Sustainable Plant Protection Systems

Agroecology and Biotechnology of Stalk Rot Pathogens of Sorghum and Millet

Project KSU-210

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Summary

In collaboration with my South African colleagues we have evaluated fumonisin levels from seven pearl millet and seven sorghum samples collected from village granaries in rural Mali. Samples (13 of 14) contained 5-70 ng/g (ppb) fumonisins, while the 14th contained > 1000 ng/g total fumonisins and could pose a human health hazard. Many of the *Fusarium* spp. from these samples do not belong to presently described *Fusarium* species. In collaboration with my Australian colleagues, we have described a new *Gibberella* species that causes stalk rot of sorghum from collections in the Philippines and Central America. This species is distinguished from other *Gibberella* species by its long, slender ascospores, and also can be recovered from diseased maize and sugarcane. We also expanded the genetic map of *Fusarium verticillioides* through the addition of approximately 500 Amplified Fragment Length Polymorphism (AFLP) markers.

Objectives, Production and Utilization Constraints

Objectives

- Increase collection of fungal samples from sorghum and millet, especially grain, and identify the species recovered.
- Develop characters, such as mating type, for assessing genetic variability in fungal populations.
- 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.
- Determine mycotoxigenic potential of *Fusarium* spp. from sorghum and millet.
- Conduct Scientific Writing and *Fusarium* identification training workshops.

Constraints

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 is limited because the strains responsible for disease often cannot be accurately identified and used repeatedly in field challenges. Correct identification of the fungi colonizing and causing disease is essential for the design of breeding and control measures. Without a thorough understanding of the pathogen's genetic diversity and population dynamics, effective control measures are difficult to design and resistant lines may have unexpectedly brief lives.

Mycotoxin contamination limits the uses 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.

Research Approach and Project Output

Research Methods

Grain samples and fungal cultures. Sorghum and pearl millet grain samples were collected in rural Mali with the assistance of IER and World Vision personnel in September 2001. The grain samples were shipped via courier to the PROMEC (Medical Research Council) laboratories in Tygerberg, South Africa for analysis for evaluation of their mycotoxin content with standard high pressure liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) protocols. Fungi were recovered from these samples at KSU by plating on a peptone-PCNB medium that enriches for *Fusarium* spp. Strains were routinely cultured on carnation leaf agar, or on modified Czapek's complete medium. Strains are maintained in long-term storage as spore suspensions in 15% glycerol frozen at -70°C.

Samples of *Gibberella sacchara* examined are from the KSU culture collection. The first of these strains received were from China and Brazil in the mid 1980s, from sugarcane in Taiwan and sorghum in Brazil, respectively. Additional confirmed isolates from sorghum include strains collected by Larry Claflin from El Salvador this past year, and strains that I collected from the Philippines in 1989. In diagnostic crosses we used the following standard *Gibberella fujikuroi* mating population mating-type tester strains from the KSU collection or from the Fungal Genetics Stock Center, Kansas City, Kansas: FGSC 7600 (*MATA-1*), FGSC 7603 (*MATA-2*), FGSC 7611 (*MATB-1*), FGSC 7610 (*MATB-2*), KSU C-01993 (*MATC-1*), KSU C-01995 (*MATC-2*), FGSC 7615 (*MATD-1*), FGSC 7614 (*MATD-2*), FGSC 7616 (*MATE-1*), FGSC 7617 (*MATE-2*), FGSC 7057 (*MATF-1*), FGSC 7056 (*MATF-2*), KSU G-05111 (*MATG-1*), KSU G-05112 (*MATG-2*), KSU H-10847 (*MATH-1*) and KSU H-10850 (*MATH-2*).

Strains used in the expanded genetic map were the same as those used for the original genetic map of this organism that was based on restriction fragment length polymorphisms (RFLPs); see the 1995 INTSORMIL Annual report for further details.

Microscopic observations. Perithecia were treated with 3% KOH and 100% lactic acid to observe any color reaction, and measured *in situ.* Asci and ascospores were mounted in water for measurement and photography. Measurements were taken of 20 each of perithecia, asci, and ascospores. Whole perithecia were fixed in 6.5% glutaraldehyde in 100 mM sodium cacodylate buffer at pH 7.6 for 4 h at room temperature, dehydrated in a graded ethanol series, and finally infiltrated and embedded in LR White resin. Sections, 1.5 m thick, were cut with a Reichert ultramicrotome, dried onto poly-l-lysine-coated glass slides, and stained with 0.5% Toluidine Blue *O* for 10 sec. Sections were mounted in immersion oil.

Amplified Fragment Length Polymorphisms (AFLPs). Cultures were grown and DNA was extracted as previously described (see 2000 INTSORMIL annual report). AFLPs were generated by using a protocol we have modified from the standard for these fungi (see 2001 INTSORMIL annual report). Most polymorphisms were characterized as presence/absence of bands although a few occurred in which the polymorphism appeared as an apparent difference in molecular weight. To analyze AFLP profiles, we manually scored the presence or absence of 486 polymorphic bands generated by 37 AFLP primer pairs (3-23 polymorphic bands per primer). We also identified polymorphic bands through bulk-segregant analysis that generated an additional 18 AFLP primer pairs that map near the gene cluster that includes the fumonisin biosynthetic genes and placed these markers on the map as well. We assumed that bands of the same molecular size in different individuals were identical. Each band was treated as a single independent locus with two alleles and unresolved bands or missing data were scored as ambiguous.

Genetic map construction. Genetic mapping of all characters was performed with Map Manager QTX11 (http://mapmgr.roswellpark.org/mmQTX) on a Macintosh G4 Power PC computer. The data were treated by the mapping program as a backcross with codominant markers, paternal parent unique, for accurate analysis of this haploid genome. The data from the map of Xu and Leslie were imported into Map Manager from Map Maker and used to reconstruct the previous linkage map. Marker data from the AFLP analysis was then imported into the program and combined with the previous data. The distribute function was used to assign map positions to the AFLP markers. Following the initial linkage group analysis, we inspected the aligned phenotype data visually to minimize linkage distance based on the assumption that single-locus double recombinants were highly unlikely and probably result from gene conversion, or from errors in scoring or bookkeeping.

Research Findings

Fumonisins in sorghum and pearl millet from Mali. The fumonisin analyses for sorghum and pearl millet are the first definitive analyses for these toxins from anywhere in the world (Table 1). The Joint FAO/WHO Expert Committee on Food Additives has set a provisional Maximum Tolerable Daily Intake of 2 µg/kg of body weight. The risk posed by these toxins depends on both quantity of grain consumed and the amount of toxin that it contains. Although the grain from sample 16 probably poses a significant health hazard, the grain from the other 13 samples probably does not, at least in terms of fumonisin toxicity. Analysis of the moniliformin levels from all 14 samples is in progress. Given these results, collection of grain samples, especially those stored on-farm or in village granaries is needed to confirm their general safety with respect to these mycotoxins.

Analysis of the fungal contaminants of these grain samples is in progress. At least four *Fusarium* species have been

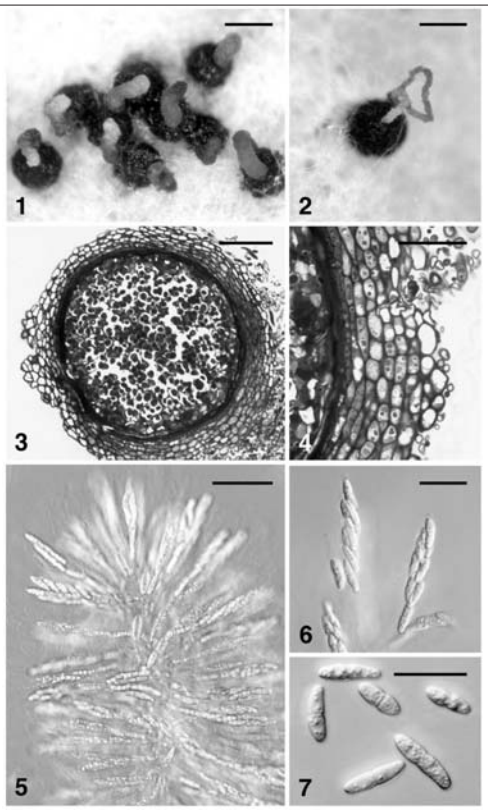
Table 1. Fumonisin and *Fusarium* species analyses of sorghum and pearl millet grain samples from Mali.

Sample	Location	Fusarium Cultures[1]	Fumonisins[b] FB_1	FB_2	FB_3	Total
Sorghum						
3	Marka Coungo market – 2000 crop	81	10	ND	ND	10
5	Fana market – 2000 crop	80	35	5	ND	40
9	Dakoumani, solarized – 2000 crop	50	10	ND	ND	10
10	Dakoumani, insecticide treated – 2000 crop	60	20	ND	ND	20
12	Douna, insecticide treated – 1997 crop	30	15	ND	ND	15
14	Kondogola – 1998 crop	47	25	ND	ND	25
16	Cinzana-Gare, "barique" storage – 2000 crop	55	360	345	320	1025
Pearl Millet						
2	Marka Coungo market – 2000 crop	58	25	ND	ND	25[c]
4	Fana market – 2000 crop	100	55	10	5	70[c]
6	Dakoumani – 1999 crop	54	17	ND	ND	17[c]
7	Dakoumani – 2000 crop	68	20	ND	ND	20
11	Douna, insecticide treated – 1995 crop	48	5	ND	ND	5
13	Kondogola – 1998 crop	64	15	ND	ND	15
15	Cinzana-Gare – 2000 crop	69	15	ND	ND	15

[a]Number of cultures of *Fusarium* spp. Cultures are not yet identified to species.
[b]Detection limit for HPLC analyses is 5 ng/g.
[c]HPLC results confirmed with LC/MS.

identified thus far, and strains belonging to at least three other species, at least one of which is undescribed, have been identified as well. The medium on which the subcultures were made is selective for *Fusarium* spp., but other fungi occasionally can be identified as well. In this set of samples, contamination with *Aspergillus* spp. was much higher than normal, and it is possible that mycotoxins produced by these fungi, e.g., aflatoxins and ochratoxin, also may be present at significant levels.

G. sacchara. English version of formal species description: Perithecia superficial, solitary to aggregated in groups of a few and seated on a minute stromatic base, obovoidal, and warty (Figs. 1 and 2); 270-390 (mean = 325) µm × 250-390 (mean = 310) µm diam; blue-black, color not changing in 3% KOH, turning red in 100% lactic acid. Perithecial wall 23.1-38.9 (mean = 30.6) µm wide laterally, formed of two obvious regions (Figs. 3 and 4). Outer wall region 18.4-33.0 (mean = 25.6) µm wide; outer cells ± angular to elliptic, 5.3-11.5 (mean = 8.4) µm length × 3.1– 5.9 (mean = 4.3) µm width, with the largest cells at the exterior and the smallest cells toward the interior of the wall, walls of cells 0.5-1.2 (mean = 0.9) µm wide and pigmented. Inner wall region 4.0-5.9 (mean = 4.9) µm wide; cells ± angular to elliptic, 7.2-16.3 (mean = 10.6) µm length × 1.1-3.2 (mean = 2.2) µm width. Cells fusiform, thin-walled and irregular, walls of inner cells 0.3-0.6 (mean = 0.4) µm wide and pigmented (Fig. 4). Outer and the inner wall regions merging imperceptibly; cells of the outer region more pigmented. Perithecial apex continuous with the outer and inner wall layers; cells at the apex smaller appearing as a reticulum; cells forming the ostiolar opening ± clavate and thick walled at the cell tips, non-pigmented merging periphyses. Cells of the apex attaining the same length to form an apical disk. Asci fusiform (Figs. 5 and 6), regularly dehiscing upon examination under the microscope and 8 spored. Ascospores



Figures. 1-7. ***Gibberella sacchara*** **produced from a cross of strains B-03852 × B-03853 on carrot agar. 1, 2. Perithecia. 3. Transverse section of a perithecium stained with toluidine blue. 4. Close up of transverse section of perithecial wall. 5, 6. Asci. 7. Ascospores. Scale bars: 1 and 2 = 200 µm; 3 and 5 = 50 µm; 4, 6 and 7 = 25 µm.**

exuded in a cirrhus (Figs. 1 and 2), ellipsoidal to obovoid with both ends rounded, 0-1 septate and slightly constricted at the septum, 3-4.5 (mean = 4.2) × 7.0-8.0 (mean = 7.6) µm (Figs. 5-7). Heterothallic species reproductively isolated from previously described species of *Gibberella*.

This species is most easily differentiated from other *Gibberella* species by its much larger and slenderer ascospores. The species is associated with sorghum stalk rot and has been recovered from leaf spots on sorghum plants. It also attacks other domesticated crops, *e.g.,* maize and sugarcane; however, no comprehensive studies of host range or pathogenicity have been conducted with this fungus. This species is most commonly seen in wet tropical and semi-tropical regions. We have no strains from temperate, arid or semi-arid regions at this time. Strains in this species are most easily confused with *Fusarium subglutinans*, and reports in the older literature of *F. subglutinans* or of *F. moniliforme* var. *subglutinans* from wet tropical and subtropical regions probably refer to this species. Strains of this species are not known to make fumonisins.

Genetic map of *G. moniliformis*. Of the AFLP bands scored in this mapping population, 25-30% were polymorphic, which is consistent with results on relatedness between two members of the same species in the *G. fujikuroi* species complex. The distribution of markers from individual primer pairs appears to be random with respect to chromosome. The major architectural features of the previous Xu and Leslie map remain unchanged (Table 2), and the present map has 636 markers that define 580 genetic loci (Fig. 8).

The update increases the total map length by approximately 50% (736 cM), while correspondingly reducing the average number of kb/cM from 32 to 21. The maps for linkage groups 11 and 12, in particular, are much denser. The average distance between identified markers has been reduced from 10 cM/interval to 3.4 cM/interval. The number of gaps greater than 20 cM in length has been reduced from 24 to five, with no interval larger than 29 cM. The remaining gaps tend to be on the distal portions of chromosomes, which remain to be identified, probably through the use of telomere probes. The distribution of the number of crossovers per chromosomes (Table 2) appears to be random (^{2} test, , $p = 0.05$). The physical size of the chromosome is not strongly correlated with the number of markers ($r = 0.72$), the number of loci ($r = 0.71$), or the length of a chromosome in map units ($r = 0.68$).

