Sustainable Plant Protection Systems

Agroecology and Biotechnology of Stalk Rot Pathogens of Sorghum and Millet

Project KSU 210
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Summary

Zearalenone is an important mycotoxin produced by some *Fusarium* species, but these species are not commonly associated with either sorghum or millet as they are most common under cool, wet conditions. Yet reports of zearalenone can be relatively common, even though species known to produce the toxin cannot be recovered from the associated grain sample. With my collaborators we examined a number of the traditional and newly described *Fusarium* species from sorghum and millet in Africa. When analyzed by thin layer chromatography, a spot that comigrates with zearalenone often can be identified. This compound also has a multi-peak UV spectrum similar to that of zearalenone, but the two compounds can be distinguished with reversed-phase HPLC. Based on mass spectral and NMR analyses, this compound was identified as 8-O-methylbostrycoidin (8-OMB), a previously identified *Fusarium* secondary metabolite of unknown, but presumably minor, mycotoxicity. Use of the correct chemical technology to identify this compound in sorghum and millet grain should permit freer trade of sorghum and millet grain and should increase the perception of sorghum and millet as wholesome foods that are relatively free of mycotoxin contaminants.

One approach to disease control is by breeding for disease resistance, but biological control is a desirable, sustainable alternative or supplement to the breeding process. Many fungi harbor dsRNA molecules, which can confer phenotypes such as hypovirulence or altered colony morphology and pigmentation. In some species of *Fusarium*, dsRNA molecules are found in every strain examined. We examined 100 *F. proliferatum* (the most widespread of the *Fusarium* species infecting sorghum) isolates, but found only four that carried dsRNAs. None of these strains had a visibly unusual phenotype. None of these dsRNAs were transmitted through sexual crosses in which the dsRNA-containing strain served as the male parent. Each isolate harbored a distinct set of dsRNAs, which ranged in size from approximately 700 bp to approximately 3,100 bp. Multiple bands were observed in three strains, and these sets of dsRNAs were transmitted as sets at a high frequency (e" 97%) to vegetatively produced microconidia. These dsRNAs are probably localized in the mitochondria, as they co-purified with mitochondria and were protected against ribonuclease A digestion in mitochondrial preparations. The fourth strain had only a single dsRNA band that was only rarely (d" 3%) transmitted to the microconidia. These data suggest that dsRNAs are unlikely to be useful as biological controls for *Fusarium* infestations of sorghum and millet.

Objectives, Production and Utilization Constraints

- 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 and Colletotrichum from Mali, Tanzania, India, 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.
- Edit Proceedings of 2000 Global Sorghum and Millet Pathology Conference.

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. Grain errantly identified as containing zearalenone can be prevented from entering international trade channels.

Fusarium spp. associated with sorghum and millet do 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. Biological control is an alternative to or supplement for resistance breeding. A common form of biological control in fungi is to use double strand RNA (dsRNA) molecules that can infect strains and behave as viruses to reduce the pathogenicity of the fungal population. In successful instances, e.g. the control of Chestnut blight caused by *Cryphonectria parasitica* in Europe, the control is sustainable in that it results in a permanent change in the fungal population and is stably maintained by dsRNA replication and transmission with the fungus on a year-in year-out basis.

Research Approach and Project Output

Research Methods

Strains and culturing techniques. We examined 100 strains of *F. proliferatum* from both symptomatic and asymptomatic plants collected across the United States for the presence of dsRNA molecules. We evaluated strains of *F. acutatum, F. andiyazi, F. begoniae, F. brevicatenulatum, F. bulbicola, F. circinatum, F. concentricum, F. denticulatum, F. guttiforme, F. konzum, F. lactis, F. nisikadoi, F. phyllophilum, F. pseudoanthophilum, F. pseudocircinatum, F. pseudonygamai,* and *F. ramigenum* for the ability to produce zearalenone and its analog.

Strains were preserved as spore suspensions in 15% glycerol and frozen at -70ºC. Vegetative cultures were grown on minimal medium (7) solidified with 2% agar in slants or petri dishes, or as liquid cultures in 125 ml Erlenmeyer flasks. Incubations on solid media were at 25Ú C under a 12 h light-12 h darkness diurnal cycle. Nucleic acids were extracted from young, rapidly growing liquid cultures. Vegetative growth measurements were made in race tubes containing either minimal or complete medium. Sexual crosses were made on carrot agar with standard tester strains from the Fungal Genetics Stock Center (University of Kansas Medical School, Kansas City, Kansas) serving as the female parents. Cultures for toxin production were grown on cracked corn.

TLC, HPLC, GC/MS, and NMR. Compounds were extracted from cracked corn cultures with acetonitrile: water (75:25) and filtered to clarify. TLC (Thin Layer Chromatography) plates were developed with chloroform: methanol (98:2), and spots identified following irradiation with UV (ultraviolet) light or treatment with concentrated H_2SO_4. Zearalenone co-eluting bands were scratched off the plates into acetonitrile: water (60:40), filtered to clarify, and the filtrate concentrated with a rotary evaporator. The compound was detected by HPLC (High Performance Liquid Chromatography) at a wavelength of 235 nm, and the eluate fraction(s) of interest were pooled and reconcentrated several times by rotary evaporation before being used for structural determination analyses. Structural analyses were made with IR (Infrared spectrometry), GC/MS (Gas Chromatography/Mass Spectrometry), NMR (Nuclear Magnetic Resonance), and ESIMS (Electrospray Ionization Mass Spectrometry) using the HPLC purified compound.

Toxicity assays. Toxicity of the zearalenone analog was evaluated in brine shrimp bioassays and in the commercially available Microtox assay. The Microtox assay measures reductions in bacterial bioluminescence as evidence of toxicity, and returns EC_{50} values, i.e. the concentration of the compound that reduces bioluminescence by 50%. Brine shrimp are cultured for 24 hours in 24-well microtier dishes containing one of 13 levels of the test compound (between 0.5 and 300 ìg/ml). Dead shrimp are counted microscopically, and then the living shrimp are killed by the addition of acetone, and the total number of shrimp in each well is counted.

Mitochondria and nucleic acid isolation. Mycelia were ground to a powder in liquid nitrogen in a mortar with buffer and â-mercaptoethanol and extracted with phenol-chloroform-isoamyl alcohol. Nucleic acids were precipitated with sodium acetate and ethanol, pelleted by centrifugation, and resuspended in sterile distilled water. Single-stranded nucleic acids were precipitated by the addition of ½ volume of 7 M LiCl, incubated on ice for 6 h, pelleted by centrifugation and discarded. Double-stranded nucleic acids were precipitated from the supernatant with ethanol at -20ÚC, pelleted by centrifugation, and resuspended in sterile distilled water. The dsDNA in each sample was digested with RNase-free DNase. The remaining dsRNAs were separated on 1.0% agarose gels and stained with

ethidium bromide. The sizes of dsRNAs were estimated by comparison with dsDNA molecular weight markers.

Mitochondria and mitochondrial nucleic acids were isolated from mycelia ground to a powder in liquid nitrogen with buffer. Mycelial debris and nuclei were removed in two slow speed centrifugations (1,000 × *g* for 10 min), and the mitochondria collected in two somewhat faster centrifugations (3,000 × *g* for 10 min), and pellets from these centrifugation steps pooled. Mitochondrial nucleic acids were extracted, purified, and evaluated as described above for total cellular nucleic acids. To determine if dsRNAs were located inside the mitochondria, ribonuclease A was added to pelleted mitochondria before they were disrupted. After incubation for one hour on ice, the reaction was stopped with the addition of Superase-In ribonuclease inhibitor and the incubation continued for another 10 minutes before the nucleic acids were extracted, purified and evaluated as described above.

Research Findings

Zearalenone analog. Seven of the strains tested produced a compound with identical migration to zearalenone on the TLC plates. In all cases this compound eluted two minutes before zearalenone on a C_{18}-HPLC column. The analog also has an absorption peak at 500 nm that is not found for zearalenone. There was no evidence for zearalenone production by any of these cultures.

The zearalenone analog was found to have an elemental composition of $C_{16}H_{13}O_5N$ and a molecular weight of 299. It contained aromatic C–H, aliphatic C–H, C=O, CH_2, C–OH, and C=CH_2 based on IR analyses, and the presence of these components was confirmed in the NMR, GC/MS, and ESIMS analyses. Based on these analyses, the zearalenone analog was identified as 8-OMB (Fig. 1). 8-OMB was first isolated from *Fusarium verticillioides* by Prof. Marasas's group in South Africa in 1979, as part of a search for the cause of equine leukoencephalomalacia; a search that culminated in the discovery of the fumonisin class of mycotoxins some 10 years later. This group did not do any TLC analyses, however, and thus failed to detect the similarity between 8-OMB and zearalenone under these simple analytical conditions. Strains of both *F. andiyazi*, which is common on sorghum, and *F. pseudonygamai*, which is common on millet, produced detectable levels of this compound. Thus, reports of zearalenone in sorghum and millet grain based on TLC analyses are likely to be false positives in which 8-OMB was present instead. For surety, reports of zearalenone in both sorghum and millet should be accompanied by a mycological analysis in which strains from at least one of the *Fusarium* species that are known to produce zearalenone was recovered.

8-OMB was moderately toxic to brine shrimp, with a LD_{50} of 38 ìg/ml. In the Microtox system, the EC_{50} of this compound was 59 ìg/ml and the EC_{20} 21 ìg/ml, again indicating moderate toxicity. 8-OMB was more toxic in the Microtox system than either zearalenone (13 ìg/ml) or some other common mycotoxins, e.g. patulin (7 ìg/ml), ochratoxin A (18 ìg/ml), penicillic acid (15 ìg/ml), and aflatoxin B_1 (23 ìg/ml). Toxicity of 8-OMB to vertebrates and other higher animals is unknown, but may be worthy of further study.

DsRNAs for biological control. Four of the 100 *F. proliferatum* isolates, D-591, D-599, D-720, and D-890, contained double-stranded nucleic acids that were susceptible to ribonuclease and resistant deoxyribonuclease establishing that these low molecular weight, double-stranded elements were dsRNA (Fig. 2). Isolate D-591 contained at least four dsRNAs (approximately 3,100, 3,000, 2,700, and 700 bp), isolate D-

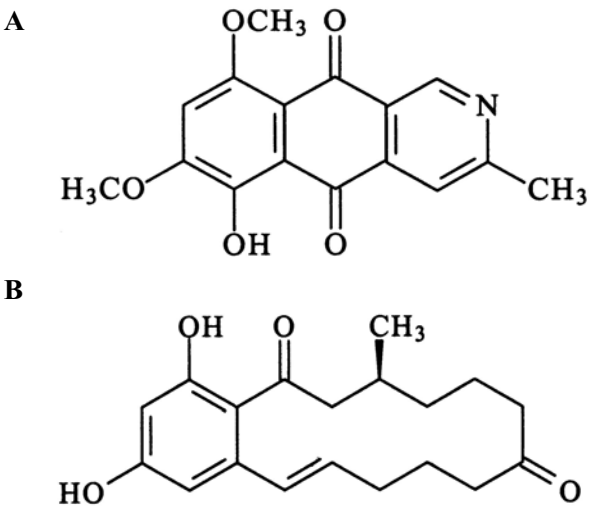

Figure 1. Chemical structures of 8-O-methylbostrycoidin (8-OMB) (A) and zearalenone (B)

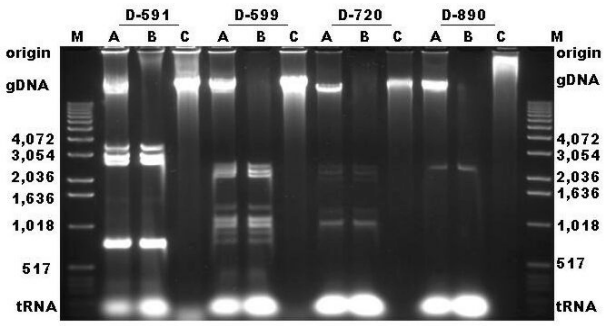

Figure 2. Nuclease digestions of total nucleic acids extracted from *F. proliferatum* strains. Strain numbers are indicated above the gel, "A" lanes are untreated total nucleic acids, "B" lanes are DNase-digested nucleic acids, and "C" lanes are RNase-digested nucleic acids. Positions of the gel origin, fungal genomic DNA (gDNA), transfer RNA (tRNA), and base-pair lengths of dsDNA molecular weight standards ("M" lanes) are indicated at the sides of the gel.

599 contained at least eight (approximately 2,400, 2,100, 2,050, 1,500, 1,050, 1,000, 800, and 700 bp), and isolate D-720 contained at least seven (approximately 2,200, 2,000, 1,900, 1,200, 1,100, 1,000, and 950 bp). Isolate D-890 contained at least one dsRNA of approximately 2,200 bp. Isogenic lines were made for D-599, D-720 and D-890 that lacked the dsRNA(s) by chance through asexual segregation during condial spore production. The phenotypes and growth rates of the dsRNA-containing strains and the dsRNA-free strains derived from them were not significantly different on either complete or minimal medium, as determined by "race-tube" assays.

Mitochondria were prepared from an isolate that lacked dsRNAs, D-599-11 (the final two digits indicate a single-conidiospore culture of the original parent). An aliquot of the preparation was "spiked" with dsRNAs extracted from isolate D-591-25 and treated with ribonuclease A to demonstrate that ribonuclease A was active enough to degrade dsRNAs outside the mitochondria (Fig. 3A). Mitochondria from isolates D-591, D-599, and D-720 contained dsRNAs that were not digested by ribonuclease A (Fig. 3), while the mitochondria from isolate D-890 did not contain dsRNA. Thus, the multiple subunit dsRNAs all are located in the mitochondria, and the presumptive single subunit dsRNA from strain D-890 has a cytoplasmic origin. These dsRNA-carrying *F. proliferatum* strains join strains of *Ophiostoma novo-ulmi*, *Rhizoctonia solani*, and *Cryphonectria parasitica* as representatives of the only four fungi known to carry mitochondrial viruses. Depending on the functionality of the dsRNAs in *F. proliferatum*, it is possible that these dsRNAs belong to a previously undescribed virus genus. This determination, however, requires the subcloning and sequencing of all of the dsRNAs from each of these strains.

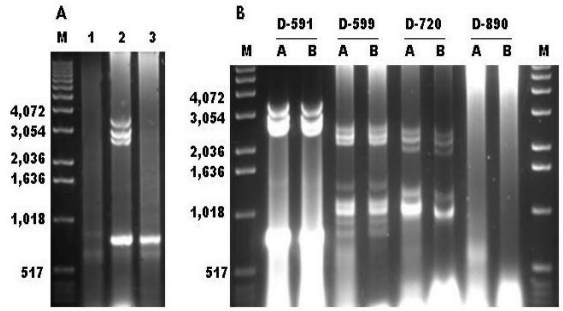

Figure 3. RNase protection of dsRNAs in mitochondria. A. Lane 1, mitochondrial nucleic acids from D-720-29; lane 2, mitochondrial nucleic acids from D-720-29 "spiked" with dsRNAs from D-591; lane 3, mitochondrial nucleic acids from D-720-29 "spiked" with dsRNAs from D-591 and treated with RNase A. Base-pair lengths of dsDNA molecular weight standards (lane "M") are indicated to the side of the gel. B. Strain numbers are indicated above the gel, "A" lanes are dsRNAs from untreated aliquots of mitochondria, and "B" lanes are dsRNAs from RNase A-treated aliquots of mitochondria. Base-pair lengths of dsDNA molecular weight standards (lanes "M") are indicated to the side of the gel.

Sixty single-conidiospore cultures of each of the four isolates were analyzed for vegetative transmission of the dsRNAs. All of the single-spore cultures of isolate D-591 contained all of the dsRNAs, although the dsRNA elements of several of the cultures had different relative staining intensities in ethidium-stained agarose gels. Only 3% (2/60) of the single-spore cultures of isolates D-599 and D-720 lacked dsRNAs, while 97% (58/60) of the single-spore cultures of isolate D-890 lacked dsRNA.

In sexual crosses, the dsRNA-containing isolates were female-sterile, preventing a direct test of the expected cytoplasmic heritability of the dsRNA molecules. Female sterility could not be directly attributed to the dsRNA molecules, however, since vegetative derivatives of dsRNA-containing cultures that contained no detectable dsRNAs also were female sterile. None of the dsRNAs were transmitted through crosses in which a dsRNA strain served as the male parent. Thus, there is no evidence to suggest that these dsRNA molecules can be successfully transmitted through the sexual cycle. How the mycovirus in D-890 is maintained under field conditions is unclear as it appears to be transmitted very poorly through vegetative reproduction, and not at all through sexual reproduction.

