Sustainable Plant Protection Systems

Agroecology and Biotechnology of Stalk Rot Pathogens of Sorghum and Millet

Project KSU 210
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Summary

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

Objectives, Production and Utilization Constraints

Objectives

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

Constraints

- Mycotoxin contamination limits the uses to which harvested grain can be put, and creates health risks for both humans and domestic animals. *Fusarium*-produced mycotoxins are among the most common mycotoxins found in cereal grains, yet have not been effectively evaluated in sorghum and millet. Since contamination often occurs on apparently sound grain, merely discarding obviously molded grain is not sufficient to avoid the mycotoxicity problems.
- *Fusarium* spp. associated with sorghum and millet do obvious damage as stalk rot, grain mold and pokkah boeng. All of these diseases can cause intermittently heavy losses in the United States and in developing countries. Breeding for resistance to *Fusarium*-associated diseases often is limited because resistant germplasm is either unavailable or has undesirable characters from which the resistance trait must be separated. The source of the pathogens in fields that have been fallow for some time has never been clearly defined.

Research Approach and Project Output

Research Methods

Recovery and culture of *Fusarium* isolates. Several species of perennial warm-season grasses, *e.g.*, *Andropogon gerardii* Vitman, *Andropogon scoparius* Michx. and *Sorghastrum nuttans* (L.) Nash, dominate vegetation on the Konza Prairie, while sub-dominant species include a diverse mixture of other warm and cool season grasses, composites, legumes, and other forbs. In October 1997, we sampled *A. gerardii*, *S. nuttans*, and *A. scoparius* from the Konza Prairie Biological Station and LTER (Long Term Ecological Research) site. We arbitrarily selected 15 plants each of *A. gerardii* and *A. scoparius*, and 14 plants of *S. nuttans* across three different experimental watersheds of the Konza Prairie that differ in the frequency in which they are burned. Flowering tillers of each plant were cut at ground level, bagged individually, and cataloged on site. Fungal isolations were made from these materials within three days of collection.

For isolation of fungi, each plant was cut into small sections (2-5 cm length) and surface sterilized in 95% ethanol for 2 minutes. Sterilized sections were rinsed briefly in sterile H_2O, and placed on a peptone-PCNB medium semi-selective for *Fusarium* spp. Cultures were incubated under fluorescent lights at 25°C for 4-7 days to allow fungal colonies to grow out onto the media. Two to five morphologically distinct *Fusarium* colonies were isolated from each plant and then transferred to complete media (CM) slants. From these colonies, cultures were started from microconidia separated by micromanipulation, and a subculture frozen for long-term storage at -70°C in 15:85 (v:v) glycerol: water. Vegetative cultures were grown on minimal medium solidified with 2% agar in slants or Petri dishes, or as liquid cultures in 125 ml Erlenmeyer flasks. Incubations on solid media were at 25°C under a 12 h light-12 h darkness diurnal cycle. Sexual crosses were made on carrot agar with standard tester strains from the Fungal Genetics Stock Center (University of Kansas Medical School, Kansas City, Kansas) serving as the female parents. Cultures for toxin production were grown on cracked corn. Harvested corn culture material was dried in a forced draft oven at 60°C for 48 h, finely ground, and stored at 4°C until used.

We extracted soluble proteins, and used the isozyme profiles to identify putative mating populations within the *Gibberella fujikuroi* species complex. Specifically, malate dehydrogenase, isocitrate dehydrogenase, fumarase, and triose phosphate isomerase profiles were evaluated for each strain. Mating population identifications were confirmed and mating type specificity (*MAT-1* or *MAT-2*) identified in crosses in which the field isolates served as males and the standard tester strains for these mating populations as the female parents. Isolates with variant isozyme profiles were crossed with testers from the mating population or set of mating populations with profiles that most closely resembled those of the field isolates. Isolates that were cross fertile with a mating-type tester strain were tested for female fertility by using the field isolate as a female in a cross with the mating type tester as the male parent. Mating type idiomorphs of isolates that were not cross fertile with any of the tester strains for the known mating populations were identified by allele-specific PCR amplification.

AFLP analyses and comparisons. Isolates for DNA extraction were cultured by inoculating approximately 1 ml of a spore suspension (typically 10^6-10^7 conidia) into 40 ml of liquid CM. Isolates were grown on a rotary shaker (150 rpm) for 2 days at room temperature (23-26°C) and harvested by filtration through milk filters. Mycelia were blotted dry between paper towels and the dried mycelia stored at -20°C until DNA extraction. DNA was extracted with a cetyltrimethylammonium bromide (CTAB) protocol as described and AFLPs generated by standard methods. *Eco*RI primers used in the final specific PCR amplifications were labeled with ^{33}ãP-ATP.

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

Initially, a single AFLP primer pair was used to group isolates that could not be identified to a specific mating population on the basis of either isozyme profile or cross fertility. Results for each group were compared to profiles from isolates of previously identified *Fusarium* species. The resulting binary data set was analyzed with the UPGMA clustering option

of PAUP (v 4.10b*) to suggest possible species associations for the unidentified isolates. UPGMA similarities were calculated based on these AFLP gels. Final UPGMA genetic distances within and between sets of species were calculated with the Dice coefficient and the CLUSTER option of SAS.

Beauvericin and fusaproliferin recovery and measurement. For beauvericin and fusaproliferin extractions, 5 g of each sample were ground and homogenized at room temperature with 50 ml of methanol and clarified by centrifugation. Samples were filtered through Whatman No. 4 filter paper and 30 ml of filtrate collected (corresponding to 3 g of sample) and then evaporated at 35-40°C under reduced pressure. The raw organic extract was resuspended in 3 ml of methanol and loaded onto a C18 column. The pre-purification column was washed with 2 ml of methanol and the eluted sample dried under vacuum. Finally, the collected residue was resuspended in 1 ml of methanol and filtered through an Acrodisk filter (0.22 mm pore diameter) before injection of 20 µl into the high performance liquid chromatograph (HPLC). Fusaproliferin was detected at 261 nm and beauvericin at 205 nm. Mycotoxins were identified by comparing retention times and UV spectra of samples with those of authentic standards and quantified by comparing peak areas from samples with a calibration curve of standards. The recovery efficiency of this extraction method was 94% and 71% for beauvericin and fusaproliferin, respectively. The detection limit was 100 ng/g for beauvericin and 25 ng/g for fusaproliferin. All analyses were run in triplicate.

Recovery and measurement of fumonisins. A 5-g sample of each fungal culture was ground and added to 50 ml of a 75:25 methanol:water solution. The samples were homogenized and then clarified by centrifugation. Thirty ml of the supernatant (corresponding to 3 g of sample) were evaporated at 35/40°C under reduced pressure, and the residue resuspended in 5 ml of methanol and dried in a centrifugal evaporator at 35-40°C. The recovery efficiency of this extraction method was 90% and 68% for FB_1 and FB_2, respectively. Fumonisins were detected by liquid chromatography-mass spectrometry (LC-MS) with resolution of 0.5 amu. The detection limit for the fumonisin standards, determined by using the protonated signal at m/z 722, was equivalent to 0.5 ng/g and the limit of quantification was equivalent to 1.2 ng/g. Measurements were made in quadruplicate within an experiment, and the data are based on the average of two experiments.

Brine shrimp assays. Toxicity to brine shrimp (*Artemia salina*) larvae was determined by exposing larvae to fungal culture extracts in 24-well cell culture plates [30 to 40 larvae per well in 500 ml of 3.3% (wt/vol) marine salt in H_2O]. The number of dead larvae was recorded after incubation at 27°C for 24 hr. The total number of larvae in each cell was counted after killing the surviving larvae by freezing at -20°C for 12 hr. Water-soluble extracts, containing fumonisins, in general had little or no toxicity towards the brine shrimp larvae. Tests in each experimental run were performed in quadruplicate. Data are based on the averages of two independent experiments. We examined correlations between toxin production by individual *Fusarium* species and mortality to *A. salina* by comparing observed toxin concentrations (ppm) to percent mortality observed with the CORR procedure of SAS.

Research Findings

Experimental rationale. *Fusarium* species are important pathogens of sorghum and millets, where they usually persist as endophytes until the end of the season when they can become serious pests causing stalk rot, head blight and grain mold. Even when these crops are planted into areas where they have not previously been cultivated, these fungi can appear on crops almost immediately. One possibility is that the pathogens are being brought in on seed and farming implements, and the other is that the pathogens are present, perhaps as pathogens or symbionts, of the native grasses in fields that have been fallowed or never planted.

We tested these hypotheses by analyzing strains from the Konza Prairie Biological Station, just south of Manhattan. The Konza Prairie is one of the largest remaining contiguous pieces of tallgrass prairie remaining in North America. This area has been grazed but has never been tilled. The present study had two objectives: (i) to determine the species composition and genetic diversity of isolates from the *Gibberella fujikuroi* species complex found on the Konza Prairie, and (ii) to examine the types and quantity of secondary metabolites produced by strains recovered from non-agricultural hosts. There has been considerable study of *Fusarium* species diversity and toxin production among isolates collected from major grain crops such as rice, maize, or sorghum. However, this study represents the first population-level characterization of a non-agricultural, grasslands *Fusarium* community to determine whether such a community differs qualitatively or quantitatively from populations associated with the agricultural crops to which these fungi are economically important pathogens on a worldwide basis.

Survey results. We examined 72 isolates that produced microconidia from a total of 241 *Fusarium* isolates recovered. Of the 52 grass stems sampled, 45 produced at least one isolate that made microconidia and that grouped morphologically within *Fusarium* sections *Liseola* or *Elegans*. More than one species from the *Gibberella fujikuroi* species complex was recovered from 16/45 plants, and members of more than two species from the *Gibberella fujikuroi* species complex were recovered from 5/45 plants indicating that multiple infections of native grasses with more than one species of *Fusarium* is common. Of the 72 *Fusarium* isolates examined, 12 belonged to *F. verticillioides*, one to *F. thapsinun*, three to *F. subglutinans*, nine to *F. konzum*, 40 to either *F. fujikuroi* or *F. proliferatum*, and seven could not be assigned readily to any of the described species in the *Gibberella fujikuroi* species complex. Isolates were characterized (Table 1) with isozymes, AFLP fingerprint profile, sexual cross-fertility, and mating type. Collectively, these characterizations did not always yield the same answer.

Table 1. Species identification for and secondary metabolite production by strains of the *Gibberella fujikuroi* species complex recovered from grasses growing on the Konza Prairie.

KSU no.	ITEM no.	Plant Host[a]	Isozyme Pattern[b]	AFLP Pattern[b]	Mating Type	FB_1 (µg/g)	FB_2 (µg/g)	FUP (µg/g)	BEA (µg/g)	% *A. salina* Mortality
A-10548	3117	*Ag*	*Fv*	*Fv*	*MAT-2*	590	420	n.d.	n.d.	93
A-10560	3122	*Ag*	*Fv*	*Fv*	*MAT-2*	1200	710	n.d.	9	97
A-10568	3126	*Ag*	*Fv*	*Fv*	*MAT-2*	760	780	n.d.	n.d.	97
A-10577	3131	*Ag*	*Fv*	*Fv*	*MAT-2*	750	670	n.d.	n.d.	68
A-10584	3136	*Ag*	*Fv*	*Fv*	*MAT-1*[c]	1000	700	n.d.	n.d.	39
A-10594	3140	*Ag*	*Fv*	*Fv*	*MAT-2*[c]	110	130	n.d.	n.d.	78
A-10605	3147	*As*	*Fv*	*Fv*	*MAT-1*	280	64	n.d.	4	100
A-10621	3152	*As*	*Fv*	*Fv*	*MAT-1*[c]	480	150	n.d.	2	100
A-10631	3158	*As*	*Fv*	*Fv*	*MAT-1*[c]	890	200	n.d.	5	48
A-10636	3161	*As*	*Fv*	*Fv*	*MAT-2*	280	97	n.d.	n.d.	100
A-10685	3181	*Sn*	*Fv*	*Fv*	*MAT-2*[c]	500	130	n.d.	n.d.	76
A-10691	3184	*Sn*	*Fv*	*Fv*	*MAT-1*	18	4	n.d.	n.d.	100
X-10626	3155	*As*	*Ff*	*Ff*-var	*MAT-1*	900	170	n.d.	180	71
X-10677	3178	*Sn*	*Fp*-var	*Ff*-var	*MAT-1*	n.d.	n.d.	n.d.	30	11
D-08384	3110	*Sn*	*Fp*	*Fp*	*MAT-1*	430	350	840	310	100
D-08387	3111	*Sn*	*Fp*	*Fp*	*MAT-2*	37	36	49	110	97
D-08392	3112	*Sn*	*Fp*	*Fp*	*MAT-1*	780	360	540	230	100
D-08403	3114	*Sn*	*Fp*	*Fp*	*MAT-1*	3600	4200	590	170	100
D-08411	3108	*Ag*	*Fp*	*Fp*	*MAT-2*	400	110	560	120	48
D-08420	3109	*Ag*	*Fp*	*Fp*	*MAT-1*	500	570	830	180	100
D-10550	3118	*Ag*	*Fp*	*Fp*	*MAT-1*	160	230	2000	450	100
D-10552	3120	*Ag*	*Fp*	*Fp*	*MAT-1*	6300	4000	260	n.d.	100
D-10557	3121	*Ag*	*Fp*	*Fp*	*MAT-2*	640	210	500	210	99
D-10563	3124	*Ag*	*Fp*	*Fp*	*MAT-1*	1300	530	490	430	100
D-10565	3125	*Ag*	*Fp*	*Fp*	*MAT-1*	610	700	150	830	100
D-10580	3133	*Ag*	*Fp*	*Fp*	*MAT-1*	280	78	390	310	100
D-10582	3134	*Ag*	*Fp*	*Fp*	*MAT-2*	620	760	550	1400	100
D-10583	3135	*Ag*	*Fp*	*Fp*	*MAT-1*	1200	830	200	1200	100
D-10587	3137	*Ag*	*Fp*	*Fp*	*MAT-1*[c]	1500	840	110	450	100
D-10590	3138	*Ag*	*Fp*	*Fp*	*MAT-2*	250	120	270	530	100
D-10591	3139	*Ag*	*Fp*	*Fp*	*MAT-2*[c]	1000	620	570	580	100
D-10599	3145	*As*	*Fp*	*Fp*	*MAT-1*	1400	360	200	1300	100
D-10609	3148	*As*	*Fp*	*Fp*	*MAT-2*[c]	1100	370	430	66	72
D-10614	3149	*As*	*Fp*	*Fp*	*MAT-2*[c]	1300	170	510	660	100
D-10616	3150	*As*	*Fp*	*Fp*	*MAT-2*[c]	3	1	200	7	28
D-10617	3151	*As*	*Fp*	*Fp*	*MAT-2*	710	190	280	1000	100
D-10625	3154	*As*	*Fp*	*Fp*	*MAT-2*	450	93	180	700	100
D-10627	3156	*As*	*Fp*	*Fp*	*MAT-1*	150	29	n.d.	66	12
D-10630	3157	*As*	*Fp*	*Fp*	*MAT-2*	520	120	51	12	3
D-10647	3165	*As*	*Fp*	*Fp*	*MAT-2*[d]	210	67	320	570	100
D-10649	3166	*Sn*	*Fp*	*Fp*	*MAT-1*	240	74	210	1200	100
D-10657	3170	*Sn*	*Fp*	*Fp*	*MAT-2*[d]	86	27	130	140	26
D-10659	3171	*Sn*	*Fp*	*Fp*	*MAT-1*[c]	n.d.	n.d.	86	270	77
D-10668	3174	*Sn*	*Ff*/*Gi*	*Fp*	*MAT-2*	810	230	560	780	100
D-10670	3175	*Sn*	*Fp*	*Fp*	*MAT-1*	170	29	170	350	89
D-10675	3176	*Sn*	*Fp*	*Fp*	*MAT-2*	340	51	630	820	100
D-10694	3185	*Sn*	*Fp*	*Fp*	*MAT-1*	860	58	45	4	35
D-08374	3107	*Ag*	*Fp* -var	*Fp*	*MAT-2*	1500	1000	450	480	100
D-10544	3115	*Ag*	*Fp* -var	*Fp*	*MAT-1*	10	23	n.d.	n.d.	5
D-10545	3116	*Ag*	*Fp* -var	*Fp*	*MAT-1*	60	820	150	n.d.	3
D-10571	3127	*Ag*	*Fp* -var	*Fp*	*MAT-2*	1500	360	200	1000	99
D-10572	3128	*Ag*	*Fp* -var	*Fp*	*MAT-2*	1700	1400	32	170	92
E-10562	3123	*Ag*	*Fs*	*Fs*	*MAT-2*[c]	trace	n.d.	300	n.d.	98