Some important gaps remain even in this relatively saturated map. One end of chromosome 5 now has eight markers that are in a cluster separated by 26 cM from the next nearest locus. Other large recombination gaps exist on chromosome 1 (21 cM) and at the ends of chromosomes 7, 8 and 10 (Fig. 8). The 10 terminal markers of chromosomes 2, 4, 7, 8 and 10 add ~120 cM to the genetic map, and elimination of these markers would reduce the average map distance to 3.2 cM/marker. Even with these large gaps the map appears to be relatively saturated as 56 loci (10%) are represented by more than one marker. The addition of more randomly selected markers is probably not an effective use of resources at this time, as only centromeres, telomeres, and a few, primarily terminal, chromosome regions remain in need of further definition at this level.

Networking Activities

Editorial and committee service (2001)

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

Research Investigator Exchange

Dr. Leslie made the following international scientific exchange visits (2001)

Australia – October 10-14
Egypt – April 25 – May 4
Ghana – September 7-12
India (ICRISAT) – September 28 – October 6

Table 2. Comparison of the linkage map of Xu and Leslie with the updated map.

		Xi amd Leslie		Updated map			Crossovers per chromosome						
Chromosome	Physical chromosome size (Mb)	No. of markers	cM	No. of markers	No. of markers	cM	0	1	2	3	4	5	Mean
1	10	15	173	77	65	241	7	32	32	27	10	13	2.5
2	6.5	15	196	53	51	189	10	39	38	14	14	6	2.1
3	4.9	15	90	58	51	190	20	44	22	13	10	12	2.0
4	4.1	14	120	62	56	214	15	27	43	18	8	10	2.2
5	4.0	11	110	48	45	189	17	20	35	28	11	10	2.1
6	3.6	13	146	68	61	195	20	40	30	11	9	11	1.9
7	3.0	12	113	50	42	169	20	38	31	20	7	5	1.8
8	2.6	14	134	49	44	184	15	35	38	21	7	5	1.7
9	2.5	10	132	49	44	173	21	41	28	18	3	10	1.8
10	2.2	14	137	60	53	216	9	39	31	15	17	10	2.0
11	2.0	6	86	42	38	150	19	50	33	11	74	4	1.6
12	0.7	4	15	20	13	78	69	27	5	12	3	1	0.8
Total	46.1	143	1452	636	568	2188	248	441	357	308	101	96	1.9

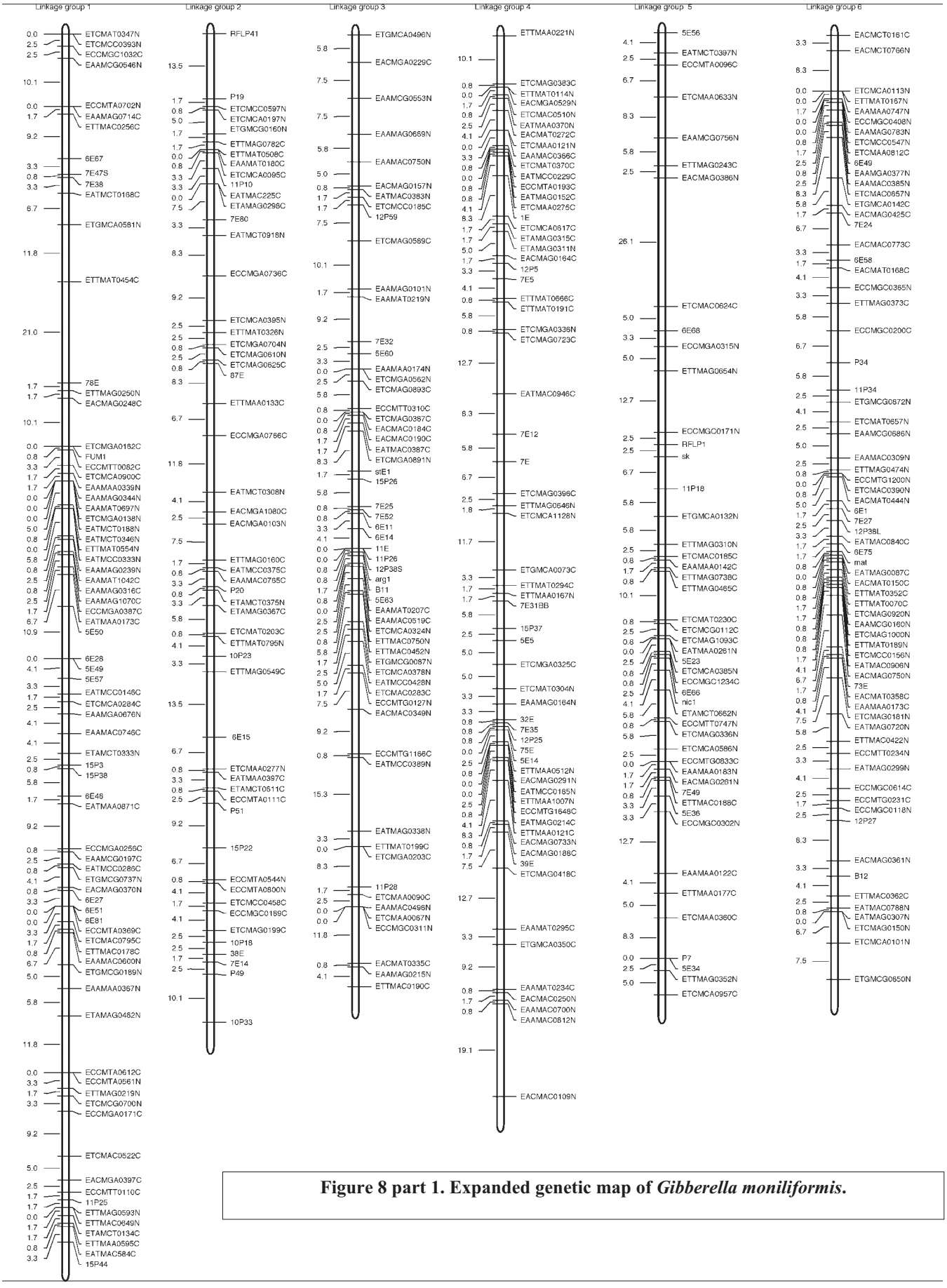


Figure 8 part 1. Expanded genetic map of *Gibberella moniliformis*.

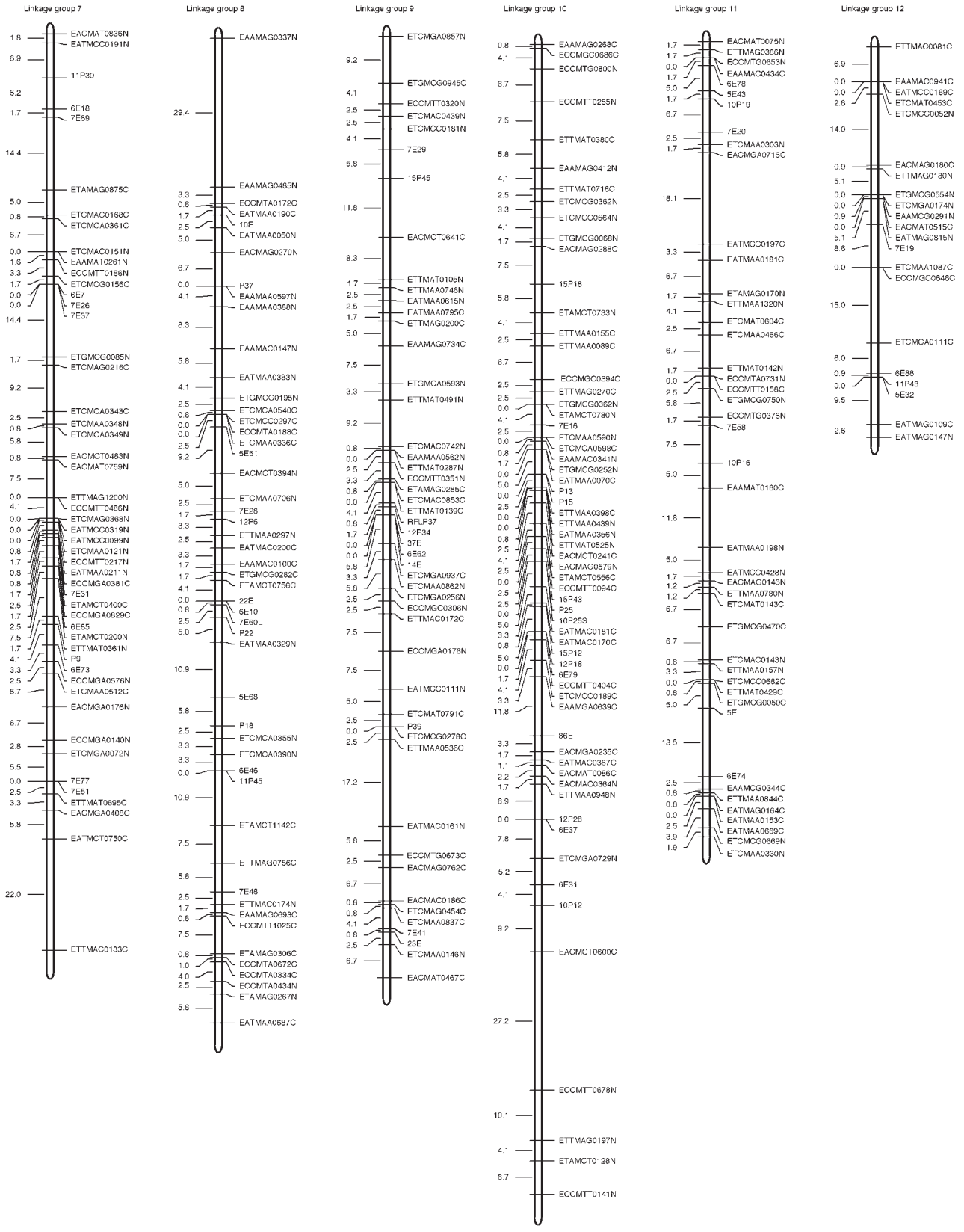


Figure 8 - part 2. Expanded genetic map of *Gibberella moniliformis*.

Mali – September 1-7
Malaysia – October 7-9
Mozambique – October 28-31

South Korea – October 14-18
South Africa – November 1-20

Seminar, Workshop & Invited Meeting Presentations (2001)

- Organized *Fusarium* Laboratory Workshop in Manhattan from June 10-15; 28 participants and five instructors from eight countries.
- Editor for Proceedings of Sorghum/Millet pathology conference in Guanjuato, Mexico.
- 22nd National Grain Sorghum Producers Conference, Nashville, Tennessee – 2/01.
- Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana – 2/01.
- Egyptian National Agricultural Library, Dokki, Egypt – 04/01.
- Savanna Agricultural Research Institute, Tamale, Ghana – 09/01.
- ICRISAT, Patancheru, India –10/01.
- Department of Plant Pathology, Seoul National University, Seoul, South Korea – 10/01.
- FABI, University of Pretoria, Pretoria, South Africa – 11/01.
- Summer Grain Crops Institute, Agricultural Research Council, Potchefstroom, South Africa – 11/01.
- Institute of Wine Biotechnology, Stellenbosch University, Stellenbosch, South Africa – 11/01.
- During 2001 *Fusarium* cultures were provided to:
- Drs. Charles Bacon and Ida Yates, USDA Russell Research Center, Athens, Georgia.
- Drs. Robert L. Bowden, Larry E. Claflin, Louis A. Heaton & Douglas J. Jardine, Department of Plant Pathology, Kansas State University, Manhattan, Kansas.
- Dr. S. Chulze, Universidad Nacional de Rio Cuarto, Rio Cuarto, Argentina.
- Drs. Anne E. Desjardines and Ronald D. Plattner, Mycotoxin Research Unit, National Center for Agricultural Utilization Research, USDA/ARS, Peoria, Illinois.
- 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.
- Dr. L. Hornok, Agricultural Biotechnology Center, Institute for Plant Sciences, Godollo, Hungary.
- Dr. Yin-Won Lee, Department of Plant Pathology, Seoul National University, Su-Won, South Korea.
- Drs. A. Logrieco and A. Moretti, Istituto Tossine e Micotossine da Parassiti Vegetali, Bari, Italy.
- Dr. W. F. O. Marasas, PROMEC, South African Medical Research Council, Tygerberg, South Africa.
- Dr. H. I. Nirenberg, Biologische Bundesantsalt für Land- und Forstwirtschaft, Berlin, Germany.
- Dr. Amir Sharon, Department of Plant Sciences, University of Tel Aviv, Tel Aviv, Israel.
- Dr. J. S. Smith, Department of Animal Sciences and Industry, Kansas State University, Manhattan, Kansas.
- Dr. Brett Summerell, Royal Botanic Gardens-Sydney, Sydney, Australia.
- Dr. Bettina Tudzynski, Westfaelische Wilhelms University, Muenster, Germany.
- Drs. M. Wingfield and B. Wingfield, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.

Other Collaborating Scientists

- Dr. Lester Burgess, Faculty of Agriculture, University of Sydney, Sydney, Australia.
- Dr. Sofia Chulze, Department of Microbiology, National University of Rio Cuarto, Rio Cuarto, Argentina.
- Drs. M. Flieger and S. Pazoutova, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
- Dr. Laszlo Hornok, Agricultural Biotechnology Center, Godollo, Hungary.
- Dr. Sandra Lamprecht, Plant Protection Institute, Agricultural Research Council, Stellenbosch, South Africa.

- Dr. Yin-Won Lee, Department of Plant Pathology, Seoul National University, Su-Won, South Korea.
- Drs. Antonio Logrieco and Antonio Moretti, Istituto Tossine e Micotossine da Parassiti Vegetali, 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.
- Drs. Charles W. Bacon and Ida Yates, USDA Russell Research Center, Athens, Georgia.
- Drs. A. E. Desjardins & R. D. Plattner, USDA National Center for Agricultural Utilization Research, Peoria, Illinois.
- Dr. K. K. Klein, Department of Biological Sciences, Mankato State University, Mankato, Minnesota.
- Dr. G. N. Odvody, Texas Agricultural Experiment Station, Corpus Christi, Texas.

Publications and Presentations

Journal Articles, Books and Book Chapters

Leslie, J. F. 2001. Population genetic level problems in the *Gibberella fujikuroi* species complex. In: Fusarium*: Paul E. Nelson Memorial Symposium* (B. A. Summerell, J. F. Leslie, D. Backhouse and W. L. Bryden, eds.), pp. 113-121. APS Press, St. Paul, Minnesota.