Mycoviruses of pathogenic fungi are of interest because some of them alter the virulence of their host, and may be useful as biological control agents. The most intensively studied case of hypovirulence is induced by *Cryphonectria hypovirus 1* (CHV1) in *C. parasitica*, the causal agent of chestnut blight. Several of the dsRNAs in *O. novo-ulmi*, the causal agent of Dutch Elm Disease, decrease virulence, and like the dsRNAs reported here, are mitochondrial. Mycovirus-infected strains of *Fusarium graminearum* with decreased virulence on wheat also are known, and may be useful as a biological control. The mycoviruses from *F. proliferatum* are unlikely to be useful as biological control agents since they do not obviously affect the phenotype of strains carrying them, although this possibility has not yet been strictly disproven. Freshly recovered isolates might be more likely to harbor dsRNAs than isolates in my collection because we routinely select for cultures that grow stably and robustly, which could eliminate many of the virus-carrying isolates.

Networking Activities

Editorial and Committee Service (2002)

- Editor of *Applied and Environmental Microbiology*
- Member of the International Society for Plant Pathology, *Fusarium* Committee

Research Investigator Exchanges

Dr. Leslie made the following international scientific exchange visits (2002)

- Malaysia – January 19-25
- Australia – January 26 – August 20
- Egypt – July 3 – August 3
- South Africa – September 22 – October 11
- Ethiopia – November 9-23
- Kenya – November 24-25

Seminar, Workshop & Invited Meeting Presentations (2002)

- Participated in *Fusarium* Laboratory Workshop in Sydney, Australia from June 23-28; 41 participants and five instructors from nine countries
- Editor for Proceedings of Sorghum/Millet pathology conference in Guanjuato, Mexico
- California Academy of Sciences, San Francisco, California – 1/02.
- Department of Biological Sciences, Stanford University, Stanford, California – 1/02.
- AustralAsian Plant Pathology Society, Mudgee, Australia – 2/02.
- CSIRO Publishing, Melbourne, Australia – 3/02.
- Department of Botany, Melbourne University, Melbourne, Australia – 3/02.
- Waite Institute, University of Adelaide, Adelaide, Australia – 3/02.
- Department of Biology, Flinders University, Adelaide, Australia – 3/02.
- Faculty of Agriculture, University of Sydney, Sydney, Australia – 3/02.
- St. Paul's College, University of Sydney, Sydney, Australia – 3/02.
- Royal Botanic Gardens – Sydney, Sydney, Australia – 5/02.
- CSIRO Plant Sciences – Canberra, Australia – 5/02.
- CRC for Tropical Plant Protection, University of Queensland, Brisbane, Australia – 5/02.
- Queensland Department of Primary Industries, Indooroopilly, Australia – 5/02.
- ATUT Final Project Review Seminar, Alexandria, Egypt – 07/02.
- FABI, University of Pretoria, Pretoria, South Africa – 10/02.
- PROMEC, Medical Research Council, Tygerberg, South Africa – 10/02.

During 2002 Fusarium cultures were provided to:

- Dr. Ranajit Bandyopadhyay, IITA, Ibadan, Nigeria
- Drs. Robert L. Bowden, Larry E. Claflin, Louis A. Heaton & Douglas J. Jardine, Department of Plant Pathology, Kansas State University, Manhattan, Kansas.
- Dr. Elhamy M. El-Assiuty, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.
- Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, Kansas.
- Dr. D. 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, Istituto Tossine e Micotossine da Parassiti Vegetali, Bari, Italy.
- Prof. Dr. W. 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.

Other collaborating scientists (Host country)

- Dr. Ranajit Bandyopadhyay, IITA, Ibadan, Nigeria
- Dr. Sofia Chulze, Department of Microbiology, National University of Rio Cuarto, Rio Cuarto, Argentina.
- Drs. M. Flieger & S. Pazoutova, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
- Dr. Laszlo Hornok, Agricultural Biotechnology Center, Godollo, Hungary
- Dr. Sandra Lamprecht, Department of Plant Pathology, Stellenbosch University, Stellenbosch, South Africa
- 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
- Dr. Anaclet S. B. Mansuetus, Department of Biological Sciences, University of Swaziland, Kwaluseni, Swaziland
- Dr. Neal McLaren, Agricultural Research Council, Potchefstroom, South Africa

- Dr. Maya Piñeiro, Mycotoxins Unit, Laboratorio Tecnologia del Uruguay, Montevideo, Uruguay
- 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.)

- Drs. A. E. Desjardins & R. D. Plattner, USDA National Center for Agricultural Utilization Research, Peoria, Illinois
- Dr. G. N. Odvody, Texas Agricultural Experiment Station, Corpus Christi, Texas

Publications and Presentations (2002)

Journal Articles, Books & Book Chapters

Fotso, J., J. F. Leslie & J. S. Smith. 2002. Production of beauvericin, moniliformin, fusaproliferin, and fumonisins B_1, B_2 and B_3 by ex-type strains of fifteen *Fusarium* species. *Applied and Environmental Microbiology* **68:** 5195-5197.

Jurgenson, J. E., R. L. Bowden, K. A. Zeller, J. F. Leslie, N. J. Alexander & R. D. Plattner. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). *Genetics* **160:** 1452-1460.

Jurgenson, J. E., K. A. Zeller & J. F. Leslie. 2002. An expanded genetic map of *Gibberella moniliformis* (*Fusarium verticillioides*). *Applied and Environmental Microbiology* **68:** 1972-1979.

Leslie, J. F., ed. 2002. *Sorghum and Millets Diseases*. Iowa State Press, Ames, Iowa. 504 pp.

Leslie, J. F. & W. F. O. Marasas. 2002. Will the real *Fusarium moniliforme* please stand up! In: *Sorghum and Millets Diseases* (J. F. Leslie, ed.), pp. 201-209. Iowa State Press, Ames, Iowa.

Porter, J. K., C. W. Bacon, W. P. Norred, E. M. Wray, G. A. Kuldau, A. E. Glenn, & J. F. Leslie. 2002. Mycotoxins from fungal-infected sorghum: *Claviceps* vs. *Fusarium* and the *Striga* connection. In: *Sorghum and Millets Pathology 2000* (J. F. Leslie, ed.), pp. 229-235. Iowa State Press, Ames, Iowa.

Book Review

Leslie, J. F. *Molecular Fungal Genetics*; eds. R. P. Oliver & M. Schweizer; Cambridge University Press, New York, 377 pp. Reviewed in *Quarterly Review of Biology* **77**(2002): 69-70.

Agroecology and Biotechnology of Fungal Pathogens of Sorghum and Millet

Project KSU 211

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Dr. John Leslie, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506
Dr. Stephen Mason, Department of Agronomy, University of Nebraska, Lincoln, NE 68583-0915
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Dr. Henry Pitre, Mississippi State University, Mississippi State, MS 39762
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Dr. Mitch Tuinstra, Department of Agronomy, Kansas State University, Manhattan, KS 66506

Summary

Eight different species of *Fusarium* were evaluated for pathogenicity in an effort to develop a screening protocol for stalk rot variability in sorghum. A strain of *F. thapsinum* (mating population F) from sorghum was the most virulent isolate tested in the experiments. *F. thapsinum* yielded higher disease scores on all three sorghum genotypes; while an isolate of mating population A (*F. verticillioides*) from corn was the least aggressive. Isolates of *F. thapsinum* are recommended for evaluating sorghum germplasm. Statistical analysis showed that scoring genotypes 14 days post inoculation with an inoculum dose of 10^3 conidia ml^{-1} or higher would differentiate differences in stalk rot resistance among genotypes. The optimum assay was a suspension of 10^4 to 10^6 conidia ml^{-1} with an incubation period of 28 days or longer. Disintegration of *C. africana* spores by freezing in liquid nitrogen followed by thawing provided the best template for PCR amplification of conidia DNA. BTX 378, 96GCPOB143 and LG 35 were very susceptible to anthracnose and lines R9113, 88 PR 1057, 90 EON 343, and 91 BE 7414 exhibited tolerance. Ergot was detected in BTX 623 at Managua and B-35 and SC 326-6 in San Blas, Nicaragua. Fungicides applied 55, 75, and 95 days after planting significantly reduced the incidence of zonate leaf spot in sorghum in El Salvador.

Objectives, Production and Utilization Constraints

Objectives

- U.S./Mexico/Nicaragua/El Salvador: Determine the prokaryotic plant pathogenic organisms responsible for unique and unusual diseases of sorghum that may pose yield constraints. These causal agents are primarily insect disseminated and a joint collaborative project was established with MSU-205.

- U.S./Mexico/Nicaragua/El Salvador: Ascertain disease incidence through surveys coupled with utilization of the ADIN nursery from Texas A & M University at various locations. Genetic variability of accessions within the ADIN will be determined if disease severity occurs.

- U.S./Nicaragua: Determine the number of races/pathotypes of *Colletotrichum graminicola*: (*C. sublineolum* and *C. falcatum*) that occur in Nicaragua with DNA fingerprint ing techniques. This is a portion of the Ph.D. thesis project of Sergio Pichardo at Mississippi State University.

- U.S./El Salvador: Continue to evaluate germplasm for genetic variability to rust and evaluate fungicides for control of various sorghum diseases..

- U.S./Central America/Africa: Develop a rapid and reliable protocol to detect *Claviceps africana* (causal agent of ergot) spores and hyphal propagules in sorghum seed and feed grains.

- U.S./Africa: Continue to evaluate germplasm and screening protocols for ascertaining genetic germplasm tolerant/ resistant to *Fusarium* stalkrot.

Constraints

Grain sorghum received limited attention in Central America in previous years as corn was the crop favored by commercial and subsistant growers. A 10-fold increase in less than 10 years in the poultry industry has provided an impetus for sorghum production. Sorghum diseases were poorly characterized and the incidence and severity were unknown. Surveys were conducted and the use of genotypes in the ADIN has shed valuable information on sorghum diseases in El Salvador and Nicaragua. Interestingly, the major diseases, anthracnose and gray leaf spot are of the highest incidence with one or the other predominating in a particular year. For example, anthracnose was predominant in 2002 and gray leaf spot in 2001.

Anthracnose is a significant constraint to yields of grain sorghum in numerous LDC's. The disease may be partially controlled by chemicals but they are either unavailable or the cost may be prohibitive for farmers. Incorporation of resistant or tolerant germplasm into acceptable cultivars would partially alleviate losses due to anthracnose. The correct species identification of the causal agent of anthracnose remains in flux.

Fusarium stalk rot is one of the most prevalent diseases of sorghum wherever the crop is grown. The causal organism is found in living plant tissues, crop debris, and soils in different geographical regions. At least 12 different *Fusarium* species have been reported as pathogens of sorghum although, for nearly 100 years, *F. moniliforme* was widely reported as the specific epithet. *F. moniliforme* consists of numerous species, strains, and isolates that are important pathogens on a wide range of economically important plants. Recently, this fungal complex was classified into eight mating populations and a large number of asexual phylogenetic species. Reducing losses due to stalk rot have been through breeding efforts to develop resistant cultivars. The complex nature of the disease coupled with the environment and lack of reliable inoculation protocols that mimic natural infection have limited the potential for screening large numbers of genotypes. Previously, *Fusarium* sp. infested toothpicks were inserted in the basal stalk to evaluated limited numbers of genotypes.

A need exists for a rapid and reliable diagnostic procedure to detect *Claviceps africana* in sorghum seeds and feed grains due to strict and rigid quarantine regulations of numerous countries. A PCR-fingerprint of this fungus was evaluated for use in detecting spores and hyphal fragments in sorghum seeds.

Research Approach and Project Output

Research Approach

Single isolates from four mating populations of *G. fujikuroi* (A, D, F, G) and four recently described species within the *G. fujikuroi* complex were evaluated for virulence against three sorghum genotypes with variable disease reactions. The causal agents included *F. verticillioides, F. proliferatum, F. thapsinum, F. nygamai, F. andiyazi, F. pseudoanthophilum, F. brevicatenulatum,* and *F. pseudonygamai*. The sorghum genotypes included SC599, BRedlan, and SU629 which are resistant, susceptible, and highly susceptible to *Fusarium* stalk rot, respectively (unpublished data). The experiments were conducted under greenhouse conditions since five of the *Fusarium* spp. have not been reported in the U.S. Seeds were treated with Captan and then planted into 1- liter pots filled with potting mix. Pots were arranged in a randomized complete block with four replications. Plants were thinned to a single plant per pot seven days after emergence and were watered and fertilized as needed. Entries were grown at 27º C with natural lighting supplemented with overhead high-pressure sodium lamps. In 2001, irrigation water was withheld for 1 week after flowering to induce leaf rolling and mild drought stress. In 2002, plants received water as needed throughout the duration of the study.

Each *Fusarium* sp. was grown (23 to 25º C) in 500 ml Erlenmeyer flasks containing potato dextrose broth and then placed on a rotary shaker (60 rpm). Conidia were separated from the mycelial mass by straining the culture suspension through four layers of cheesecloth. Conidial concentrations were ascertained with a hemacytometer. Inocula suspensions were adjusted to 5×10^4 conidia ml^{-1} with 10 mM (pH 7.2) phosphate buffered saline (PBS). *Fusarium proliferatum* (strain KSLM) was used to determine the optimum inoculum concentration and period of time after inoculation to evaluate germplasm. KSLM is an isolate from a *Fusarium* stalk rot infected sorghum plant in Kansas.

Fourteen days after anthesis, an Idico filler-plug gun equipped with a stainless steel modified needle was used to deliver one ml of inoculum of each isolate into the basal portion of the stalk of individual plants. Twenty-eight days after inoculation, plants were harvested, split lengthwise, and rated for disease incidence and severity by measuring the internal length of necrotic lesions and counting the number of nodes crossed by the lesion.

In 2000, two sorghum hybrids SA3042 x SC35 (susceptible) and Redlan x SC599 (resistant) were tested against inocula concentrations of 0, 10^3, 10^4, 10^5 and 10^6 conidia ml^{-1} and 14, 21, 28, 35 and 42 days PI. In 2001, the number of test genotypes were diversified and increased from two to ten hybrids.

At flowering, 25 plants from each whole plot unit were tagged with different colored tape. Fourteen days after flowering, the tagged plants (five plants for each inoculum dose in each whole plot unit) were inoculated in the basal stalk using an Idico filler-plug gun equipped with a modified syringe and needle that was calibrated to deliver 1.2 ml of inoculum. Fourteen days after inoculation, one plant for each of the inoculum dose levels was harvested in each whole plot unit and scored for disease severity. Plants were harvested every seven days until 42 days after inoculation. Disease severity was scored by splitting stalks lengthwise and measuring lesion lengths (cm) and counting the number of nodes crossed by *F. proliferatum*.

Ergot conidia obtained from naturally infected sorghum was mixed with healthy sorghum seeds at a concentration of 10^5 spores/ml. DNA from ergot spores was obtained by the following treatments with the spore suspension:

Sonicated for one minute;
Placed in a microwave until the suspension boiled;
Centrifuged at 14,000 rpm for 5 minutes, water was discarded and the pellet resuspended in 4N NaOH overnight at room temperature. HCl was added to lysate and adjusted to pH 7; DNA precipitated with 2 vol EtOH and centrifuged at 14,000 rpm for 10 min and supernatant discarded. Pellet was air dried and dissolved in water.

Approximately 100 Fl of spores suspension was frozen in liquid nitrogen and then immediately thawed. One Fl of conidial suspension was placed on a glass slide, covered with a plastic cover slip and crushed by applying pressure on the slip. Slide and cover slip were washed with 100Fl of water and then placed in a 1.5ml tube. One hundred Fl of a 2X lysis buffer (20mM Tris HCl, pH 7.3, 1.5 mM $McCl_2$, 50 mM KCl, 0.01% proteinase K, 0.01% SDS) was added. The sample was incubated at 37 C for 1 hr, 95 C for 10 min and then cooled to 40 C. DNA was precipitated by adding 2 vol of EtOH and centrifuging. The sample was air dried and dissolved in water.

The above procedures were also used with one and two-day-old germinated spores.

Four Fl of intact ergot spores in a suspension and each of the above treaments were used as templates for PCR amplification utilizing PCR Master mix (Promega) and PCR temperature profiles adjusted for each specific pair of primers. PCR products were separated in 2% agarose gels with EtBr and visualized with UV light and photographed.

Primers used included: ITS5, 10R, OPA 01, CGA RAM and a pair that was designed in the laboratory for the trihydrophobine sequence which has GN-rich areas that may vary in length for different ergot geographical areas. A pair of primers designated CPMK2 and obtained from another ergot fungus, *Claviceps purpurea* (causal agent of ryegrass ergot), was also used.