Table 1. Cont'd - Species identification for and secondary metabolite production by strains of the *Gibberella fujikuroi* species complex recovered from grasses growing on the Konza Prairie.

KSU no.	ITEM no.	Plant Host[a]	Isozyme Pattern[b]	AFLP Pattern[b]	Mating Type	FB_1 (μg/g)	FB_2 (μg/g)	FUP (μg/g)	BEA (μg/g)	% *A. salina* Mortality
E-10646	3164	*As*	*Fs*	*Fs*	*MAT-1*	n.d.	n.d.	190	5	24
E-10688	3182	*Sn*	*Fs*	*Fs*	*MAT-1*	n.d.	n.d.	1300	10	100
F-10597	3142	*Ag*	*Ft*	*Ft*	*MAT-1*	6	9	n.d.	n.d.	6
I-08373	3106	*Ag*	*Fs*-var	*Fk*	*MAT-1*	10	12	540	120	79
I-10595	3141	*Ag*	*Fs*-var	*Fk*	*MAT-2*	17	12	210	160	80
I-10638	3162	*As*	U	*Fk*	*MAT-2*	120	24	ND	5	2
I-10653	3168	*Sn*	*Fs*-var	*Fk*	*MAT-1*[c]	n.d.	n.d.	250	91	28
I-10663	3173	*Sn*	*Fs*	*Fk*	*MAT-1*	n.d.	n.d.	310	4	17
I-10676	3177	*Sn*	*Fs*	*Fk*	*MAT-1*	n.d.	n.d.	210	230	80
I-10678	3179	*Sn*	*Fs*-var	*Fk*	*MAT-2*	n.d.	n.d.	50	59	32
I-10689	3183	*Sn*	*Fs*	*Fk*	*MAT-2*	ND	ND	140	89	43
I-10681	3180	*Sn*	U	*Fk*	*MAT-2*	ND	ND	78	320	78
I-10578	3132	*Ag*	*Fs*-var	*Fk*-like	*MAT-2*[d]	trace	trace	160	650	99
X-10622	3153	*As*	U	Type-α	ND	18	5	ND	80	100
X-10635	3160	*As*	U	Type-α	ND	ND	ND	ND	32	100
X-10661	3172	*Sn*	U	Type-α	ND	ND	ND	ND	190	57
X-10576	3130	*Ag*	*Fv*	Type-β	ND	9	12	ND	ND	0
X-10634	3159	*As*	*Fv*	Type-β	ND	ND	ND	ND	10	1
X-10551	3119	*Ag*	U	Type-γ	*MAT-1*[d]	13	13	ND	32	13

ND – not detected.

[a]Species abbreviations: *Ag – Andropogon gerardii*, *As – Andropogon scoparius*, and *Sn - Scoparius nuttans*

[b]Species abbreviations: *Ff – F. fujikuroi*, *Fk – F. konzum*, *Fp – F.proliferatum*, *Fs – F. subglutinans*, *Ft – F. thapsinum*, *Fv – F. verticillioides*, and U – unique. Patterns that are substantially the same as one species but differ at one or a few bands (usually new) are designated as "var" for variant. Strains with a "var" designation need not have the same banding pattern.

[c]Female fertile.

[d]Mating type determined via mating-type allele specific PCR.

At least 53 of the 72 isolates are potentially pathogenic to sorghum, which is consistent with the hypothesis that native grasslands can serve as refuges for *Fusarium* strains pathogenic on agriculturally important crops. The three isolates of *F. subglutinans* are unlikely to be sorghum pathogens, and the pathogenic potential of the remaining 16 isolates of *F. konzum* and unidentified *Fusarium* spp. towards sorghum is unknown. In general, the range of species found in the prairie grasses paralleled that recovered from the maize or from sorghum crops grown in the adjacent area. The only species that collected that had not been typically reported from either of these two crops is *G. konza*, and the only species commonly recovered from either maize or sorghum that we did not recover from the Konza Prairie was *F. andiyazi*. The *Fusarium* species most commonly reported from maize and from sorghum are *F. verticillioides* and *F. thapsinum*, respectively. Although both *F. verticillioides* and *F. thapsinum* were present on the Konza Prairie, 17% and 2% of the isolates, respectively, neither was found at the high levels (often > 70%) frequencies at which they can be recovered from agricultural fields of maize or sorghum. *F proliferatum* often is viewed as a "generalist", as it has been recovered from a broad range of agricultural hosts, including asparagus, banana, maize, mangos, millet, pine, rice, sorghum, and tobacco. When *F. proliferatum* is recovered it often is relatively infrequent (5-15% of the total population), especially in the Great Plains region in the United States. This species also is not considered generally to show any significant host preference or specialization, although in commercial sorghum in Egypt, *F. proliferatum* often is the most commonly recovered fungus. The dominance of *F. proliferatum* on the Konza Prairie is consistent with a hypothesis in which a generalist species is expected to be at an advantage in a native ecosystem, even if this species can survive and persist in a more specialized agricultural ecosystem.

Mycotoxin production and toxicity to *Artemia salina*. Toxin production by the Konza Prairie isolates was neither qualitatively, nor quantitatively different from isolates of those same species drawn from agricultural settings. Isolates of *F. verticillioides* generally produced fumonisins, but neither beauvericin nor fusaproliferin. In general the isolates of *F. proliferatum* produced as much or more fumonisins as did the isolates of *F. verticillioides*. Six of the isolates of *F. proliferatum* produced > 2000 μg/g total fumonisins, including D-08403

which produces ~8000 µg/g total fumonisins and D-10552 which produces > 10,000 µg/g, and is one of the highest producers of these toxins ever reported.

In general, the toxicity, *i.e.*, LD_{50}, of strains from species in the *G. fujikuroi* species complex to *A. salina* is correlated with the concentrations of beauvericin and/or fusaproliferin in the corresponding organic extracts. For the isolates of *F. proliferatum* examined, toxicity was positively correlated with both fusaproliferin ($p = 0.02$) and beauvericin ($p < 0.001$), but was not correlated with fumonisin production. The toxin production pattern by *F. proliferatum* strain D-08411 suggests that additional toxigenic compounds are synthesized by this species and remain to be identified.

Evolution of new pathogenic species. One way of creating new pathogens is analogous to the manner in which new traits are imported into agricultural crops, *i.e.,* through wide crosses with related species. We found evidence that such wide crosses may be occurring under natural conditions on the Konza Prairie. Native areas are a more likely place for such wide crosses to occur as they usually contain more species than those found in a comparable agroecosystem. Selection pressure in a native system, which has more and usually fragmented ecological niches, often is different from that in an agroecosystem, which usually is relatively uniform and may contain only a single ecological niche, making it more likely for the progeny of a wide cross to be able to survive and become a significant component of the native system.

One of the strains identified from the Konza Prairie survey, X-10626, is of particular interest as its molecular markers are consistent with it being a hybrid between *F. fujikuroi* (usually a rice pathogen) and *F. proliferatum*. Such a hybrid could be the origin of the capability of some strains of *F. proliferatum* to cause pokkah boeng disease, since *F. fujikuroi* strains are capable of producing various plant growth promoters, most notably gibberellic acid. Genes for the production of the plant growth promoters could be transferred between species in such a cross.

Results of interspecific *Fusarium* crosses. Thirty-two progeny were collected from a cross between X-10626 and FGSC 7614 (*F. proliferatum* standard) and scored for 70 segregating AFLP markers. Approximately 19% of the AFLP markers did not segregate in a 1:1 manner, and the mean frequency of the allele derived from the *F. proliferatum* parent amongst the progeny was 54%. Eighteen progeny were collected from the cross between X-10626 and FGSC 8932 (*F. fujikuroi* standard) and scored for 24 segregating AFLP markers. Approximately 17% of the AFLP markers were not segregating in a 1:1 manner, and the mean frequency of the allele derived from the *F. fujikuroi* parent amongst the progeny was 56%. A cross also was made between the *F. fujikuroi* and *F. proliferatum* tester strains. Forty-seven progeny were collected from this interspecific cross and scored for 80 segregating AFLP markers. Approximately 66% of the AFLP markers were not segregating in a 1:1 manner, and the mean frequency of the allele derived from the *F. proliferatum* parent amongst the progeny was 61%. Only ~75% of the progeny of this cross were fertile in a backcross to the standard *F. proliferatum* tester strains.

The existence of X-10626 under field conditions suggests that crosses between isolates of different *Fusarium* species can occur in nature and are not just laboratory artifacts. If the proportion of loci at which segregation is not 1:1 is used as a measure of the genetic distance between the parental strains, then the testers for *F. fujikuroi* and *F. proliferatum*, in whose intercross approximately two thirds of the loci do not segregate 1:1, are more distant from each other than either of these testers is from KSU X-10626, for which < 20% of the loci are not segregating in a 1:1 manner. One interpretation of these results is that the standard testers for *F. fujikuroi* and *F. proliferatum* represent distinct species, but that one, or more, hybrid swarms, perhaps separated geographically or specialized to a particular host, exist between these species. As *F. fujikuroi* normally is associated with rice and is easily confused with *F. proliferatum* on the basis of morphology, the hybrid nature of many field strains might easily be missed unless relatively extensive crossing and/or molecular analyses were conducted. Indeed the entity currently identified as *F. proliferatum*, which is known to have a broad host range and geographic distribution, could be a series of hybrid swarms that share taxonomically important morphological characters. The number, pathogenicity and relatedness of such putative hybrid swarms remain important questions for further study and analysis.

Networking Activities

Editorial and Committee Service (2003)

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

Fusarium Committee

- Senior Fulbright Scholar Review Panel (U.S. – Australia – New Zealand)

Research Investigator Exchanges

Dr. Leslie made the following international scientific exchange visits (2003):

Australia – January 20 – February 1
Malaysia – February 1-4
South Korea – February 4-9
Nigeria – April 25 – May 4
Egypt – September 12-18
Italy – September 18-26
Nigeria/Burkina Faso/Ghana – October 9-25
South Africa – October 31 – November 21

Seminar, Workshop & Invited Meeting Presentations (2003)

Organized in *Fusarium* Laboratory Workshop at KSU – Manhattan from June 22-27; 43 participants and five instructors from 17 countries.

Editor for Proceedings of Sorghum/Millet pathology conference in Guanjuato, Mexico.

9th International Fusarium Workshop, Sydney, Australia.
School of Biological Sciences, Science University of Malaysia, Penang, Malaysia.

School of Agricultural Biotechnology, Seoul National Univ., Suwon, South Korea.

Department of Plant Pathology, University of California-Davis – 02/03.

22nd Fungal Genetics Conference, Asilomar, California – 03/03.

International Institute for Tropical Agriculture, Ibadan, Nigeria – 04/03.

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Institute of the Science of Food Production, CNR, Bari, Italy.

FABI, University of Pretoria, Pretoria, South Africa.
Institute of Wine Biotechnology, Stellenbosch University, Stellenbosch, South Africa.

Workshop on Technologies to Produce Mycotoxin-Free Agricultural Commodities in Developing Countries, St. Louis, Missouri.

During 2003 Fusarium *cultures were provided to:*

Dr. Ranajit Bandyopadhyay, IITA, Ibadan, Nigeria
Drs. Robert L. Bowden, Larry E. Claflin, & Mitch Tuinstra, Kansas State University, Manhattan, Kansas.
Dr. Elhamy M. El-Assiuty, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.
Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, Kansas.
Dr. D. Geiser, Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania.
Prof. Dr. Laszlo Hornok, Agricultural Biotechnology Center, Institute for Plant Sciences, Godollo, Hungary.
Prof. Dr. Yin-Won Lee, Department of Plant Pathology, Seoul National University, Su-Won, South Korea.
Dr. Antonio Logrieco, Istituto Tossine e Micotossine da Parassiti Vegetali, Bari, Italy.
Prof. Dr. W. F. O. Marasas, PROMEC, South African Medical Research Council, Tygerberg, South Africa.
Dr. J. Scott Smith, Department of Animal Sciences & Industry, Kansas State University, Manhattan, Kansas.
Dr. Brett Summerell, Royal Botanic Gardens-Sydney, Sydney, Australia.
Dr. Bettina Tudzynski, Westfaelische Wilhelms University, Muenster, Germany.
Drs. Mike Wingfield & Brenda Wingfield, Forestry & Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa.
Dr. Baharuddin bin Salleh, School of Biological Sciences, Science University of Malaysia, Penang, Malaysia.
Dr. Kerry O'Donnell, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, Illinois.