Leslie, J. F. and W. F. O. Marasas. 2001. *Fusarium* in sorghum: Life in interesting times. *Proceedings of the 22nd Sorghum Improvement Conference of North America (Nashville, Tennessee)*, pp. 76-83.

Leslie, J. F., K. A. Zeller and B. A. Summerell. 2001. Icebergs and species in populations of *Fusarium*. *Physiological and Molecular Plant Pathology* 59: 107-117.

Marasas, W. F. O., J. P. Rheeder, S. C. Lamprecht, K. A. Zeller and J. L. Leslie. 2001. *Fusarium andiyazi* sp. nov., a new species from sorghum. *Mycologia 93:* 1203-1210.

Summerell, B. A., J. F. Leslie, D. Backhouse, W. L. Bryden and L. W. Burgess, eds. 2001. Fusarium*: Paul E. Nelson Memorial Symposium*. APS Press, St. Paul, Minnesota. 392 pp.

Abstracts

Alexander, N. J., R. D. Plattner, R. L. Bowden and J. F. Leslie. 2001. Linkage of molecular markers with trichothecene genotypes in *Gibberella zeae*. *Fungal Genetics Newsletter* 48(Suppl.)**:** 158.

Leslie, J. F., K. A. Zeller, A. Logrieco and A. Moretti. 2001. Species diversity and genetic variation among *Fusarium* isolated from prairie grasses. *Phytopathology* 91**:** s54.

Vargas, J. I., R. L. Bowden, K. A. Zeller and J. F. Leslie. 2001. Comparisons of North and South American populations of *Gibberella zeae*. *Phytopathology 91:* s91.

Zeller, K. A., J. E. Jurgenson, and J. F. Leslie. 2001. Simultaneous mapping of multiple *vic* loci in *Gibberella fujikuroi* Mating Population A (*Fusarium verticillioides*). *Phytopathology* 91: s99.

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
Dr. Stephen Mason, Department of Agronomy, University of Nebraska, Lincoln
Mr. Henry Nzioki, National Dryland Farming Research Center-Katumani, P. O. Box 340, Machakos, Kenya
Dr. Gary Peterson, Texas A&M Agricultural Experiment Station, RR 3, Box 219, Lubbock, TX
Ing. Sergio Pichardo, UNA, Apartado Postal 453, Managua, Nicaragua
M.C. Jesus Narro Sanchez, INIFAP, Apdo. Postal 112., C.P. 38010, Celaya, Guanajuato, Mexico
Dr. Mitch Tuinstra, Department of Agronomy, Kansas State University, Manhattan, KS

Summary

A sorghum growth model was coupled with ergot prediction model developed in South Africa to evaluate ergot potential on a specific maturity genotype at different planting dates in a specific environment. Thirty years (1970-1999) of consecutive climatic data from weather stations in Concordia, Dodge City, Manhattan, Russell, Topeka, and Wichita, Kansas and Grand Island, Nebraska were utilized in the model. Ergot potentials varied among locations primarily due to planting date and/or mean maximum RH during anthesis. Most planting dates recommended for each location would result in limited or no incidence of ergot based on our model. Late planting dates increased the risk of infection.

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 has been implemented with project MSU-205.

- U.S./Kenya: Continue to screen for genetic variability of sorghum germplasm to covered kernel smut and ergot diseases that continually occur and affect the nutrition of people and animals.

- U.S./Mexico/Nicaragua/El Salvador: Ascertain the prevalence of diseases through surveys and use of the ADIN nursery from Texas A&M University.

- U.S./Mexico: Collaborate with Mr. Jesus Narro on a bacterial disease project of sorghum in the Bahel region of Mexico.

- Determine if physiological resistance rather than pollen management is the mechanism for tolerance to *Claviceps africana*.

- Continue to evaluate screening protocols for determining genetic variability of grain sorghum to sooty stripe disease (*Ramulispora sorghi*). Determination of various environmental parameters to maximize incidence and severity of disease will also be included.

Constraints

Ergot (sugary disease) of sorghum (*Sorghum bicolor* (L.) Moench), caused by *Claviceps africana* was only a problem in grain sorghum in Africa and Asia prior to 1996 when the disease was first detected in Brazil and Argentina. In 1997, ergot was found in Colombia, Honduras, Nicaragua, El Salvador, Mexico, numerous islands in the Caribbean, and in the U.S. (Kansas, Nebraska, and Texas). The sudden, widespread appearances of ergot in recent years demonstrate the potential impact of *C. africana* on the sorghum industry worldwide.

Ergot is a disease of unfertilized sorghum ovaries The stigma is the principal site of the infection. Susceptibility to infection is from floral gaping, just before or at anthesis. Infected spikelets exude sweet, sticky drops of fluid honeydew containing three types of conidia: macroconidia, secondary conidia, and microconidia. Wind dissemination

of secondary conidia is the most important mode of dispersal for local and long distance spread of *C. africana* and may explain the rapid, long-distance disease spread in Australia and the Americas.

Germplasm resistant to ergot have been identified in numerous reports, but these lines are mostly photoperiod-sensitive, tall and agronomically undesirable. Environmental interactions have also limited the use of these accessions in breeding because germplasm reported as resistant in one environment was susceptible in a different geographical location or environment. We evaluated various accessions of *Sorghum* spp. and potential grass hosts for their reaction to ergot under greenhouse conditions at Manhattan, KS.

The development and use of a disease forecasting system based on weather data (temperature, humidity, rainfall, solar radiation, etc.) and host factors and performance (pollen production and viability, stigma receptivity, nicking, etc.) could be most useful in managing ergot. Use of predictive models have been used for other crops to optimize chemical applications. Models to predict ergot in sorghum were developed by McLaren and Flett in South Africa and Wang et al., in Australia. McLaren's model may not be applicable to geographical regions other than those in South Africa because the relationships were based on a fixed number of days to characterize the start and duration of both the flag leaf stage and anthesis. Starting date and length of sorghum growth stages are known to vary within a location due to climatic conditions such as daily temperatures. Predictive models could be used to adjust ergot severity data to differentiate genotypes. There is also evidence that relationships between ergot severity and environmental parameters differs for various genotypes which complicates a predictive model. Wang's model in Australia is based on the physiological relationships of sorghum growth and development and ergot infection and climate. For this model, an infection factor and the mean relative humidity at 9:00 hours during flowering were the primary factors influencing ergot. This model cannot be used to predict ergot severity for a particular genotype although it may be used in the relative comparison of ergot incidence in different geographical regions.

A growth model for sorghum (SORKAM) was developed by researchers at Texas A&M and Kansas State to evaluate growth and development of grain sorghum. The model describes the morphological development of a well-fertilized single plant in response to the environment. SORKAM can be used to evaluate sowing date alternatives, plant populations, irrigation scheduling, evaluation of yield variation for years with above and below normal rainfall, and assesses the impact of stress on grain yield.

Grain sorghum received limited attention in Central America in previous years as corn was the crop favored by commercial and subsistent growers. Unfavorable climatic conditions and soils coupled with 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.

Covered kernel smut is one of the more important diseases of grain sorghum in LDC's. The disease is easily controlled by chemical seed treatments but these chemicals may not be available or the cost may be prohibitive for purchase by farmers. Incorporation of resistant or immune germplasm into acceptable cultivars would partially alleviate concerns about covered kernel smut.

Sooty stripe is a major disease of sorghum in those areas where the crop is primarily grown under limited or no-till cultural practices. Sooty stripe is also important in other countries such as Mali where yield reductions are common (D. T. Rosenow, personal communication).

The causal agent, *Ramulispora sorghi*, has been difficult to increase in culture due to finite growth conditions. Previously in this project, we were able to ascertain growth media and temperature requirements to increase inoculum for a screening protocol. Conditions that enhance disease incidence and severity remain unknown. A misting system to increase relative humidity was installed. The misting system is controlled by leaf moisture sensors that are connected to a controller regulated by a computer software program. It is believed that relative humidity is an important component for disease development. In addition, a dew chamber was purchased to determine the optimum epidemiological parameters for optimum disease severity under growth chamber conditions.

Research Approach and Project Output

Research Approach

The SORKAM model was coupled with the model of McLaren and Flett to evaluate ergot potential on a specific maturity genotype at different planting dates in a specific environment. Thirty years (1970-1999) of consecutive climatic data from weather stations in Concordia, Dodge City, Manhattan, Russell, Topeka and Wichita, Kansas and Grand Island, Nebraska were utilized in the model. These cities are located in regions with extensive acres of sorghum and ergot disease was previously reported.

The weather parameters used were solar radiation, maximum and minimum temperature, moisture, latitude, longitude, and elevation for each location. Three planting days were selected as based on recommendations from Kansas State University for each location and two planting days outside the suggested planting dates (May 1=day 121, and July 1= day 182) were chosen to represent early and late planting date events. All locations shared the same planting dates except for Wichita which has different recommended planting dates.

All inputs including soil type, planting depth, hybrid, plant population, planting depth, row spacing, etc. were similar for the locations. Analysis of variance (ANOVA) and Unequal variance F-test and T-tests were performed for simulated ergot potential responses, using years as replications. Least square means were used to evaluate the effect of planting date on ergot potential. Significant differences were determined at p= 0.05. The two levels of relative humidity (80 and 95%) were used to evaluate the effect of relative humidity on ergot potential. Estimated ergot potential was expressed in terms of percentage of years (30 years) showing severity of ergot greater than 0 and 15% for the planting dates at 80 or 95% relative humidity for each location.

We evaluated various accessions of *Sorghum* spp. and potential grass hosts for their reaction to ergot under greenhouse conditions at Manhattan, KS. Inflorescences were inoculated by spraying a suspension of 2.0×10^5 macroconidia/ml at anthesis, panicles were covered with clear plastic bags, and inoculation was repeated after five days. Ergot symptoms were not observed on several accessions from *S. bicolor* ssp. *arundinaceum*, and *S. bicolor* ssp. *drummondii.* Within *S. bicolor* ssp. *arundinaceum*, IS 14257 and IS 14357, representing race *verticilliflorum* and IS 14301 and PI 185574 representing race *arundinaceum* were free of ergot infection. *S. bicolor* spp. d*rummondii* (IS 14131) expressed high levels of resistance to ergot.

IS 14131 and IS 14357 were crossed to A3TX430 to produce male-sterile testcross hybrids to evaluate the physiological basis of resistance in these accessions. Parent lines, male-sterile hybrids, and susceptible lines were evaluated for genetic variability to *C. africana* at the winter nursery in Guayanilla, Puerto Rico and under greenhouse conditions at Manhattan, KS.

Sorghum accessions: F_1 sorghum plants from resistant and susceptible gene combinations were obtained from the sorghum breeding program of Dr. Mitch Tuinstra and are as follows:

TXARG1 = SxS; IS14131 = SxR (resistant); IS8525 = SxR (tolerant).

Inoculation Protocol: F_1 plants were inoculated by dipping panicles into a suspension of *C. africana* conidia and then covered with plastic bags. Four flowers per panicle from each of five plants from each cross were harvested after 6, 13, 18, 24, 36, 48, 72 and 96 hr post inoculation. The collected florets were fixed and stained with cotton blue-lactophenol. Stigmas were sliced with a scalpel to permit observation of single layered stylar hair. Florets were examined with a compound microscope utilizing 25X and 63X objectives.

Ergot model: The model proposed by McLaren for predicting ergot potential under South African conditions is as follows: $Y= (aX_1) + EXP(b X_2 + c X2 + d) + (e* X_3)$; Y= (-3.229 x X1) + EXP (-0.0029 x X2 x X2 + 0.0936 x X2 + 3.061) + (0.379 x X3); Y = expected mean ergot severity in a genetically broad based sorghum population (ergot potential);

X_1= mean minimum temperature (^oC), 23 to 27 days before flowering;

X_2= mean daily maximum temperature (^oC), 1-5 days after flowering;

X_3= 80% or 95% relative humidity (^oC), 1-5 days after flowering.

a, b, c, d ,e= regression coefficients

Research Output

Determination of physiological resistance: Susceptible checks were heavily infected with ergot. The male-sterile crosses were nearly as resistant as the parent lines. Since resistance was expressed in male sterile genetic backgrounds, the mechanism of resistance appears to be physiological in nature. ATX623 had 90% of the florets infected whereas the accessions described as resistant above were nearly free of ergot. Previously, IS 8525 was the only known accession in a male-sterile cross that exhibited tolerance to *C. africana*.

Conidial germination was detected 6 hr after infestation. The 12- hr time frame was similar to the percentage of germinated conidia as observed for 6 hr. More conidia germinated on stigmas of susceptible plants than on resistant ones (Table.1). No conidial germination occurred after 12 hr post inoculation due to extremely high temperatures in the greenhouse (July, 2001). Honeydew was not observed in this experiment.

The experiment was repeated and plants inoculated in April, 2002. Four florets were collected from 6-7 plants of each cross at the same time intervals as above. Environmental conditions in the greenhouse were nearly perfect. Honeydew exuded between the outer glumes eight days after inoculation. Results (Table 1) reveal that lower numbers of conidia germinated on resistant sorghum stigmas than on susceptible plants. Florets are continuing to being analyzed to determine if physiological resistance is the mode of action for sorghum immunity to ergot.

Our ergot predictive model utilizing SORKAM and the ergot model developed for South Africa utilized consecutive climatic data over a 30-year period with five planting dates. Ergot potentials varied among locations mostly due to planting date and/or mean maximum RH during anthesis (Table 2). Most planting dates recommended for each location showed limited or no incidence of ergot based on our model. Late planting dates increased the risk of infection especially in Grand Island, NE. Russell, KS and Grand Island, NE were locations that presented a low risk of ergot within

Table 1. Conidial germination on susceptible, intermediate, and resistant sorghum stigmas 6 hr and 12 hours after inoculation.