ADIN: The All Disease and Insect Nursery (ADIN) accessions (courtesy of D.T. Rosenow, TAM, Lubbock, TX) were planted at San Blas and Managua, Nicaragua and Izalco and San Andres in El Salvador. The ADIN in El Salvador were utilized in a control study with fungicides (Cycosin and Elosal) applied at 35, 55, 75 and 95 days after planting. The target diseases included zonate leaf spot, anthracnose, rust and northern leaf blight.

Research Output

Prokaryotes: Considerable effort has been devoted in Nicaragua and El Salvador to detect prokaryotic plant pathogens that are disseminated by insects. This is a joint effort with MSU-205. The association of prokaryotes with insects is common in the tropics with other crops and is assumed to also occur in sorghum. Rainfall has been inconsistent in Nicaragua and prolonged droughty conditions have occurred for the past two years. The insect-pathogen relationship is normally found when climatic conditions are favorable for producing lush plants.

Ergot: Disintegration of *C. africana* spores by freezing in liquid nitrogen followed by thawing provided the best template for PCR amplification of conidia DNA. Primers tested showed different patterns of amplification which depended on method of DNA isolation and age of conidia (dormant, days after germination). Research continues on selecting primers, optimum spore morphology and use of hyphae as DNA templates.

***Fusarium*:** The average pathogenicity scores were highest for the *F. thapsinum* isolate followed by isolates from *F. pseudonygamai* and *F. andiyazi* while the *F. verticillioides* isolate was generally the least virulent. Variations in disease scores among pathogens ranged from 6.53 cm to 10.08 cm in lesion length and 1.61 to 2.24 nodes crossed by the disease lesion. Comparisons of means revealed that the *F. thapsinum* isolate produced significantly longer lesions than isolates of *F. verticillioides* and *F. nygamai*. Mean disease scores for the pathogen species were generally consistent between the two experiments and few interactions were detected.

Mean lesion length of *F. proliferatum* inocula concentrations for the year 2000 ranged from 6.31 (control) to 18.15 cm (10^6 conidia ml^{-1}) and days PI from 7.22 cm (14 days) to 17.31 cm (42 days). Similarly in 2001, mean lesion length ranged from 5.13 cm (control) to 20.46 cm (10^6 conidia ml^{-1}) for inocula concentrations, and from 8.01 cm (14 days) to 16.52 cm (42 days) for days PI. A similar trend was observed for the number of nodes crossed (data not shown). Mean number of

nodes crossed in 2000 ranged from 0.61 (control) to 3.21 after inoculated with the highest inoculum concentration and from 1.11 (14 days PI) to 3.03 after 42 days PI.

Similarly, in 2001, the number of nodes crossed ranged from 0.41 (control) to 3.42 (10^6 conidia ml^{-1}) for inocula concentrations and from 1.00 to 2.79 for days PI. The two-way interaction effects were generally not significant except for genotype by inocula concentrations and genotype by days PI for lesion length in 2001 although these interactions failed to change rankings. The increase of disease severity with increasing inocula levels was comparatively lower among entries that involved resistance sources (SC1154, SC1039, and SC134) resulting in significant genotype by inocula interactions. Genotype x concentration x incubation interaction effect was not significant except for lesion length in 2000. In general, interaction effects were much lower than the main effect of the factors.

The mean square for the highest inoculum dose and 42 days PI was relatively lower than the preceding levels in the first year of the experiment. Lesion length mean square for inoculum dose ranged from 400 (control) to 2381 (10^5 conidia ml^{-1}) in 2000, and from 192 (control) to 1359 (10^6 conidia ml^{-1}) in 2001. Similarly, mean square for days PI ranged from 1286 (14 days PI) to 1778 (28 days PI) in 2000, and from 284 (14 days PI) to 905 (35 days PI) in 2001.

Significant differences in mean disease score were detected among genotypes for both scoring methods. Inoculum concentration and days PI significantly ($P \leq 0.01$) affected the severity of Fusarium stalk rot in both testing seasons. Inoculum concentration effects were more significant than days PI. Disease severity generally increased with higher inocula levels and days PI.

Significant differences in disease severity were detected among the genotypes tested. SC599 was the most resistant variety and SU629 and BRedlan were the most susceptible in each experiment. The lesion lengths for SC599 averaged only 3.37 cm compared to 11.61 cm for SU629. A significant genotype by experiment interaction was detected for the two disease severity traits; however, the pattern of disease reactions among genotypes in each experiment was similar. Remarkably, the genotype by pathogen interaction was not significant indicating that the genotypes responded similarly to infection by each pathogen isolate.

ADIN Nicaragua: The all disease and insect nursery was planted at two locations in Nicaragua, Managua (Table 1) and San Blas. The San Blas location was extremely dry and the incidence and severity of sorghum diseases were extremely low (data not shown). Anthracnose was the major disease found in Managua. The lines BTX 378, 96GCPOB143 and LG 35 were very susceptible to anthracnose and the lines R9113, 88 PR 1057, 90 EON 343, and 91 BE 7414 exhibited less than 10% severity (Table 1). Ergot was detected in BTX 623 at Managua and B-35 and SC 326-6 in San Blas. Downy mildew was detected by Ing. René Clará at Esteli, Nicaragua which is about 150 km North of Managua. The incidence and severity remain unknown, however, UNA and INTA personnel will monitor the situation closely.

Table 1. ADIN results from UNA/INTA, Managua, Nicaragua (2002)[a]

Accession	Anthracnose (% severity)
B-35	20
SC 326-6	22.5
SC 414-12E	20
SC 630-11E(II)	20
R 9188	42.5
86 EO 366	17.5
90 EON 328	15
90 EON 343	5
91 BE 7414	5
87 BH 8606-6	10
88 BE 2668	17.5
94 CW 5045	17.5
96 CA 5986	17.5
96 CD 635	27.5
96 CD 677	17.5
99 BD 3726/98CD187	17.5
99 CA 2244	40
99 CA 2519	40
99 CA 1422	17.5
99 PR 1159/B LD6	20
LG 70	10
LG 35	55
B8 PR 1011	17.5
B8 PR 1059	37.5
B8 PR 1051	17.5
98 BRON 125	40
B8 PR 1013	35
B8 PR 1057	8
TX 2880	17.5
GR 108-90M24	17.5
95 BRON 155	35
95 BRON 151	22.5
96 GCP OB 124	17.5
96 GCP OB 143	60
96 GCP OB 157	20
96 GCP OB 160	20
96 GCP OB 172	15
MB 108 B	17.5
97 BRON 179	17.5
98 BRON 122	20
88 B 928	20
R 9113	5
97 BRON 304	9
B 9104	27.5
B 9107	30
87 EO 109	25
B 9601	40
88 B 943	25
94 B 1055	25
B 9105	27.5
R 9603	27.5
B 9307	30
R 9120	35
91 B 2978	42.5
TX 2911	42.5
R 9618	32.5
R 9519	22.5
Malisor 84-7	35
SRN 39	32.5
Sureño	32.5
TX 2783	25
TX 2767	5
TX 2783	37.5
BTX 635	25
BTX 623	40
BTX 631	45
TAM 428	35
TX 430	40
TX 7078	40
BTX 378	67.5

[a]Results recorded by Ings. Sergio Pichardo and Yanet Guttierrez.

El Salvador: Fungicides proved effective in reducing incidence of zonate leaf spot (*Gloeocercospora sorghi*) at Izalco and San Andres. Control was significant at 55, 75, and 95 days after planting but not significant when fungicides were applied 35 days after planting. In addition, significant differences were noted in reducing incidence of anthracnose, northern leaf blight and rust when fungicides were applied 95 days after planting.

Networking Activities

Workshops

Yanet Gutierrez, Sergio Pichardo and Jesus Narro visited Kansas State University in June, 2003 to attend the Fusarium shortcourse and discuss forthcoming collaborative research plans.

Research Investigator Exchanges

Yanet Gutierrez from UNA, Managua, Nicaragua worked in the lab of Dr. L. E. Claflin on a training session from July 29 - August 13, 2002.

Dr. L. E. Claflin surveyed sorghum fields and discussed mutual research in El Salvador and Nicaragua in December, 2002.

Research Information Exchange

The All Disease and Insect Nursery (ADIN) that was graciously provided by Dr. D. T. Rosenow was planted in two locations in both El Salvador and Nicaragua to determine disease incidence and severity.

Numerous extension publications, compendia, and textbooks were furnished to Reina Guzman and Ing. Sergio Pichardo. In addition, speciality equipment and supplies were purchased with funds from KSU 211 and distributed to the laboratories.

KSU 211 provided the necessary funds for the following collaborators to attend the Fusarium shortcourse at Kansas State in June, 2003:

Yanet Gutierrez (Nicaragua)
Sergio Pichardo (Nicaragua)
Jesus Narro (Mexico)

KSU 211 provided funds to Sergio Pichardo for travel to Starksville, M.S. to initiate a Ph.D. program at Mississippi State University.

Forty copies of each ICRISAT compendia entitled, "Manual Para la Identificacion de las Plagas Insectiles del Sorgo, and Manual Para la Identificacion de las Enfermedades del Sorgo y Mijo" were purchased and sent to El Salvador and Nicaragua for distribution.

Publications and Presentations

Reed, J. D., M. R. Tuinstra, N. W. McLaren, K. D. Kofoid, N. W. Ochanda, and L. E. Claflin. 2002. Analysis of combining ability for ergot resistance in grain sorghum. Crop Sci. 42:1818-1823.

Reed., J. D., B. A. Ramundo, L. E. Claflin, and M. R. Tuinstra. 2002. Analysis of resistance to ergot in sorghum and potential alternate hosts. Crop Sci. 42:1135-1138.

Claflin, L. E., and L. M. Giorda. 2002. Stalk Rots of Sorghum. Pages 185-190 in: Sorghum and Millets Diseases. J. F. Leslie, ed. Iowa State Press, Ames, Iowa, 516 pp.

Tuinstra, M. R., T. T. Teferra, L. E. Claflin, R. G. Henzell, A. Borrell, N. Seetharama, G. Ejeta, and D. T. Rosenow. 2002. Breeding for Resistance to Root and Stalk Rots in Sorghum. Pages 281-286 in: Sorghum and Millets Diseases. J. F. Leslie, ed. Iowa State Press, Ames, Iowa, 516 pp.

Presentations

Italy (9/10/02-9/20/02). Organized and presented a session on control at the 6th International Conference on Pseudomonas syringae Pathovars and Related Pathogens, Maratea, Italy and discussed bioterriorism programs with FAO scientists (Rome).

Nicaragua (12/1/02-12/4/02). Delivered supplies and evaluated ADIN nurseries for disease incidence and severity as part of the INTSORMIL program.

El Salvador (12/4/02-12/8/02). Delivered equipment, books, supplies, evaluated ADIN nurseries, and research plots under the auspices of INTSORMIL.

Miscellaneous Publications

Claflin, L. E. 2002. Agroecology and biotechnology of fungal pathogens of sorghum and millet. Pp. 11-17 in INTSORMIL Ann. Repts., A Technical Res. Rept. of the Grain Sorghum/Pearl Millet Collaborative Research Support Program (CRSP), University of Nebraska, Lincoln.

Enhancing the Utilization of Grain Sorghum and Pearl Millet through the Improvement of Grain Quality via Genetic and Nutrition Research

Project KSU 220
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Summary

Improve Nutrition

The emphasis of this project is to develop high yielding sorghum varieties and hybrids with enhanced nutritional and grain quality characteristics for use as human food and in animal feed. Recent nutritional studies have focused on comparisons of large-seeded hybrid sorghum genotypes with conventional hybrid sorghum and maize varieties for differences in feed value. Poultry feeding trials were conducted using broiler chicks to provide information on the metabolizable energy value of the various cereal grains. These analyses indicated that certain large-seeded hybrid sorghums were equivalent in feeding value to maize and were significantly better than conventional sorghum varieties. Breeding efforts have been initiated to transfer these enhanced feed quality characteristics into improved sorghum varieties.

Other research efforts to improve grain quality characteristics of sorghum and millet have focused on the characterization and utilization of genes to improve resistance to grain mold and tolerance to weathering. Studies evaluating the role of known defense response pathways have shown that factors other than the activation or accumulation of defense genes account for the differences in sorghum genotypes with contrasting host-plant resistance characteristics. Marker-assisted selection studies evaluating the expression of grain mold resistance genes tagged in the variety Sureño indicated that a subset of these resistance genes is expressed across environments and in diverse genetic backgrounds. These genes represent excellent candidates for utilization in crop improvement programs via marker-assisted selection.

Increase Yield

Natural tolerance to heat and drought permit sorghum to be grown in areas unsuited for production of other cereal crops. Past breeding efforts have significantly enhanced yield potential in semi-arid environments, but little attention has been focused on feed value and grain quality. Germplasm sources to improve the nutritional value of sorghum have been identified. The focus of this project is to deliver these traits to sorghum producers and end-users through the development of sorghum cultivars with enhance feed-value and grain-quality characteristics. Breeding efforts have been initiated to transfer these

enhanced feed quality characteristics into high-yielding sorghum varieties adapted for production in Africa, Central America, and the United States. This will be accomplished through conventional breeding strategies and by adapting marker-assisted selection technologies as appropriate.

Improve Institutional Capacity

A training program is being developed to transfer the technology and knowledge needed to effectively utilize improved sorghum and millet cultivars for animal feeding and human food. Technical assistance and technology transfer in Central America is being pursued through interactions with Dr. Carlos Campabadahl, one of the leading nutritionists in Central America. Dr. Hancock has lectured with Dr. Campabadahl at LANCE and RAPCO short courses for animal nutritions. These week-long short courses in animal feeding and nutrition include leaders in the animal feeding industry from Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, the Dominican Republic, Columbia, Venezuela, and Ecuador. Technology transfer efforts in West Africa were initiated by hosting Dr. Salissou Issa, Head of the Animal Husbandry Unit at the INRAN Rainfed Crops Program in Niger, on a tour of the animal research facilities at Kansas State University to plan training programs directed towards the needs of key poultry producers and feed millers in West Africa. These efforts will include demonstration experiments and workshops in Africa in cooperation with Dr. Salissou Issa.

Promote Economic Growth

The marketing and utilization of sorghum grain often has been limited by lower grain quality and feed value compared to other cereals. Given the complexity of these traits, plant breeders traditionally have placed little emphasis on end-use value of sorghum for human and animal consumption. Our research project attempts to address this weakness in sorghum and millet crop improvement through the integration of traditional plant breeding with biotechnology to develop elite hybrids and cultivars with improved nutritional and grain quality traits. Sorghum genotypes with enhanced feed-value and grain-quality characteristics have been identified and these genes are being incorporated into improved genetic backgrounds for deployment in regions of Africa, Central America, and the United States.

Objectives, Production and Utilization Constraints

Objectives

- Identify and map genes associated with improved grain and feed quality characteristics.
- Develop robust biotechnology tools for tagging genes that contribute to grain mold resistance and enhanced nutritional value.
- Develop techniques to rapidly quantify feed quality of sorghum and millet for poultry and to quantify food quality of sorghum.
- Develop high-yielding sorghum cultivars with improved feed quality and grain mold resistance using both conventional breeding techniques and marker-assisted selection technology.
- Provide technology transfer and technical assistance in promoting the use of improved sorghums and millet in poultry feeding in the developing regions of West Africa and Central America.

Constraints

New entrepreneurial opportunities for production of animal feeds and products in developing countries including meat and eggs are needed to move sorghum and millet from subsistence crops to value-added commodities. However, the marketing and utilization of sorghum grain often has been limited by lower grain quality and feed value than other cereals. Sorghum kernels are exposed to the environment as they mature and grain mold problems are common; however, even in the absence of contaminating fungi, sorghum grain typically has lower digestibility and metabolizable energy values as compared to other cereals.

Research efforts are needed to address food quality and feed efficiency traits in sorghum and millet. Components of feed quality are frequently defined in terms of animal performance or metabolizable energy value. These traits can be measured in animal feeding trials, but these experiments are costly and not amenable to high-throughput testing as required in a plant breeding program. This research project attempts to address this weakness in sorghum and millet through the integration of laboratory assays for feeding quality, traditional plant breeding, and biotechnology to develop elite hybrids and cultivars with improved nutritional and grain quality traits. The recognition of the true nutritional value of grain sorghum by animal producers will lead to greater health and productivity in regions of the world where hunger and poverty are major issues.

Genes for grain quality and host-plant resistance can be identified and tagged with DNA-based markers to facilitate crop improvement. Some good resistance sources have been identified but the inheritance of these traits is complex and screening under field conditions is environmentally dependent and often unreliable. Breeding projects to assemble these genes into improved cultivars should proceed rapidly with the aid of marker-assisted selection complemented by performance tests made in multiple environments.