Other collaborating scientists (Host country)

Dr. Sofia Chulze, Department of Microbiology, National University of Rio Cuarto, Rio Cuarto, Argentina.
Drs. M. Flieger & S. Pazoutova, Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
Dr. Laszlo Hornok, Agricultural Biotechnology Center, Godollo, Hungary
Dr. Yin-Won Lee, Department of Plant Pathology, Seoul National University, Su-Won, South Korea
Drs. Antonio Logrieco, Antonio Moretti & Giuseppe Mulé, Institute of the Science of Food Production, CNR, Bari, Italy
Dr. Anaclet S. B. Mansuetus, Department of Biological Sciences, University of Swaziland, Kwaluseni, Swaziland
Dr. Neal McLaren, Agricultural Research Council, Potchefstroom, South Africa
Prof. Baharuddin Salleh, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia
Dr. Brett A. Summerell, Royal Botanic Gardens, Sydney, Australia
Drs. Michael and Brenda Wingfield, FABI, University of Pretoria, Pretoria, South Africa

Other collaborating scientists (U.S.)

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

Publications and Presentations

Journal Articles.

Saleh, A. A., K. A. Zeller, E. M. El-Assiuty, A.-S. M. Ismael, Z. M. Fahmy & J. F. Leslie. 2003. Amplified fragment length polymorphism (AFLP) diversity in *Cephalosporium maydis* from Egypt. *Phytopathology* **93:** 853-859.

Seifert, K. A., T. Aoki, R. P. Baayen, D. Brayford, L. W. Burgess, S. Chulze, W. Gams, D. Geiser, J. de Gruyter, J. F. Leslie, A. Logrieco, W. F. O. Marasas, H. I. Nirenberg, K.

O'Donnell, J. P. Rheeder, G. J. Samuels, B. A. Summerell, U. Thrane & C. Waalwijk. 2003. The name *Fusarium moniliforme* should no longer be used. *Mycological Research* **107:** 643-644.

Summerell, B. A., B. Salleh & J. F. Leslie. 2003. A utilitarian approach to *Fusarium* identification. *Plant Disease* **87:** 117-128.

Wu, X., J. F. Leslie, R. A. Thakur & J. S. Smith. 2003. Preparation of a fusaproliferin standard from the culture of *Fusarium subglutinans* E-1583 by high performance liquid chromatography. *Journal of Food and Agricultural Chemistry* **51:** 383-388.

Zeller, K. A., R. L. Bowden & J. F. Leslie. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. *Phytopathology* **93:** 874-880.

Zeller, K. A., B. A. Summerell, S. Bullock & J. F. Leslie. 2003. *Gibberella konza* (*Fusarium konzum*) sp. nov., a new biological species within the *Gibberella fujikuroi* species complex from prairie grass. *Mycologia* **95:** 943-954.

Abstracts

Bowden, R. L., J. E. Jurgenson, J.-K. Lee, Y.-W. Lee, S. H. Yun, K. A. Zeller, & J. F. Leslie. 2003. A second generation genetic map of *Gibberella zeae*. *Fungal Genetics Newsletter* **50**(Suppl.): 102.

Cumagun, C. J. R., R. L. Bowden, J. E. Jurgenson, J. F. Leslie & T. Miedaner. 2003. Mapping of quantitative trait loci associated with pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) causing head blight of wheat. *Phytopathology* **93**:S19.

Jeon, J.-J., H. Kim, H.-S. Kim, K. A. Zeller, T. Lee, S.-H. Yun, R. L. Bowden, J. F. Leslie & Y.-W. Lee. 2003. Genetic diversity of *Fusarium graminearum* from maize in Korea. *Fungal Genetics Newsletter* **50**(Suppl.): 142.

Saleh, A. A. & J. F. Leslie. 2003. Molecular phylogenetic analysis indicates *Cephalosporium maydis* is a distinct taxon in the *Gaeumannomyces-Phialophora* species complex. *Fungal Genetics Newsletter* **50**(Suppl.): 142.

Saleh, A. A. & J. F. Leslie. 2003. Biological species in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*) recovered from maize and sorghum in Egypt. *Fungal Genetics Newsletter* **50**(Suppl.): 142.

Zeller, K. A. & J. F. Leslie. 2003. When species concepts collide. *Phytopathology* **93**:S93.

Zeller, K. A., J. I. Vargas, G. Valdovinos-Ponce, J. F. Leslie & R. L. Bowden. 2003. Population genetic differentiation and lineage composition among *Gibberella zeae* in North and South America. *Fungal Genetics Newsletter* **50**(Suppl.): 143.

Zeller, K. A., M. A. Wohler, L. V. Gunn, S. Bullock, B. A. Summerell & J. F. Leslie. 2003. Interfertility and marker segregation in hybrid crosses of *Gibberella fujikuroi* and *Gibberella intermedia*. *Fungal Genetics Newsletter* **50**(Suppl.): 144.

Agroecology and Biotechnology of Fungal Pathogens of Sorghum and Millet

Project KSU 211

Larry E. Claflin
Kansas State University

Principal Investigator

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Collaborating Scientists

Dr. Jeff Dahlberg, USGSPA, Lubbock, TX 79408
Ing. Reina F. de Serrano and Carlos Borja, CENTA, Apartado Postal 885, San Andres, La Libertad, El Salvador
Dr. John Leslie, Dept. of Plant Pathology, Kansas State University, Manhattan, KS 66506
Dr. Stephen Mason, Dept. of Agronomy, University of Nebraska, Lincoln 68583-0915
Mr. Henry Nzioki, National Dryland Farming Research Center-Katumani, P. O. Box 340, Machakos, Kenya
Ing. Sergio Pichardo, UNA, Apartado Postal 453, Managua, Nicaragua (Now a graduate student in entomology & plant pathology, Mississippi State University)
Dr. Henry Pitre, Mississippi State University, Mississippi State, MS 39762
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 66506

Objectives, Production and Utilization Constraints

Objectives

- U.S./Mexico/Nicaragua/El Salvador: Determine the prokaryotic plant pathogenic organisms responsible for unique and unusual diseases of sorghum that may pose yield constraints. These causal agents are primarily insect disseminated and a joint collaborative project was established with MSU-205.
- U.S./Mexico/Nicaragua/El Salvador: Ascertain disease incidence through surveys coupled with utilization of the ADIN nursery from Texas A & M University at various locations. Genetic variability of accessions within the ADIN will be determined if disease severity occurs.
- U.S./Nicaragua: Determine the number of races/pathotypes of *Colletotrichum graminicola*: (*C. sublineolum* and *C. falcatum*) that occur in Nicaragua with DNA fingerprinting techniques. This is a portion of the Ph.D. thesis project of Sergio Pichardo at Mississippi State University.
- U.S./El Salvador: Continue to evaluate germplasm for genetic variability to rust and evaluate fungicides for control of various sorghum diseases.
- U.S./Central America/Africa: Develop a rapid and reliable protocol to detect Claviceps africana (causal agent of ergot) spores and hyphal propagules in sorghum seed and feed grains.
- U.S./Africa: Continue to evaluate germplasm and screening protocols for ascertaining genetic germplasm tolerant/resistant to Fusarium stalkrot.

Constraints

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

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

Fusarium stalk rot is one of the most prevalent diseases of sorghum wherever the crop is grown. The causal organism is found in living plant tissues, crop debris, and soils in different

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

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

Networking Activities

Research Investigator Exchanges

L. E. Claflin traveled to Leon (Celaya) Mexico September 16-19, 2004 to evaluate sorghum germplasm at the INIFAP station with Jesus Narro. Considerable time was devoted to bacterial disease diagnosis and detection. The Baheel area of Mexico is often plagued with bacterial streak disease. Several compendia, specialty chemicals and antisera (for bacterial disease diagnoses) were given to Mr. Narro.

L. E. Claflin surveyed sorghum fields and discussed mutual research in El Salvador and Nicaragua from November 15 - 22.

L. E. Claflin visited Mississippi State University July 8 - 10 to assist in planning the Ph.D. program for Sergio Pichardo.

L. E. Claflin was granted an adjunct Professorship and appointed to the graduate faculty in the Department of Entomology and Plant Pathology at Mississippi State University for the purpose of serving on the committee of Sergio Pichardo.

L. E. Claflin attended the 50th annual PCCMCA meeting (4/18 - 4/23) in El Salvador and discussed future research projects with collaborators in El Salvador and Nicaragua. Cooperative on-farm projects in Nicaragua were finalized with Octavio Menocal (INTA).

Research Information Exchange

The All Disease and Insect Nursery (ADIN) was planted in three locations in El Salvador including an area in Northern El Salvador that has incurred losses due to white flies. This is the first report of white flies colonizing sorghum. Three locations were also planted in Nicaragua including an area plagued with downy mildew to determine disease incidence and severity and possibly, determine the pathotype.

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

Publications and Presentations

de Serrano, R. S., Claflin, L. E., Jaco, M. P., and A. Moran. 2004. Evauacion del Dano Ocasionado por Roya (*Puccinia* sp) en sorgos comerciales y criollos en El Salvador. LaCalera 3:23-27.

de Serrano, Morales, C. A. B., and L. E. Claflin. 2004. Evaluacion de tolerancia a enfermedades e insectos en viveros ADIN (All Disease and Insect Nursery) en El Salvador. PCCMCA 218 (abstract).

Melara, C. B., de Serrano, R. F., and L. E. Claflin. 2004. Respuesta de variedades criolla & jejoaradas de sorgo (Sorghum bicolor) a la aplicacion de fungicidas 2003. PCCMCA:219 (abstract).

Gao, Z., Jayaraj, J., Muthukrishnan, S., Claflin, L. E., and G. H. Liang. 2004. Efficient genetic transformation of sorghum using a visual screening marker. Genome (accepted)

Ramundo, B. A., and L. E. Claflin. 2004. Identification of *Burkholderia andropogonis* with a Repetitive Sequence BOX element and PCR. Current Microbiology (In Press).

Tesso, T. T., Tuinstra, M. R., and L. E. Claflin. 2004. Analysis of stalk rot resistance and genetic diversity among drought tolerant sorghum genotypes. Crop Science (in press).

Tesso, T. T., Tuinstra, M. R., and L. E. Claflin. 2004. Estimation of combining ability for resistance to Fusarium stalk rot in grain sorghum. Crop Science 44:1195-1199.

Zhou, B., Ardales, E., Brasset, E., Claflin, L. E., Leach, J. E., and S. H. Hulbert. 2004. The Rxo1/Rba1 locus of maize controls resistance reaction to pathogenic and non-host bacteria. Theor Appl Genet (In Press).

Presentations

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

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

Miscellaneous Publications

Claflin, L. E. 2003. Agroecology and biotechnology of fungal pathogens of sorghum and millet. Pp. 9-13 in INTSORMIL Ann. Repts., A Technical Res. Rept. of the Grain Sorghum/Pearl Millet Collaborative Res. Support Prog. (CRSP), University of Nebraska, Lincoln.

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

Project KSU 220
Mitchell Tuinstra and Joe Hancock, Kansas State University
William Rooney and Clint Magill, Texas A&M University

Principle Investigators

Dr. Mitch Tuinstra, Kansas State University, Dept. of Agronomy, Manhattan, KS 66506
Dr. Joe Hancock, Kansas State University, Dept. of Animal Sciences and Industry, Manhattan, KS 66506
Dr. William Rooney, Texas A&M University, Dept. of Soil & Crop Sciences, College Station, TX 77843
Dr. Clint Magill, Texas A&M University, Dept. of Plant Pathology & Molecular Biology, College Station, TX 77843

Collaborating Scientists

Dr. Carlos Campabadahl, Centro de Investigaciones en Nutricion Animal, Universidad de Costa Rica, San Jose, Costa Rica
Dr. Medson Chisi, Private Bag 7, Mt. Makulu Research Station, Chilanga, Zambia
Ing. René Clará, Centro Nacional de Technologia, Agricola de El Salvador, San Salvador, El Salvador
Dr. Salissou Issa, INRAN Rainfed Crops Program, INRAN, BP 429, Niamey, Niger
Dr. Issoufou Kapran, INRAN Rainfed Crops Program, INRAN, BP 429, Niamey, Niger
Dr. Chris Little, Dept. of Biology, UT-Pan American University, Edinburgh, TX 78541
Dr. Paul Marley, Natl Ag Res Project Office, Inst Ag Research, Ahmadu Bello Univ, PMB 1044, Samaru, Zaria, Nigeria
Dr. Paco Sereme, Director General, INERA, 01 B.P. 476, Ouagadougou, Burkina Faso
Dr. Aboubacar Touré, IER/Sotuba Research Station, BP 262, Bamako Mali
Dr. Eva Weltzien Rattunde, ICRISAT, P.B. 320 Bamako, Mali
Mr. Adama Neya, INERA, Station de Farako-Ba, BP 910, Bobo Dioulolasso, Burkina Faso
Mamourou Diourte, IER/Sotuba Research Station, BP 262, Bamako, Mali

Summary

The marketing and utilization of sorghum grain often has been limited by lower grain quality and feed value compared to other cereals. This research project attempts to address this weakness through plant breeding to develop elite varieties and hybrids with improved nutritional and grain quality traits and through development and transfer of animal feed and production technologies to developing countries. Breeding efforts continue with the exchange and testing of new germplasm and improved varieties through collaboration of scientists around the world. Animal feed workshops and seminars as well as poultry feeding demonstrations are being conducted with collaborators in numerous countries in Africa and Central America.

The major emphasis of this project is to develop sorghum varieties and hybrids with enhanced nutritional and grain quality characteristics. Large-seeded sorghum genotypes with enhanced feed-value and grain-quality characteristics have been identified and these genes are being incorporated into improved genetic backgrounds for deployment in regions of Africa, Central America, and the United States. We also are cooperating with TAM 224 to determine if high protein digestibility and grain mold resistance can be combined. Currently, small populations have been developed to test this relationship and we have begun to create larger populations in order to completely characterize this relationship.