Germplasm	No. of conidia examined	No. of germinated conidia	No. of conidia/100,000
6 hr.			
TXARG1	18,960	5	26[1]
IS8525	32,880	1	2
IS14131	47,880	1	3
12 hr			
TXARG1	18,000	6	33[a]
IS8525	23,880	1	2
IS14131	41,040	0	0

Table 2. Maximum ergot potential utilizing 30 years of climatic data for five planting dates

	Maximum Ergot Potential	
	Relative humidity	
Location	80%	95%
Manhattan, KS	1.8 July 1*	7.5 July 1
Concordia, KS	14.6 July 1	20.2 July 1
Grand Island, NE	22.8 July 1	28.5 July 1
Topeka, KS	20.9 July 1	26.6 July 1
Wichita, KS	0	0.7 May 1
Dodge City, KS	14.0 July 1	19.7 July 1
Russell, KS	22.4 June 20	28.0 June 20

*Planting date with highest potential of ergot.

the suggested sorghum planting dates if mean (95%) maximum relative humidity occurred during anthesis. The greatest threat of ergot could occur if sorghum was planted on June 20. Grand Island presented the highest percentage of 30 years (16.67) with risk of ergot incidence if also planted on June 20. Significant differences in ergot potential among RH levels were not observed at four of the locations (Concordia, Russell, Topeka and Wichita, KS) for any of the planting dates. Increasing RH from 80 to 95% at Dodge City and Manhattan, KS and Grand Island, NE significantly increased ergot potentials.

Both panicle and foliar diseases were observed on accessions within the All Disease and Insect Nursery (courtesy of D. T. Rosenow, TAM, Lubbock, TX). For panicle diseases, B35 was particularly susceptible to both *Curvularia* and anthracnose (Table 3). TX 7078, BTX 378, R9188 and R9519 were susceptible to *Curvularia*. Resistant accessions included SC 326, LG 35, 97 BRON 304, B9105 and TX 2911 (Table 3).

Rust was particularly severe in El Salvador and anthracnose was the major foliar disease in Nicaragua (data not shown). Accessions susceptible to rust included SC 630, 87 BH 8606, TX 2880, 95 BRON 151, 88 B 943, and TX 2911 (Table 4). Resistant germplasm included 86 EO 366, 96 GCP OB 124 and BTX 631.

Networking Activities

Workshops

Implemented and conducted a workshop in conjunction with Dr. Pitre (MSU-205) on sorghum pests and diseases in Managua, Nicaragua for approximately 40 attendees from INTA/CINIA, Nicaragua, CENTA, El Salvador and private industry from June 10-14, 2002.

Research Investigator Exchanges

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

L. E. Claflin surveyed sorghum fields and discussed mutual research in El Salvador and Nicaragua in December, 2001.

Various equipment and supplies provided to Reina Guzman in CENTA and Sergio Pichardo in UNA through passthrough funds of KSU-211.

A Zeiss microscope from UNA was carried to the US, cleaned, eyepieces replaced, adjusted and then returned to Nicaragua. Repair of the microscope resulted in a potential savings of $10,000.

Research Information Exchange

Antisera specific to *Xanthomonas campestris* pv. *holcicola* (causal agent of bacterial streak disease of sorghum) was provided to Ranijit Bandyopadhya (ICRISAT) and Jesus Narro (Mexico)

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, the following speciality equipment

Table 3. Incidence and severity of panicle diseases in the All Disease and Insect Nursery - El Salvador (2001).

	Panicle Diseases				
Accession	Anthracnose	Curvularia	Fusarium	Phoma	Bird damage
B-35	5	5	1	0.8	1
SC 326-6	1.3	1.3	1	1	2
SC 414-12E	2	2.3	1	1.3	1
SC 630-11E(ii)	1	1.7	2	1	1
R 9188	1	4	1	1.4	1
86 EO 366	1	2.7	1	1	1.3
90 EON 328	1.3	1.7	1.3	1	1
90 EON 343	1.1	2	1	1	1
91 BE 7414	1	2.3	1	1	1.3
87 BH 8606-6	1.3	2.7	1.3	1.3	1
88 BE 2668	1	2	1	1	1
94 CW 5045	1	2	1	1	1
96 CA 5986	1	2	1	1.3	1.74
96 CD 635	1	2.3	1	1.3	1
96 CD 677	1.4	2	1	1.3	1
99 BD 3726/98CD187	1.3	1.7	1	1	1.3
99 CA 2244	1	1.7	2	1	1
99 CA 2519	1	2.3	1.3	1	1.7
99 CA 1422	1	1.4	1.3	1	1
99 PR 1159/B LD6	1	2.5	1.5	1	1
LG 70	1	2	1.7	1	1
LG 35	1.3	1.7	1	1.3	1
B8 PR 1011	2.3	2.7	1.3	1.3	1
B8 PR 1059	1	3	1.7	1.7	1
B8 PR 1051	1	2.3	1	1.3	1
98 BRON 125	1	2	1.3	1	1
B8 PR 1013	1	3.7	1	1.3	1
B8 PR 1057	1	1.3	1.7	1	1.3
TX 2880	1	2.5	1.5	1	1
GR 108-90M24	1.3	1.3	1.3	1	1
95 BRON 155	1	1.7	1.3	1	1
95 BRON 151	1	2.3	1.3	1	1
96 GCP OB 124	1	2	1	1	1
96 GCP OB 143	1	1.7	2	1	1.3
96 GCP OB 157	1.3	2	1	1.3	1
96 GCP OB 160	1.7	2.3	1.3	1	1
96 GCP OB 172	1	2.7	1.3	1	1
MB 108 B	1	2	1	1	1.3
97 BRON 179	1	2	1.3	1	1
98 BRON 122	1	1.7	1.7	1	1.7
88 B 928	1	2	1.3	1	2
R 9113	1	2	1.3	1	1
97 BRON 304	1	2	1	1.3	1
B 9104	1	2.3	1.3	1	1
B 9107	1	3	1	1.3	1
87 EO 109	1	1.3	2.3	1	1.7
B 9601	1	2.5	1	1.1	1.5
88 B 943	1	2.7	1	1.3	1
94 B 1055	1.1	3	1	1.8	1
B 9105	1	1.7	1.7	1	1.3
R 9603	1	3.3	1	1.7	1
B 9307	1.4	2	1	1.7	1
R 9120	1	3	2	1.3	1
91 B 2978	1	1.7	1.3	1.3	1.3
TX 2911	1	2	1	1	1
R 9618	1.3	1.7	1	1.3	1.7
R 9519	1	3.7	1	1.3	1
Malisor 84-7	1.7	1.7	1	1.3	1
SRN 39	1	2.3	1	1.3	1
Sureño	1	1.7	1.3	1.3	1.7
TX 2783	1	2.7	1	1.7	1
TX 2767	1	2.7	1.3	1	1
TX 2783	1	2	1	1	1.3
BTX 635	1.3	2	1	1	1
BTX 623	1	2	1	1	2
BTX 631	2	1.7	1.3	1	2.3
TAM 428	1	1.3	2.3	1.3	.2.3
TX 430	1	3	1.3	1.3	1
TX 7078	1.3	4	1.3	2	1
BTX 378	1.3	3.3	1.3	2.3	1

and supplies were purchased with funds from KSU-211 and distributed to the laboratories:

Incubator – Boekel Incubator – No. 133000, Pseudomonas Agar F, Potato Dextrose Broth, Agar, Gram Stain Kit Solutions, Autoclavable Biohazard Bags, Printer HP Office Jet G55xi, Sharp Microwave –Model R209EK, Fisher Latex Examining Gloves, Moldex Respirator Masks, O/F Basal Media, Inoculating Turn Table, Dryslide Oxidase Test, Difco Agar, Gelatin Agar, Nitite Broth, Multicolor Tape Rolls and dispenser, Automatic Slide Dispenser, Lactose Monohydrate, Dextrose, Sorbitol, Mannose, Gram +/- Slides, Nutrient Broth, Yeast Extract, Cellobiose, CD Burner - Plexwriter, Zip Drive – Iomega 250MB

Publications and Presentations.

Abstracts:

Tuinstra, M. R., T. Teferra, L. E. Claflin, R. G. Henzell, N. Seetharama, G. Ejeta, and D. T. Rosenow. 2001. Sorghum Industry Conference, Nashville, TN, February 18-20, 2001.

Ramundo, B. A, M. R. Tuinstra, and L. E. Claflin. 2001. Genetic variability of various plant hosts to *Claviceps africana*. KS-NE Sorghum Conference, Clay Center, NE August 30-31.

Reed, J. D. M. R. Tuinstra, N. W. Ochanda, K. D. Kofoid, and N. W. McLaren. Analysis of ergot resistance in sorghum. 2001. ASA-CSSA-SSSA An. Mtg. Charlotte, NC. Oct 21-25.

Teferra, T. T., M. R. Tuinstra, R. L. Vanderlip, and K. D. Kofoid. 2001. Analysis of genetic diversity for Fusarium stalk rot resistance in sorghum. ASA-CSSA-SSSA An. Mtg. Charlotte, NC Oct 21-25.

Journal Articles

Claflin, L. E., M. R. Tuinstra, and B. A. Ramundo. 2001. Ergot: A new disease of sorghum in the Western Hemisphere, pp. 114-127. *In* Proc. Am Seed Trade Assoc. Mtg., Chicago, Il, December 5-8, 2000.

Tuinstra, M. R., T. Teferra, L. E. Claflin, R. G. Henzell, A. Borrell, N. Seetharma, G. Ejeta and D. T. Rosenow. 2001. Root and Stalk Rot Resistance in Sorghum. Proc. 22nd Biennial Grain Sorghum Research Utilization Conference. Nashville, TN, Feb 18-20, 2001. Pgs 32-34.

Presentations

Claflin, L. E. 2001. Pokkah boeng disease of corn and sorghum. Universidad Nacional Agraria, Managua, Nicaragua, Nov 27.

Claflin, L. E. 2001. Pokkah boeng disease of corn and sorghum. Centro Nacional de Technologia (CENTA), La Libertad, El Salvador, Nov. 29 .

Claflin, L. E. 2001. Pokkah boeng disease of corn and sorghum. Pioneer Hi-Bred Int (and others), Guadalajara, Mexico, Dec. 3.

Miscellaneous Publications

Claflin, L. E. 2001. 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 Res. Support Prog. (CRSP), University of Nebraska, Lincoln.

Table 4. Incidence and severity of foliar diseases of sorghum accessions in the All Disease and Insect Nursery - El Salvador (2001)

Accession	Foliar Diseases		Grey Leaf	Rust	Ergot	Anthracnose	Bacteria
	Zonate	Leaf Blight					
B-35	1	0.8	3	2.3	3	5	1
SC 326-6	1.3	1.3	1.3	1.7	1	2	1
SC 414-12E	1.7	1.3	2	2.3	2	1.7	1
SC 630-11E(II)	1	1.8	1	3.4	1	0.8	1
R 9188	1	0.8	1	3	1.5	0.8	1
86 EO 366	1	2.3	1	1	2	1.7	1
90 EON 328	1	2.3	1	2	1	1	1.3
90 EON 343	1	1.8	1	2	1	1.3	1
91 BE 7414	1	1.7	1	2.3	1	1.3	1
87 BH 8606-6	1	1.3	1.3	3.7	1	1	1
88 BE 2668	1	1.3	1.3	2.3	1	1.7	1.3
94 CW 5045	1.5	1.5	1	2.6	1	1.1	1
96 CA 5986	1	2	1.3	1.3	1	1.3	1
96 CD 635	1.3	2.3	1	2	1	1.3	1
96 CD 677	1.3	1.7	1.3	2.3	1.3	1.7	1
99 BD 3726/98CD187	1	1.3	1	3	1	1	1
99 CA 2244	1	2.3	1.3	1.7	1	1	1
99 CA 2519	1	1.7	1	1.3	1	1.7	1
99 CA 1422	1	1.3	2.3	1.7	1	1.7	1
99 PR 1159/B LD6	1	2.1	1	1.6	1	2.1	1
LG 70	1.7	1.3	1.32	3	1.7	1.3	1
LG 35	1	1.3	1	1.3	1	4	1
B8 PR 1011	1.3	1.3	1	2.7	1	2.7	1
B8 PR 1059	1	2	1.3	2	1	2	1
B8 PR 1051	1	1.7	1	1.3	1	2.3	1
98 BRON 125	1	2	1.3	1.3	1	1.7	1
B8 PR 1013	1.3	1	1.3	2.3	1	1.7	1
B8 PR 1057	1	1	1.3	2	1	2.7	1
GR 108-90M24	1	1.3	1.7	2.3	2.3	2.3	1
95 BRON 155	1	1	1.3	1.7	1.3	2.3	1
95 BRON 151	1	1.3	1	4.7	1	1.7	1
96 GCP OB 124	1	2.3	1	1	1	1.3	1
96 GCP OB 143	1	1.7	1	2	1	1	1
96 GCP OB 157	1	1.7	1.3	2	1.3	3.3	1
96 GCP OB 160	1	1	1	1.7	1.3	2.7	1
96 GCP OB 172	1	1	1	1.3	2	4	1
MB 108 B	1	2	2	2.7	1	2	1
97 BRON 179	1	1	1	2.3	1	3	1
98 BRON 122	1.3	1.7	1.3	2.3	1.3	1.3	1
88 B 928	1	1.7	1	2.3	1.3	2	1
R 9113	1.3	2.3	1	2	1	1.3	1
97 BRON 304	1.7	2.3	2	2.7	1	1	1
B 9104	1	1.3	2	1.7	1	1	1
B 9107	1	1	1	2.7	1.3	3.7	1
87 EO 109	1	1.7	1	3.3	1	1.3	1
B 9601	1.5	1.1	1	1.9	1	3.1	1
88 B 943	1	1.3	1	4	1.3	2.3	1
94 B 1055	1	1.8	1	2.3	1	2	1
B 9105	1	1	1.7	2.7	1	3	1
R 9603	1.3	1.3	1	2.3	1	1.7	1
B 9307	1	2	1	2	1	1.7	1
R 9120	1	1.3	1	2.3	1	1.7	1
91 B 2978	1.7	1	1.3	2	1	1.3	1
TX 2911	1	1	1	4	1	1	1
R 9618	1	1.7	1	2	1	1	1
R 9519	1	1.7	1	3	1	2.3	1
Malisor 84-7	1.3	2	1.7	1.7	1	1.3	1
SRN 39	1	1.3	1	3.3	1	1	1
Sureño	1	2	1	2.3	1	1.3	1
TX 2783	1	1.3	1	3.3	1	1	1
TX 2767	1.3	1.7	1.3	3.3	1	2	1
TX 2783	1.7	1.3	1.7	3	1.7	2	1
BTX 635	1	1.7	1	1.7	1.3	1.3	1
BTX 623	1.3	1	1.7	1.7	1	2	1
BTX 631	1	2	1	1	1	1	1
TAM 428	1.3	1.7	1.3	2	1	2	1.3
TX 430	1.3	1	1	3	1.7	1	1
TX 7078	1	1	1.1	3	1	1	1
BTX 378	1.3	2	1	3.3	1	1.3	1
TX 2880	1	1.1	1	4.1	1	2.1	1

Rating scale: 1= trace - 2%; 2 = 2 - 10%; 3 = 11- 25%; 4 = 26 - 50; 5 = 51 - 75%; 6 = death.