Research Approach and Project Output

Research Methods

Collaborative research efforts in Africa and Central

America are supported through short and long-term training programs, germplasm exchange and evaluation, and complementary basic research support activities. These research efforts are conducted in three regional programs including West Africa, Southern Africa, and Central America. Current training activities include graduate student education, short-term information exchange, training visits to the United States for collaborating researchers, and workshop activities in animal production and nutrition. Several collaborating researchers from Niger, Zimbabwe, and Mozambique were hosted in research exchange and planning activities in 2002-2003.

Crop improvement efforts to develop cultivars adapted to environments in West Africa, Southern Africa and Central America utilize elite varieties and cultivars that are adapted to each of the regions. The lines used to create these populations are selected through evaluations of elite U.S. and host country germplasm in the target region. This material is evaluated in the target region in conference with collaborating plant breeders. Improvement efforts in Western and Southern Africa focus on the development of early-maturing, drought-tolerant cultivars and hybrids while efforts in Central America are on improved food-type and Macio Criollos cultivars. These efforts are focused on the development of photoperiod sensitive hybrids using Ma5 and Ma6.

The underlying objective for research to identify and map genes related to grain quality is to develop a better understand the genetic control of important quality traits and generate genetic markers that can be used by sorghum improvement programs in the near future. Combining these traits into one genotype is a significant challenge that could be facilitated by the use of molecular technology. The development of these technologies should enhance the efficiency of combining grain quality factors including feed quality characteristics and grain mold resistance into varieties with high yield potential. Mapping populations are being developed and characterized in cooperation with collaborators at domestic and international sites. These populations are being genotyped in laboratories in the U.S. using various types of genetic markers.

Technical assistance and technology transfer efforts in poultry production and nutrition are currently focused on workshop and short course activities. In 2003, Dr. Hancock contributed to the LANCE Short Course, a week-long short course in animal nutrition. The participants included 30 industry leaders in animal feeding/nutrition with representatives from Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, the Dominican Republic, Columbia, Venezuela, and Ecuador. Plans are being made to expand these technical assistance activities to key poultry producers and feed millers as in West Africa.

Research Findings

Analysis of Sorghum and Maize for Differences in Poultry Feed Quality

Raw sorghum germplasm sources that allow for improvement of seed size via increased grain-fill rate and duration have been publicly released and are currently being utilized to produce hybrids of increased seed size and yield potential. Recent genetic studies have indicated that normal-seeded hybrids generally produced lower crude protein, slightly lower crude fat, and higher starch values than that of the large-seeded hybrids; however, the impact that these differences in composition might have on metabolizable energy (ME) values in feed rations is not clear.

The objective of this study was to compare the feed quality of large-seeded grain sorghum hybrids with hybrids of normal seed size. The varieties evaluated in this study included eight sorghum hybrids produced from crosses between two commercial U.S. females and two large-seeded and two normal-seeded male parent lines. The female parent lines used in this study were ASA3042 and AWheatland. The male parents included two normal-seeded lines (Tx2737 and Tx435) and two large-seeded lines (KS115 and Eastin-1). These hybrid sorghums and a hybrid maize check were produced under dryland conditions at two locations in Kansas in 2000 and 2001. The resulting 36 grain samples were evaluated for crude fat, protein, fiber, moisture, nitrogen, ash, and gross energy content. Feeding assays using broiler chicks were conducted to provide information on the ME content of the various cereal grains.

A combined analysis of seed weight and composition across the four locations revealed significant differences among sorghum hybrids (Table 1). Hybrids produced from crosses with male lines Eastin-1 and KS115 had seed weights ranging from 2.69 to 4.14 grams per 100 seed and were significantly greater than hybrids produced from crosses with Tx435 and Tx2737 whose seed weights ranged from 2.25 to 2.53 grams. Hybrids produced using KS115 averaged 3.95 grams per 100 seeds and were significantly greater than the other hybrids. Maize hybrids averaged 28.35 grams per 100 seeds and were nearly 10 times larger than the sorghum hybrids.

Analysis of composition differences among the hybrid sorghum and maize varieties indicated that crude protein for the sorghum samples ranged from 12.1 to 14.1% (Table 1). The hybrid sorghums were generally greater in protein than hybrid maize with a few exceptions. The hybrid maize check had the greatest crude fat content and was significantly higher than most of the hybrid sorghums (Table 1). Hybrids produced from KS115 were the exception and did not significantly differ from maize for fat content. Notable differences or trends among grain samples were not apparent for fiber, ash, nitrogen free extract, or gross energy content.

Significant hybrid, environment, and hybrid by environment interaction effects were noted for ME in the combined analysis. The most significant factor influencing ME values was the hybrid effect. However, given hybrid by environment interactions, differences in ME were reported on an individual environment and combined average basis (Table 2). Individual

hybrid ME values across the four environments ranged from 3.121 Mcal kg-1 for AWheatland x Tx435 up to 3.736 Mcal kg-1 for ASA3042 x KS115. In the combined analysis, the ASA3042 x KS115 hybrid had the greatest average ME content of 3.591 Mcal kg-1, followed by the hybrid check at 3.508 Mcal kg-1. The average ME content of the KS115 hybrids was higher than the other male hybrid groups with a ME value of 3.484 Mcal kg-1. The Tx2737 hybrids had an average ME content of 3.387 Mcal kg-1, followed closely by the Tx435 hybrids, which averaged 3.362 Mcal kg-1. The Eastin-1 hybrids had the lowest average ME value of 3.313 Mcal kg-1.

Comparisons of the hybrids used in this study indicated that KS115 hybrids produced grain with exceptionally large seed size and an increased level of fat content. These seed characteristics appear to be beneficial to sorghum-based poultry diets, resulting in increased animal performance that was comparable to that of maize. Results from this study indicate that the use of KS115 as a means of increasing seed size and yield potential in grain sorghum improvement programs may also contribute to enhanced feed quality.

Grain Mold Resistance

A critical component of the KSU220 project was the improvement of grain mold resistance using marker assisted selection (MAS). Previous work had detected five QTL influencing grain mold resistance from Sureño in the recombinant inbred line progeny from the cross of RTx430 x Sureño. From this work, experiments were initiated to determine the efficacy of MAS for grain mold resistance in sorghum.

Table 1. Combined analysis of seed weight, crude protein, and crude fat for hybrid sorghum and maize varieties produced at two Kansas locations in 2000 and 2001

Entries	Seed weight	Crude protein	Crude fat
	g 100seeds-1	*%*	*%*
ASA3042 x KS115	3.76	13.5	3.6
AWheatland x KS115	4.14	12.9	3.8
ASA3042 x Eastin-1	2.69	14.1	3.4
AWheatland x Eastin-1	2.93	13.3	3.5
ASA3042 x Tx435	2.39	13.2	3.4
AWheatland x Tx435	2.53	12.1	3.1
ASA3042 x Tx2737	2.25	12.7	3.4
AWheatland x TX2737	2.36	12.3	3.4
Hybrid maize	-	10.2	3.8
GRAND MEAN	2.88	12.7	3.5
LSD (0.05)	0.26	2.2	0.4

Table 2. Metabolizable energy (ME) contents for individual location and hybrid treatments

	Environment				
Entries	Ottawa ME	Manhattan ME	Belleville ME	Manhattan ME	Average ME
	Mcal kg-1				
ASA3042 x KS115	3.623	3.736	3.425	3.581	3.591
AWheatland x KS115	3.519	3.478	3.200	3.310	3.376
ASA3042 x Eastin-1	3.426	3.334	3.204	3.187	3.288
AWheatland x Eastin-1	3.416	3.367	3.340	3.227	3.337
ASA3042 x Tx435	3.146	3.615	3.398	3.277	3.359
AWheatland x Tx435	3.121	3.268	3.512	3.363	3.316
ASA3042 x Tx2737	3.450	3.586	3.463	3.318	3.454
AWheatland x TX2737	3.275	3.325	3.272	3.407	3.320
Hybrid maize	3.322	3.495	3.728	3.489	3.508
GRAND MEAN	3.366	3.467	3.393	3.351	3.394
LSD (0.05)	0.259	0.273	0.234	0.205	0.196

Five populations were developed to test the efficacy of MAS for grain mold resistance. In each population, Sureño was used as the grain mold resistant parent with one of five elite parental lines (Tx430, Tx436, Tx2903, Tx635, and Tx631). From each cross, F2 progeny were selected based on maturity and short plant height. A total of 1,000 F2:3 lines (approximately 200 lines per population) were evaluated for agronomic desirability and grain mold resistance in Weslaco, Beeville and College Station, Texas. From this evaluation, a total of 100 F3:4 lines were selected and advanced in a winter nursery. In the F_4 generation, DNA samples from each line were taken for QTL marker analysis.

In the summer of 2002, 87 $_{F4:5}$ lines were evaluated in six locations across Texas in randomized complete block with two replications per location. Parental lines and standard grain mold resistant lines were included as checks in all environments. The trials were located in Beaumont, Beeville, College Station (2), Corpus Christi (2), Victoria, and Weslaco. In College Station and Corpus Christi, two trials were planted in separate fields for a total of eight environments. In addition to agronomic traits, grain mold ratings were collected on each genotype in all locations.

Since five distinct populations were evaluated in this study, it was clear that there would be varying degrees of polymorphism between Sureño and the respective adapted parents. An array of molecular markers linked to the sorghum grain mold QTL were screened for polymorphism using DNA from the parental lines. Those markers that proved to be polymorphic between 'Sureño' and any of the adapted parents were subsequently amplified, visualized, and scored in the respective populations. Both SSR and AFLP markers were utilized in this studied, and the method of visualization depended upon both the particular marker system and the nature of the polymorphism itself. All AFLP markers were visualized using the LiCor gel system. SSR markers were either visualized via super fine resolution agarose gel or silver stained polyacrylamide.

Table 3. Grain mold rating means of homozygous classes within each linkage group associated with a grain mold resistance QTL from Sureño by Klein et al. (2001) using grain mold resistance ratings from eight environments in Texas in 2002

Population	Allele Source	QTL Marker LG-D	LG-E	LG-F	LG-G	LG-I
		grain mold rating† (0 to 9)				
Tx430 x Sureño	Tx430	4.72 *	5.02 *	5.40 *	5.15 *	5.15 **
	Sureño	4.10	4.48	4.35	4.66	4.31
	LSD	0.05	0.05	0.05	0.05	0.01
Tx436 x Sureño	Tx436	4.44	4.68	4.61	4.57	-
	Sureño	4.59	4.56	4.64	4.66	-
	LSD	ns	ns	ns	ns	
Tx631 x Sureño	Tx631	4.80	4.80	4.48	4.77	4.55 *
	Sureño	4.87	4.64	4.62	4.71	4.83
	LSD	ns	ns	ns	ns	0.05
Tx635 x Sureño	Tx635	4.16	-	4.32	4.00 *	-
	Sureño	4.18	-	4.31	4.32	-
	LSD	ns	-	ns	0.05	-
Tx2903 x Sureño	Tx2903	5.08 *	5.52	5.20 *	5.21 *	5.07
	Sureño	5.22	4.91	4.76	4.91	5.13
	LSD	0.05	ns .	0.05	0.05	ns
Combined	Adapted	4.68	4.68	4.88 *	4.75	4.95
	Sureño	4.73	4.71	4.57	4.64	4.85
	LSD	ns	ns	0.05	ns	ns

† Grain mold rating, 1 = resistant to 9 = susceptible

To test the effectiveness of MAS, lines from each population were classified for QTL marker alleles at each of the five loci. Comparisons in level of grain mold resistance were then made between classes with Sureño or adapted parent allele for each locus (A, B, C, D, and E). Comparisons were made within populations and across the entire study. If MAS enhances grain mold resistance, then the group with the Sureño allele should have greater grain mold resistance than the group with the adapted parent allele.

Grain mold pressure varied widely among environments (Table 3), ranging from a mean of 2.937 in Beaumont (least grain mold pressure) to 7.345 in Beeville where the most disease pressure was encountered. When the mold scores within all environments were compared, it was found that the eight respective environments effectively formed four groups of environments, which had mean grain mold scores that were not statistically different. Since selection had been practiced for various phenotypic traits (for height and agronomic desirability) in earlier generations, the populations used in this study were not of a suitable structure for the construction of a linkage map. As would be expected, the five adapted parents varied to a large extent with respect to the proportion of the molecular markers that were polymorphic between them and Sureño. RTx430 showed the greatest amount of polymorphism, while BTx635 showed the least.

Comparisons of the marker allele classes across all populations indicated that only one of the five QTL enhanced selection for grain mold resistance (Table 3). The presence of the Sureño allele in LG-F enhanced mold resistance across all environments. The remaining three QTL showed no effect on mold incidence in the combined analysis. However, when the data were analyzed by specific population, MAS was clearly effective in the population derived from crosses with RTx430 (Table 3), but in the remaining four populations, the markers did not particularly enhance selection efficiency, producing significant improvements in only 5 of 17 contrasts (Table 3).

These results indicate that while MAS can be effective, there are limitations to the application of the technique. The utility of MAS was clearly valuable in the Tx430 population, which is not surprising since these QTL were mapped in this population. However, these QTL are clearly less valuable in the other populations evaluated in this study. Therefore, while selection efficiency maybe enhanced through MAS, its utility is limited to populations in which mapping is completed. A positive result from the current work is that the markers did appear to be applicable across environments. In fact certain QTL appeared to have stronger effects in specific types of environments. If the applicability across populations were solved, the use of specific QTLs based on the environment could prove particularly useful.

Other supporting grain mold resistance research activities have focused on an analysis of the role of defense response pathways in preventing grain mold of sorghum. Panicles of four cultivars were inoculated at anthesis with conidial suspensions of *F. thapsinum* and *C. lunata*, the fungi most often found in naturally molded grain. RNA was extracted from the immature floral tissues at various times following inoculation. Levels of mRNA for four known defense-response genes, phenylalanine ammonia lyase, chalcone synthase, b-1,3-glucanase and chitinase were examined by hybridization to PCR generated clones of the respective genes. Expression of each gene increased rapidly following inoculation with either fungus. Although differences were seen in response to the two pathogens, the general pattern was similar in Sureño (resistant), RTx2911 (highly resistant), SC170 (intermediate resistance), and RTx430 (very susceptible) to grain mold. The results imply that factors other than the timing of activation or level of expression of these particular defense response genes account for cultivar differences seen when the plants are challenged at the time of flowering.

Highly conserved sequences within cloned disease resistance genes from other species were also used to search for homologs in the sorghum EST database and to generate candidate resistance gene analogs (RGAs) via PCR. Thus far, 13 of the clones sequenced have features characteristic of resistance genes. Hybridization of these clones to two sorghum BAC libraries has identified 19 different BAC clones that include RGAs.

Evaluation of Tan Plant Hybrids

The improvement of grain quality remains an important goal of many sorghum breeding programs. The TPHT (Tan Plant Hybrid Test) and IFST (International Food Sorghum Test) are grown to test adaptation and productivity of new commercial and experimental tan plant hybrids with improved grain quality. The TPHT was grown in multiple locations in Texas and Kansas. The IFST was grown in locations in Africa, Latin America and Texas. The results of the IFST (Table 4) indicate that many food quality hybrids are competitive with traditional hybrids in the full season category, but further improvements are still needed to develop early- and mid-season food quality hybrids. (Results of the TPHT are not presented, but are available at http://sorghum.tamu.edu). These will be continued to further the development of this material into Central America and Southern Africa.

Networking Activities

Workshops and Meetings

INTSORMIL Principal Investigators Conference – November 17-20, 2003, Addis Abbaba, Ethiopia.