Natural tolerance to heat and drought permit sorghum to be grown in areas unsuited for production of other cereal crops. Past breeding efforts have significantly enhanced yield potential in semi-arid regions of the world, but little attention has been focused on feed value and grain quality in these production environments. Tan-plant sorghum hybrids with improved drought tolerance are being developed to address this problem. In the United States, food-grade hybrids are now commercially available in all maturity groups. These hybrids are high yielding and well adapted to dryland and limited-irrigation environments.

Our training program focuses on the transfer of technology and knowledge to allow development and utilization of improved sorghum and pearl millet cultivars for animal feeding and human food. A key component of technical assistance and technology transfer in Central America is the RAPCO Short Course for animal nutrition. This week-long short courses in

animal feeding and nutrition is held each year and includes participants from Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, the Dominican Republic, Columbia, Venezuela, Peru, and Ecuador. Additionally, for the 2003-2004 budget year, short courses were held in Managua and San Salvador from January 11-17, 2004. These short courses were designed specifically to address issues (real and perceived) that limit the expanded use of sorghum as a feedstuff for poultry farming in Nicaragua and El Salvador. Technology transfer efforts in West Africa were initiated in 2003 through interaction with Dr. Salissou Issa, Head of the Animal Husbandry Unit at the INRAN Rainfed Crops Program in NIGER. Farm visits were accomplished in the Niamey area during August of 2003 and animal feeding trials (accomplished in Niger) were evaluated during a second visit to Niger in April of 2004. Additional feeding trials are currently being conducted in Niamey to demonstrate the relative feed value of local and improved sorghum varieties in comparison to traditional corn-based feed rations.

In addition to providing new cultivars and the technology to utilize them effectively, graduate students and visiting scientists with interest in crop improvement, crop utilization, and molecular biology are being hosted for short-term and graduate training at Kansas State University and Texas A&M University. Student projects are strongly multidisciplinary and provide opportunities for collaboration with investigators from different departments and universities. The focus of this training is to enhance the human and institutional capacity of research institutions in developing countries.

Objectives, Production and Utilization Constraints.

Objectives

Research

- Identify and map genes related to grain quality, including but not limited to grain mold resistance, seed size, protein content, anthracnose resistance, and grain quality parameters per se. Strategies will be developed to use these tools and this information to improve the efficiency of crop improvement efforts.

Germplasm Development

- High-yielding, locally-adapted sorghum varieties and hybrids with improved grain quality, grain mold resistance, and resistance to other disease (such as anthracnose) will be developed using conventional breeding techniques and marker-assisted selection strategies.

Training and Institutional Development

- Short-term and graduate education programs will be conducted to train U.S. and international scientists in plant breeding and genetics as well as animal nutrition.
- Technical assistance will be provided to promote the use of improved sorghum and millet grains in poultry feeding in the developing regions of West and Southern Africa and Central America.

Constraints

Poultry and egg production is increasing in many developing countries as economies grow and demand for higher-protein diets increase. This change in diet provides an opportunity for farmers to market grain as a cash crop to feed millers instead of the traditional view of cereal grains as a subsistence crop for human consumption. The grain of choice in poultry feed in many countries is corn. This decision makes sense when local and inexpensive stockpiles are available for use in feed manufacturing. However, many sorghum-producing countries are using more expensive, imported corn in formulating poultry rations. This decision is often based on the belief that sorghum and millet are not suitable for poultry production even though considerable information is available showing good feed-value of sorghum and millet. Furthermore, it is known that variety selection and adoption of appropriate milling technologies can enhance the value of sorghum so that it is nearly equal to the feed value of corn.

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

Research and technology transfer efforts are needed to address food quality and feed efficiency traits in sorghum and millet. Our research efforts will address this weakness in sorghum and millet through the integration of laboratory assays for feeding quality, traditional plant breeding, and biotechnology to develop elite hybrids and cultivars with improved nutritional and grain quality traits. Technology transfer efforts are underway to train and inform poultry producers, nutritionists, and feed mill operators as to the feed value of sorghum and millet and the best-management practices required to maximize the profitability and efficiency of poultry production in developing countries. The recognition of the true nutritional value of grain sorghum by animal producers will lead to greater health and productivity in livestock and humans in regions of the world where hunger and poverty are commonplace.

Research Approach and Project Output

The KSU 220 research project is multidisciplinary in nature. Principle investigators at Texas A&M University and Kansas State University coordinate this research project. These

institutions are located in two of the largest sorghum producing states in the United States. Research efforts involve interdisciplinary collaborations on each campus as well as regional collaboration among institutions. Research efforts at Kansas State University focus heavily on development of sorghum varieties and hybrids with improved agronomic performance and nutritional value and technology transfer efforts to improve the utilization of sorghum and millet in poultry rations. Research at Texas A&M University focuses heavily on development of sorghum varieties and hybrids with improved grain mold and weathering resistance for use as human food and animal feed.

Our crop improvement efforts in the United States are focused on the development of improved parent lines for hybrid seed production. This involves both applied plant breeding activities as well as germplasm characterization and enhancement for improved agronomic performance and enhanced food and feed value. These research activities are coordinated through extensive nursery and field-testing programs in Texas and Kansas. The programs in each state use a rich and diverse pool of sorghum and millet germplasm assembled from crop improvement programs in the United States and internationally. Crosses and populations derived from elite lines or germplasm sources are evaluated in multi-location nurseries and regional yield trials. Crop improvement efforts to develop cultivars adapted to environments in West and Southern Africa and Central America utilize elite varieties and cultivars that are adapted to each of the regions. The lines used to create these populations are selected through evaluations of elite U.S. and host country germplasm in the target region. This material is evaluated in the target region in conference with collaborating plant breeders. Improvement efforts in Western and Southern Africa focus on the development of early-maturing, drought-tolerant cultivars and hybrids while efforts in Central America are on improved food-type and Macio Criollos cultivars. These efforts are focused on the development of photoperiod sensitive hybrids using Ma5 and Ma6.

More basic research efforts are focused on the identification and utilization of genes that contribute to improved grain quality. Combining various grain quality attributes into one genotype is a challenge that could be facilitated by the use of molecular technology. Results from these studies will provide a better understanding of the genetic control of important quality traits and will provide genetic markers that can be used by sorghum improvement programs in the near future. One aspect of this research focuses on disease resistance (R) genes isolated from a variety of plant species to identify similar genes in sorghum. Many of these genes are likely to control variation in host-plant resistance to important sorghum diseases. Development of these technologies should enhance the efficiency of combining grain quality factors including feed quality characteristics and grain mold resistance into varieties with high yield potential. Mapping populations are being developed and characterized in cooperation with collaborators at domestic and international sites. These populations are being genotyped in laboratories in the U.S. using various types of genetic markers.

Collaborative research efforts in Africa and Central America are supported through short and long-term training programs, germplasm exchange and evaluation, and complementary basic research support activities. These research efforts are conducted in three regional programs including West Africa, Southern Africa, and Central America. Current training activities include graduate student education, information exchange workshops and meetings, short-term training visits to the United States for collaborating researchers, and workshop activities in animal production and nutrition. Technical assistance and technology transfer efforts in poultry production and nutrition are currently focused on workshop and short course activities. In 2003 and 2004, Dr. Hancock contributed to the RAPCO Short Course, a weeklong short course in animal nutrition. The participants included industry leaders in animal feeding/nutrition with representatives from Mexico, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, the Dominican Republic, Columbia, Venezuela, Peru, and Ecuador. Additionally, Dr. Hancock collaborated with Dr. Lloyd Rooney and René Clará to present short courses in Managua and San Salvador from January 11-17, 2004. Those short courses were designed specifically to address issues (real and perceived) about expanding the use of sorghum as a feedstuff for poultry farming in Nicaragua and El Salvador. Technology transfer efforts in West Africa were initiated in 2003 through collaborative interaction with Dr. Salissou Issa, Head of the Animal Husbandry Unit at the INRAN Rainfed Crops Program in NIGER. Farm visits were accomplished in the Niamey area during August of 2003 and animal feeding trials (accomplished in Niger) were given "on-site" evaluation in April of 2004. Additional feeding trials are currently being conducted near Niamey to demonstrate the relative feed value of local and improved sorghum varieties in comparison to corn-based feed rations. Finally, plans are being made to expand these technical assistance activities to key poultry producers and feed millers as in Southern Africa and West Africa this fall and/or next spring in collaboration with John Taylor and Salissou Issa, respectively.

Research Findings

Grain Mold Resistance

This focus of this research is to develop a better understanding of the genetic control of disease resistance, particularly grain mold, in sorghum. One aspect of this research focuses on disease resistance (R) genes isolated from a variety of plant species to identify similar genes in sorghum. The great majority of known R-genes possess a putative nucleotide-binding site (NBS), a domain that spans approximately 300 amino acids at the N-terminus end of the encoded protein. All NBSs are composed of distinctive, highly conserved, short amino-acid motifs. Two approaches were used to identify analogs of known resistance genes (RGAs) present in the sorghum genome. One strategy focused on the development and use of degenerate primers matching conserved regions (P-loop and GLPL region) of NBS genes was to amplify sorghum genomic DNA via

the polymerase chain reaction (PCR). Any bands of the appropriate size were cloned and sequenced for comparison to known resistance gene NBS sequences. In a different approach, the NBS sequences from 18 previously cloned R-genes were used to search for sequence homology among all available sorghum DNA sequences using the programs BLAST or "TBLASTN". The NBS sequences from 18 cloned disease-resistance genes (6-Arabidopsis, 3-flax, 3-tomato, 1-potato, 3-rice, 1-maize, 1-barley) were used to search for homology with all available sorghum DNA and protein sequences. Each sorghum sequence in the NCBI GenBank and TIGR databases was examined for homology to each NBS. Likewise, all the sequences in a large set of expressed sequence tags (sequences derived from mRNA present in control or treated tissues) available from the University of Georgia were compared to each NBS sequence. More that 80 resistance gene analogs have been identified and cloned based on database searches of sorghum expressed sequence tags and by PCR amplification using primers derived from motifs conserved in resistance genes cloned from other species (Table 1). All of the matching EST clones and PCR-generated fragments have been used as probes versus the sorghum-mapping parents (BTx623 X IS3620C) to identify restriction endonucleases that generate polymorphisms. Those with clear-cut banding patterns will be used to locate the position of the RGA on the sorghum map. Forty-two restriction fragment length polymorphism markers have been placed on the sorghum genetic map to date. Because resistance genes are often clustered in plant genomes, the ability to identify prospective R-genes may greatly speed the search for gene tags for use in marker assisted selection, one of the goals of this INTSORMIL project. Based on reports from other species, it is likely that at least a portion of the RGAs will be genes that confer disease resistance to fungal, bacterial, and viral pathogens and even to insects.

Table 1. Source and number of sorghum resistance gene analogs identified based on homology to nucleotide binding site (NBS) sequences.

Database	Tissue source	Library code	Number of clones
Sorghum EST	Pathogen-induced:incompatible	PI	14
	Pathogen-induced:compatible	PIC	12
	Immature pannicles	IP	9
	Light-grown seedlings	LG	7
	Heat-shocked seedlings	HS	3
	Dark-grown seedlings	DG1	2
	Embryos	EM	2
	Iron-deficient seedlings	FE	2
	Oxidatively-stressed leaves and roots	OX	2
	Drought-stressed	WS	2
	Drought-stressed after flowering	DSAF	1
	Drought-stressed before flowering	DSBF	1
	Ethlene-treated seedlings	ETH	1
	Ovaries	OV1 2	1
	Phosphorus-deficient seedlings	PH	1
	Acid- and alkaline-treated roots	RHOH	1
	Abscisic acid-treated seedlings	ABA1	0
	Callus culture/cell suspension	CCC1	0
	Nitrogen-deficient seedlings	NIT1	0
	Pollen	POL1	0
	Salicylic acid-treated seedlings	SA1	0
	Salt stressed seedlings	SS1	0
	Wounded leaves	WOUND 1	0
Sorghum Genomic Sequences	GSS (Genome Survey Sequences)		43
	NR (Non-redundant)		14

Primers and probes for quantification of chalcone synthase and chitinase mRNA have also been developed and are being used to compare responses to inoculation with spores of *F. thapsinum* and *C. lunata*. Four inbred cultivars, RTx2911, Sureño, SC170 and RTx430, that range from highly resistant to susceptible are being evaluated for differences in reaction. The real-time PCR protocol is sensitive enough for use on specific floral tissues, so will permit testing the hypothesis that differences in resistance result from differential levels or timing of expression of these defense-response genes.

Applied plant breeding efforts to enhance grain mold resistance focused on five breeding populations that were created by crossing elite U.S. sorghum parental lines (RTx430, RTx436, BTx631, BTx635, and Tx2903) with 'Sureño', a dual-purpose grain mold resistant sorghum cultivar. Molecular markers associated with five previously reported quantitative trait loci (QTL) for grain mold resistance originating in 'Sureño' were used to determine if their presence enhanced selection for grain mold resistance in these populations. The allelic status of 87 F4 lines, with respect to these QTL, was determined using both simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP) markers. All 87 F4:5 lines and their parental lines were evaluated for grain mold resistance in replicated trials in eight diverse environments in south and central Texas during the summer of 2002. The effects of each allele from the grain mold resistant parent 'Sureño' were determined across and within all five populations, within individual environments, and in each population x environment combination. With a few exceptions, the QTL were effective in reducing grain mold susceptibility only within the RTx430/Sureño progeny. These studies indicate that while these alleles do confer additional grain mold resistance, they are only selectable in the original mapping population. This fact limits their potential usefulness in an applied breeding program.

Anthracnose Resistance

Breeding for stable host plant resistance to anthracnose has been difficult because of the variable nature of the pathogen and an incomplete understanding of host/pathogen interaction. To develop new lines with possibly more durable forms of resistance, different sources of genetic resistance must be identified and characterized. The objectives of this study were (1) to determine if different sources with anthracnose resistance possess different genes for resistance, (2) to determine the inheritance of anthracnose resistance in the groups identified in objective 1, and (3) to identify which sources provide resistance across environments. Populations created from hybrid-

izing resistant by resistant lines were evaluated to determine if segregation for resistance occurred within a family. The presence of segregation (susceptible plants) within a population indicated that the parents have different resistance genes. In the eleven germplasms evaluated, six different sources of resistance were identified. Segregation ratios in resistant × susceptible F2 populations were consistent with the expectations of simply inherited traits and resistance was dominant in some lines and recessive in others. Evaluation of the sources of resistance across environment indicated that one source, SC748-5, provided resistance in all evaluation environments. Efforts are currently underway to map the resistance in SC748-5.