Enhancing the Utilization of Grain Sorghum and Pearl Millet through the Improvement of Grain Quality Via Genetic and Nutritional Research

Project KSU-220
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Summary

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, but little attention has been focused on grain quality. Grain development under heat and drought stress results in variable seed size and composition whereas development under high rainfall and humidity favors grain mold and weathering. Both conditions lead to lower food and feed value.

The marketing and utilization of sorghum grain often has been limited by poor grain quality. Grain mold is a common problem because sorghum kernels are exposed to the environment as they mature. However, even in the absence of contaminating fungi, sorghum grain typically has lower digestibility and metabolizable energy values when compared to other cereals. Numerous researchers have reported differences in growth performance of poultry and swine when fed grain from different varieties of sorghum; however, little information is available about inheritance of food and feed

quality components and their associations with digestibility and nutritional quality. Research and germplasm development activities in KSU-220 attempt to address these questions and problems.

Our research efforts are focused on identification and characterization of germplasm sources that have improved grain quality and nutritional value characteristics. Breeding projects to assemble these genes into improved cultivars should proceed rapidly with the aid of marker-assisted selection and with performance tests made in multiple environments. The results from these studies will contribute to the development of value-enhanced sorghum and millet grains and the transfer of animal feeding technologies will promote the development of new entrepreneurial opportunities for production of meat and other animal products in Africa and Central America.

In the past year, we initiated several studies to identify and characterize sources of grain and nutritional quality. We have also initiated theoretical studies to evaluate the most effective strategies for implementing marker-assisted selection in sorghum crop improvement. Given the recent implementation of this project, most of these studies were only just initiated; however, we will be providing preliminary results from a poultry feeding trial to evaluate nutritional characteristics of sorghum hybrids with variable seed size.

Objectives, Production and Utilization Constraints

Objectives

Research

- Study the inheritance of seed size and feed quality components in sorghum.
- Determine the metabolizable energy content of sorghum hybrids differing in seed size versus corn in poultry rations.
- Identify, clone, and map genes for grain mold resistance, anthracnose resistance, and improved nutritional characteristics.

Germplasm Development

- Develop sorghum varieties and hybrids with improved yield potential and food quality characteristics.
- Develop recombinant inbred (RI) sorghum mapping populations to identify markers for grain mold resistance, anthracnose resistance, and improved nutritional characteristics.
- Evaluate tan-plant food sorghum hybrids for differences in grain quality and food processing characteristics.
- Evaluate the feasibility of marker-assisted selection for grain mold resistance.

Training, Networking, and Institutional Development

- Identify graduate students from Central America and Africa through the aid of collaborators.
- Establish formal and working collaborations and plans for work in Central America and Africa.

Constraints

Sorghum and millet production around the world is constrained by the lack of high-yielding cultivars with superior food and feed quality characteristics. This interdisciplinary research project attempts to address this problem through research to develop a better understanding of the genetic traits and physical properties that contribute to grain and nutritional quality and through crop improvement efforts via biotechnology and traditional plant breeding approaches.

Breeding efforts to directly address nutritional quality of grain sorghum have been slowed by the lack of routine screening procedures for this trait. Accurate methods for determination of chemical composition are well documented (AOAC, 1990), but tend to be complicated and are not acceptable predictors of nutrient bioavailability. Research strategies must be developed to measure food or feed efficiency traits. Components of feed quality are frequently defined in terms of animal performance such as weight gain per unit of grain fed or in terms of metabolizable energy per unit of grain. 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. Therefore, rapid and less hazardous screening methods need to be developed and applied to prediction of nutritional characteristics for grain sorghum and millet.

Genes for grain and nutritional quality can be identified and tagged with DNA-based markers to facilitate crop improvement. Breeding projects to assemble these genes into improved cultivars should proceed rapidly with the aid of marker-assisted selection and with performance tests made in multiple environments. The combined expertise of the team assembled for this proposal will permit identification, verification, and implementation of genes that contribute to various aspects of grain quality.

Research Approach and Project Output

Analysis of feed quality and metabolizable energy content of sorghum and corn hybrids.

Research Methods

Simultaneous genetic improvement for grain size and feed quality requires evaluation of seed weight and the major characteristics of feed: contents of protein, fat, and starch

and digestibility. Several studies have investigated the chemical composition of grain sorghum; however, little information is available about inheritance of that composition and digestibility and their associations with feed quality. Knowledge of genetic variability for feed quality characteristics, digestibility, seed weight, and their associations is desirable for designing optimal breeding strategies to improve feed quality.

Germplasm sources with a dominant mode of inheritance for seed size have been identified. In our research, one of the best germplasm sources for increased seed size and yield potential was the breeding line KS115. Given the dominant pattern of inheritance for these traits in this line, this germplasm source should be extremely useful in development of sorghum varieties and hybrids with increased seed size and crude protein content and better protein quality. However, feeding trials were needed to measure the impact of these traits on animal performance characteristics and metabolizable energy (ME) content.

Poultry feeding trials are currently being conducted to evaluate the potential effect of seed size on feed efficiency and ME content of sorghum. Eight sorghum hybrids differing in seed size and feed quality characteristics were grown at two locations in Kansas in 2000. Bulk grain samples (20 kg) for each hybrid were harvested for determination of feed quality. A bulk sample of corn produced at each location was also obtained for comparison with the sorghum samples. Differences in feed quality characteristics among hybrids were determined using a randomized complete block design with environments as blocks.

Newly hatched chicks were obtained for feeding trials. The experiment was conducted using a randomized complete block design with six chicks per cage and six cages per feed treatment. Diets were prepared according to NRC guidelines using hammer milled grain sample (2.4 mm). The chicks were fed a common diet for 14 days and were then changed to diets with the various grain treatments for seven days. Feed and water were consumed on an *ad libitum* basis. Chicks were allowed to adjust to the experimental diets for five days then fecal samples were collected for two days. The excreta and diets were dried and analyzed for gross energy, nitrogen, and chromium to allow calculation of ME for the test ingredients. Differences in weight gain and gain/feed were measured to evaluate growth performance.

Research Findings

When combined with improved yield potential, increased seed size and uniformity should enhance utilization attributes of sorghum grain. More consistent seed size should improve the physical and mechanical handling of grain during processing and may also improve flour yield for food-grade sorghums. Sorghum varieties with large grain also tend to be preferred for food use in many developing countries where sorghum is used for human consumption.

Although multi-year studies will ultimately be required to determine the effect of seed weight on nutritional value, differences in feed and nutritional quality characteristics of corn and sorghum hybrids were detected in grain samples produced in 2000 (Table 1). In general, the larger seeded hybrids developed using KS115 were generally higher in crude protein and fat as compared to normal sorghum hybrids. Large differences in metabolizable energy content were also noted among grain samples (Table 2). Again, hybrids produced using KS115 were generally higher in metabolizable energy content than conventional sorghum hybrids. Surprising, SA3042 × KS115 was significantly higher in metabolizable energy content than the corn samples in the combined analysis. Although preliminary, these results indicate great promise for improving metabolizable energy content of sorghum by integrating KS115-type grain characteristics into improved genetic backgrounds.

Table 1. Analysis of feed quality characteristics of sorghum and corn hybrids grown at two locations in Kansas in 2000.

Pedigree	Seed weight g 100 seed $^{-1}$	Crude protein %	Crude fat %
Wheatland × KS115	4.04	12.56	4.01
SA3042 × KS115	3.03	12.83	3.70
Wheatland × Eastin-1	2.81	12.62	3.55
SA3042 × Eastin-1	2.68	12.46	3.43
Wheatland × Tx2737	2.26	11.53	3.60
SA 3042 × Tx2737	3.01	11.67	3.46
Wheatland × Tx 435	2.53	11.24	3.13
SA3042 × Tx 435	2.49	12.19	3.47
Bulk Corn		10.27	3.82
LSD (0.050)	1.30	1.47	0.42

Table 2. Metabolizable energy content of sorghum and corn grain samples produced at Manhattan and Ottawa, Kansas in 2000.

	Metabolizable energy		
Hybrid	Manhattan	Ottawa	Combined
	---------- kcal ----------		
Wheatland × KS115	3.48	3.52	3.50
SA3042 × KS115	3.74	3.63	3.668
Wheatland × Eastin-1	3.37	3.42	3.39
SA3042 × Eastin-1	3.34	3.43	3.38
Wheatland × Tx2737	3.33	3.28	3.30
SA 3042 × Tx2737	3.59	3.45	3.52
Wheatland × Tx 435	3.27	3.12	3.20
SA3042 × Tx 435	3.61	3.15	3.38
Bulk Corn	3.49	3.33	3.41
LSD (0.050)	0.26	0.26	0.27

Networking Activities

Workshops /Conferences

Drs. Rooney and Hancock attended INTSORMIL Central American research planning and coordination meeting in Managua, Nicaragua, February 26-28, 2002. During this meeting, work plans were established with Ing. Rene Clara, Hector Deras and Rafael Obando.

Dr. Tuinstra attended the Sorghum Industry Conference and the Sorghum Germplasm Committee Meeting in San Francisco, CA, February 18-20, 2002.

Germplasm and Research Information Exchange

Germplasm was sent to collaborating scientists in El Salvador, and Zambia for evaluation in these locations during the 2002 growing season.

Two hybrid sorghum trials were developed and distributed to cooperators in 2002. The IFSAT (International Food Sorghum Adaptation Test) is compiled of tan plant sorghum hybrids predominantly from the TAM220C, TAM222 and TAM223 for evaluation worldwide. The TPHT (Tan Plant Hybrid Trial) is a cooperative test between Texas A&M and Kansas State University, funded by PROFIT and the National Grain Sorghum Producers to evaluate commercial tan plant hybrids for agronomic and grain quality in U.S. production systems.

Dr. Rooney released two sets of sorghum germplasm (Tx2912-2920 and Tx2921-Tx2928) in February 2002. These germplasms were made available to sorghum improvement programs throughout the world. In addition, standard hybrid tests of food quality (IFSAT) hybrids were prepared and distributed to cooperators in the U.S., Mexico, Central America and Southern Africa.

Publications and Presentations

Journal Articles

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Klein, R.R., R. Rodriguez-Herrera, J.A. Scheulter, P.E. Klein, Z.H. Yu, and W.L. Rooney. 2001. Identification of genomic regions that affect grain mold incidence and other traits of agronomic importance in sorghum. Theor. Appl. Genet. 102:307-309.

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Tuinstra, M.R., G.L. Liang, C. Hicks, K.D. Kofoid, and R.L. Vanderlip. 2001. Registration of KS 115 sorghum. Crop Science 41: 932-933.

Books, Book Chapters, and/or Proceedings

Tuinstra, M.R., T.D. Kriegshauser, R.L. Vanderlip, K.D. Kofoid, and J.D. Hancock. 2001. Can long grain-fill duration improve yield potential and grain quality of sorghum? Pp. 185-195. In Proceedings of the 56th Corn and Sorghum Research Conference, 2001. American Seed Trade Association. Chicago, IL, Dec. 5-7, 2001. Alexandria, VA. USA.

Low Input Ecologically Defined Management Strategies for Insect Pests on Sorghum

Project MSU-205

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Summary

MSU-205 sorghum plant protection research and institution building activities in Honduras were de-emphasized in 2001. Entomological research was expanded in Nicaragua and El Salvador, 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 15 years when this project worked with low input, subsistence farming systems in Honduras. Collaborative research activities in Nicaragua with the Instituto Nicaraguense de Technologia (INTA), the Universidad Nacional Agraria (UNA), and the Universidad Autonoma de Nicaragua (UNAN) 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 programs for specific pests or complex of pests. Crop protection information was published for distribution into farm communities in Honduras, as the result of research on a complex of insect pests that limits sorghum production in this country. Similar 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. Collaborative participation in this research with scientists at INTA, UNA, UNAN, CENTA and the Nicaraguan National Sorghum Producers Association (ANPROSOR) has 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

Honduras

- Conduct on-farm survey to determine farmer acceptance and utilization of improved sorghum production and insect pest management practices in traditional and improved intercropped sorghum production systems in hillside and coastal plain fields in southern Honduras.
- Complete graduate student research and academic program for Master of Science degree in entomology at Mississippi State University.
- Prepare manuscripts for publication in scientific journals.

Nicaragua

- Complete graduate student research and academic program for Master of Science degree in entomology at Mississippi State University.
- 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 first year of research and academic programs for MSU-205 PhD 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

- Conduct 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

Honduras

Research in Honduras for the past 23 years emphasized biology, ecology behavior, population dynamics and pest control tactics for soil insects, foliage feeders and stem borers that were identified as the principal insect pest constraints to intercropped sorghum and maize production on subsistence farms in southern Honduras. Information obtained was incorporated into insect pest management programs appropriate for this region. The results of collaborative research with scientists at the Panamerican School of Agriculture was published in 1999 by Zamorano Academic Press in a popular article, "La Langosta del Sorgo y el Maiz". This publication was distributed into farm communities to provide recommendations to farmers for management of the complex of insects that is devastating to these crops annually. Cultural, chemical and biological control practices have been identified for use in integrated insect pest management programs for the principal pests on these grain crops. The benefits of this information have been reported in previous INTSORMIL annual reports and numerous scientific journal papers. For example, an economic evaluation of integrated insect pest management tactics in intercropped sorghum and maize production systems in southern Honduras indicated that sorghum production was increased by 20% and maize by 35% at the farm level. These increases could return $2.9 million a year to production of these crops in this area when market prices are high.

The on-farm survey conducted in June 2002 in southern Honduras included subsistence farmers in hillside and coastal plain sorghum production systems. The survey included farmers that cooperated with MSU-205, as well as several that were not cooperators. A survey instrument was prepared and administered in Spanish in person by the MSU-205 graduate student. Farmers were selected at random for interviews. The survey was particularly interested in information on changes in crop production practices used by farmers in this region, as well as the extent of technologies that were provided to the farmers by agricultural professionals in the region.

Several things became apparent after only a limited number of interviews. The subsistence farmers in this region have little contact with agricultural professionals, possibly only once a year or even less; the small amount of chemicals used are generally given to them by agriculture related private organizations, but occasionally they have to purchase the materials if they use the materials over time and do not use improved sorghums developed for this region; and they have poor access to crop production literature or exposure to agricultural professionals at meetings, either on the farm or in a nearby community.