2003 Sorghum Industry Conference – February 16-18, 2003, Albuquerque, New Mexico.

Table 4. Combined analysis of performance characteristics of hybrids evaluated in the 2002 International Food Sorghum Adaptation Test at six locations in the U.S. and Central America

Hybrid	SD†	PL	GL	GM	DY	HT	EX	DS	LO	MS	TW	YD
ATX631*RTX437	W	T	b	4.0	70.8	47.6	3.8	3.3	1.0	13.3	55.8	6,257
ATX642*RTX430	R	P	-	4.8	70.3	43.8	5.5	4.1	1.0	13.4	55.2	6,085
A0PR59*5BRON154	R	T	t	1.3	78.5	47.6	3.7	3.6	1.0	13.6	58.9	5,832
ATX378*RTX430	R	P	-	3.6	68.8	48.8	4.8	4.9	1.8	13.0	54.9	5,797
A8PR1057*5BRON139	R	T	t	1.3	55.6	44.1	1.7	3.7	1.1	14.6	59.8	5,784
ATX631*RTX436	W	T	b	3.1	72.8	47.1	3.8	3.5	1.3	14.0	57.5	5,770
ATX2752*RTX430	R	P	-	4.0	69.5	44.1	3.4	4.3	1.3	14.2	54.7	5,729
ATX623*RTX430	W	P	-	4.0	70.1	50.9	4.5	5.0	1.6	12.2	54.7	5,722
AHF14*RTX436	W	T	st	4.0	72.0	44.0	3.4	3.2	1.0	13.6	54.0	5,623
AHF14*96GCPobs124	R	T	st	2.6	72.0	43.9	2.7	3.4	1.1	13.7	56.9	5,595
A9701*RTX437	R	T	t	3.0	68.5	45.0	4.0	4.4	1.0	13.7	53.3	5,590
ATX631*R9818	W	T	b	3.1	74.6	48.7	3.7	3.4	1.3	14.0	57.5	5,587
AHF14*96GCPobs172	R	T	t	2.8	71.8	41.8	2.9	3.4	1.1	14.9	60.1	5,484
AHF8*96GWO92	R	T	st	1.3	76.3	42.5	3.7	4.0	1.0	14.9	59.9	5,480
ATX631*R9317	W	T	t	3.6	73.9	46.7	3.5	3.4	1.5	14.1	58.7	5,455
ATX631*R9528	W	T	lb	3.3	73.6	48.1	3.7	2.9	1.4	14.0	57.4	5,447
ATX631*R9693	W	T	st	3.3	72.5	48.1	3.8	4.1	2.8	14.0	58.5	5,395
AHL8*R9317	W	T	st	4.5	72.8	41.8	4.7	3.0	1.3	13.9	43.2	5,263
A9614*RTX436	W	T	st	3.8	74.9	49.2	4.6	4.2	2.4	14.1	56.9	5,243
A8PR1057*Tx436	R	T	t	3.5	74.1	43.8	3.4	3.7	1.1	14.5	59.2	5,201
A9717*RTX2903	R	T	st	2.6	72.6	42.9	4.3	3.5	1.4	14.0	57.1	5,180
ATX631*R9759	W	T	t	2.6	71.8	51.6	3.7	4.6	3.5	13.9	55.4	5,116
ATXARG-1*R9818	W	T	t	3.3	73.8	43.7	3.6	3.6	1.1	13.6	39.2	5,048
A0PR59*5BRON139	R	T	t	1.6	78.8	43.9	3.0	3.9	1.0	14.7	55.6	5,046
A9601*RTX436	W	T	lb	3.8	73.1	46.5	5.8	4.1	2.6	13.9	56.1	5,010
ATX635*RTX436	W	T	st	3.8	76.5	51.1	4.2	4.2	1.4	14.4	59.9	5,009
A9701*RTX436	R	T	st	2.5	70.6	44.6	5.0	4.0	1.3	13.6	57.0	4,949
ATX631*R9839	W	T	t/sn	3.3	55.5	50.0	2.9	4.9	1.8	14.1	58.6	4,920
AHL8*R9528	W	T	st	3.8	73.1	45.3	4.1	4.3	1.0	14.2	41.7	4,906
A9717*RTX436	W	T	st	5.1	69.9	42.0	4.7	3.6	1.3	13.1	56.7	4,899
A8PR1049*Tx436	W	T	st	4.3	74.5	44.4	3.8	3.6	1.4	13.7	56.3	4,827
ATX631*RTX2903	R	T	lb	1.8	74.6	45.0	3.7	3.4	1.4	14.3	56.2	4,743
ATX631*R9752	W	T	t	3.5	75.4	48.9	3.6	3.9	2.3	13.6	55.3	4,741
AHF8*5CA4625	R	T	t	2.8	74.8	42.9	3.0	4.0	1.0	14.5	59.5	4,724
ATXARG-1*R9759	W	T	t	2.6	74.0	45.6	4.0	3.8	2.3	13.9	57.3	4,719
ATXARG-1*RTX436	W	T	t	4.0	75.3	42.2	3.5	3.8	1.4	13.8	41.7	4,687
ATX631*R9809	R	T	st	2.5	75.0	48.0	3.2	5.4	2.6	13.6	55.5	4,681
ATX631*5CA4213-1	R	T	t	2.8	52.8	46.1	2.6	4.4	1.3	15.1	58.6	4,668
ATXARG-1*R9693	W	T	t	4.0	73.9	43.2	3.9	4.4	1.9	13.8	58.0	4,660
AHF14*5CA4205-3	R	T	st	2.5	75.8	42.0	3.2	3.9	1.0	14.6	60.0	4,644
ATXARG-1*R9839	W	T	t	4.1	57.9	43.2	2.1	4.7	1.3	13.8	40.2	4,546
AHF8*R9752	W	T	sn	3.5	74.3	45.5	4.0	3.9	1.3	13.9	58.1	4,460
AHF14*5CA4208	R	T	t	2.3	75.9	43.5	3.0	3.5	1.0	14.7	42.6	4,426
ATXARG-1*R9805	R	T	t	3.0	74.0	43.9	4.7	4.4	2.0	13.6	57.2	4,425
ATX631*R9840	W	T	sn	3.1	74.5	49.3	3.3	4.2	3.3	14.3	57.7	4,386
ATX631*5CA4631	W	T	t	2.5	53.9	49.9	3.2	3.9	2.1	15.0	59.5	4,346
A8PR1057*5BRON155	R	T	t	1.8	54.9	42.1	2.9	4.2	1.0	14.8	41.4	4,316
ATX631*R9805	R	T	bf	2.5	73.0	47.5	5.2	4.9	2.6	13.7	59.4	4,253
ATX635*R9840	W	T	st	3.3	67.8	49.5	4.2	5.1	2.8	14.0	57.7	4,049
ATX635*R9809	R	T	t	2.1	78.1	52.2	5.1	6.4	2.9	13.7	59.3	3,883
GRAND MEAN				3.1	71.2	45.9	3.8	4.0	1.6	14.0	55.1	5,052
CV				23.0	7.0	4.6	28.4	21.4	67.9	9.4	24.7	17
LSD (0.05)				1.1	4.1	2.3	0.9	0.9	1.0	1.2	13.2	665

† SD = seed color (R = red, W = white, Y = yellow); PL = plant color (P = purple, R = red, T = tan); GL = glume color (t = tan, b = brown, r = red, sn = siena, st = straw, lb = light brown); GM = grain mold rating (1 = none to 9 = susceptible); DY = mid-anthesis (days); HT = plant height (inches); EX = head exsertion (inches); UN = uniformity (1 to 4 scale); DS = desirability (1 to 9 scale); LO = root and stalk lodging (1 to 9 scale); TW = test weight (lbs/bushel); YD = grain yield (lb/acre)

Participated in the Sorghum Germplasm committee meetings in Wichita, KS and Albuquerque, NM in October 2002 and February 2003, respectively.

Research Investigator Exchanges

Hosted Mr. Leo Mpofu (Zimbabwe) for sorghum breeding training in College Station in July 2002.

Hosted Mr. Joaquim Mutaliano (Mozambique) during his English Language training at Texas A&M from August to December 2002.

Hosted Drs. Issofou Kapran and Salissou Issa (INRAN-Niger) during a visit to Kansas State, Texas A&M, and Purdue Universities in October 2002.

Hosted Dr. R. G. Henzell, Sorghum Breeder from Hermitage Research Station in Queensland, Australia, during a visit to Kansas State and Texas A&M Universities during October 2002.

Germplasm and Research Information Exchange

Coordinated the Tan Plant Hybrid Trial. This trial is designed to evaluate commercially available tan plant (improved grain quality) sorghum hybrids for agronomic adaptation and grain quality parameters. The test included 40 hybrids from eight companies and was grown in nine locations across Kansas and Texas. Included in this trial were breeding lines from TAM223, TAM222 and TAM220C.

Distributed germplasm from TAM220C for evaluation in Central America and Southern Africa.

Distributed germplasm from KSU220A for evaluation in Niger and South Africa.

Publications and Presentations

Journal Articles

Moran, J.L., and W.L. Rooney. 2003. Comparative agronomic performance of iso-cytoplasmic grain sorghum hybrids. Crop Sci. 43:777-781.

Prom, L.K., R.D. Waniska, A.I. Kollo, and W.L. Rooney. 2003. Response of eight sorghum cultivars inoculated with Fusarium thapsinum, Curvularia lunata, and a mixture of the two fungi. Crop Protection (in press).

Rooney, W.L. 2003. Registration of Tx2912 to Tx2920 sorghum germplasm. Crop Sci. 43: 442-443.

Rooney, W.L. 2003. Registration of Tx2921 to Tx2928 sorghum germplasm. Crop Sci. 43: 443-444.

Rooney, W.L., F.R. Miller, and L.W. Rooney. 2003. Registration of RTx437 sorghum parental Line. Crop Sci. 43: 445-446

Yu, J., and M.R. Tuinstra. 2003. Analysis of cold tolerance in sorghum under controlled environment conditions. Field Crops Research (in press).

Hicks, C., M.R. Tuinstra, J.F. Pedersen, F.E. Dowell, and K.D. Kofoid. 2002. Genetic analysis of feed quality and seed weight of sorghum inbred lines and hybrids using analytical methods and NIRS. Euphytica 127:31-40.

Islam-Faridi, M.N., K.L. Childs, G. Hodnett, M.A. Menz, R.R. Klein, P.E. Klein, W.L. Rooney, J.E. Mullet, D.M. Stelly, and H.J. Price. 2002. A Molecular Cytogenetics Map of Sorghum Chromosome 1: FISH Analysis with Mapped BACs. Genetics 161:345-353.

Morgan, P.W., S.A. Finlayson, K.L. Childs, J.E. Mullet, and W.L. Rooney. 2002. Developmental physiology of grasses: Opportunities to improve adaptability and yield. Crop Sci. 42:1791-1799.

Reed, J.D., M.R. Tuinstra, B.A. Ramundo, and L.E. Claflin. 2002. Analysis of resistance to ergot in sorghum and potential alternate hosts. Crop Sci. 42:1135-1138.

Reed, J.D., M.R. Tuinstra, N.W. McLaren, K.D. Kofoid, N.W. Ochanda, and L.E. Claflin. 2002. Analysis of combining ability for ergot resistance in sorghum. Crop Sci. 42:1818-1823.

Books, Book Chapters, and Proceedings

Moran, J.L., W.L. Rooney, G.N. Odvody, and R.A. Frederiksen. 2003. Differences in ergot vulnerability among sorghum genotypes and the relationship between stigma receptivity and ergot vulnerability. pp. 113-120. In J.F. Leslie (ed.), Sorghum and Millets Diseases. Iowa State Press, Ames, IA.

Rooney, W.L., S.D. Collins, R.R. Klein, P.J. Mehta, R.A. Frederiksen and R. Rodriquez-Herrera. 2003. Breeding sorghum for resistance to anthracnose, grain mold, downy mildew and head smuts. pp. 273-280. In J.F. Leslie (ed.), Sorghum and Millets Diseases. Iowa State Press, Ames, IA.

Tuinstra, M.R., T.T. Teferra, L.E. Claflin, R.G. Henzell, A. Borrell, N. Seetharama, G. Ejeta, and D.T. Rosenow. 2002. Breeding for resistance to root and stalk rots in sorghum. pp. 281-286. In J.F. Leslie (ed.), Sorghum and Millets Diseases. Iowa State Press, Ames, IA.

Dissertations and Theses

Kriegshauser, T.D. 2003. Genetic analysis of large-seeded sorghum hybrids with increased grain-fill duration and effects of increased seed size on feed quality. M.S. Thesis. Kansas State University, Manhattan, KS.

Stamm, M.J. 2003. Effects of a genetically longer grain fill duration on seed weight and composition of grain sorghum. M.S. Thesis. Kansas State University, Manhattan, KS.

Tesso, T.T. 2002. Analysis of resistance to Fusarium stalk rot in grain sorghum [Sorghum bicolor (L.) Moench]. Ph.D. Dissertation. Kansas State University, Manhattan, KS.

Abstracts

Kriegshauser, T.D., M.R. Tuinstra, and J.D. Hancock. 2002. Feed quality analysis of large-seeded sorghum hybrids. INTSORMIL Principle Investigators Conference, Addis Ababa, Ethiopia, November 16-23.

Rooney, W.L., D.T. Rosenow, G.C. Peterson, and M.R. Tuinstra. 2002. Application of molecular marker technology to sorghum improvement programs. INTSORMIL Principle Investigators Conference, Addis Ababa, Ethiopia, November 16-23.

Stamm, M.J., and M.R. Tuinstra. 2002. Analysis of protein partitioning in large-seeded sorghum. ASA-CSSA-SSSA Conference, Indianapolis, IN, November 11-14.

Low Input Ecologically Defined Management Strategies for Insect Pests on Sorghum

Project MSU 205

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Summary

With the conclusion of research activities in Honduras during the past 23 years, crop pest management research was expanded in Nicaragua and El Salvador in 1999-2002, with emphasis on insect pest constraints to sorghum production in improved cropping systems on large agricultural farms on the Pacific coastal plain. Unlike the activities of the past 23 years when this project worked with low input, subsistence farming systems in Honduras, activities in Nicaragua and El Salvador emphasize development of insect pest management tactics and strategies on sorghum in improved technology production systems. Collaborative research activities with the Instituto Nicaraguense de Technologia (INTA), the Universidad Nacional Agraria (UNA), the Nicaraguan National Sorghum Producers Association (ANPROSOR) in Nicaragua, and the Centro de Technologia de Agricola (CENTA) in El Salvador have included investigations on insect biology, behavior, ecology and population dynamics of the sorghum midge and fall armyworm, the two principal insect pests on sorghum in this region of Central America. Information from these investigations is used in developing cultural, biological and chemical control tactics for implementation in insect pest management systems for specific pests or complex of pests. Popular articles have been published for farmer utilization in the application of sorghum midge pest management in Nicaragua and El Salvador. Complementary research on insect pest behavior and damage to sorghum is in progress in the United States for improving sorghum midge and fall armyworm pest management strategies. The collaborative research activities among INTSORMIL and research and farmer organizations have been fruitful in developing greater research capacity and furthering institution building activities in this ecogeographic zone. Graduate student education and professional workshops have increased agricultural capabilities of professionals in this region of Central America. The MSU 205 principal investigator will continue to support graduate student education, to conduct sorghum research in Central America and the United States, to collaborate with scientists in governmental organizations and agricultural universities, and to work with non-governmental organizations to develop improved insect pest management practices for sorghum production.

Objectives, Production and Utilization Constraints

Nicaragua

- Refine investigations to determine fall armyworm occurrence, seasonal population levels, and extent of damage to sorghum by this pest. Conduct pest management studies with fall armyworm.
- Prepare manuscripts for publication in scientific journals and popular article for distribution into farm communities.

- Meet with Central America collaborator scientists in INTA, UNA UNAN and ANPROSOR to develop collaborative sorghum crop protection research plans for 2002.
- Complete second year of research and academic programs for MSU 205 Ph.D. student.

El Salvador

- Collaborate with scientists in CENTA to evaluate insecticides and application procedures for fall armyworm management and evaluate sorghums for resistance to this lepidopterous pest.

United States

- Continue experiments to evaluate the effectiveness and economical benefit of insecticide spray programs and refine the economic thresholds for fall armyworm and sorghum midge on sorghum.

Research Approach and Project Output

Nicaragua

MSU 205 initiated research activities in Nicaragua in 1999, after initially developing collaborative relationships with scientists at INTA in Managua in 1998. Unlike research activities in Honduras during the past 23 years in subsistence farming situations, entomological research in Nicaragua emphasized insect pest constraints to sorghum production in large, improved technology systems on the Pacific coastal plain. The principal insect pest constraints to sorghum production on the coastal plain are recognized to be sorghum midge, fall armyworm and chinch bug. Research was completed on seasonal occurrence of sorghum midge on host plants and oviposition behavior on specific hosts. Tactics for management of the midge were evaluated and included planting date, crop variety and insecticide efficacy. A manuscript, representing this research was published in the international journal, *Tropical Agriculture*. A second journal paper considering the occurrence of sorghum midge on sorghum during the second crop-growing season on the Pacific coastal plain of Nicaragua was published in *La Calera* the scientific journal of the National Agricultural University of Nicaragua. A popular article, "La Mosquita De La Panoja Del Sorgo", was published by INTA and prepared for distribution into farm communities in 2002. The information in this publication will assist farmers in sorghum midge pest management.

The student from Nicaragua that completed the Master of Science degree in entomology is continuing entomology studies for the Ph.D. degree at Mississippi State University. This research emphasizes economic thresholds and evaluations of fall armyworm and sorghum midge management practices in monoculture sorghum in the United States.