Breeding for Improved Feed- and Food-grade Characteristics in Sorghum

Genetic sources that contribute to improved seed size have been publicly released and currently are being used in crop improvement to increase seed size and yield potential. Recent genetic studies indicated that these seed characteristics appear to be beneficial to sorghum-based poultry diets, resulting in increased animal performance that was comparable to that of birds fed maize-based diets. Although these large seeded sorghum hybrids produce grain with enhanced nutritional value, genetic improvement in grain mold resistance and agronomic adaptation are still needed. Five inbred lines with improved seed size and acceptable grain mold resistance characteristics were identified in 2004 (Table 2). These lines combine large seed size with improved agronomic characteristics. Early generation materials also are being evaluated and several F4 lines were identified that integrate large seed with improved grain mold resistance. Three large mapping populations currently are being developed to determine the genetic bases for variation in seed size. We also are cooperating with TAM224 to determine if high protein digestibility and grain mold resistance can be combined. Several small populations have been developed to test this relationship and we have begun to create larger populations in order to completely characterize this relationship.

Table 2. Sorghum lines with improved seed size and agronomic characteristics.

Entry	Plant color	Grain color	Seed weight *g seed*$^{-1}$
KS115 – Large-seeded Check	Purple	Yellow	0.055
02MN5035T	Tan	Yellow	0.054
02MN5035P	Purple	Yellow	0.054
02MN5398	Tan	Yellow	0.044
02MN5453	Tan	Yellow	0.043
Tx430 – U.S. Check	Purple	Yellow	0.027
90SN7 – Niger Check	Tan	White	0.033

Crop improvement efforts to further improve the yield potential and grain quality of tan-plant sorghum hybrids are continuing. Elite tan-plant parent lines and hybrids are evaluated across the Central Great Plains to determine specific zones of adaptation and grain quality attributes. The regional tan-plant hybrid test has been conducted in Texas, Kansas, and Nebraska during the past three years. The number of commercial entries continues to rise and hybrids are now available in all maturity groups. Several full-season, tan-plant hybrids have been identified with high yield potential and acceptable grain quality in limited irrigation environments. Certain regions of Texas, especially the Winter Garden and High Plains areas, are optimally suited for production of food-grade hybrids. Many of these hybrids also are adapted to areas of Kansas and Nebraska, where small premiums have been received for the improved grain quality. Of the 40 entries in the 2003 test, most were experimental hybrids from private and public sorghum breeding programs. The majority of the entries were white-grain, tan-plant hybrids, but two red-grain, tan-plant hybrids also were included. A comparison of entries indicated that tan-plant hybrids tended to be later in maturity but similar in yield and plant height (Table 3). Several full-season, tan-plant hybrids were identified that combined high yield potential and good grain quality; however, only a few early- and mid-season tan-plant hybrids were identified with similar characteristics (Table 4). Our crop improvement efforts will continue to be focused on development of early- and medium-maturity hybrids. The grain quality of most tan-plant hybrids was good and could be used in processing for food, animal feed, or industrial applications. Several of the experimental hybrids evaluated in 2003 had lower grain quality, but these entries will not be commercially released. Red-grain, tan-plant hybrids will continue to be emphasized because of improved grain weathering resistance in these materials.

Sorghum hybrids with waxy endosperm (either homozygous or heterozygous waxy) generally have better processing and/or nutritional value but lower grain yield potential compared to non-waxy endosperm hybrids. The cause of this yield reduction is not known. From a genetic perspective, the yield reduction could be caused by pleiotrophy or genetic linkage between the *wx* locus and other loci that influence grain yield. The specific cause of this relationship is important because an effective breeding program can alleviate the problem if it is because of linkage. The objective of this study was to determine whether linkage or pleiotrophy is causing the negative relationship between grain yield and waxy endosperm. From each of two F_2 breeding populations segregating for waxy endosperm, between 40 and 50 inbred lines were derived, with equal numbers of waxy and non-waxy endosperm lines. No selection for yield was practiced during the development of these lines. The lines from these two populations and a set of testcross hybrids (derived from one population) were evaluated in four environments in Texas from 1998 to 2000. Across all tests and environments, the combined yield of the waxy genotypes was 17% lower than non-waxy genotypes. While yields were lower in waxy genotypes, analysis of the individual in-

Table 3. Comparison of tan-plant and pigmented-plant hybrids in the 2003 regional tan-plant sorghum trials in Texas.

Location	Hybrid type	Plant color	Plant height *inch*	Panicle exsertion *inch*	Anthesis *days*	Desirability rating *1 to 10*	Grain yield *lbs/acre*
College	Conventional	Purple	52	3	74	5.1	5,036
Station	Food-type	Tan	52	2	77	4.7	4,622
	LSD (P<0.05)		ns	ns	***	ns	ns
Gregory	Conventional	Purple	48	5	69	4.5	3,279
	Food-type	Tan	45	4	73	4.8	2,303
	LSD (P<0.05)		ns	ns	***	ns	*
Hondo	Conventional	Purple	55	4	67	4.3	5,868
	Food-type	Tan	52	4	70	3.9	5,275
	LSD (P<0.05)		ns	ns	***	*	ns
Halfway	Conventional	Purple	46	3	73	4.3	7,024
	Food-type	Tan	47	4	74	3.6	6,643
	LSD (P<0.05)		*	ns	*	**	ns
Perryton	Conventional	Purple	53	4	78	4.5	7,123
	Food-type	Tan	53	5	79	3.9	7,073
	LSD (P<0.05)		ns	*	**	**	ns
Combined	Conventional	Purple	51	4	72	4.5	5,666
	Food-type	Tan	50	4	75	4.2	5,183
	LSD (P<0.05)		*	ns	***	**	ns

Table 4. Agronomic characteristics and average grain yields of hybrids evaluated in the regional tan-plant sorghum trials in Kansas in 2002-2003.

Hybrid Entry	Plant color	Maturity class	Harvey County	Republic County	Thomas County	Finney County	Average
			----------bu/acre----------				bu/acre
MMR Genetics Jowar 1	Tan	Full	51	168	176	116	128
Tx2752*Tx2783	Purple	Full	54	155	193	104	127
Tx635*Tx436	Tan	Full	47	175	185	98	126
Warner 902W	Tan	Full	47	172	172	111	126
NC+ 7W92	Tan	Full	47	169	162	107	121
Wheatland*Tx430	Purple	Medium	56	135	176	115	121
Tx631*Tx436	Tan	Full	51	163	169	98	120
Tx631*Tx437	Tan	Medium	48	166	165	89	117
Sorghum Partn. NK8828	Tan	Full	40	152	169	104	116
Dekalb DKS44-41	Tan	Early	48	160	158	96	116
TxArg1*Tx436	Tan	Full	33	146	143	115	109
Tx623*Tx430	Purple	Medium	42	146	166	83	109
Asgrow Eclipse	Tan	Early	47	134	155	90	107
Sorghum Partn. 1486	Tan	Full	39	93	135	88	89

bred lines and hybrids revealed that several waxy inbred lines were not statistically different from the best non-waxy inbreds. These results imply that selection of high yielding waxy genotypes is possible, but a significant breeding emphasis on their development is required to effectively identify those genotypes.

Sorghum germplasm characterization efforts also continue in an effort to identify new germplasm sources to enhance the elite sorghum germplasm pool. In cooperation with Drs. Medson Chisi and Neal McClaren, sorghum cultivars and breeding lines from Southern African are being used as pollinators to create a set of hybrids to determine the level of heterosis present

in this germplasm. This trial was conducted in Zambia during 2003-04 and it currently is under evaluation in Texas.

Networking Activities

Workshops and Meetings

Dr. Mitch Tuinstra participated in the KSU Fall Cereal Conference, Manhattan, KS, July 31, 2003.

Dr. Joe Hancock lectured about feedstuffs and feed manufacturing to nutritionists, veterinarians, and feed manufacturers (30 to 35 people representing 12 to 14 Central/South American and Caribbean countries) at the week-long RAPCO (Cursos Regionales en Produccion Animal) Short Course in Atenas, Costa Rica, August, 2003.

Dr. Mitch Tuinstra participated in the INTSORMIL Regional Program Review, West Africa (Niger and Mali), Oct 10-18, 2003.

Drs. Tuinstra and Rooney participated in the American Seed Trade Association Meetings, Chicago, IL, Dec 10 – 12, 2003.

Drs. Tuinstra and Rooney participated in the ARS Sorghum Germplasm Committee, Chicago, IL, Dec 10, 2003.

Dr. Mitch Tuinstra participated in the Plant and Animal Genome Meeting, San Diego, CA, January 11-14, 2004.

Dr. Joe Hancock gave lectures to research scientists from INTA/CINA and academics from the University of Nicaragua (total of 45 people) to share sorghum grain data from his lab concerning the effects of particle size, extrusion, expansion, UPC, steam flaking, white seed/tan plant, kernel hardness and size, waxy endosperm, ergot, tannins, distillers grains, etc., in diets for poultry and swine, Managua, Nicaragua, January 12, 2004.

Dr. Joe Hancock collaborated with Dr. Lloyd Rooney and Ing. René Clará to present a seminar on myths about sorghum feeding value, processing properties, chemical composition, and tannins to 35 members of the El Salvador Poultry Producers Association in San Salvador, January 14, 2004.

Drs. Tuinstra, Hancock, and Magill participated in the West Africa Regional Planning Meeting, Ouagadougou, Burkina Faso, April 18-21, 2004.

Dr. Mitch Tuinstra participated in the Annual INTSORMIL Technical Committee Meeting, Kansas City, MO, May 6-7, 2004.

Research Investigator Exchanges

Dr. Joe Hancock traveled to Niger to evaluate research facilities and develop plans for poultry feeding studies in August of 2003.

Dr. D. Booyens, Pannar Seed Company, South Africa was hosted for a two day visit to Kansas State University.

Dr. Darrell Rosenow was hosted at KSU to evaluate cooperative nursery plots and review research activities, Aug 28, 2003.

Dr. Rooney traveled to El Salvador and Nicaragua in November to plan activities and evaluate germplasm in cooperative trials with Ing. René Clará and Rafael Obando.

Dr. William Rooney traveled to Zambia and South Africa to participate in the External Review of the Southern Africa program in March 2004.

Dr. William Rooney traveled to El Salvador in April 2004 to participate in the PCCMCA meeting and to make selections in breeding populations being grown in Santa Cruz Porilla, El Salvador.

Dr. Farid Waliyah, ICRISAT, was hosted for a visit to KSU to discuss potential for collaborative research activities regarding mycotoxins in feed rations, particularly for poultry, in West Africa.

Dr. Joe Hancock traveled to Niger to set up poultry feeding demonstrations in April of 2004.

Germplasm and Research Information Exchange

Coordinated the 2003 Tan Plant Hybrid Trial. This trial is designed to evaluate commercially available tan plant (improved grain quality) sorghum hybrids for agronomic adaptation and grain quality parameters. The test included 40 hybrids from nine companies that were grown in 11 locations across Kansas and Texas. Included in this trial were breeding lines from TAM220, TAM222, and TAM223.

Distributed germplasm from KSU220A for evaluation in Niger, Burkina Faso, Ghana, Mali, and Senegal.

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

Planted, evaluated, and increased seed for potential germplasm releases for TAM222.

Publications and Presentations

Journal Articles

Little CR, and C Magill. 2004. Elicitation of defense response genes in sorghum floral tissues infected by Fusarium

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Tesso TT, MR Tuinstra, and LE Claflin. 2004. Analysis of stalk rot resistance and genetic diversity among drought tolerant sorghum genotypes. Crop Sci (in press).

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Zhana X, D Wang, MR Tuinstra, S Bean, PA Seib, and XS Sun. 2003. Ethanol and lactic acid production as affected by sorghum genotype and location. Industrial Crops and Products 18: 245-255.

Agrama HA and MR Tuinstra. 2003. Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. African J. Biotech. 2:334-340.

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Rooney WL, FR Miller, and LW Rooney. 2003. Registration of RTx437 Sorghum Parental Line. Crop Sci. 43:445-6.

Dissertations and Theses

Satchidanand Pandravada. 2004. Genetic analysis of early season cold tolerance in sorghum. Ph.D. Dissertation. Kansas State University, Manhattan, KS.

Ahmed Sabry. 2003. QTL mapping of resistance to Sorghum Downy Mildew in Maize. Ph.D. Dissertation Texas A&M University, College Station, TX.

Aparna Viswinathan. 2003. Phylogenetic analysis of Sclerospora graminicola using internal transcribed spacer region-2. M.S. Thesis. Texas A&M University, College Station, TX.

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

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

Abstracts

Bean SR, Dixon CE, Tuinstra MR, Pedersen J. and Erpelding J. 2003. Relationships between endosperm protein composition and grain hardness in sorghum. AACC Conf. Portland, OR, Sept. 28-Oct. 2.

White PM Jr, Rice CW, and Tuinstra MR. 2003. Potential for enhanced soil carbon sequestration using lodging-resistant grain sorghum varieties. ASA-CSSA-SSSA Conf. Denver, CO, Nov. 10-12.

Pandravada A, Tuinstra MR, St Amand PC, Mullet JE, and Klein PE. 2003. A genetic map of sorghum based on 567 AFLP and SSR markers. ASA-CSSA-SSSA Conf. Denver, CO, Nov. 10-12.