There appears to be little currently available insect pest management and crop production technology transferred from professional crop production specialists to subsistence farmers in this region of Honduras. Several farmers indicated that their source of certain improved crop production technology was obtained from individuals in the MSU-205 project. It was apparent from this interview that an educational program is needed and that it must be conducted at a level of educational understanding for the illiterate farmers in this region. Sorghums have been developed, agronomic crop production practices have been identified and insect pest management programs have been designed for improved sorghum yield in this region of Honduras. These technologies are available to crop production specialists, but they are not transferred to the farmers.

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 15 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, the midge being most destructive. Research was conducted to determine 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 Master of Science thesis was completed and one manuscript representing this research was prepared for publication in the international journal, "Tropical Agriculture". This paper involved management of the sorghum midge on sorghum on the coastal plain of Nicaragua. A second journal paper considered the occurrence of sorghum midge on sorghum during the second crop-growing season on the Pacific coastal plain of Nicaragua is to be published in "LaCalera" 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 that completed the Master of Science degree in entomology is continuing entomology studies for a 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. These studies were initiated in 2001.

The MSU-205 PI and MSU-205 Ph.D. graduate student participated in the Central America Sorghum Workshop in Managua in February 2002. Collaborative crop protection research was discussed and plans were made for the 2002 growing season. Particular emphasis was given to developing plans for collaborative, multidisciplinary, on-farm crop protection investigations with MSU-205, INTA, UNA and ANPROSOR collaborating. A work plan has been prepared and will be implemented in 2002.

The MSU-205 PI (Pitre) and KS-210B PI Claflin) conducted a five-day sorghum plant protection workshop in Managua, June 10-14, 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. Participants were presented a workshop manual that included pictures of the insect pests and associated plant damage to be considered in the entomological presentations. Field trips were taken to observe insects and related plant damage, as well as plant diseases on sorghum.

El Salvador

Entomological research with scientists in CENTA was planned and coordinated for the 2001 sorghum growing season in El Salvador, when the MSU-205 PI visited CENTA in November, 2000. 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 involved identification of the complex of soil insect pests and determining the extent of damage and economic significance of these insects on sorghum. This objective included elucidation of the occurrence and aspects of population dynamics of these pests. Plans were made to obtain this information from sampling programs in different crop agroecosystems. A second planned objective involved the principal insect defoliator, the fall armyworm. Observations on populations of this caterpillar on and damage to sorghum in the All Disease and Insect Nursery (ADIN) was made during the 2001 crop growing season. This was coordinated with collaborating sorghum breeder and plant pathologists in CENTA and Kansas State University (KSU-210B), respectively.

Insecticide evaluations for efficacy on fall armyworm larvae on sorghum four days after application indicated that the chiten inhibitors, Lufenuron and tefubenzuron, provided the greatest mortality (92%), Lorsban provided 63% mortality, whereas nuclear polyhedrosis virus and a fungus, *Bauveria bassiana*, provided low mortality (< 5%), with the botanical insecticide Neem providing 17% mortality. Mortality of fall armyworm larvae in plots treated with Lorsban insecticide at two rates in two different volumes of water was significantly influenced by two applications compared with one application, but was not significantly influenced by volume of water. Insecticide treatment plots yielded more than the untreated plots. Infestations of fall armyworm larvae on sorghum treated with Lorsban was lower with each additional spray application, but yield of plots was not significantly different among treatments; all insecticide treatments had higher yield than the untreated. These results 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.

Sorghums were evaluated in the "All Disease and Insect Nursery" (ADIN) in collaboration with entomologist and plant pathologist at CENTA and KSU-210B PI (Claflin). No significant differences in damage were recorded for varieties tested, although several varieties had lower damage ratings than other varieties; these varieties are recommended for the breeding program. Yields were similar for all varieties except a local variety, which had lower yield.

These and similar studies will be conducted in 2002. Particular emphasis will be given to studies to determine economic damage and economic threshold levels for fall armyworm on sorghum in different stages of plant development. Similar studies will be conducted in the United States (Mississippi).

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 were conducted in 2001 with fall armyworm to determine infestation levels suitable for artificial infestations, survival of fall armyworm larvae in three stages of development at infestation and over time after infestation, time of day most suitable for infestation, and other infestation procedures. This information is being 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 will be recorded for treatments. This research will be duplicated by CENTA in El Salvador. Information from these studies can improve the application of pest management practices for fall armyworm on sorghum.

Replicated field tests were conducted in 2001 to determine the optimum procedures for infesting sorghum in the whorl stage with fall armyworm larvae. Mechanical infestation using the "bazooka application" provided satisfactory infestation levels when first, second or third instar larvae were used. Infestation levels were compared to determine survival of larvae over time. Results indicated that regardless of the number of larvae placed on plants in the whorl stage generally only one insect was present on each plant when larvae reached the last instar. Time of day when plants were infested did not appear to influence the success of the infestation method.

Economic threshold studies will be conducted with sorghum midge on sorghum in 2002 and 2003.

Networking Activities

Collaborator scientists and administrators at INTA in Nicaragua expressed interest in supporting a sorghum crop protection workshop emphasizing pest management. This workshop was successfully conducted in June 2002 and included aspects of integrated insect and plant disease management. MSU-205 and KS-210B PI's were presenters. The workshop was successful because of detail coordination by scientists and administrators at INTA and UNA.

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 midge in Nicaragua, prepared by INTSORMIL MSU-205 and INTA, provides information for farmers to manage this insect pest on sorghum to improve yield. This publication is distributed by INTA into farm communities with assistance from local agricultural professionals.

Publications and Presentations

Journal Articles

Vergara, O.R. and H.N. Pitre. 2001. Planting data, weed management and insecticide application practices for control of lepidopterous pests in intercropped sorghum and maize in southern Honduras. Trop. Agric. 78: 182-189

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Dissertations and Theses

Carrillo, Mario. 2001. Insect populations and pest management strategies in traditional and improved sorghum and maize production systems in foothill and coastal plain fields in southern Honduras. M.S. thesis. Mississippi State University 71 pp.

Miscellaneous Publications

Presentations

Pitre, H.N. 2002. Insect pests on sorghum and related crop management practices. Presentation at Central America Sorghum Workshop. Managua, Nicaragua. February.

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Striga Biotechnology Development and Technology Transfer

Project PRF-213

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Summary

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

In Year 23, we summarize part of a Ph.D. research by a former student, Abdalla Mohamed of Sudan, who has discovered that certain sorghum genotypes are resistant to *Striga* because of a unique mechanism where they show necrotic tissue at the point of parasitic attachment thereby discouraging normal development of the parasite. This expression called a hypersensitive response is typical in many pathogens where further development and expansion of pathogenic infection is curtailed by the defense response caused by necrosis developing at the site of infection. We developed new laboratory assays that target the disruption of a particular signal exchange or that detect a precise defense response at a given point in the life cycle of the parasite to define and characterize mechanisms of resistance. The major premise of our research thrust in *Striga* is based on the fact that field *Striga* resistance is quantitatively inherited and is influenced by confounding environmental factors, and is therefore difficult to manipulate. On the other hand, individual interactions between host and parasite in the early stages of the infection process appear to be simply inherited. Characterization of these more qualitative interactions between host and parasite, allows us to dissect the complex trait of *Striga* resistance into more manageable components based on the nature or action of signals exchanged between the parasite and its hosts. As a result, selection efforts directed to pooling these variations of signal exchanges between host and parasite may lead to a more durable *Striga* resistance.

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 be-

yond 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:

- Develop effective assays for resistance-conferring traits and screen breeding materials assembled in our *Striga* research program for these traits.
- Elucidate basic mechanisms for *Striga* resistance in crop plants.
- Combine genes for different mechanisms of resistance, using different biotechnological approaches, into elite widely adapted cultivars.
- 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.
- Develop integrated *Striga* control strategies, with our LDC partners, to achieve a more effective control than is presently available.
- Assess (both *ex ante* and *ex post*) of the adaptation and use of these control strategies, in cooperation with collaborating agricultural economists.
- 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

Hypersensitive Response as a Mechanism of Striga Resistance in Sorghum

Appearance of the parasitic weed *Striga* (*Striga* spp.) on host plants in the field is the eventual expression of a series of interactive events between the parasite and its hosts. Empirical breeding for *Striga* resistance in field crops has relied on selection of host plants that allow emergence of few parasitic plants and show little or no loss in productivity of the crop. Plant breeders in several programs around the world have identified sorghum [*Sorghum bicolor* (L) Moench] varieties with good levels of *Striga* resistance using this approach. However, the specific mechanisms of many of these resistance sources have not been properly characterized because of lack of appropriate laboratory procedures that reveal the specific interactions in the early stages of infection. In this study, we report on the identification of sorghum variants with hypersensitive response (HR) to parasitic infection characterized by expression of necrotic lesions at attachment sites discouraging further penetration of the parasite into host roots. We examined the HR reaction of known *Striga* resistant and susceptible sorghum cultivars using an *in vitro* assay developed in our laboratory. All susceptible sorghum cultivars showed no necrosis. In contrast, resistant cultivars, Framida, Dobbs, and a wild sorghum accession, P47121, showed necrosis in over 70% of attached *Striga.* In each of these lines, attached *Striga* were discouraged from penetration and further development. The HR reaction of these genotypes appears to be graded and varied in intensity. The reaction in P47121 was almost twice the intensity of the reaction of Framida. Over 83% of the *Striga* attached on P47121 exhibited discouraged penetration compared to 49% for Framida. These results suggest that P47121 would be a good candidate as a donor parent for an introgression of HR gene(s) for resistance into adapted sorghum cultivars.

Significant progress has been made in breeding for *Striga* resistance in several crops. However, there has been limited understanding of the basic mechanisms associated with resistance to *Striga*. Several hypotheses on possible host resistance mechanisms have been proposed. Most are based on cytological studies and observation of production of exudates *in vitro*. Nevertheless, there appears to be a general parallel between host-pathogen interactions in plant diseases and defense responses triggered during *Striga* invasion. The major limitation to making precise determination of these observations during the development of the parasite appears to be the lack of appropriate bioassays that reveal early interactions between the host and parasite.

One host-resistance mechanism, the hypersensitive response (HR), has been extensively studied in several plant pathogens. The HR generally refers to the appearance of a necrotic region around the site of attempted infection, followed closely by death of the affected host cells within hours of the attack. The HR can be phenotypically diverse ranging from a single cell response to large and spreading necrotic areas in a tissue accompanying parasitic colonization. Necrosis of the affected tissue has been shown, in some situations, to be directly related to the accumulation, oxidation, and polymerization of phenolic compounds. The objective of this study was to identify *Striga* resistant sorghum lines that express HR using an *in vitro* system, the extended agar gel assay (EAGA), a modification of a procedure we had described earlier.

A collection of sorghum cultivars, wild accessions, and breeding lines from our sorghum breeding program were sampled for this study. Seven cultivated sorghum lines (SRN-39, Framida, IS9830, 555, Dobbs, IS4225, and Shan Qui Red) with known field reaction to *Striga* infection, five wild sorghum accessions (P47121, P1885, P14539, P14529, and P12-26), and 95 BC_3F_4 progenies, derived from a cross between P47121 and two male sterile based populations (CK60 and KP9) were evaluated for HR to *Striga* invasion. All seeds were from plants grown at the Purdue University Agronomy Research Center, West Lafayette, IN.

Striga (*S. asiatica* (L) Kuntze) seeds were obtained from the USDA/APHIS, Whiteville, Methods Development Center, Whiteville, NC, courtesy of Drs. Robert Eplee and Rebecca Norris. *Striga* seeds were stored and handled under quarantine restrictions in an approved quarantine laboratory on the campus of Purdue University. All experiments involving *Striga* seeds and seedlings were performed in this facility.

The EAGA is a modification of the agar gel assay. In this assay, large (150 mm) Petri dishes with a thick agar layer were used to support growth of seedlings for a longer period than in the agar gel assay. Because seedlings can be grown for longer periods, effects of signal exchanges between host and parasite as well as host defense responses beyond germination could be observed using the EAGA. With the agar gel assay, we were only able to detect *Striga* germination in response to exudates from host roots with no opportunity to observe post attachment parasitic reactions. Three days following inoculation, each dish was observed for germination, parasitic attachment and host root development, then treated with ethylene to remove any difference in host roots for inducing *Striga* seed germination. The HR was observed when a clear necrotic lesion develops around the attachment site. The reaction normally starts a few hours after attachment and the lesion becomes more intense in 24 h. *Striga* seedling discouragement was observed 3 days following the attachment. Whereas *Striga* seedlings attached on susceptible host roots penetrate and develop, those on resistant roots are discouraged and never penetrate or develop beyond attachment.

All known susceptible sorghum cultivars had what appeared to be a normal association with *Striga* when observed under the conditions of the EAGA. *Striga* seedlings developed successfully without any apparent sign of stress

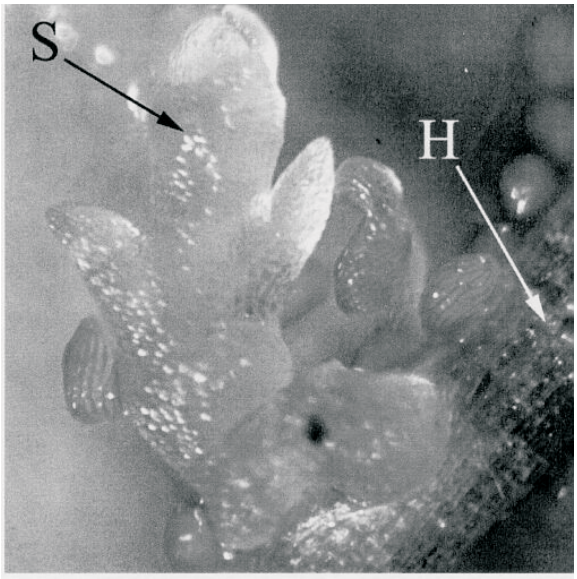
or damage. However, in some *Striga* resistant sorghum cultivars and wild sorghum accessions, a key host-defense response was observed. In these genotypes, necrotic areas appeared at *Striga* attachment sites on the sorghum root. These necrotic lesions most often start as red spots, which turn brown with time. The lesions were often large and some spread up to 2 mm from the center of attachment, but most remained localized. Attached *Striga* at these necrotic sites often did not develop further (discouraged from penetration), and eventually died on the host.