Entomological investigations were initiated with UNA, with additional participation with ANPROSOR in 2002 following meetings to discuss constraints to sorghum production in Nicaragua. Collaborative crop protection research was discussed and plans were made for the 2002-2005 growing seasons, which begins in August. There was specific interest in crop protection training. Particular emphasis was given to developing plans for collaborative, multisciplinary, on-farm crop protection investigations with MSU 205, INTA, UNA and ANPROSOR collaborating. Collaborative research activities among these organizations was limited in 2002, although meetings among scientists from each organization to discuss research plans for the future did occur. This was evident in the joint planning and conduct of meetings among scientist and with producers.

Studies were conducted by INTA scientists to evaluate alternative treatments for managing sorghum midge and leaf-footed bugs on sorghum panicles. Treatments included biological organisms, plant and insecticide chemicals, and crop barriers. Sorghum midge infestations and damage were less in insecticide treatment compared with the barrier crop treatment; the biological treatments provided interesting comparisons, but more research is needed to substantiate the results obtained.

The MSU 205 PI (Henry Pitre) and KSU 211 PI (Larry Claflin) conducted a five-day sorghum plant protection workshop in Managua in 2002. The workshop was sponsored by INTA and UNA and was attended by 38 agricultural professionals from INTA, UNA and ANPROSOR (in Nicaragua) and CENTA (in El Salvador). Technical presentations included entomology and plant pathology pest management principles, pest management tactics and strategies, defining integrated pest management programs and specific insect and disease agent pest constraints to sorghum production in Nicaragua and the region and related pest management programs. Field trips were taken to observe insects and related plant damage, as well as plant diseases on sorghum. This workshop provided the stimulus for several sorghum pest management meetings that were conducted by UNA, INTA and ANPROSOR in a collaborative effort. Two meetings were held, one with producers from the hills and the coastal plain, and the other with farmers from the coastal plain. Forty producers attended each workshop. Production problems were identified to include specific insect pests, fungal diseases, and mycotoxins in grain. Specific needs were addressed to include: workshops on IPM and post harvest problems, and information on identification, weed control, variety selection and fertilization.

Plans were made for a collaborating scientists at UNA to begin a Ph.D. program in June, 2003 at Mississippi State University. This scientists will receive additional training in plant pathology, with specialized training in insect pest management. Research activities will be collaboratively directed by scientists at Mississippi State University and Kansas State University. The student participated in the Fusarium Laboratory Workshop held at Kansas State University in June.

El Salvador

Entomological research with scientists in CENTA in El Salvador was initiated in 2001. Insects of greatest interest and thought to be the most damaging to sorghum crops in El Salvador include the complex of soil inhabiting insects and defoliators (particularly fall armyworm). The objectives of research for 2001-2002 involved determining the extent of damage and economic significance of fall armyworm on sorghum. This objective included elucidation of the occurrence and aspects of population dynamics and control tactics for this pest. Observations on populations of this caterpillar on and damage to sorghum in the All Disease and Insect Nursery (ADIN) was made during the crop growing season. This was coordinated with collaborating sorghum breeder and plant pathologists in CENTA and Kansas State University (KSU 211), respectively. The results of these investigations were reported in the MSU 205 Year 23 annual report, and published as three separate papers in the scientific journal *La Calera*. The results of the investigations indicate that sorghum plants damaged by fall armyworm in early vegetative stages can compensate for this damage during later stages of plant development. This further indicates that insecticides should be used with complete knowledge of the stage of plant development at the time of fall armyworm infestation and potential for this pest to cause irreversible feeding damage to the developing plants. The infestation level at critical times during sorghum development should be given particular attention in recommending fall armyworm control measures using recommended insecticides. Additional research is needed to refine the recommendations for fall armyworm pest management on sorghum during different phenological stages of the crop.

Studies involving the examination of economic threshold levels for fall armyworm on whorl-stage sorghum in 2002 corroborated the results of similar studies conducted in 2001. These studies were conducted at three locations. Plants infested with fall armyworms at levels ranging from 0 to 80 percent had similar yields at harvest.

Sorghums in the ADIN were again evaluated in 2002 in El Salvador and sorghum fields were sampled to determine occurrence of insect pests during the growing season. A stem borer was recognized to cause damage to sorghum in some areas; the extent of damage was not determined. Also, a lepidopterous larvae, the pink scavenger caterpillar, was observed to infest sorghum panicles. Plans have been made to conduct research on these pests in 2003.

Plans were completed for the collaborating entomological scientists and CENTA to begin formal English language training in August 2003 at the University of Nebraska. The scientists will begin a Ph.D. program in entomology at Mississippi State University in January 2004.

United States

The economic threshold for caterpillar pests on whorl stage sorghum and sorghum midge on panicles is not clearly identified for sorghum in different growth stages. Preliminary studies conducted with fall armyworm to determine infestation levels suitable for artificial infestations, survival of fall armyworm larvae in stages of development at infestation and over time after infestation, time of day most suitable for infestation, and other infestation procedures. This information was used in 2002 to observe fall armyworm larval behavior and to refine economic threshold levels using two strategies, one involving number of insects per plant and the other percentage of plants infested. Yield data was recorded for treatments. Information from these studies can improve the application of pest management practices for fall armyworm on sorghum.

Sorghum fields for treatment infestations including one, two, three or four inoculations were compared with un-infested plants in 2002. No significant differences in yield were recorded among treatments regardless of the number of larval infestations. This information further suggests that FAW larvae damage to sorghum throughout plant development (whorl to boot stages) may not significantly reduce yield when plants are not stressed. Insecticide application to control this pest on sorghum may not be warranted.

Further investigations to evaluate the total effects of FAW feeding damage and economic thresholds for this pest on sorghum are planned for 2003. These studies include 1) Effect of multiple infestations of FAW on sorghum yield and 2) FAW damage to sorghum panicles.

Networking Activities

The sorghum crop protection workshop in Nicaragua organized by INTSORMIL (MSU 205) KSU 211, INTA, UNA and ANPROSOR served as the stimulus for the development of meetings between scientists, farm organizations and sorghum producers. Such meetings were conducted in 2002-2003 and additional meetings are planned for 2003. They included aspects of integrated insect pest and plant disease management. The workshops were successful because of detail coordination by scientist and administrators at INTA, UNA and ANPROSOR.

Research investigator exchanges involved shipment of supplies and small equipment for research purposes.

Networking with ANPROSOR provides opportunities to conduct on-farm research with cooperation from many farmers associated with this national sorghum producers association.

The popular articles on sorghum insect pests provide information for farmers to manage these pests on sorghum to

improve yield. Publications are distributed by INTA into farm communities with assistance from local agricultural professionals.

Publications and Presentations

Journal Articles

Carrillo, M.A. and H.N. Pitre. 2002. Observations on diapause in *Metaponpneumata rogenhoferi*: Moschler in southern Honduras and notes on other lepidopterous species. Ceiba. 43(2): 2-10.

Zeledon, J. and H.N. Pitre. 2002. Management of sorghum midge on sorghum on the coastal plain of Nicaragua. Trop. Agric. 79(2): 114-119.

Cordero, R.J. and H.N. Pitre. 2002. Development of *Metaponpneumata rogenhoferi* on maize, sicklepod and wheat germ diet. Trop. Agric. 79(2): 133-136.

Carrillo, M.A., H.N. Pitre and R.J. Cordero. 2002. Development and reproduction by *Metaponpneumata rogenhoferi* Moschler. Trop. Agric. 79(3): 1-5.

Vergara, O.R., H.N. Pitre and D.W. Parvin. 2002. Economic evaluation methods for integrated pest management in intercropped maize and sorghum production systems in southern Honduras. Ceiba. 43(2): 35-45.

Presentations

Jaco, M.P., H. Pitre and J. Zeledon. 2002. Control of Fall Armyworm (*Spodoptera frugiperda)* on Sorghum in El Salvador. INTSORMIL Principal Investigators Conference. Addis Ababa, Ethiopia. Nov. 18-20. (Poster)

Pitre, H.N. Pest Management Principles Applicable to Entomology. Sorghum Plant Protection Workshop. 2002. Managua, Nicaragua.

Pitre, H.N. Insect Pest Management Tactics and Strategies for Sorghum Production. Sorghum Plant Protection Workshop. 2002. Managua, Nicaragua.

Pitre, H.N. The Sorghum Plant and Sorghum Insect Pests. Sorghum Plant Protection Workshop. 2002. Managua, Nicaragua.

Pitre, H.N. Insect Pests on Sorghum: Laboratory Identification. Sorghum Plant Protection Workshop. 2002. Managua, Nicaragua.

Pitre, H.N. Insect Pests on Soybeans and Sorghum. Miss. Coop. Ext. Serv. Commercial Applicators Training Program. 2002. Mississippi State University.

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 S*triga*, 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 recent activities in the area of research on breeding for durable resistance to *Striga* in sorghum. Our program emphasizes identification and characterization of genetic variants of sorghum with known inheritance and expression of biological defense responses. We employ simple laboratory bioassays and molecular markers in identifying new variants and introgressing genes for *Striga* resistance from various sources into desired genotypes. Sorghum cultivars with single as well as multiple mechanisms of *Striga* resistance have been generated. Field evaluations are conducted in Africa to test efficacy of each putative *Striga* resistance mechanism as well as level and durability of the resistance acquired by pyramiding genes from several sources. In 2002, after extensive testing in multi-location tests, one of our elite lines was officially released for commercial cultivation in the Amhara region of Ethiopia. The cultivar was recommended and seed disseminated under a local name, "Brhan", translated as "light in the midst of the darkness", *Striga*.

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 objective 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

Breeding for Durable Resistance to Striga in Sorghum

The use of crop cultivars with resistance to *Striga* has been

widely acknowledged as the most practical and economically feasible control measure, particularly for subsistence farmers in *Striga* endemic regions of Africa and India. Host-plant resistance is also central to integrated *Striga* management approaches when combined with other innovative and input-based farming practices. However, progress from past efforts in breeding *Striga* resistant crops has been rather limited. The reasons for the slow progress vary from complexity of the trait to lack of research support as well as lack of a functional and rational approach to selection strategy. In recent years, significant advances have been made in sorghum, maize, and cowpeas leading to development of cultivars with good levels of *Striga* resistance. Nevertheless, crop cultivars with durable resistance to *Striga* have not yet been identified.

With a research paradigm that focuses on dissecting *Striga* resistance into simple highly heritable components, development of bioassays that allow identification of disrupted host-parasite associations, and adoption of molecular marker technologies, it is now possible to develop crop cultivars with multiple mechanisms of *Striga* resistance. Crop genotypes that possess multiple genes for *Striga* resistance, based on distinct mechanisms, are likely to have genetic resistance that is durable across several environmental conditions as well as across ecological variants of the parasite. In this report, we discuss the strategy we have adopted and the progress we have made in breeding sorghum cultivars with durable resistance to *Striga* in a collaborative research approach engaging a number of scientists from several organizations.

The central theme of our *Striga* research program at Purdue University is to develop a better understanding of the biology of host-parasite interaction in the life cycle of *Striga* parasitism. We focus on the characterization of evidences for signal and resource exchange between the host and parasite. Our objective is to isolate a specific signal exchange or interaction between the host and parasite and, based on such an observation, develop appropriate laboratory assays for screening genetic variants of sorghum that may lack or produce an essential signal or a defense response. Our working hypothesis is that mechanisms of resistance could be defined on the basis of host-dependent developmental processes and the essential signals exchanged. Hence, disruption of one of these signals or resources results in failure to establish parasitism. The basic rationale and theoretical assumptions behind our approach have been outlined in an earlier report

The lack of simple, rapid, and reliable laboratory procedures for evaluating crop germplasm has hampered progress in breeding for *Striga* resistance. In the last few years, reliable bioassays have been developed for germplasm screening and generation of genetic information. In our breeding program we routinely employ the Agar Gel Assay for germplasm screening as well as for examining host-parasitic interactions during the early infection process. Disruption in signal exchange at the important stages of germination and haustorial initiation early in life cycle can be detected by this assay. We have more recently developed two other in vitro techniques, the Extended Agar Gel Assay and the Paper Roll Assay described at this conference. These new assays allow critical observation of genetic differences among host genotypes at later stages during attachment, penetration, and development of the parasite on host tissue. We have used these three assays to identify genetic variants and characterize mechanisms of *Striga* resistance associated with: a) low germination stimulants (*lgs*) production, b) low haustorial factor (*lhf*) production, c) hypersensitive response (*HR)*, and d) incompatible response (*IR*) to infection.

We generated several recombinant inbred populations segregating for genes conditioning resistance due to each of the mechanisms of resistance identified. These populations are genotyped using SSR markers to identify molecular markers linked with genes controlling *Striga* resistance. Introgression and pyramiding of genes for multiple mechanisms of *Striga* resistance is facilitated by both the bioassays as well as these molecular markers. Evaluation of field *Striga* resistance of genetic stocks, segregating populations, and recombinant inbred lines have been conducted in *Striga* infested fields in several countries. Although we had earlier tested against *Striga asiatica* at Whiteville, NC, all our recent field-tests have been conducted against *Striga hermonthica* in Africa in collaboration with several national research programs.

With availability of simple laboratory bioassays, isolation of genetic variants of host plants that disrupt parasitic association at critical stages has been made possible. We have particularly exploited the mechanism of *Striga* resistance based on the low production of host plant root exudates required for germination. Diverse sorghum genotypes with little or no stimulant production capacity have been identified. The genetics of low germination stimulant (*lgs*) production has been established. A number of improved sorghum varieties with *Striga* resistance due to *lgs* have been developed and effectively disseminated to *Striga* endemic areas in several African countries. Introgression of genes for low haustorial factor (*lhf*), hypersensitive response (*HR*), and incompatible response (*IR*) to *Striga* infection into improved sorghum germplasm are currently all underway in our sorghum breeding program.

The difficulty of empirical breeding of *Striga* resistance in the field, associated with the complexity of the trait and its interaction with the environment, coupled with the gradual improvement in the cost effectiveness of PCR technologies have made use of molecular markers a viable alternative in *Striga* resistance breeding worldwide. In our sorghum research program, we have generated a sorghum linkage map of 1628 centiMorgans (cM), with an average interval of 9.5 cM between adjacent loci. Putative QTL associated with field resistance to both *S. asiatica* and *S. hermonthica* have been detected. Two of these QTL are mapped on the same linkage group with *lgs*, but independently from factors conditioning *Striga* resistance due to other mechanisms. Several studies are underway to test different mapping populations at a number of locations with an international network of collaborators to identify key QTL that

are universal for use in marker assisted selection for *Striga* resistance.

There appears to be a paucity of major gene sources of *Striga* resistance among germplasm pools of cultivated sorghums. Among landraces and improved sorghum lines that we screened using the different bioassays, variants with *lhf, HR,* and *IR* have been rare, but sources of *lgs* have been more commonly obtained. In contrast, there has been a greater preponderance of genetic variation for many of these traits among sorghum germplasm in wild and related species. This has opened great opportunities for introgressing genes from these wild species into cultivated sorghum lines for greater protection, either sequentially or via gene stacking approaches.

Improved sorghum varieties with resistance to *Striga* have been developed and released in several African countries. However, the wide use of these cultivars by farm communities is limited primarily because of lack of strong seed production and distributing agencies. Furthermore, some ecologies in Africa favor local landraces that are uniquely adapted to these niches where introduced cultivars do not do as well. In the highlands of Ethiopia and in the high rainfall areas of West Africa where long duration Dura and Guinea sorghums, respectively, are cultivated, improved *Striga* resistant Caudatum are not accepted because of problems of grain deterioration even when agronomically adapted. Such niches also exist in Tanzania where the so-called rice-type Guinea sorghums are favored. In these specialized niches, there is a need to incorporated *Striga* resistance genes into favored local landrace cultivars. Some of these varieties have been farmer-selected for *Striga* tolerance. We are currently undertaking an introgression of genes for *Striga* resistance and tolerance into selected landraces for Ethiopia, Sudan, and Tanzania in East Africa, and Niger and Mali in West Africa.

In addition to combining *Striga* tolerance from African landraces with major gene resistance sources from our genetic stocks, we have also embarked on stacking major genes resistances based on different mechanisms into improved sorghum lines. We hypothesize that pyramiding genes for *Striga* resistance based on different mechanisms would enhance durability of resistance sources as well as stability of performance under changing environmental conditions. Preliminary evidence, from field testing under *Striga hermonthica* in Africa, of experimental sorghum lines that combine mechanisms of *Striga* resistance based on *lgs, HR,* and *IR* suggest that there is stronger field resistance expression in those lines than in single mechanism resistance sources.

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 of 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 visitors from India, Ethiopia, Uganda, Burkina Faso, 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, Uganda, Kenya, Eritrea, Niger, Mali, and Zimbabwe.

Publications

Refereed Papers

Mohamed, A., A. Ellicott, T. L. Housley and G. Ejeta. 2003. Hypersensitive response to *Striga* infection in sorghum. Crop Sci. 43: 1320-1324.