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Low Input Ecologically Defined Management Strategies for Insect Pests on Sorghum

Project MSU 205

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Summary

Research activities in MSU 205 during the past few years has concentrated on principal insect pest problems on sorghum grown on relatively large commercial farms on the Pacific coastal plains of Nicaragua and El Salvador. These activities differed somewhat from MSU 205 research conducted in Honduras during the previous 20 years in that in Honduras almost all of the entomological research was conducted on insect situations on intercropped sorghum and corn grown on very small subsistence farms on the hillsides and on the coastal plains. Low input, inexpensive insect pest management technology was the emphasis on the subsistence farms in Honduras, whereas the commercial operations in Nicaragua and El Salvador can involve a much higher level of insect pest management technology with greater costs to the farmer. Collaborative research activities with the Instituto Nicaraguense de Technologia (INTA) the Universidad Nacional Agraria (UNA), the Nicaraguan National Sorghum Producers Association (ANPROSOR) in Nicaragua, and the Centro de Technologia de Agricola (CENTA) and the University of El Salvador in El Salvador have included investigations on insect biology, behavior, ecology and population dynamics of the sorghum midge, stalk borers and fall armyworm, the principal insect pests on sorghum in this region of Central America. With the first outbreak of whiteflies on sorghum, corn and rice in El Salvador in 2003, this pest problem was given considerable attention by entomologists at the University of El Salvador. Information from the basic investigations on the insect pests in used in developing cultural, biological and chemical control tactics for implementation in insect pest management systems for the specific pests or complex of pests. Research has been published in scientific journals and popular articles have been published for farmer utilization in the application of sorghum midge pest management in Nicaragua and fall armyworm management in El Salvador. Workshops and seminars have been organized and presented to emphasize integrated insect and disease management in sorghum. Complementary research on insect pest behavior and management, and damage to sorghum by specific insect pests is in progress in the United States for improving sorghum midge, stalk borer and fall armyworm pest management strategies. The collaborative research activities among INTSORMIL and research and farmer organizations have been fruitful in developing greater research capacity and furthering institution building activities in this ecogeographic zone. Graduate student education and professional workshops have increased agricultural capabilities of professionals in this region of Central America. The MSU 205 principal investigator will continue to support graduate student education to conduct sorghum research in Central America and the United States, to collaborate with scientists in governmental organizations and agricultural universities, and to work with non-governmental organizations to

develop improved insect pest management and integrated crop management practices for sorghum production.

Objectives, Production and Utilization Constraints

Nicaragua

- Collaboration among INTA and UNA scientists to investigate on-farm cultural and non-chemical methods for management of insect pests and plant diseases on sorghum.
- MSU 205 PI to meet with Central America collaborator scientists and NGO personnel in INTA, UNA and ANPROSOR to develop collaborative sorghum production research plans for 2003.
- Conduct IPM workshops for agricultural professionals and local sorghum producers.

El Salvador

- Evaluate the efficacy of insecticide programs on sorghum varieties for control of stem borers.
- Evaluate the efficacy of insecticide programs on sorghum varieties for control of sorghum webworm.
- Establish research relationships with entomologist at the University of El Salvador to collaboratively investigate the serious whitefly problem encountered in El Salvador in 2003.

United States

- Continue experiments to refine the economic thresholds for fall armyworm on sorghum.
- Investigate the influence of sorghum-soybean rotational cropping systems on insect pest occurrence and population densities, and damage to the crops. Work collaboratively with plant pathologist in describing these relationships for plant diseases on both crops.
- Complete research and academic programs for MSU 205 Ph.D. student.

Research Approach and Project Output

Nicaragua

Investigations were conducted on commercial farms at 2 locations (Chanandega and Tisma) in Nicaragua where replicated sorghum plots were planted to examine the effects of chemical (diazinon spray), non-chemical (Neem spray and the fungus biological agent *Beauveria bassiana*), and a cultural planting system (pigeon pea barrier) on the incidence and severity of insect pests and diseases. The MSU 205 PI traveled to Nicaragua in November 2003 with Dr. Larry Claflin, plant pathologist, INTSORMIL KSU 211, to observe progress in the on-farm research and demonstration interdisciplinary IPM program. Plots were maintained and sampled by the investigators during the period from August through December. The insect pests included in a sampling program were fall armyworm, *Mocis* (a looper), sorghum midge and leaf-footed bug. Natural enemies, plant diseases, plant damage, and yield were recorded. The results obtained for cumulative fall armyworm and *Mocis* infestations during the different plant growth stages indicated that there were no differences in numbers of larvae in the different treatments. No other insect pests infested the crop in damaging levels. Treatment plots were too small and Neem and *B. bassiana* were not effective on sorghum midge, and the pigeon pea barrier planting was not used in these small plots in a manner that might be practical in having any influence on insects that fly some distances. Plant diseases were investigated in this interdisciplinary on-farm investigation and are reported by the collaborating plant pathologists. These investigations (on-farm demonstrations) are important in the effective transfer of improved crop production technology. The on-farm IPM demonstrations will be conducted again in 2004 with modifications in methodology to improve scientific design of the work plan.

Two professional and farmer educational workshops emphasizing sorghum production problems were conducted in two regions in Nicaragua with contributions from scientists and administrators at UNA, INTA and ANPROSOR. Participants participated in the exchange of information and particularly by identifying and discussing specific agronomic, insect, and disease problems encountered in crop production situations. Particular attention was given to insect and disease identification, crop fertilization, weed control, alternative pest control methods, aflatoxins, post harvest technology and technical training assistance. Technical training workshops are planned and will be conducted in Nicaragua in 2004 and in the future.

A plant pathologist (M.S. degree) with UNA began his PhD program in MSU 205 in the Entomology and Plant Pathology Department at Mississippi State University in August 2003.

El Salvador

Entomological research in CENTA consisted of investigations to apply insect pest control tactics on sorghum for management of stalk borers in stalks and sorghum webworm on panicles in intercropped sorghum and corn in two locations in El Salvador. Insecticide applications were made on two sorghum hybrids at appropriate times to control the larvae of these insect pests. Similar results were obtained for each pest in that the control tactics had little influence on crop yield, primarily because the insect infestations were too low to obtain significant damage to the crops. However, sorghum was infested with fewer stalk borer larvae than corn in this intercropped system, suggesting that stalk borers may not reduce yield of sorghum when sorghum is intercropped with corn. The application of insecticide may have significantly influenced the stalk borer populations if the pest was at greater infestation levels. Sorghum webworms infested one variety (Soberano) in greater numbers than the other variety (RCV), with some reduced yield

for Soberano. This may suggest some resistance to this pest in RCV. Insecticide had little effect on the sorghum webworm infestation. Because these insects have been identified by farmers to contribute to apparent sorghum yield losses, similar investigations will be conducted in 2004 in areas experiencing significant stalk borer and sorghum webworm problems in 2003.

The All Disease and Insect Nursery (ADIN) was established as in the past years with CENTA plant pathologists observing and recording technical information. The ADIN will be established in 2004 with entomologist participation.

An entomologist (M.S. degree) with CENTA began his Ph.D. program in the Entomology and Plant Pathology Department at Mississippi State University in May 2004.

United States

The goals of entomological research activities in the United States have been and continue to emphasize the refinement of IPM tactics and strategies for management of the principal whorl (fall armyworm) and panicle (sorghum midge, sorghum webworm, fall armyworm and corn earworm) pests of sorghum. Research to redefine the economic threshold (ET) for fall armyworm on whorl stage sorghum is considered necessary to elucidate pest infestation levels required to warrant the practical use of insecticide. The generally recommended ET of 1 larvae per plant was confirmed for whorl stage 2 (5 leaves) sorghum. This ET was determined to be too low for subsequent whorl stages.

The influence of sorghum-soybean rotational cropping systems on insect pests populations and incidence of plant diseases is under investigation (2003-2004). Insect pest (root and vegetable feeders) diversity and density, seasonal infestation levels, and incidence of root, stem and foliage diseases on sorghum and soybeans (6 rotational cropping systems) are being investigated. The influence of the cropping systems on mycotoxin-forming fungi in both crops will be determined.

A student from Nicaragua has completed the Ph.D. degree in entomology. A second student (with M.S. degree in plant pathology) from Nicaragua is conducting the rotational cropping (sorghum-soybean systems) multidisciplinary research in Mississippi with co-advisory by the MSU 205 PI and a plant pathologist in the Entomology and Plant Pathology Department at Mississippi State University.

The MSU 205 PI will continue to advise graduate students, travel to host countries, provide advice for research activities, participate in collaborative research and technology transfers in host countries and the United States, and publish in scientific journals, as well as prepare popular crop production articles for distribution into farm communities.

Networking Activities

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

Networking with ANPROSOR in Nicaragua provides opportunities to conduct on-farm integrated insect pest and disease management research with cooperation from many farmers associated with this National Sorghum Producers Association.

Popular articles on sorghum insect pests published during the past two years provide information for farmers to use in managing these pests on sorghum to improve yield. Publications are distributed by INTA into farm communities with assistance from local agricultural professionals.

Publications and Presentations

Journal Articles

Zeledon, J. and H.N. Pitre. 2003. Occurrence of sorghum midge (*Stenodiplosis sorghicola*) (Coq.) (Diptera: Cecidomyiidae) during the second crop growing season on the Pacific coastal plain of Nicaragua. La Calera. 3: 27-29.

Jaco, M.P. and H.N. Pitre. 2003. Insecticides evaluated for control of fall armyworm (Lepidoptera: Noctuidae) on sorghum. La Calera. 3: 8-11.

Jaco, M.P. and H.N. Pitre. 2003. Application of insecticide in various volumes of water at stages of sorghum development for control of fall armyworm (Lepidoptera: Noctuidae). La Calera. 3: 12-14.

Jaco, M.P. J.A. Moran and H.N. Pitre. 2003. Frequency of insecticide spray application for control of fall armyworm (Lepidoptera: Noctuidae) on sorghum. La Calera. 3: 20-22.

Dissertations and Theses

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

Miscellaneous Publications

Zeledon, J.J. and H.N. Pitre. 2003. Evaluation of insecticides for management of fall armyworm, corn earworm and sorghum webworm on sorghum panicles, 2002. Arthropod Management Tests. 28: F-94.

Presentations

Insect pests on soybeans and sorghum. Mississippi Agronomic Professional Continuing Education Workshop. Mississippi State University (2003).

Striga Biotechnology Development and Technology Transfer

Project PRF 213

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Summary

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

In this report, we summarize our recent activities in screening wild sorghum accessions for their potential as sources of powerful *Striga* resistance genes for sorghum breeding. A small collection of wild sorghums screened for potential *Striga* resistance mechanisms allowed us to identify some unique reactions that prevent the parasitic invasion. The bioassays we used were designed to take a quick look at the earliest steps in parasitic establishment. Among the germplasm we studied were sorghums around which *Striga* did not germinate. Accessions were also identified that had reduced capacity to elicit haustorial induction of *Striga asiatica*. To our knowledge, this is the first report of low haustorial initiation activity. Up to now, this potentially useful trait has not been found among any of the *Striga* resistant sorghums. Thus, low haustorial initiation capacity may be a good trait to transfer from wild to cultivated sorghums. None of these wild sorghum accessions has yet been field tested in *Striga* sick plots so at this point we cannot correlate these phenomena observed in the laboratory with actual *Striga* resistance. Chemical and genetic characterization of the traits reported here for PQ-434 are currently underway.

Objectives, Production and Utilization Constraints

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

Striga spp. is economically important parasites of sorghum, millets and other cereals in tropical Africa and Asia. Yield losses of sorghum due to *Striga* infestation, coupled with poor soil fertility, low rainfall, and lack of production inputs, all contribute to survival difficulties for subsistence farmers. Eradication of *Striga* has been difficult to the unique adaptation of *Striga* to its environment and the complexity of the host-parasite relationship. Suggested control measures including mechanical or chemical weeding, soil fumigation, nitrogen fertilization, have been costly and beyond the means of poor subsistence farmers. Host plant resistance is probably the most feasible and potentially durable method for the control of *Striga*. Host resistance

involves both physiological and physical mechanisms. Our goal is to unravel host resistance by reducing it to components based on the signals exchanged and disrupt their interactions at each stage of the *Striga* life cycle. The specific objectives of our collaborative research project are as follows:

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

Research Approach and Project Output

Research Methods

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

The working hypothesis is that an intricate relationship between the parasite and its hosts has evolved exchange of signals and interruption of one or more of these signals result 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

Unique Sources of Striga Resistance in Wild Relatives of Sorghum

Witchweed (*Striga* spp.) infestation in crops is a major constraint to crop production across much of Africa and part of Asia. Crop damage is most severe where drought and low soil fertility already limit productivity. Often mechanical or chemical control options are too expensive or ineffective against witch-

weeds, and farmers with infested land have no other choice than to change their crop or abandon their fields. A more practical control measure for subsistence farmers to insure productivity in a *Striga* -infested field is to grow crops with resistance to *Striga*.

It is plausible that the severe devastation wrought by witchweed species may be slowed or even halted by the flow of *Striga* resistance genes from the wild to an agricultural ecology. This flow to some extent has naturally occurred in *Sorghum* but will become less likely as modern agriculture continues to separate the crop from its wild companions. The selected advantages of wild sorghum that evolved under pressure from *Striga* spp. may be exploited in their cultivated relatives by deliberate introgression. As more is learned about the interactions between *Striga* and its hosts, the search for specific opportunities to disrupt parasitic association can be narrowed. Wild relatives of cereal hosts can be screened for the phenotypes corresponding to these opportunities. Although *Striga* resistance in wild and related species has not been fully exploited, a few surveys of wild sorghums for *Striga* resistance have been reported.

In this study, we screened 55 wild sorghums and 20 improved and landrace cultivars for potential mechanisms of *Striga* resistance using in vitro procedures developed in our laboratory. Our focus was on events observed early in the process of parasitic establishment, before *Striga* attaches to sorghum. The objective of the study was to determine if new sources of preattachment *Striga* resistance can be found within the primary gene pool of sorghum.

Sorghum seed used in this study were obtained from a collection maintained at the Purdue University sorghum research program. Sorghum seed was deglumed and surfaced sterilized with 25 ml 1.3% sodium hypochlorite solution for 1 hr. Bleach was removed by several washes of sterile water. Sorghum seed was then treated overnight in 10 ml 5% w/v captan slurry (active ingredient: *N*-[trichloromethyl]thio-4-cyclohexene-1,1-dicarboimiide, 39%), a non-systemic fungicide. After washing twice with sterile water, seed was transferred to sterile petri plates containing filter paper thoroughly wetted with sterile water. Sorghum was germinated in covered plates in the dark overnight at 28°C.