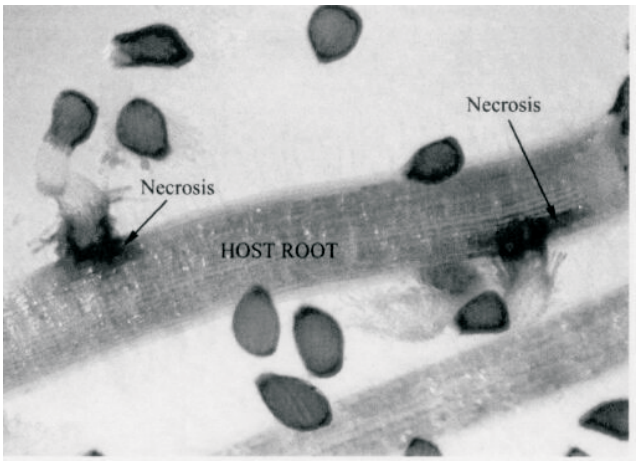
Among sorghum cultivars tested, Framida and Dobbs showed necrosis in about 70% of attached *Striga* and almost 50% of the attached *Striga* were discouraged from penetration. No necrosis was observed on roots of susceptible sorghum cultivars. Some *Striga* resistant sorghum cultivars, including SRN39, IS9830, and 555, also did not show necrotic lesions. These genotypes are resistant to *Striga* because they are low producers of the *Striga* germination signal, as indicated by germination distance. Among the wild sorghum accessions, P47121 (*Sorghum verticilliflorum*) showed necrotic lesions on 89% of the attached *Striga* seedlings. In this genotype, 83% of the total attachment points were discouraged from penetration and further development. Cultivars and accessions screened in this study were clearly and consistently classified into two categories, those exhibiting HR response and those with no necrosis. In both cultivated and wild sorghum accessions, susceptible genotypes induced germination and supported normal growth and development of the parasite.

The EAGA is an effective tool for demonstrating that different *Striga* resistant sorghum cultivars may possess different resistance mechanisms. A widely used sorghum cultivar Framida, with strong field resistance, possesses two mechanisms; it expresses an HR reaction in addition to a low germination stimulant production. Cultivars with moderate field resistance expressed one mechanism, either the low germination stimulant production (555, IS 9830) or a mild HR reaction (Dobbs). Contrary to an earlier report, resistance in sorghum cultivar SRN-39 could not be characterized as involving HR. The HR reaction of genotypes appears to be graded and variable in intensity. A single infected root may show reddening in most but not necessarily all attachment sites. Some attachment sites may appear necrotic early and fade with *Striga* growing normally. Overall, however, lines possessing HR reaction to *Striga* showed greatly reduced percentages of *Striga* attachments and reduced parasitic association relative to susceptible genotypes.

In each of the genotypes that exhibited HR, necrosis was observed at attachment sites as early as three days after infection. Discouragement of parasitic development was evident seven days following infection, reaching a maximum in 12 days after infection. In general, sorghum genotypes that showed necrosis at attachment sites also showed parasitic discouragement. A positive and significant correlation ($r = 0.84$) was observed between the presence of necrosis and *Striga* seedling discouragement. While parasitic discouragement can be noted, observation made on necrotic tissue is more readily apparent and reliable since this symptom appears early when the health of the host tissue is not of concern.

Hypersensitive response to *Striga* invasion is a readily observable trait with the EAGA reported here as well as with the paper roll assay both of which could be effectively used for germplasm screening. Since HR expression is associated with discouraged attachment of the parasite, it could serve as a powerful *Striga* resistance mechanism.





Deployment of Striga Control Packages

Collaborative international testing of *Striga* resistant sorghum cultivars, developed under the INTSORMIL PRF-213 project, has led to the identification of a number of sorghum varieties to be officially released for commercial cultivation in several countries. In Ethiopia, two *Striga* resistance cultivars were officially released, in 1999, for wide

cultivation in *Striga* endemic regions of northwest Ethiopia. In 2002, yet another one of our varieties was identified and recommended for official release in the Amhara region. This cultivar, released under the local name , Brhan (translated as Light) is expected to bring significant relief to the widespread and overwhelming darkness, that is *Striga*, in this region. The *Striga* resistance attributes of Brhan have been found to be superior to our previous releases particularly in the Amhara region.

To promote the extensive use of these varieties and rapid multiplication and distribution of seed in an organized farmer-to-farmer seed multiplication effort, we implemented a pilot project in Ethiopia, as an Integrated *Striga* Management (ISM) package with funds provided by the Office of Foreign Disaster Assistance (OFDA) at the USAID. The package includes seed of *Striga* resistant sorghum, nitrogen fertilizer, and the use of tied ridging as a water conservation measure. The combined use of moisture conservation, improved fertilization, and *Striga* resistant cultivars is expected to provide better control of *Striga* than use of any one of the individual packages. A total of 5.7 tons of seed of the two *Striga* resistant INTSORMIL varieties were produced at Melkassa Research Station. An estimated 1000 farmers in four *Striga* endemic regions of the country have been targeted to receive a package of technologies that includes fertilizer and tied-ridges.

In Tanzania, two of our *Striga* resistant varieties were also recommended for official release and cultivation in the sorghum growing environments of Tanzania where *Striga* has been a significant production constraint. Also in Eritrea, four of our *Striga* resistant cultivars have been identified for verification and wide demonstration in several locations in the country. Furthermore, plans have been made to plant approximately 33 hectares of these four varieties for seed increase in the 2002 crop season to be used in a large scale pilot project in the summer of 2003.

Networking Activities

Workshop and Program Reviews

Participated in the evaluation of a Food Security Project for the Amhara Region in Ethiopia at the invitation of USAID/Ethiopia, May 5-12, 2001, Addis Ababa, Ethiopia.

Participated in a study on survey of available technologies for use in development of drought tolerant crops in Eastern Africa for the Inter-Governmental Agency for Development, May 12-18, 2001, Nairobi, Kenya.

Attended and chaired two sessions at the 7th International Parasitic Weed Conference, 6-8 June, 2001, Nantes, France.

Attended International Conference on Contemporary Development Issues in Ethiopia, 16-18 August 2001.

Organized a stakeholders conference to discuss findings of regional study on state of technologies for drought tolerant crops in East Africa, 27-31 October 2001, Nairobi, Kenya.

Traveled to Ethiopia to initiate a program on community-based improved sorghum seed multiplication and integrated *Striga* management program for Ethiopia and Eritrea, 10-22 December, 2001.

Research Investigator Exchange

Visited University of Paris and the Tropical Ag Res Centre (CIRAD) and held discussions with staff at both institutions regarding collaborative research on sorghum *Striga* resistance, 10-12 June, 2001, France.

Hosted international visitors from Ethiopia, Tanzania, Zimbabwe, and Australia.

Germplasm Exchange

We continue to provide an array of sorghum germplasm from our breeding program to national research programs in developing countries. Our germplasm is provided in either a formally organized nursery that is uniformly distributed to all collaborators that show interest or upon request by a national program of specific germplasm entries or groups from our germplasm pool. Germplasm was distributed to cooperators in 25 countries in 1996, 15 countries in 1997, 10 countries in 1998, and 7 countries in 1999.

Three new *Striga* resistant varieties of sorghum from our program in 2001 were recommend for commercial cultivation in two African countries, one in Tanzania and two in Ethiopia.

Publications

Refereed Papers

Mohammed, A., G. Ejeta, and T. Housley. 2000. *Striga* asiatica seed conditioning and 1-aminoacylopropane-1- carboxylate oxidase activity. Weed Research 41:165-176.

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Published Abstracts

Gunaratna, N. and G. Ejeta. 2001. Selection of seedling cold tolerance in sorghum. Agronomy Abstracts, Charlotte, North Carolina.

Phillips, F., G. Ejeta, G. Shaner, and G. Buechley. 2001. Inheritance of rust resistance in sorghum. Agronomy Abstracts, Charlotte, North Carolina

Grenier, C., G. Ejeta, P. Bramel, J. Dahlberg, E. El-Ahmadi, M. Mahmond, G. Peterson, and D.T. Rosenow. 2001. Sorghums of the Sudan: Importance and diversity. Agronomy Abstracts, Charlotte, North Carolina.

Invited Research Lectures

Ejeta, G. 2001. Introgression of genes from landraces and wild relatives of sorghum. Presented at the American Seed Trade Association Annual Conference. 6-8 December, Chicago, Illinois.

Ejeta, G. 2001. Exploiting global genetic variation in sorghum improvement. Presented at Kansas State University, Invited seminar, Department of Agronomy. 5 September, Manhattan, Kansas.

Ejeta, G. 2001. The State of Agricultural Research in Sub-Saharan Africa. A keynote address, presented at the International Conference on Contemporary Development Issues in Africa. 16 August, Western Michigan University, Kalamazoo, Michigan.

Ejeta, G. 2001. An African Success Story: the control of a noxious weed. Presented at the Wabash Area Center for Lifetime Learning, 6 November, W. Lafayette, IN.

Sustainable Management of Insect Pests

Project WTU-200

Bonnie B. Pendleton
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Summary

The PI traveled to Botswana, Zambia, and South Africa in April to review sorghum research and establish collaborative projects to manage sugarcane aphid, *Melanaphis sacchari*, sorghum midge, *Stenodiplosis sorghicola*, and other major insect pests and develop integrated pest management (IPM) approaches for insect pests of sorghum in the field and storage. Research was planned with Dr. Munthali, entomologist at the Botswana College of Agriculture, who also will collaborate with current INTSORMIL collaborators from the Department of Agriculture (DAR) in Botswana. Research to develop IPM tactics for managing major insect pests was planned and begun with Drs. Diarisso, Doumbia, and Youm in Mali and with Mr. Abdou Kadi Kadi in Niger. Extract from a Malian plant, *Acacia nigricans*, was used to prevent infestation of stored sorghum grain by lesser grain borer, *Rhyzopertha dominica*. Sorghum lines were evaluated for resistance to sorghum midge and panicle-infesting bugs and will be evaluated again during the 2002 cropping season. Standard plant differentials were used to biotype greenbugs, *Schizaphis graminum*, infesting sorghum and wheat in the Panhandle and South Texas. DNA from sorghum lines developed for resistance to greenbug biotypes E and I was extracted and amplified fragment length polymorphism (AFLP) used to try to locate and map genes for resistance to different biotypes of greenbug. A Malian scientist was identified to come to West Texas A&M University to learn English and begin graduate studies. Graduate education programs of four students were directed and their research begun during 2002. One student assessed effects of temperature on fecundity and longevity of different biotypes of greenbug on sorghum. A student from India evaluated fitness of greenbug biotype I on resistant and susceptible sorghums and wild grass hosts. Another student from India studied effects of different amounts of soil moisture and nitrogen on abundance and longevity of greenbugs on sorghum. A third student from India began research to establish procedures for producing male greenbugs and viable eggs for use in determining genetic differences among biotypes of greenbug. The PI advised extension personnel and the National Grain Sorghum Producers organization on management of insect pests of sorghum and pearl millet. Greenbugs and advice for evaluating newly developed sorghums for resistance to greenbugs were provided to a commercial seed company and to molecular biologists at the Texas Agricultural Experiment Station.

Objectives, Production and Utilization Constraints

Objectives

West Africa

- Establish collaborative research with scientists in Mali and Niger to develop and transfer strategies, especially non-chemical methods, to manage major 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

- Establish collaborative research to identify and 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 major insect pests so effective management strategies and longer lasting plant resistance can be developed. Determine the distribution of biotypes of greenbug in Texas. Assess fitness of greenbugs on wild and cultivated hosts to better understand insect-plant interactions. Assess effects of temperature on the biology of different greenbug biotypes to determine the optimum temperature for evaluating resistance to greenbug in sorghum and better understand how biotypes of greenbug develop.

- Assess effects of agronomic practices on abundance of and damage caused by insect pests. Study effects of soil fertility and moisture on abundance of greenbugs on sorghum.

- Collaborate with breeders to evaluate sorghum germplasm for greater yield potential and tolerance to major insect pests.

- In collaboration with molecular biologists, use biotechnology techniques to study insect genetics and locate genes for resistance in improved sorghum lines so the genetic relationship between insects and resistant plants can be understood and durability of sorghum resistance increased.

- Provide technical advice and assistance on major insect pests and IPM tactics and how they function to assist extension personnel, commodity organizations, and the sorghum industry to transfer pest management information to sorghum and pearl millet farmers.

- Supervise graduate student research in entomology and IPM. A student from the United States is studying the effect of temperature on fecundity and longevity of greenbug biotypes on sorghum. A student from India is evaluating fitness of greenbugs on susceptible and resistant sorghum and wild hosts. Another student from India is evaluating the effect of different amounts of soil moisture and nitrogen on abundance of greenbugs on sorghum. A third student from India began testing procedures to produce male greenbugs and fertile eggs so different biotypes can be mated and polymorphism of the progeny studied to identify genetic differences among biotypes.

- Participate in Entomological Society of America and other professional and scientific meetings and activities. The PI was asked to organize sorghum entomology symposia at several up-coming meetings.

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 sorghum midge, aphids, panicle-infesting bugs, and stalk borers on sorghum, and millet head miner, *Heliocheilus albipunctella*, on pearl millet. Sorghum midge can destroy 100% of sorghum kernels in the field. Recently, greenbugs destroyed 60% of sorghum in southern Mali. Damage by panicle-infesting bugs and associated infection by pathogens reduce grain yield and quality and render the grain unusable for human consumption. Stalk borers bore into sorghum and kill the central shoot, causing "deadheart", or break the peduncle. Larvae of millet head miner cut flowers and tunnel in kernels of spikes of pearl millet. Other insects could become pests when agronomic practices are changed and new varieties of sorghum or pearl millet are used. Effective management of insect pests requires a multi-disciplinary team with knowledge of entomology, plant pathology, plant breeding, and cereal quality.

Southern Africa

In Southern Africa (mainly Botswana and South Africa), sugarcane aphid; sorghum shoot fly, *Atherigona soccata*; sorghum midge, and stalk borers infest and reduce yield of sorghum in the field. Beetles destroy stored sorghum grain. Few taxonomic keys are available for identification of insect pests in Southern Africa. This project will assist a team to improve production and develop IPM strategies for sorghum insect pests in South Africa.

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 insect 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 being transferred to extension personnel, farmers, and others.