Rich, P.J., C. Grenier and G. Ejeta. 2003. Sources of *Striga* resistance mechanisms in wild relatives of sorghum. Crop Sci. (In Press).

Conference Proceedings

Grenier, C. A. Mohamed, P.Rich, T. Housley and G. Ejeta. 2002. Bioassays to characterize and dissect mechanisms of resistance to *Striga*. *In*: Devries et al (eds) Proc Int. Conf. On Biotechnology, Breeding and Seed Systems for Africa. INTSORMIL, Kamapala, Uganda. (In Press).

Kapran, I., C. Grenier, A. Ellicott, A. Toure, Z. Gutema, A. Babiker, H. Sadaan and G. Ejeta. 2002. Introgression of genes for *Striga* resistance into African landraces of sorghum. *In*: Devries et al (eds) Proc of Int. Conf. On Biotechnology and Seed Systems for Africa, Kampala, Uganda (In Press).

Published Abstracts

Mohamed, A., A. Ellicott, T. L. Housley and G. Ejeta. 2002. Hypersensitive response to *Striga* infection in sorghum. INTSORMIL Int. Principal Investigators Conference, Addis Ababa, Ethiopia.

Invited Presentations

Ejeta, G. 2002. Advances in *Striga* biotechnology and control at INTSORMIL, Principal Investigators Conference, 17-20 November, Addis Ababa, Ethiopia.

Ejeta, G. 2002. Increasing crop yields through hybrids: Prospects for sorghum hybrids in Ethiopia. First National Workshop on Sorghum and Millets in Ethiopia, 11-14 November, Nazret, Ethiopia.

Ejeta, G. 2002. Biotechnology based approaches to the study of host plant resistance to *Striga* in sorghum. First National Workshop on Sorghum and Millets in Ethiopia, 11-14 November, Nazret, Ethiopia.

Ejeta, G. 2002. Host-plant resistance to *Striga* in sorghum. Training Workshop on *Striga* Resistant Sorghum Seed Seed Production and Dissemination. Melkassa, Ethiopia.

Sustainable Management of Insect Pests

Project WTU 200

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West Texas A&M University

Principal Investigator

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Collaborating Scientists

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Mr. Hamé Abdou Kadi Kadi, Entomologist, INRAN/CERRA, BP 240, Maradi, Niger
Dr. D. C. Munthali, Entomologist, Private Bag 0027, Botswana College of Agriculture, Gabarone, Botswana
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Summary

The PI traveled to Mali in October to review collaborative research to manage insect pests and develop integrated pest management (IPM) approaches for sorghum and pearl millet in the field and storage. The biology of and resistance to insect pests of sorghum and pearl millet was researched with Mr. Abdou Kadi Kadi in Niger. A Malian came to West Texas A&M University to learn English and begin graduate studies. The graduate program of one student from the United States was completed. Graduate students assessed effects of temperature on fecundity and longevity of different biotypes of greenbug on sorghum, fitness of greenbug biotype I on resistant and susceptible sorghums and wild grasses, and effects of different amounts of soil moisture and nitrogen on the biology of greenbugs. Graduate students began assessing effects of resistant sorghum on coccinellids feeding on greenbugs from the sorghum and began assessing resistance of sorghum and cowpeas to storage weevils. Sorghums and a new insecticide developed by commercial companies were evaluated against greenbugs. The PI advised agricultural consultants, extension personnel, and the National Grain Sorghum Producers on management of insect pests. The PI and graduate students participated in sorghum and entomology meetings.

Objectives, Production and Utilization Constraints

Objectives

West Africa

- Assist scientists from Mali and Niger with collaborative research to develop and transfer strategies, especially non-chemical methods, to manage insect pests and improve yield and income from sorghum and pearl millet.
- Identify a Malian to begin graduate studies in IPM and entomology in the United States.

Southern Africa

- Assist scientists in Botswana and South Africa with research to evaluate resistance and develop IPM strategies for insect pests of sorghum in the field and storage.

United States

- Study the biology, ecology, and population dynamics of insect pests so effective management strategies and longer-lasting plant resistance can be developed. Assess fitness of greenbugs on wild and cultivated grasses to better understand insect-plant interactions. Assess effects of temperature on the biology of greenbug biotypes to determine the optimum temperature for evaluating resistance to greenbug in sorghum.
- Assess effects of agronomic practices on the abundance of and damage caused by insect pests. Study effects of soil moisture and fertility on abundance of greenbugs on sorghum.
- Collaborate with breeders, commercial seed industry, and molecular biologists to develop sorghum germplasm for greater yield potential and tolerance to major insect pests.

- Supervise graduate student research in entomology and IPM.
- Advise agricultural consultants, extension personnel, commodity organizations, and the sorghum industry with managing insect pests of sorghum.
- Participate in professional and scientific meetings and activities.

Production Constraints

West Africa

Abiotic stresses and such biotic constraints as insects, diseases, and *Striga* limit production of sorghum and pearl millet in West Africa. The most damaging insect pests are panicle-infesting bugs; sorghum midge, *Stenodiplosis sorghicola*; sugarcane aphid, *Melanaphis sacchari*; and stalk borers on sorghum, and millet head miner, *Heliocheilus albipunctella*, on pearl millet. Sorghum midges can destroy 100% of kernels. Panicle-infesting bugs and associated infection by pathogens reduce yield and quality and render grain unusable for human consumption. Stalk borers bore into sorghum and kill the central shoot or break the peduncle. Larvae of millet head miner cut flowers and tunnel in kernels of pearl millet. Other insects can become pests when agronomic practices are changed and new crop varieties are used. Effective management of insect pests requires a multi-disciplinary team with knowledge of entomology, plant pathology, agronomy, plant breeding, and cereal quality.

Southern Africa

Sugarcane aphid, sorghum shoot fly, *Atherigona soccata*; sorghum midge, stalk borers, and termites infest and reduce yields of sorghum in the field. Beetles destroy stored sorghum grain. Few taxonomic keys are available for correct identification of insects from the region.

United States

Major insect pests include greenbug, sorghum midge, and panicle-infesting bugs and caterpillars. Ecosystem disruption caused by monoculture of sorghum increases the severity of pests and results in increased production costs and reduced yield. Insecticides prevent damage and yield loss, but overuse results in increased production costs, disruption of the ecosystem, outbreaks of secondary arthropod pests, resurgence of the targeted pest, and environmental contamination. Biology, insect-plant interactions, amounts of damage, and economic and ecological costs associated with the use of chemicals to control insect pests need to be understood better. Biological and cultural management tactics such as use of resistant cultivars are needed to prevent damage by insect pests. Development of resistant sorghums requires collaboration among plant breeders, entomologists, and molecular biologists.

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. For effective IPM, the insect pest must be identified correctly; its biology, ecology, and population dynamics understood; abundance determined in relation to crop damage and yield loss; economic threshold determined; and direct control tactics used, especially conservation of natural enemies, agronomic practices, resistant varieties, and chemicals only when necessary. Information and technology from the research is transferred to extension personnel, farmers, and others.

West Africa

Tiecoura Traore from Mali came to West Texas A&M University to learn English and begin graduate studies in IPM and entomology in fall 2003. From 18-29 October, sorghum research was reviewed and collaborative research projects planned with scientists in Mali. Dr. Diarisso evaluated ash and extracts from local plants to control panicle-infesting bugs in the field and beetles of stored sorghum grain. Twenty-five kilograms of leaves from neem trees and from *Calotropus procera* were pounded and mashed in 30 liter of water. The juice was filtered and sprayed on bug-resistant Malisor92-1 and susceptible S34 sorghums as seedlings, at the end of flowering, and at the hard-dough stage. Bugs were counted the day before and week after treatment. Sorghum plots were infested with 51.2, 31.8, and 37.5 bugs in the plots not treated, treated with neem, and treated with *Calotropus*, respectively. There was no significant difference in the number of bugs on plants sprayed with juice from neem or *Calotropus*. Damage was greater on nontreated sorghum than treated Malisor92-1. Leaves of the two plants contained flavonoids, sterols, triterpenes, and other chemical compounds.

In collaboration with Dr. Peterson, Dr. Diarisso, and Mr. Abdou Kadi Kadi, sorghum lines and landraces were evaluated for resistance to insect pests in Mali and Niger. Resistance of 74 sorghums to panicle-feeding bugs and sorghum midge was evaluated at Samanko, Mali. At 50% flowering, sorghum midge larvae per 10 rachis branches per panicle of sorghum in each plot were counted. The percentage of damaged kernels per panicle was calculated. At maturity, the kernels were assessed using a scale of 1 to 9 for damage by sorghum midge. Damage ranged from 1 to 4.5. Four sorghums with scores of 1 for sorghum midge, 2 to 2.5 for bugs, and 2 to 2.5 for grain mold were only slightly damaged. Numbers of bugs ranged from 2.6 to 8.1 per panicle.

Southern Africa

In collaborative research with the PI and Dr. Peterson, Dr. Munthali evaluated 100 sorghum lines from the United States

for resistance to sugarcane aphid, stalk borers, and termites at the Botswana College of Agriculture. Each sorghum was planted in two single-row plots 7 m long. Seeds were sown with 30 cm between plants and 50 cm between rows. A total of 25 seeds was planted per plot. Plants were examined every two weeks. The number of plants infested with sugarcane aphids was recorded four and eight weeks after plant emergence. Sugarcane aphids were more abundant on sorghum in January than March. Abundance in January ranged from 14.8% infested plants of 02CG6312-1 to 100%. Only 02L227-BK sorghum was resistant to both sugarcane aphids and termites, while 02L264 was resistant only to termites. Percentage of plants attacked by stalk borers in January ranged from 10% of 02CG6239-BK and 02L286-BK to 88.6%. The proportion of plants with deadhearts ranged from 0% of 02CG6372-BK to 85.7%. Nine and 14 sorghums had only 0-25% of plants with damaged leaves and deadhearts caused by stalk borers.

Dr. Munthali also evaluated 19 sorghums developed in the SADC region. Each sorghum was planted in four 7- by 7-m plots in a randomized complete block design. Abundance of insects was assessed using the method of House (1985). Coccinellids were counted on all plants in each plot. Abundance of and damage by stalk borers also were assessed on all plants in each plot during the vegetative growth to determine damage to leaves and at grain filling to evaluate damage to stalks. All plants in each plot were dissected and larvae were counted by species. Plants infested with sugarcane aphids decreased from 38.6 to 9.3% from January to March, but all sorghums were equally susceptible (Table 1). Significantly more coccinellids were found in January (5.2 per plant) than March (1.5), but abundance did not vary significantly among the sorghums. The most abundant coccinellid was *Hippodamia variegata* (4.5 beetles per plot). The number of stalk borers did not vary significantly among the sorghums, but infestation was significantly greater in January than March. Damage to Marupantse (2.2) was significantly least in March. Overall, 64.2% of plants were infested per plot. Damage to leaves and stalks was great (78 and 54.2%, respectively), and 40% of plants had deadhearts. The sorghum significantly affected the number of emergence holes per stalk; Marupantsi had fewest holes. Spotted stem borer, *Chilo partellus*, was the most abundant (54.5%) of the stalk borers.

United States

Boot-stage sorghum in Moore County, Texas was used to evaluate 0.56 kg ha^{-1} of Lorsban® (chlorpyrifos) and 0.040 to 0.099 kg/ha of experimental F1785 (FMC Corporation) for suppression of greenbug and effect on coccinellids. Greenbugs and coccinellids on 10 consecutive whole plants in a row were counted. Treated sorghum was infested with significantly fewer greenbugs 3 and 7 days after treatment than was nontreated sorghum. Coccinellids were not influenced by insecticide. Chemically treated leaves in petri dishes were used to assess resistance of field-collected Banks grass mites, *Oligonychus pratensis*. Mites from frequently treated fields on the Texas High Plains were as much as 7,000-fold more resistant to bifenthrin but not dimethoate or propargite than were mites from fields that had never been treated in New Mexico. The PI also evaluated 237 sorghum lines developed by Pioneer Hi-Bred International, Inc. for resistance to greenbug biotype I.

Master's student Kishan Sambaraju assessed the fitness of biotype I greenbugs on wild grasses and resistant and suscep-

Table 1. Effects of sorghums on sugarcane aphid, stalk borers, and coccinellids in Botswana.

	Sugarcane aphid			Stalk borer		
Sorghum	% attacked plants/plot	Damage score	Coccinellids/plot	Damage score	Emergence holes	Larvae/plot
SDSR 91014	23.8 a	2.6 a	2.3 a	4.6 ab	6.6 ab	5.5 abc
BSH 1	25.4 a	2.8 a	2.4 a	4.8 ab	6.8 ab	9.2 ab
SDSH 98009	26.5 a	3.2 a	3.1 a	4.8 ab	8.8 a	8.0 ab
ICR 89028	16.8 a	2.8 a	1.9 a	4.1 ab	6.5 ab	9.0 ab
Segaolane	22.6 a	2.5 a	2.7 a	5.0 a	8.4 a	11.0 a
Tegemeo	31.5 a	3.4 a	4.0 a	5.0 a	6.7 ab	4.9 bc
Marupantsi	18.5 a	2.6 a	2.0 a	3.6 b	2.5 b	3.2 c
Masie	23.9 a	2.8 a	2.0 a	4.9 ab	8.0 a	4.7 bc
SDSR 91039	27.6 a	3.2 a	2.7 a	5.0 a	6.8 ab	6.9 ab
SV 2	24.0 a	3.4 a	2.8 a	4.6 ab	10.5 a	5.2 bc
ICSH 93107	14.6 a	2.2 a	2.7 a	5.0 a	7.4 ab	5.0 bc
Town	24.8 a	2.9 a	4.3 a	4.9 ab	12.6 a	5.7 abc
SV 1	34.7 a	3.4 a	3.6 a	5.0 a	7.8 a	11.1 a
SDSH 98012	29.3 a	3.2 a	5.4 a	5.0 a	13.3 a	9.4 ab
SDS 6013	29.4 a	3.0 a	3.5 a	5.0 a	6.0 ab	6.6 ab
Mmabaitse	27.6 a	3.6 a	3.7 a	5.0 a	10.0 a	8.0 ab
Mahube	11.6 a	2.8 a	2.9 a	4.9 ab	4.8 ab	2.9 c
LARSVYT 46-85	23.9 a	3.2 a	2.1 a	4.9 ab	9.5 a	11.2 a
Phofu	18.3 a	2.4 a	2.3 a	4.8 ab	7.5 ab	5.5 abc
Overall average	23.9	2.9	2.8	4.7	7.5	6.5

tible sorghum and wheat. Greenbugs are thought to survive on wild grasses when cereal crops are not available. Seeds of susceptible RTx430 sorghum; resistant LG-35 sorghum; susceptible Custer wheat; resistant GRS1201 wheat; barnyardgrass, *Echinocloa crus-galli*; Johnsongrass, *Sorghum halepense*; jointed goatgrass, *Aegilops cylindricum*; and Arriba western wheatgrass, *Agropyron smithii*; were sown in a greenhouse. Three, 2.5-cm^3 plastic cages each containing a single greenbug were clipped onto leaves of plants in each of six pots, for a total of 18 clip cages on each kind of grass. The original greenbug was removed after it produced a nymph. The nymph was retained until it produced offspring, which were counted and removed daily. The number of days the greenbug lived was recorded. The experiment was done three times. The pre-reproductive period of the greenbug was longest on barnyardgrass (7.5 days) and western wheatgrass (6.4 days) and shortest (5.2 days) on resistant wheat and jointed goatgrass (Table 2). Average fecundity was only 13.9 nymphs per greenbug on barnyardgrass and 22.4 nymphs on western wheatgrass, but 4.4 and 2.7 times that many on susceptible wheat (62.2 nymphs) and sorghum (61.5 nymphs). The total number of nymphs produced per greenbug differed significantly between susceptible versus resistant sorghum or wheat. Each greenbug lived only 14.8 days on barnyardgrass. Greenbugs lived longest on grasses of the genus *Sorghum* (29.4, 28.8, and 27.5 days on Johnsongrass, RTx430, and LG35, respectively). The eight grasses were adequate to good hosts for biotype I greenbugs. The worst wild host was barnyardgrass, and the best were Johnsongrass and jointed goatgrass, which are related to sorghum and wheat. The resistance mechanism in the resistant sorghum and wheat probably is antixenosis or tolerance, rather than antibiosis.