Striga asiatica seed received under quarantine conditions prescribed by USDA-APHIS and the Indiana Department of Natural Resources into our parasitic weed containment facility under permit. *Striga asiatica* seed was washed upon receipt with 1% Tween-20 (polyoxyethylenesorbitan monolaurate), a surfactant, and several rinses of water to remove sand and debris. Cleaned weed seed was air dried and stored in a desiccator for at least two weeks before conditioning. Conditioning of *Striga* began 10%14 days prior to infection in 0.5 g batches. Conditioning involved washing seed with 25 ml 75% ethanol for two minutes in a sonicator. This was followed by three rinses in sterile water of one minute each with sonication. The seed was then washed in a sonicator for two minutes with 25 ml Metricide 28 (Metrex, Inc., active ingredient: glutaraldehyde, 2.5%), a disinfectant, followed by three sterile water rinses. The seed was then washed with 25 ml 0.525% sodium hypochlorite for two minutes with sonication followed by three water rinses. Finally, surface sterilized seed was incubated at 28°C in 32 ml freshly prepared 50 ppm benomyl (Dragon, Inc., active ingredient: methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate, 50%), a systemic benzimidazole fungicide. The benomyl solution was changed after 2 days and subsequently every 3%4 days during the conditioning period.

Screening the collection of sorghum germplasm with the Extended Agar Gel Assay yielded several interesting results. Several wild sorghums displayed low germination stimulation of *Striga* seed in agar. The majority of wild accessions examined, however, were high *Striga* germination stimulators. The overall range of MGD values measured in Experiment 1 was greater among the wild accessions than among the cultivars. Fourteen putative low germination stimulators were identified among the wild sorghums. Thirteen of the twenty cultivars were low germination stimulators. One accession of *S. b. drummondii* (PQ-434) from Group 1 showed no germination of *Striga* in agar before ethylene treatment. A comparison of the very low germination of *Striga* near the roots of PQ-434 was made to that near a high stimulant cultivar CK60. After three days in agar with the growing roots of PQ-434, conditioned *Striga* seed did not germinate, whereas those with CK60 showed high germination.

Roots of PQ-434, SRN39 and Shanqui Red reached an average of 15 cm, 10 cm and 12 cm, respectively, after 3 days in the agar system. Although root weight was not measured, it appears that the three were similar since SRN39 had the shortest but thickest root and the wild sorghum PQ-434 had the longest and thinnest of the three. The degree to which root branching occurred within the observation period was low for all three entries. Ethylene inhibited sorghum root elongation so the position of the apical end changed little over the two days between measurements. The mean MGD for PQ-434 was not significantly ($p<0.008$) lower than that of the low stimulant producer SRN39. When germination stimulant activity, however, was measured as the percentage of weed seed germinated along the sorghum root, PQ-434 gave the lowest values of the three sorghums assayed. The mean of the *Striga* germination percentages near the roots of PQ-434 were significantly ($p<0.008$) lower than the germination percentage near SRN39. MGD was highly correlated with the percent germination of nearby *Striga* seed measured in Experiment 3 before ethylene treatment (r=0.96), very similar to the high correlation between similar measurements (r=0.93) we had reported earlier. Germination percentages of *Striga* near the sorghum roots were measured again two days after treating plates with ethylene and compared to *Striga* in plates containing no sorghum. *Striga* in agar without sorghum did not germinate during the three days before ethylene treatment. Two days after ethylene treatment, an average of 48% of the weed seed had germinated on these plates without sorghum. *Striga* germination percentages mea-

sured from blank plates in other experiments were similar. In Experiment 1, 44±12% (mean ± one standard deviation) of the weed seed germinated two days after the ethylene treatment and 49±15% for Experiment 2. *Striga* seed within 3 mm of the roots of PQ-434 germinated to a slightly, but significantly, lower degree (36±6%) after ethylene treatment relative to the *Striga* in the blank plate. Weed seed germination near the roots of SRN39 and Shanqui Red occurred at 44±10% and 52±14%, respectively, neither significantly different than on the plate without sorghum. This suggests that PQ-434 has a slight inhibitory effect on *Striga* seed germination.

The most distinguishing attribute found in this collection of wild sorghums is the apparent low haustorial initiating activity of some accessions. In Experiment 1, twenty accessions were classified as low haustorial initiators based on mean MHD comparisons with the cultivars. The lowest MHD values were measured on those accessions put into separate groups. Only one of the cultivated sorghums, SRN39, fell into either of these groups. All other cultivars tested were classified as high haustorial initiators. Wild accessions PQ-434, IS14313, IS18803, IS14301 and IS14264 had the lowest MHD values. All of these were also low germination stimulators (Group1) with the exception of IS14264 that contained a mixture of high and low germination stimulators (Group 5). The low haustorial initiation activity of PQ-434 is apparent *Striga* seeds artificially germinated with ethylene around this accession rarely formed haustoria, defined by a lack of radicle hairs on germinated *Striga*. This contrasts with the high stimulant line CK60 that forms recognizable haustoria within two days after ethylene treatment. The percentage of germinated *Striga* with haustoria along the sorghum root was taken as an additional measure of haustorial initiation activity. PQ-434 was compared with the low stimulant cultivar SRN39 and high stimulant cultivar Shanqui Red. Although the differences between the mean values of PQ-434 and SRN39 are significant ($p<0.008$), the greater distinguishing feature of the wild sorghum from the cultivars is the very low percentage of *Striga* near its roots that form haustoria. By this measure, the *Striga* -resistant cultivar SRN39 did not significantly differ from the *Striga* susceptible Shanqui Red in haustorial initiation activity. In contrast to the high correlation ($r=0.96$, $p<0.0001$) found in this experiment between MGD and near-root percent germination before ethylene treatment, MHD was only partly correlated with percentage of germinated *Striga* that formed haustoria ($r=0.65$). It is possible that screening for low haustorial initiation activity using only MHD might miss sorghums that produce a haustorial initiation signal but inhibit haustorial formation near their roots. Such sorghums may possess a very effective *Striga* resistance mechanism.

The survey of the wild sorghum collection revealed accessions with a wide range of MHD values, such as IS18874 and HD#758 with mean MHD > 4 mm and the low haustorial initiators PQ-434, IS14301, IS14313 and IS18803 with mean MHD < 0.5 mm. None of the known *Striga* -resistant cultivars had such low MHD values as the latter group, but many exceeded the highest values measured among the wild sorghums assayed. In light of the xenognostic quinone model of haustorial initiation, both high and low types are quite interesting. The high MHD values may reflect a varied cell wall composition in these sorghums that when digested with peroxidases release a more active or diffusible xenognosic compound than DMBQ. The low MHD sorghums may possess traits that interfere with H_2O_2 production, have lower levels of peroxidases, or altered root epidermal cell wall structure such that the xenognostic quinones are not produced. Alternatively, quinones released are inhibitory of the semiquinone redox response at the *Striga* binding site in a manner similar to those compounds.

Heritability of the low haustorial initiation capacity has not been clearly established. Preliminary results from progeny of PQ-434 crossed with high germination stimulant lines indicate that the low haustorial initiation trait is simply inherited with dominant gene action. If the low MHD trait is transferable and can effectively prevent haustorial initiation in any *Striga* spp., then improved sorghums could be developed that would presumably avoid parasitic attack. More significantly, a low haustorial initiation trait could be combined with high germination stimulant production in a *Striga* resistant sorghum that would also help to rid the surrounding soil of viable *Striga* seed, eliminating the need to sacrifice a sorghum season to trap crops. MHD, or percentage of haustoria formed in the extended agar gel assay, may be useful measures of a currently unexploited *Striga* resistance mechanism of low haustorial initiation activity. The predictive value of MHD and percentage of haustorial initiation to actual *Striga* resistance remains to be tested. It is evident from the survey of the twenty cultivars that other mechanisms beyond low germination stimulation and low haustorial initiation contribute to their *Striga* resistance since some of those with high MGD and MHD values are reported to have field resistance. Potential post-attachment reactions in sorghum against *Striga* have been reported including the hypersensitive and incompatible responses. The former was identified in wild accession #47-121 from our collection. By combining genes conferring pre-attachment with post-attachment mechanisms, aided by assays for each, sorghums with durable *Striga* resistance can be developed. Wild sorghums may be sources of unique resistance traits lacking in cultivars since they have evolved under selective pressures imposed by *Striga* spp.

Networking Activities

Workshop and Program Reviews

We have been involved in a number of pilot projects associated with *Striga* research and development this past year. Three major initiatives have been underway in Ethiopia, Eritrea, and Tanzania via a pilot project directed at the promotion of an integrated *Striga* management using a mix of technologies, including *Striga* resistant sorghum cultivars, nitrogen fertilization, as well as tied-ridges as a water conservation measure. In Ethiopia, over three thousand demonstration plots have been planted in four regions of the country with very exciting and

promising results. Plots planted to the IPM technology yielded consistently higher and up to four times the yield of the untreated farmer-managed plots. Project efforts in Eritrea were similar, but scaled down in number with only one hundred demonstration plots this first year. A similar project in Tanzania focused on three regions each with over fifty on-farm demonstrations. The second, but very important, objective of each of these pilot projects focuses on promoting a functional seed multiplication efforts based on sale of good quality seed for a premium price. Keen farmers were identified, trained, and encouraged to engage in seed business. While early results of the quality of seed from these organized multiplication efforts have been good, it is too early to judge if the concept of seed as a business entity has taken hold yet.

A training workshop was held in Eritrea to kick of the demonstration and seed multiplication activities. A mid-term review was conducted to assess progress in activities that have been underway in Ethiopia. In addition, presentations on the *Striga* biotechnology research were made both at the First Sorghum and Millet Improvement Workshop in Nazret, Ethiopia and at the 2002 INTSORMIL PI Conference in Addis Ababa, Ethiopia.

Research Investigator Exchange

Over the last two years, Dr. Dale Hess was associated with our program as a visiting scientist conducting *Striga* research. He left recently having moved into a teaching and research position at Goshen College. Also, Dr. Hamidou Traore, a Fulbright fellow who spent a year at Purdue conducting *Striga* research in our facility returned to his home country of Burkina Faso. His work in our laboratory focused on identification of sorghum lines with multiple mechanisms of *Striga* resistance.
Germplasm Exchange

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

Publications

Refereed Papers

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

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

Conference Proceedings

Grenier, C., Deressa, A., Z. Gutema, G. Gebeyehu, H. Shewayerga, M. Mekuria, A. Belay, T. Tadesse, N. Mengistu, O. Oumar, A. Adugna, B. Tsegaw, and G. Ejeta. 2004. Integrated *Striga* Management (ISM) in East Africa. Proc. Consultation Workshop on Millet and Sorghum Based Systems in West Africa, McKnight-ICRISAT-INRAN, Niamey, Niger.

Toure, A., B. Dembele, M. Kayentao, and G. Ejeta. 2004. Genetic Improvement of *Striga* in Sorghum. Proc. Consultation Workshop on Millet and Sorghum Based Systems in West Africa, McKnight-ICRISAT-INRAN, Niamey, Niger.

Kapran, I., C. Grenier, and G. Ejeta. 2004. Introgression of Genes for *Striga* Resistance into African Landraces of Sorghum. Proc. Consultation Workshop on Millet and Sorghum Based Systems in West Africa, McKnight-ICRISAT-INRAN, Niamey, Niger.

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Ellicott, A. and G. Ejeta. 2003. Inheritance of hypersensitive response to *Striga* in sorghum. Agronomy Abstracts, Denver Colorado.

Ejeta, G., A. Deressa, H. Shewayerga, A. Belay, M. Mekuria, T. Hussein, A. Fanta, C. Grenier, and Z. Gutema. 2003. Integrated *Striga* Management in Sorghum. Agronomy Abstracts. Denver, Colorado.

Invited Presentations

Ejeta, G. 2003. The biology and control of *Striga*. Training Workshop on *Striga* Management and Control in Eritrea. Asmara, Eritrea.

Ejeta, G. 2003. Genetic control of *Striga*. Training Workshop on *Striga* Management and Control in Eritrea. Asmara, Eritrea.

Ejeta, G. 2003. *Striga* biology and Control. Workshop on mid-term review of the ISM pilot project in Ethiopia. Melkassa, Ethiopia

Sustainable Management of Insect Pests

Project WTU 200

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Summary

The PI traveled to Ghana and Mali in October 2003 and Botswana and South Africa in March 2004 to review collaborative research to manage insect pests and develop integrated pest management (IPM) approaches for sorghum and pearl millet. In April, the PI organized and participated in an African Sorghum and Millet Entomology Workshop for entomologists from nine countries after the West Africa INTSORMIL meeting in Burkina Faso. Sorghum from crosses of Malisor84-7 and improved lines were resistant to panicle bugs and grain mold in Mali. Sorghum sprayed with extracts from local plants were less damaged by sorghum midge than the nontreated check. More stalk borers infested millet planted after sorghum, especially when sorghum residue was left in the field after harvest in Mali. More borers infested millet than maize or sorghum. Abundance of plants infested by stalk borers or sugarcane aphids was not affected, but abundance of termites was 6-fold greater on susceptible than resistant genotypes of 19 SADC sorghums in Botswana. A graduate student evaluated resistance of sorghum and cowpeas to storage weevils and will graduate in August 2004. Another graduate student assessed tritrophic effects of resistant sorghum on coccinellids feeding on greenbugs from sorghum and will graduate in August 2004. Graduate students graduated in August 2003 who finished assessing fitness of greenbug biotype I on different grasses and effects of different amounts of soil moisture and nitrogen on the biology of greenbugs. A Malian came to West Texas A&M University to learn English and began graduate studies in 2003. Sorghums developed by INTSORMIL project TAM 223 and Pioneer Hi-Bred, International, Inc. were evaluated for resistance to different biotypes of greenbugs. The PI advised extension personnel and the National Grain Sorghum Producers on management of insect pests. The PI and graduate students presented research results at entomology and other scientific meetings.

Objectives, Production and Utilization Constraints

Objectives

West Africa

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

Southern Africa

- Support scientists from Botswana and South Africa with research to evaluate resistance and develop IPM strategies

for such sorghum insect pests as sugarcane aphid, stalk borers, and termites.

United States

- Study biology, ecology, and population dynamics of insect pests so effective management strategies and longer-lasting plant resistance can be developed. Assess fitness of greenbugs on different grasses to better understand insect-plant interactions. Use standard and molecular techniques to identify biotypes of greenbugs from the field.
- Assess agronomic practices on abundance of and damage by insect pests. Evaluate effects of soil moisture and fertility on greenbugs on sorghum. Assess tritrophic effects of resistant sorghum on beneficial lady beetles feeding on greenbugs from the sorghum.
- Collaborate with breeders, commercial seed industry, and molecular biologists to develop sorghum germplasm for greater yield potential and resistance to major insect pests.
- Supervise graduate student research and education in entomology and IPM.
- Advise extension and commodity organizations on managing insect pests of sorghum.
- Participate in professional meetings to transfer insect pest management information.