West Africa

Collaborative research to manage insect pests of sorghum and pearl millet was planned with Drs. Diarisso, Youm, and Doumbia from Mali and Mr. Abdou Kadi Kadi from Niger. Sorghum has been planted to assess the effect of different crop residue practices on the abundance of sorghum midges and stalk borers that survive after diapause in Mali. Sorghum crop residue practices in Mali will be inventoried and evaluated for effectiveness against sorghum midge and stalk borers. In collaboration with Mr. Abdou Kadi Kadi, a survey will be conducted in sorghum production areas of Niger to assess damage caused by sorghum midge. In collaboration with Mr. Abdou Kadi Kadi, abundance and causes of mortality of different life stages of naturally occurring millet head miner will be assessed and sampling methodology verified so natural enemy-host interactions can be understood for pearl millet in Niger.

In collaboration with Dr. Peterson, Dr. Diarisso, and Mr. Abdou Kadi Kadi, sorghum lines and landraces will continue to be evaluated for resistance to insect pests in Mali and Niger. Percentage of infestation by sorghum midge, panicle-infesting bugs, and aphids; damage rating score; and yield were assessed of 67 sorghums from the Mali breeding program, INTSORMIL plant breeders, and ICRISAT during the 2001 growing season at Sananko, Cinzana, and Kita, Mali (refer to the West Africa country reports). In Niger, Mr. Abdou Kadi Kadi found sorghum genotypes 99SSDF9-18, 99SSDF9-21, 99SSDF9-29, 99SSDF9-33, 99SSDF9-35, DJ6514, F10SSDF9-35, ICSV197, ICSV745, ICSV90001-02, ICSV90011, ICSV90013, and ICSV93077 resistant to sorghum midge. ICSV90001, ICSV90002, ICSV90011, and ICSV90013 were highly resistant, with grain losses of 16.7, 18.2, 6.4, and 2.1%, respectively. The experiments will be repeated during 2002.

Tiecoura Traore from Mali was identified to come to West Texas A&M University to learn English and begin graduate studies in IPM and entomology.

In collaborative research, Dr. Diarisso used five grams of powder from local *Acacia nigricans* plants to treat 10 grams of kernels of each of six sorghums (Bibalawili – resistant to lesser grain borer, ICSH89002 – susceptible, BOPR11, BTx378, LG2CG3S, and LG21CG3C) in three replications. Kernels were infested with 30 lesser grain borer adults on 14 February 2002. The insects were removed two weeks later. Adults that emerged were counted each week. During peak emergence five weeks after the insects were removed, most lesser grain borers (48) emerged from nontreated kernels of ICSH89002. Fewer insects emerged from treated than from nontreated kernels, except for LG21CG3C from which the same number of insects emerged in both treatments (Table 1). Lesser grain borers attacked more kernels of nontreated LG2CG3S (36.4%), but fewest kernels when that sorghum genotype was treated with *Acacia* (11.8%). Least weight was lost from kernels of treated BOPR11 (4.0%), while most weight was lost from nontreated kernels of ICSH89002 (22.9%).

Southern Africa

From 31 March through 14 April, sorghum research was reviewed and collaborative research projects planned with scientists in Southern Africa. Sorghum was evaluated for resistance to sugarcane aphid and sorghum midge in Botswana and South Africa. Research was planned with Dr. Munthali to assess natural enemies and population dynamics of major insect pests of sorghum in Botswana. Collaboration in entomology was proposed among Dr. Munthali at the Botswana College of Agriculture, scientists at the Department of Agriculture (DAR), and INTSORMIL. Sorghum lines will be evaluated for resistance to sugarcane aphid, sorghum midge, sorghum shoot fly, stalk borers, and stored grain insect pests. In South Africa, sorghum genotypes were identified with excellent resistance to sugarcane aphid in the greenhouse (seedling stage) and field (adult stage).

United States

Greenbugs infesting sorghum, small grains, and wild hosts were collected in the Panhandle and South Texas, and standard plant differentials were used to identify the greenbugs to biotype. All greenbugs collected in fields in Texas were identified as biotype I. In collaboration with Drs. Burd and Shufran, additional samples of greenbugs will be collected and biotypes determined.

Table 1. Lesser grain borers that emerged per week, percentages of kernels attacked, and proportion of weight of damaged versus nondamaged kernels when sorghum was treated with powder of *Acacia nigricans* at Sotuba, Mali.

Sorghum	Treatment	Adults emerged per week	Percentage of kernels attacked	Weight of damaged vs. noninfested kernels (%)
Bibalawili	*Acacia*	9.9	23.5	12.1
Bibalawili	Check	6.1	13.8	6.1
ICSH89002	*Acacia*	6.6	15.3	7.9
ICSH89002	Check	17.1	29.7	22.9
toBOPR11	*Acacia*	9.1	12.6	4.0
BOPR11	Check	13.8	13.8	8.9
BTx378	*Acacia*	8.5	15.6	9.2
BTx378	Check	9.7	24.8	15.2
LG21CG3C	*Acacia*	7.8	17.5	11.4
LG21CG3C	Check	7.8	20.8	9.1
LG2CG3S	*Acacia*	8.9	11.8	6.5
LG2CG3S	Check	14.5	36.4	22.2

In collaboration with Dr. Zhu-Salzman, AFLP polymorphic markers that differentiate sorghum greenbug biotypes were developed for use in identifying unknown greenbugs from the field. The PI evaluated resistance of 300 sorghum lines Dr. Peterson developed for resistance to greenbug biotypes E and I, and Dr. Zhu-Salzman extracted DNA from the sorghum and is using AFLP to try to locate and map genes for resistance to different biotypes of greenbug.

Master's student, Kishan Sambaraju, evaluated fitness of greenbug biotype I on resistant and susceptible sorghums and wild grass hosts. Greenbugs are thought to survive on wild grasses when grass crops are not available. Seeds of susceptible RTx430 sorghum; resistant LG-35 sorghum; Johnsongrass, *Sorghum halepense*; Arriba western wheatgrass, *Agropyron smithii*; and jointed goatgrass, *Aegilops cylindricum*, were sown in a greenhouse. A biotype I greenbug was placed in a 2.5-cm^3 plastic cage clipped onto a plant leaf. The original greenbug was removed after it produced a nymph. The nymph was retained until it produced offspring, which were counted and removed each day. The number of days the greenbug lived was recorded. The number of nymphs produced per day differed significantly among the grass hosts (Wilk's Lambda = 0.045, $F_{(32,52)} = 34.7$, $P < 0.0001$). Significantly fewer nymphs were produced on western wheatgrass than on the other grasses (Table 2). Only 35% as many nymphs (22.8) was produced per greenbug on western wheatgrass as on susceptible sorghum (64.4 nymphs). Average longevities of greenbugs were significantly less on western wheatgrass and jointed goatgrass (19.2 days) than on grasses of the genus *Sorghum.* The experiment will be replicated two more times using several additional species of wild grasses.

Master's student, Anastasia Palousek, assessed effects of 14-27 and 22-35° C temperatures (daily low-high cycle) on the biology of greenbug biotypes E and I on sorghum to determine the optimum temperature for evaluating resistance and try to understand how greenbug biotypes develop.

Table 2. Number of nymphs produced and longevity per biotype I greenbug on grass hosts.

Host	Total number of nymphs	Longevity (days)
RTx430 sorghum	64.4 a	26.9 a
Johnsongrass	60.1 a	27.6 a
LG-35 sorghum	57.4 a	28.2 a
Jointed goatgrass	61.0 a	19.2 b
Western wheatgrass	22.8 b	19.2 b

Means followed by the same letter within a column are not significantly different at $p = 0.05$ (Fisher's LSD)

Twenty plants of RTx430 sorghum were used for each combination of temperature and greenbug biotype. A single greenbug enclosed in a 2.5-cm^3 clear plastic cage was attached to each of two leaves on a sorghum plant that had seven true leaves. The infested sorghum kept in an environmental chamber. 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. Each biotype E greenbug produced significantly more nymphs per day (1.5) and overall (33.8), especially at warmer temperatures, than did biotype I greenbugs (1.2 nymphs per day and 27.8 overall) (Table 3). Significantly fewer greenbugs were produced per day at 14-27 than at 22-35° C. Greenbugs lived four times longer and produced 3.4 times more nymphs at the cooler than the warmer temperature, but longevity did not differ between the two biotypes (approximately 23 days). The experiment will be repeated using 10-23 and 18-31° C temperatures.

Master's student, Suresh Veerabomma, studied effects of amounts of soil moisture (-1/3, -1/2, and –3 bars) and nitrogen (50, 100, and 150 ppm) on abundance and longevity of greenbug biotype I. 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. Numbers of

Table 3. Effect of temperature on greenbug biotypes E and I.

Temperature (°C)	Nymphs produced per day	Fecundity	Longevity (days)
14-27	1.3 a	47.6 a	37.5 a
22-35	1.5 b	14.0 b	9.4 b

Means followed by the same letter within a column are not significantly different at $p = 0.05$.

greenbug nymphs produced per day and total longevity were assessed. Soil moisture, but not nitrogen, significantly affected greenbug fecundity, with almost twice as many nymphs produced per greenbug on sorghum in soil with –1/3 bar (44.2 nymphs) as with –3 bars of moisture (24.3 nymphs) (Table 4). Greenbug longevity was affected significantly by different amounts of soil moisture and nitrogen. Fecundity and longevity were most per greenbug on sorghum planted in soil with –1/3 bar of moisture and 100 ppm of nitrogen.

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

	Total number of nymphs produced per greenbug	Number of days each greenbug lived
Moisture (bars):		
-1/3	44.2 a	23.9 a
-1/2	42.0 a	23.4 a
-3	24.3 b	19.0 b
Nitrogen (ppm):		
50	34.8 a	21.6 ab
100	39.3 a	23.5 a
150	36.5 a	21.2 b

Means followed by the same letter for a treatment within a column are not significantly different at $p = 0.05$ (Fisher's LSD).

Networking Activities

Workshops

The PI participated in the Workshop on Sustainable Agroecosystems in Semiarid Regions (14-27 June 2002, Canyon, Texas) and the 50th Annual Meeting of the Southwestern Branch of the Entomological Society of America (24-27 February 2002, Guanajuato, Mexico) and gave invited presentations at the Entomology Science Conference (30-31 October 2001, College Station, Texas) and 49th Annual Agricultural Chemicals Conference (19 September 2001, Lubbock, Texas).

Research Investigator Exchanges

Botswana, South Africa, and Zambia – 31 March–14 April 2002. In Botswana, the PIs of TAM-222, TAM-223, and this project met with four INTSORMIL collaborators in the Department of Agricultural Research and with faculty and administrators of the Botswana College of Agriculture to discuss INTSORMIL and establish collaborative research. Entomology studies at Sebele Research Station were viewed. In Zambia, sorghum breeding and evaluation nurseries were viewed, and we met with the Director of U.S.-A.I.D./Zambia, Chief Agriculture Research Officer of the Ministry of Food, Agriculture, Director of the Golden Valley Agricultural Research Trust, and Permanent Secretary to the Minister of Agriculture to discuss potential contributions of INTSORMIL collaboration and the importance of sorghum to the food security of Zambia. In South Africa, we met with the entomologist and plant pathologist from the Agricultural Research Corporation-Grain Crops Research Institute and personnel and students of cereal quality at the University of Pretoria to discuss present and future INTSORMIL collaboration in sorghum and pearl millet research and technology transfer. Sorghum was evaluated for resistance to sugarcane aphid and sorghum midge.

Research Information Exchange

The PI assisted extension entomology specialists with management of insect pests of sorghum and pearl millet and advised the National Grain Sorghum Producers organization on IPM of sorghum insect pests. Information was provided to the Intensive Sorghum Committee of the U.S. Grains Council for sending food sorghums to Southern Africa.

Mr. Barry Miller with Pioneer Hi-Bred International, Inc. at Plainview, Texas, was assisted with evaluating newly developed sorghums for resistance to greenbugs. Greenbugs and information on how to evaluate resistance were provided to Dr. Yiqun Weng, a Texas Agricultural Experiment Station molecular biologist who is using biotechnology techniques to locate wheat genes resistant to greenbugs.

Supplies were provided for Dr. Diarisso to conduct research in Mali. Insect-rearing cages, preservation supplies, and identification books were purchased for Dr. Munthali in Botswana.

Publications and Presentations

Publications

Brown, E.D., J. Trybom, W.A. Colette, R.C. Thomason and B.B. Pendleton. 2002. Effects of systemic seed treatment insecticides imidacloprid and thiamethoxam on sorghum hybrids. International Sorghum and Millets Newsletter (in press).

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 (submitted).

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 (submitted).

Peterson, G.C., B.B. Pendleton and G.L. Teetes. 2002. PROFIT – Productive Rotations On Farms In Texas. In J. Leslie (Ed.). Sorghum and Millet Pathology III, Iowa State University Press (in press).

Peterson, G.C. and B.B. Pendleton. 2001. PROFIT – Productive Rotations On Farms In Texas: a new paradigm for sorghum research and information delivery in Texas. Pp. 82-92. In Proceedings of the 56th Annual Corn and Sorghum Research Conference. Chicago, IL. December 5-7, 2001. American Seed Trade Association, Inc., Alexandria, VA.

Presentations

Gary C. Peterson and Bonnie B. Pendleton. 2001. PROFIT - Productive Rotations On Farms In Texas: a new paradigm for sorghum research and information delivery in Texas. 56th Annual American Seed Trade Association Corn and Sorghum Research Conference, 5-7 December 2001, Chicago, IL.

Bonnie B. Pendleton. Greenbug biotypes. Entomology Science Conference, 30-31 October 2001, College Station, TX.

Bonnie B. Pendleton. Update on "PROFIT." Entomology Science Conference, 30-31 October 2001, College Station, TX.

Edward D. Brown and Bonnie B. Pendleton. Effects of systemic seed treatment insecticides Cruiser and Gaucho on sorghum hybrids. Entomology Science Conference, 30-31 October 2001, College Station, TX.

Bonnie B. Pendleton. What's new for sorghum pest management? 49th Annual Agricultural Chemicals Conference, 19 September 2001, Lubbock, TX.

Edward D. Brown, Bonnie B. Pendleton, W. Arden Colette, Ronald C. Thomason and James Trybom. Effect of systemic seed treatment insecticides imidacloprid and thiamethoxam on sorghum hybrids. 49th Annual Agricultural Chemicals Conference, 19 September 2001, Lubbock, TX.