Master's student Anastasia Palousek assessed effects of 10-23, 14-27, 18-31, and 22-35° C temperatures (daily low-high cycle) on the biology of greenbug biotypes E and I to determine the optimum temperature for evaluating resistance of sorghum to greenbugs. Twenty plants of RTx430 sorghum were used for each combination of temperature and biotype. A single greenbug enclosed in a clip cage was attached to each of two leaves on a sorghum plant that had seven true leaves. The infested sorghum was kept at 14:10 light:dark hours in an incubator. The original greenbug was discarded after it produced a nymph which was retained. When the greenbug in each cage began producing offspring, the nymphs produced per day were counted and removed. The greenbug was monitored until death. The pre-reproductive period was significantly longest for greenbugs at the coolest temperature of 10-23° C (9.4 days) and shortest at the warmest temperature of 22-35° C (Table 3). Greenbug biotypes E and I at the coolest temperature produced 2.8 and 5.1 times more nymphs (51.4 and 50.9) than did greenbugs at the warmest temperature (18.1 and 9.9). Greenbug biotypes E and I at the coolest temperature lived 5.0 and 7.2 times longer (60.3 and 61.9 days) than at the warmest temperature (12.1 and 8.6 days). Length of the pre-reproductive period, total fecundity, and longevity per greenbug differed significantly by the biotype of greenbug only at the warmest temperature of 22-35° C.

Table 2. Pre-reproductive period, fecundity, and longevity of biotype I greenbugs on grasses.

Grass	Pre-reproductive period (days)	Total fecundity (nymphs) per greenbug	Longevity (days)
Barnyardgrass	7.5 ± 0.3 a	13.9 ± 2.4 d	14.8 ± 1.0 cd
Western wheatgrass	6.4 ± 0.2 ab	22.4 ± 2.2 cd	17.7 ± 1.0 c
Jointed goatgrass	5.2 ± 0.1 b	54.1 ± 2.6 b	19.3 ± 0.8 bc
Johnsongrass	5.7 ± 0.1 b	57.1 ± 2.7 ab	29.4 ± 1.4 a
GRS1201 wheat	5.2 ± 0.1 b	47.3 ± 2.8 b	22.0 ± 1.2 bc
Custer wheat	5.4 ± 0.1 b	62.2 ± 3.1 a	22.9 ± 1.0 bc
LG35 sorghum	5.5 ± 0.1 b	48.9 ± 2.5 b	27.5 ± 1.4 ab
RTx430 sorghum	5.3 ± 0.2 b	61.5 ± 2.7 a	28.8 ± 1.3 ab

Means followed by the same letter in a column are not significantly different (Tukey's HSD).

Master's student Suresh Veerabomma assessed effects of different soil water potentials (-33, -50, -100, and -300 kPa) and nitrogen (21, 50, 100, and 150 ppm) on abundance and longevity of biotype I greenbugs. A greenbug enclosed in a clip cage was attached to a leaf of each of 90 sorghum plants, 10 per treatment combination, in a greenhouse. The experiment was done twice. The length of the pre-reproductive period, daily number of nymphs produced, and longevity were assessed. Soil water potential, but not nitrogen, significantly affected greenbug fecundity, with almost twice as many nymphs produced per greenbug on sorghum in soil with -33 kPa of water potential (44.5 nymphs) as with -300 kPa (28.5 nymphs) (Table 4). Greenbug longevity was affected by different soil water potentials but not nitrogen. Longevity was 22.2 and 28.6 days on sorghum in soil with -300 and -33 kPa of water.

Table 3. Effects of temperature and biotype on greenbug biotypes E and I on sorghum.

Temp.	Pre-reproductive period (days)		Total fecundity (nymphs) per greenbug		Longevity (days)	
(° C)	Biotype E	Biotype I	Biotype E	Biotype I	Biotype E	Biotype I
10-23	9.4 ± 0.1aA	9.4 ± 0.2aA	51.4 ± 2.8aA	50.9 ± 2.7aA	60.3 ± 2.8aA	61.9 ± 2.7aA
14-27	6.6 ± 0.2bA	6.8 ± 0.1bA	50.2 ± 3.2aA	43.4 ± 2.4abA	38.0 ± 1.8bB	44.6 ± 1.5bA
18-31	6.5 ± 0.2bA	6.0 ± 0.3cA	40.2 ± 2.7bA	42.2 ± 4.4bA	26.5 ± 1.3cA	23.3 ± 1.2cA
22-35	5.0 ± 0.2cA	3.9 ± 0.2dB	18.1 ± 3.0cA	9.9 ± 2.3cB	12.1 ± 1.1dA	8.6 ± 1.0dB

Means followed by the same lower-case letter in a column or upper-case letter within a treatment in a row are not significantly different (LSD, $P = 0.05$).

Table 4. Effect of soil water and nitrogen on biotype I greenbugs on sorghum.

	Number of days of pre-reproductive period	Nymphs produced per greenbug	Number of days each greenbug lived
Water potential (kPa)			
-33	6.5 a	44.5 a	28.6 a
-50	6.4 a	40.6 a	27.5 a
-100	6.3 a	39.9 a	28.3 a
-300	6.4 a	28.5 b	22.2 b
Nitrogen (ppm)			
21	6.7 a	35.3 a	26.1 a
50	6.4 a	35.9 a	25.5 a
100	6.3 a	40.3 a	27.9 a
150	6.3 a	38.9 a	25.3 a

Means followed by the same letter for a treatment within a column are not significantly different (Tukey's HSD, $P = 0.05$).

Networking Activities

Workshops

The PI participated in and co-authored four posters presented at the 51th Annual Meeting of the Southwestern Branch of the Entomological Society of America (22-27 February 2003, Oklahoma City, Oklahoma). For the Biennial Conference of the National Grain Sorghum Producers (16-18 February 2003, Albuquerque, New Mexico), the PI served as entomology discipline chair, organized and moderated the entomology symposium, and co-authored five presentations. The PI gave three presentations at the Entomology Science Conference (6-8 November 2002, College Station, Texas), at the 50th Annual Agricultural Chemicals Conference (28 August, Lubbock, Texas), and at the Joint Meeting of the Greenbug Research Consortium and U.S. Department of Agriculture Western Coordinating Committee (WCC-066) on Integrated Management of Russian Wheat Aphid and Other Cereal Aphids (9-10 September 2002, Stillwater, Oklahoma). The PI participated in the INTSORMIL Principal Investigators' Conference (18-20 November, Addis Ababa, Ethiopia). During two sessions, 10 entomologists involved with INTSORMIL discussed aphids, armyworms, panicle-infesting bugs, shoot fly, sorghum midge, spittle bugs, stalk borers, white grubs, and storage insects as major pests of sorghum. Millet head miner, blister beetles, panicle-infesting bugs, shoot fly, stalk borers, and storage insects are pests of pearl millet. Better storage, biological control, biotechnology, botanical insecticides, cultural practices, habitat management, IPM, and plant resistance were discussed as ways to manage insect pests.

Research Investigator Exchanges

From 18-29 October 2002, the PI traveled in Mali and discussed and reviewed research and needs with scientists and administrators of the Institut d'Economie Rurale (IER) at Bamako, Cinzana, Samanko, Sikasso, and Sotuba and with ICRISAT sorghum breeders. Entomological emphasis was on managing panicle-infesting bugs, sorghum midge, stalk borers, sugarcane aphid, and storage beetles. Collaborative research in agronomy, breeding, entomology, plant pathology, maintaining sorghum diversity, and quality of stored grain was viewed at Sotuba. A project at Cinzana and Samanko used neem, *Calotropus*, and diazinon against *Erystylus* bugs on Malisor92-1 and S-34 sorghums. A cooperative project between Drs. Diarisso and Mamourou Diourte (plant pathologist) on stalk borers and anthracnose was seen at Sikasso. The sorghums in the All Disease and Insect Nurseries (ADIN) at the different research stations looked bad because of scarce rainfall and pests.

Research Information Exchange

The PI advised agricultural consultants, extension, and the National Grain Sorghum Producers on management of sorghum insect pests. The PI is assisting Dr. John Burd, USDA-ARS, Stillwater, Oklahoma, with a multi-year, multi-state study of greenbugs on wild and cultivated grasses. Two hundred thirty-seven sorghums developed for resistance to biotype I greenbug were evaluated for Pioneer Hi-Bred International, Inc. An experimental insecticide developed by FMC Corporation was evaluated for effect on greenbugs and coccinellids in the field. Supplies were provided for entomological research for Drs. Diarisso and Doumbia in Mali, Mr. Abdou Kadi Kadi in Niger, and Dr. Munthali in Botswana. Sorghum entomology reference materials were sent to Dr. Paul Tanzubil in Ghana and Dr. Johnnie van den Berg in South Africa.

Publications and Presentations

Publications

Peterson, G.C., B.B. Pendleton and G.L. Teetes. 2002. PROFIT – Productive Rotations On Farms In Texas. In J. Leslie (Ed.). Sorghum and Millets Diseases, World Agriculture Series, Iowa State Press: A Blackwell Publishing Company, Ames, IA. Pp. 365-370.

Bowling, R., R. Shufran, B. Pendleton, C. Copeland, and S. Cox. 2003. Insecticide evaluations for greenbug (Homoptera: Aphididae) management in sorghum on the Texas High Plains. P. 93. In J.A. Dahlberg, R. Kochenower, R. Klein, B. Rooney, S. Bean, B. Pendleton, J. Stack, and B. Maunder (eds.). Proceedings of the 23rd Biennial Grain Sorghum Research and Utilization Conference. February 16-18, 2003. Albuquerque, NM.

Palousek, A.L., B.B. Pendleton, B.A. Stewart, G.J. Michels, Jr., and C.M. Rush. 2003. Fecundity and longevity of greenbug (*Schizaphis graminum*) affected by biotype and temperature. P. 98. In J.A. Dahlberg, R. Kochenower, R. Klein, B. Rooney, S. Bean, B. Pendleton, J. Stack, and B. Maunder (eds.). Proceedings of the 23rd Biennial Grain Sorghum Research and Utilization Conference. February 16-18, 2003. Albuquerque, NM.

Pendleton, B. 2003. Roundtable discussion by producers on management of sorghum insect pests and future research needs. P. 91. In J.A. Dahlberg, R. Kochenower, R. Klein, B. Rooney, S. Bean, B. Pendleton, J. Stack, and B. Maunder

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Sambaraju, K.R., B.B. Pendleton, C.A. Robinson, R.C. Thomason, and M.D. Lazar. 2003. Greenbug fitness on wild and cultivated hosts. P. 99. In J.A. Dahlberg, R. Kochenower, R. Klein, B. Rooney, S. Bean, B. Pendleton, J. Stack, and B. Maunder (eds.). Proceedings of the 23rd Biennial Grain Sorghum Research and Utilization Conference. February 16-18, 2003. Albuquerque, NM.

Veerabomma, S., B.B. Pendleton, B.A. Stewart, C.A. Robinson, and G.J. Michels, Jr. 2003. Effect of different amounts of soil moisture and nitrogen on greenbug fecundity and longevity on sorghum. P. 100. In J.A. Dahlberg, R. Kochenower, R. Klein, B. Rooney, S. Bean, B. Pendleton, J. Stack, and B. Maunder (eds.). Proceedings of the 23rd Biennial Grain Sorghum Research and Utilization Conference. February 16-18, 2003. Albuquerque, NM.

Palousek, A.L. 2003. Effect of biotype and temperature on the fitness of greenbug, *Schizaphis graminum* (Rondani), on sorghum. M.S. thesis. West Texas A&M University, Canyon, TX.

Palousek, A.L., B.B. Pendleton, B.A. Stewart, G.J. Michels, Jr. and C.M. Rush. 2002. Fecundity and longevity of greenbug, *Schizaphis graminum*, affected by biotype and temperature. International Sorghum and Millets Newsletter 43:54-55.

Sambaraju, K.R., B.B. Pendleton, C.A. Robinson, R.C. Thomason and M.D. Lazar. 2002. Greenbug fitness on sorghum and non-cultivated hosts. International Sorghum and Millets Newsletter 43:55-56.

Presentations

51st Annual Meeting of the Southwestern Branch of the Entomological Society of America, February 22-27, 2003, Oklahoma City, OK – *Fecundity and longevity of greenbug, Schizaphis graminum, affected by biotype and temperature* presented by A.L. Palousek, B.B. Pendleton, B.A. Stewart, G.J. Michels, Jr., and C.M. Rush; *Greenbug fitness on sorghum and non-cultivated hosts* presented by K.R. Sambaraju, B.B. Pendleton, C.A. Robinson, R.D. Thomason, and M.D. Lazar; *Response of Banks grass mite populations to bifenthrin and dimethoate* presented by R. Shufran, R. Bowling, B. Pendleton, P. Porter, G. Cronholm, C. Patrick, and B. Lewis; and *Effect of different amounts of soil water and nitrogen on greenbug fecundity and longevity on sorghum* presented by S. Veerabomma, B.B. Pendleton, B.A. Stewart, C.A. Robinson, and G.J. Michels, Jr.

23rd Biennial Grain Sorghum Research and Utilization Conference, February 16-18, 2003, Albuquerque, NM – *Insecticide evaluations for greenbug (Homoptera: Aphididae) management in sorghum on the Texas High Plains* presented by R. Bowling, R. Shufran, B. Pendleton, C. Copeland, and S. Cox; *Fecundity and longevity of greenbug (Schizaphis graminum) affected by biotype and temperature* presented by A.L. Palousek, B.B. Pendleton, B.A. Stewart, G.J. Michels, Jr., and C.M. Rush; *Roundtable discussion by producers on management of sorghum insect pests and future research needs* by B. Pendleton; *Greenbug fitness on wild and cultivated hosts* presented by K.R. Sambaraju, B.B. Pendleton, C.A. Robinson, R.C. Thomason, and M.D. Lazar; and *Effect of different amounts of soil moisture and nitrogen on greenbug fecundity and longevity on sorghum* presented by S. Veerabomma, B.B. Pendleton, B.A. Stewart, C.A. Robinson, and G.J. Michels, Jr.

INTSORMIL Principal Investigators' Conference, November 18-20, 2002, Addis Ababa, Ethiopia – *Use of local plants to control Rhizopertha dominica on stored sorghum in Mali* presented by N.Y. Diarisso, O. Youm, A. Togola, and B.B. Pendleton; *Status of sorghum midge research in Niger* presented by H.A. Kadi Kadi, I. Kapran, and B.B. Pendleton; *Fecundity and longevity of greenbug, Schizaphis graminum, affected by biotype and temperature* presented by A.L. Palousek, B.B. Pendleton, B.A. Stewart, G.J. Michels, Jr., and C.M. Rush; *Greenbug fitness on sorghum and non-cultivated hosts* presented by K.R. Sambaraju, B.B. Pendleton, C.A. Robinson, R.C. Thomason, and M.D. Lazar; and *Effect of different amounts of soil nitrogen and water on greenbug fecundity and longevity on sorghum* presented by S. Veerabomma, B.B. Pendleton, B.A. Stewart, C.A. Robinson, and G.J. Michels, Jr.

Annual Meeting of the Entomological Society of America, November 17-20, 2002, Ft. Lauderdale, FL – *Response of Banks grass mite populations to bifenthrin, dimethoate, and propargite* presented by R. Bowling, P. Porter, G. Cronholm, C. Patrick, B. Lewis, R. Shufran, and B. Pendleton.

Entomology Science Conference, November 6-8, 2002, College Station, TX – A. Palousek and B. Pendleton. *Greenbug biology affected by biotype and temperature* presented by A. Palousek and B. Pendleton; *Greenbug fitness on wild and cultivated grasses* presented by K. Sambaraju and B. Pendleton; and *Effect of soil moisture and fertility on greenbugs* presented by S. Veerabomma and B. Pendleton.

Joint Meeting of the Greenbug Research Consortium and U.S. Department of Agriculture Western Coordinating Committee (WCC-066) on Integrated Management of Russian Wheat Aphid and Other Cereal Aphids, September 9-10, 2002, Stillwater, OK – *Effect of biotype and temperature on greenbug biology* presented by A.L. Palousek and B.B. Pendleton, *Fitness of biotype I greenbug on different grass hosts* presented by K. Sambaraju and B.B. Pendleton, and *Effect of soil moisture and nitrogen on fitness of greenbug biotype I on sorghum* presented by S. Veerabomma and B.B. Pendleton.

50th Annual Agricultural Chemicals Conference, August 28, 2002, Lubbock, Texas – *Fecundity and longevity of greenbug, Schizaphis graminum, affected by biotype and temperature* presented by A.L. Palousek, B.B. Pendleton, B.A. Stewart, G.J. Michels, Jr., and C.M. Rush; *Greenbug fitness on sorghum and non-cultivated hosts* presented by K.R. Sambaraju, B.B. Pendleton, C.A. Robinson, R.C. Thomason,

and M.D. Lazar; and *Effect of different amounts of soil nitrogen and water on greenbug fecundity and longevity on sorghum* presented by S. Veerabomma, B.B. Pendleton, B.A. Stewart, C.A. Robinson, and G.J. Michels, Jr.