of ATx631, CE151, and SC630-11E11 was 37.2-46.8% at 105 days after infestation. Sureño was most resistant, while SC630-11E11 was least resistant. Grain weight loss was correlated to the number of maize weevils produced per gram and score of damage to grain, but not to grain size, hardness, or protein.

Michael Pendleton and E. Ann Ellis at the Microscopy and Imaging Center at Texas A&M University used light and scanning electron microscopy to relate morphology of seed coats of the 20 sorghum genotypes with resistance to maize weevils. A razor blade and hammer were used to split dry sorghum grains. The split grains were exposed to osmium vapor and coated with gold-palladium using a Hummer sputter coater. The cross-section of the seed coat of each genotype was observed by scanning electron microscopy using a JEOL JSM 6400 at 15 KeV, 12-mm working distance, and magnifications of 500-2000x. Pieces of seed coat were dried, fixed, and embedded in epoxy resin and sectioned for observation by using a Zeiss Axiophot compound light microscope at bright field magnifications of 100-600x. Grains of the different genotypes were different in cross-section, and the difference was related to resistance to maize weevil. The seed coat of the most resistant (Sureño) was twice as thick as that of susceptible SC630-11E11.

Networking Activities

Workshops

The PI presented two posters at the 24th Biennial Grain Sorghum Research and Utilization Conference, Reno, NV, 19-22 February 2005; two posters at the 53rd Annual Meeting of the Southwestern Branch of the Entomological Society of America and Annual Meeting of the Society of Southwestern Entomologists, Albuquerque, NM, 28 February - 3 March

2005; attended the 52nd Annual Meeting of the Entomological Society of America, Salt Lake City, UT, 14-17 November 2004; and gave four presentations at the Entomology Science Conference, College Station, TX, 26-28 October 2004. The PI and M.S. students presented four posters at the Summer Crops Field Day, Bushland, TX, 31 August 2004; and the PI presented information on biotyping greenbugs at the Northern Panhandle Field Day, Stratford, TX, 25 May 2005.

Research Investigator Exchanges

From March 10-21, 2005, the PI discussed and reviewed research with scientists from Botswana College of Agriculture, INIA in Mozambique, and ARC and North West University in South Africa and met with prospective Ph.D. students. Research Information Exchange

The PI advised extension, National Grain Sorghum Producers, and commercial seed companies on management of sorghum insect pests. Two hundred twenty-one and 135 sorghums developed for resistance to biotype I greenbug were evaluated for Pioneer Hi-Bred International, Inc. and Milo Genetics. The PI assisted Dr. John Burd, USDA-ARS, Stillwater, OK, with collecting greenbugs from wild and cultivated grasses. Reference materials and/or supplies were provided to Mr. Chitio in Mozambique, Dr. Munthali in Botswana, Dr. van den Berg in South Africa, and Dr. Yaro Diarisso in Mali.

Publications and Presentations

Publications

- Chitio, F.M., B.B. Pendleton, and G.J. Michels, Jr. 2004. Resistance of stored sorghum grain to maize weevil (Coleoptera: Curculionidae). *International Sorghum and Millets Newsletter* 45:35-36.
- Sambaraju, K.R., and B.B. Pendleton. 2005. Fitness of greenbug (Homoptera: Aphididae) on wild and cultivated grasses. *Southwestern Entomologist*.
- Ayyanath, M.M., B.B. Pendleton, and G.J. Michels, Jr. 2004. Effect of greenbugs (Homoptera: Aphididae) from resistant sorghum on the life cycle of convergent lady beetle (Coleoptera: Coccinellidae). Pp. 108-111. In *Proceedings of the Summer Crops Field Day*. Bushland, TX. August 31, 2004.
- Bowling, R.A., R. Bowling, B. Pendleton, and G.J. Michels, Jr. 2004. Alternatives to organophosphates and carbamates for managing aphids in wheat and sorghum. Pp. 81-83. In *Proceedings of the Summer Crops Field Day*. Bushland, TX. August 31, 2004.
- Chitio, F.M., B.B. Pendleton, and G.J. Michels, Jr. 2004. Resistance of stored sorghum grain to maize weevil (Coleoptera: Curculionidae). Pp. 129-131. In *Proceedings of the Summer Crops Field Day*. Bushland, TX. August 31, 2004.
- Palousek Copeland, A.L., B.B. Pendleton, and G.J. Michels, Jr. 2004. Fecundity and longevity of greenbug (Homoptera:

- Aphididae) affected by biotype and temperature. Pp. 132-133. In *Proceedings of the Summer Crops Field Day*. Bushland, TX. August 31, 2004.
- Sambaraju, K.R., and B.B. Pendleton. 2004. Fecundity and longevity of greenbug on wild and cultivated grasses. Pp. 140-143. In *Proceedings of the Summer Crops Field Day*. Bushland, TX. August 31, 2004.
- Veerabomma, S., B.B. Pendleton, and G.J. Michels, Jr. 2004. Effects of soil water and nitrogen on fitness of greenbug (Homoptera: Aphididae) on sorghum. Pp. 144-146. In *Proceedings of the Summer Crops Field Day*. Bushland, TX. August 31, 2004.
- Almas, L.K., W.A. Colette, and B.B. Pendleton. 2005. Grain sorghum production and profit optimization with alternative water management strategies in the Texas Panhandle. P. 21. In J. A. Dahlberg, B. Bean, S. Goldman, B. Rooney, S. Bean, J. Burd, T. Isakiet, and R. Kochenower [eds.], *Proceedings of the 24th Biennial Grain Sorghum Research and Utilization Conference*. Reno, NV. February 19-22, 2005.
- Bowling, R.A., B. Pendleton, R. Bowling, and G. Michels. 2005. Alternatives to organophosphates and carbamates for managing aphids in wheat and sorghum. Pp. 40-41. In *Proceedings of the 53rd Annual Meeting of the Southwestern Branch of the Entomological Society of America and Annual Meeting of the Society of Southwestern Entomologists*. Albuquerque, NM. February 28 – March 3, 2005.
- Pendleton, M., and B.B. Pendleton. 2005. Comparison of morphology of sorghum grain to resistance to maize weevil (Coleoptera: Curculionidae). P. 37. In *Proceedings of the 53rd Annual Meeting of the Southwestern Branch of the Entomological Society of America and Annual Meeting of the Society of Southwestern Entomologists*. Albuquerque, NM. February 28 – March 3, 2005.
- Pendleton, M.W., E.A. Ellis, F.M. Chitio, and B.B. Pendleton. 2005. Comparison of morphology of sorghum grain to resistance to maize weevil. Pp. 76-78. In J. A. Dahlberg, B. Bean, S. Goldman, B. Rooney, S. Bean, J. Burd, T. Isakiet, and R. Kochenower [eds.], *Proceedings of the 24th Biennial Grain Sorghum Research and Utilization Conference*. Reno, NV. February 19-22, 2005.
- Ayyanath, M.M. 2004. Effect of greenbugs (Homoptera: Aphididae) from resistant sorghum on the life cycle of convergent lady beetle (Coleoptera: Coccinellidae). M.S. thesis. West Texas A&M University, Canyon, TX.
- Chitio, F.M. 2004. Resistance of stored cowpea to cowpea weevil (Coleoptera: Bruchidae) and sorghum to maize weevil (Coleoptera: Curculionidae). M.S. thesis. West Texas A&M University, Canyon, TX.

Presentations

- Summer Crops Field Day, Bushland, TX, August 31, 2004: Alternatives to organophosphates and carbamates for managing aphids in wheat and sorghum by Roxanne Bowling, Robert Bowling, Bonnie Pendleton, and G. J. Michels, Jr.
- Fecundity and longevity of greenbug, *Schizaphis graminum*, affected by biotype and temperature by Anastasia L.

- Palousek, Bonnie B. Pendleton, Bobby A. Stewart, G. J. Michels, Jr., and Charles M. Rush
- Fecundity and longevity of greenbug on wild and cultivated grasses by Kishan R. Sambaraju and Bonnie B. Pendleton
- Effects of different amounts of soil nitrogen and water on greenbug fecundity and longevity on sorghum by Suresh Veerabomma, Bonnie B. Pendleton, Bob A. Stewart, Clay A. Robinson, and G. J. Michels, Jr.
- Randall County Ag Day, Canyon, TX, October 7, 2004: Fecundity and longevity of greenbug, *Schizaphis graminum*, affected by biotype and temperature by Anastasia L. Palousek, Bonnie B. Pendleton, Bobby A. Stewart, G. J. Michels, Jr., and Charles M. Rush
- Effects of different amounts of soil nitrogen and water on greenbug fecundity and longevity on sorghum by Suresh Veerabomma, Bonnie B. Pendleton, Bob A. Stewart, Clay A. Robinson, and G. J. Michels, Jr.
- Entomology Science Conference, College Station, TX, October 26-28, 2004: Alternative management strategies for greenbugs by Roxanne Bowling, Robert Bowling, Bonnie Pendleton, and Jerry Michels
- Tritrophic interaction of resistant sorghum, greenbug, and convergent lady beetle by Bonnie Pendleton and Murali MohanAyyanath
- Resistance of stored sorghum to maize weevil by Bonnie Pendleton and Fernando Chitio
- Greenbug biotypes by Bonnie Pendleton and Tiecoura Traore
- North American Grain Congress, Reno, NV, February 19-22, 2005: Comparison of morphology of sorghum grain to resistance to maize weevil by M. W. Pendleton, E. A. Ellis, F. M. Chitio, and B. B. Pendleton
- Grain sorghum production and profit optimization with alternative water management strategies in the Texas Panhandle by L. K. Almas, W. A. Colette, and B. B. Pendleton
- 53rd Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists, Albuquerque, NM, February 28 - March 3, 2005: Alternatives to organophosphates and carbamates for managing aphids in wheat and sorghum by Roxanne Bowling, Bonnie Pendleton, Robert Bowling, and Jerry Michels
- Comparison of morphology of sorghum grain to resistance to maize weevil (Coleoptera: Curculionidae) by Michael W. Pendleton, E. Ann Ellis, Fernando M. Chitio, and Bonnie B. Pendleton
- Invited seminar, Department of Entomology, Texas A&M University, College Station, TX, April 14, 2005: Bioecology and management of greenbug. Northern Panhandle Field Day, Stratford, TX, May 25, 2005 – Biotyping greenbugs.