Production Constraints

West Africa

The most damaging insect pests of sorghum in West Africa are panicle-infesting bugs; sorghum midge, *Stenodiplosis sorghicola*; stalk borers; and beetles in stored grain. Sorghum midges can destroy 100% of kernels. Panicle bugs and associated infection by pathogens reduce yield and quality and render grain unusable for human consumption. Stalk borers bore into sorghum and kill the central shoot or break the peduncle. Storage pests consume and contaminate grain. The worst insect pests of pearl millet are millet head miner, *Heliocheilus albipunctella*, and *Coniesta ignefusalis* stalk borer.

Southern Africa

Stalk borers; sugarcane aphid, *Melanaphis sacchari*; panicle-infesting bugs; termites; and sorghum midge infest and reduce yields of sorghum. Beetles destroy stored sorghum grain.

United States

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

Research Approach and Project Output

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

West Africa

Tiecoura Traore from Mali finished English training and began graduate studies at West Texas A&M University. From 8-19 October 2003, sorghum and pearl millet research was viewed and collaborative research projects planned with scientists in Ghana and Mali. In Mali, the PI participated in the External Evaluation Panel review of INTSORMIL activities. Drs. Diarisso and Doumbia evaluated crosses between Malisor 84-7 and introduced, improved sorghums for resistance to panicle bugs at Sotuba, Mali. Abundance of bugs was assessed on five panicles per genotype at hard-dough. At maturity, damage by bugs was rated 1-9, where 1 = all kernels developed with few feeding punctures, to 9 = most kernels brown and/or withered and barely visible between the glumes. Grain mold was rated 1-5. Grain hardness, weight of 200 kernels, and germination were determined. Forty-six, 29, and 17 sorghums from three preliminary nurseries were resistant to bugs and grain mold. Panicles of 99-CZ-F5P-136-2, 99-CZ-F5P-131-2, 99-CZ-F5P-97-2, 99-SB-F5DT-170-1, 99-SB-F5DT-49-2, and Malisor84-7 were infested artificially with 20 pairs of bugs to confirm resistance. Bugs were counted 20 days after infestation. All six sorghums were resistant to bugs and mold, with damage scores of 1-1.33. Abundance of bugs ranged from 0.0, to 7.3 for Malisor84-7. Weight of 200 kernels ranged from 4.0 for Malisor84-7 to 5.0 for 99-SB-F5DT-170-1 nonprotected panicles, 3.8 for Malisor84-7 to 4.9 for 99-SB-F5DT-170-1 protected by cages, and 4.2 for 99-SB-F5DT-49-2 to 5.0 for Malisor84-7 protected by pollination bags.

Efficacy of extracts from local plants was assessed against bugs, sorghum midge, and grain mold on bug-susceptible S34 sorghum in the field in Mali. Bugs were counted a day before and week after treatment. Damage by bugs and mold were rated at maturity. Panicles protected by bags were not infested

by bugs, sorghum midge, or mold. Damage to and weight of protected panicles did not differ from that of panicles sprayed with Dursban. Panicles protected by bags or sprayed with Dursban were less infested with insects and mold than were panicles treated with extracts from local plants, which were better than the check. The different rates of neem seed jelly did not result in differences in damage by bugs or sorghum midge, but less mold was on panicles sprayed with the high dose than on panicles sprayed with the low dose. Weight of protected kernels was greater than that of grain from the other treatments. Kernels treated with juice of *Calotropis procera* leaves weighed least. (Table 1)

Drs. Diarisso and Mamourou Diourté evaluated crop residue management practices against stalk borers and anthracnose in sorghum at Finkolo, Mali. A randomized complete block was used with three replications of six treatments (crop residue removed from the sorghum field after harvest and before planting (check); crop residue left in the sorghum field, then chopped and buried when sorghum was planted the following year; crop residue removed from the sorghum field after harvest and before planting maize; crop residue left from sorghum then chopped and buried when maize was planted in the field; crop residue removed from the sorghum field after harvest and before planting millet; and crop residue left in the sorghum field, then chopped and buried when millet was planted). Leaves and stems from seedling to mature plants were checked for stalk borers. At maturity, 10 plants per treatment were dissected for larvae, pupae, and tunnels. Virtually no borers were found in sorghum rotated with maize, and only a few larvae and tunnels were found in sorghum when crop residues were removed after harvest and before planting (check) or when residues were left in the sorghum field and chopped and buried when the next sorghum crop was planted. Millet was more vulnerable than maize or sorghum. Approximately 10 times more larvae and three times more pupae and tunnels were found when residues were removed from sorghum after harvest and before millet was planted than were found in the check plots. More than 20 times more larvae and appoximately six times more pupae and tunnels were found when residues were left in the sorghum field and chopped and buried when millet was planted. Removal of residue from the field after harvest and before planting reduced abundance of stalk borers. *Coniesta ignefusalis* was the dominant species.

Southern Africa

From 4-14 March 2004, the PI participated in the External Evaluation Panel review of INTSORMIL activities in southern Africa, viewed sorghum research, planned collaborative projects with scientists, and met prospective graduate students in Botswana and South Africa.

In collaborative research at Botswana College of Agriculture, Dr. Munthali and Mr. Obopile evaluated for resistance to sugarcane aphid, stem borers, and termites, 19 sorghums developed by National Research Systems and SADC Regional Research Projects. Each genotype was planted in four, 7- by 7-m plots in a randomized complete block. Seeds were sown with 30 cm between plants and 50 cm between rows. Plots were examined every two weeks for pests and natural enemies. Percentages of infested plants were determined per plot. Coccinellids were counted on all plants in a plot. Adult coccinellids and ichneumonid wasps were collected for identification. Abundance of plants infested by sugarcane aphids was not affected by sorghum genotype. Infested plants per plot during 2002-3 and 2003-4 were 23.9 and 42.8% (Table 2). Abundance of predators per plot was 6.5-fold less during 2002-3 (2.8 coccinellids) than 2003-4 (18.3 predators and 8.8 coccinellid adults). Coccinellids were abundant when sugarcane aphids were abundant in 2003-4. Pests and predators were assessed only once, early in 2003-4. Natural enemies are most abundant on sorghum early in the season. The 19 sorghum genotypes were equally susceptible to stalk borers. In 2003-4 and 2002-3, 78.3 and 64.2% of plants were infested per plot. In 2003-4 and 2002-3, 17.3 and 40.4% of plants had deadhearts. Although many plants were infested, a smaller proportion had severe deadheart symptoms in 2003-4. Abundance of termites was similar and great on SDSR91039, ICR89028, Segaolane, Phofu, Tegemeo and SV2, with 92.2, 91.1, 83.8, 82.9, 79.9 and 79.0% infested plants per plot, respectively. Plants of Town, BSH1, SDSH98009, SDSH98012, LARSVYT46-48, SV1,

Table 1. Damage by sorghum midge, panicle bugs, and grain mold and kernel weight of S34 sorghum treated with extracts from local plants.

Treatments	Damage by midge/10 panicles	Damage by bugs/10 panicles	Amount of grain mold/10 panicles	Weight (g) of grain from center rows
Check without spray	3.6 a	5.1 a	2.7 ab	633.3 bc
Neem seed jelly 80 g/liter	3.2 ab	5.4 a	2.9 a	733.3 bc
Neem seed jelly 160 g/liter	2.3 bc	5.0 a	2.6 b	766.6 bc
Juice from *Calotropis procera* leaves 25 kg/30 liters	2.6 abc	5.3 a	2.7 ab	500.0 c
Dursban 80 ml/15 liters	1.8 cd	3.3 b	2.5 b	966.6 ab
Panicle protected by bag	1.0 d	1.0 b	1.0 c	1200.0 a
CV%	36.73	14.96	6.77	22.72
Probability	0.048	0.00	0.00	0.005
Significance	S	HS	HS	HS

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

Table 2. Effects of sorghums on sugarcane aphid, coccinellids, stalk borers, and termites in Botswana during 2003-2004.

Sorghum	% sugarcane aphid-infested plants/plot	Coccinellids		Stalk borers		Termites	
		Adults	All stages	% plants attacked	% plants with dead hearts	% plants infested	% plants severely damaged
SDSH98012	35.8[NS]	14.5[NS]	29.0[NS]	44.4[NS]	7.1[NS]	11.9b	3.8c
SDSR91014	36.5	2.5	13.8	59.3	5.9	17.2b	6.9c
SDSH98009	41.0	21.5	42.5	74.4	3.4	11.2b	3.4c
Mmabaitse	37.1	7.5	15.0	64.8	16.2	14.6b	4.1c
SDS6013	36.2	8.8	17.8	65.8	22.8	14.8b	3.3c
BSH1	48.6	21.8	43.5	91.0	5.0	9.2b	3.4c
ICSH93107	46.5	16.0	38.5	79.4	11.1	17.0b	4.7c
Town	44.6	7.0	13.2	91.3	17.9	7.9b	2.2c
SV1	45.4	0.0	0.0	84.2	27.8	13.3b	5.4c
Mahube	58.1	15.0	30.0	70.4	20.7	19.6b	7.4c
LARSVYT46-48	41.2	4.8	9.5	86.5	35.9	12.3b	7.5c
Macia	41.6	12.5	22.0	89.9	34.3	14.9b	5.9c
Marupantsi	52.1	3.5	7.0	77.5	32.0	27.1b	8.3c
Segaolane	34.9	18.5	38.5	83.8	1.7	83.8a	1.7c
ICSR89028	40.2	3.2	6.0	91.1	3.4	91.1a	3.3c
SV2	36.4	7.5	13.8	92.2	9.0	79.9a	19.3c
SDSR91039	41.7	2.0	4.2	79.9	19.3	92.2a	9.0c
Tegemeo	33.2	0.8	3.0	79.0	26.3	79.0a	26.3c
Phofu	61.8	0.2	0.8	82.9	28.8	82.9a	28.7c
Overall average	42.8	8.8	18.3	78.3	17.3	36.8	8.1
CV	33.5	166.7	169.0	28.17	99.40	39.16	150.47

[NS] Means in a column are not significantly different (ANOVA, $P < 0.05$); [a,b] Means in a column followed by the same letter are not significantly different (Tukey, $P < 0.05$).

Mmabaitse, SDS6013, Macia, ICSH93107, SDSR91014, Mahube, and Marupantsi infested by termites were 7.9, 9.2, 11.2, 11.9, 12.3, 13.3, 14.6, 14.8, 14.9, 17.0, 17.2, 19.6, and 27.1%, respectively. Abundance of infested plants was 6.2-fold greater for susceptible than resistant sorghum. Termites severely damaged 8.1% of the plants per plot.

United States

Master's student Fernando Chitio from Mozambique evaluated resistance of 20 genotypes of stored sorghum grain to maize weevil, *Sitophilus zeamais*, and 20 genotypes of cowpea to cowpea weevil, *Callosobruchus maculatus*. Three female and two male newly emerged weevils were put with five g of sorghum grain in each of 10 vials. Vials of each kind of sorghum were sequentially set up and evaluated every three weeks for 105 days. Each day, each grain in the 10 vials of one kind of sorghum was evaluated for damage, numbers of live and dead weevils were counted, and grain in each vial was weighed. A scale of 1-5 was used to score damage, where 1 = no evidence of damage, 2 = some feeding on the surface, involving 1-25% or one shallow hole in a kernel, 3 = two tunnels, causing 26-50% damage to a kernel, 4 = 51-75% damage or more than two holes in a kernel, 5 = 76-100% damage and many tunnels in a kernel. Fewest maize weevils emerged from Sima, Macia, and Sureno — 1.7, 2.8, and 3.1 per gram of grain at 105 days after infestation, respectively (Table 3). Most emerged from CE151, SRN39, SC630-11E11, and ATx631 — 14.2, 12.4, 12.1, and 12.0 per gram of grain at 105 days after infestation. Sureno, Sima, Malisor-84-7-167, and Macia were least damaged, with scores of 1.5, 1.6, 1.7, and 1.8 at 105 days after infestation. CE151, SC630-11E11, and ATx631 were most damaged, with scores of 4.0, 3.9, and 3.9 at 105 days after infestation. Of the original 5.0 g of grain per vial, grain in vials of Sureno, Sima, Macia, Malisor-84-7-167, and Tegemeo weighed most at 105 days after infestation — 5.0, 4.8, 4.7, 4.7, and 4.6 g. Percentages of weight loss of grain of Sureno, Sima, Macia, and Malisor-84-7-167 were 0.8-6.6%. Grain of SC630-11E11, CE151, and ATx631 weighed least at 105 days after infestation, with 2.7, 2.8, and 3.1 g remaining of the original 5.0 g per vial. Percentages of weight loss of grain of ATx631, CE151, and SC630-11E11 were 37.2-46.8%. Grain weight loss was correlated to the cumulative total number of maize weevils produced per gram and with the score of damage to the grain, but not to grain size, hardness, or protein content. Cowpea weevils destroyed 34-65% of grain of the 20 genotypes of cowpeas.

Master's student Murali Ayyanath assessed tritrophic effects of biotype I greenbugs from resistant PI550607 versus susceptible RTx430 sorghum on the life cycle of convergent lady beetles at 23 and 30°C. Lady beetles from the field were paired in condiment cups. Larvae produced were reared individually in condiment cups at 23 or 30°C and a photoperiod of 14:10 light:dark hours in an incubator. Each day, a known number of greenbugs from RTx430 sorghum in a greenhouse was fed to half of the larvae, and the same number of greenbugs from PI550607 sorghum was fed to the other half of the larvae. The number of greenbugs remaining was counted and discarded the next day. Adult lady beetles that emerged were paired in one of four ways depending on the sorghum source of green-

Table 3. Number of maize weevil adults per gram, score of damage, and percentage of weight loss (± SEM) at 105 days after infestation of sorghum grain

Sorghum	Total maize weevils/gram	Damage score	% weight loss
Sureno	3.1 ± 0.50g	1.5 ± 0.10i	0.8 ± 0.07j
Sima	1.7 ± 0.66g	1.6 ± 0.19i	3.8 ± 0.09ij
Macia	2.8 ± 1.17g	1.8 ± 0.26hi	5.4 ± 0.18h-j
Malisor84-7-167	3.9 ± 0.81fg	1.7 ± 0.14hi	6.6 ± 0.12hi
Tegemeo	3.5 ± 1.02fg	2.0 ± 0.20g-i	8.2 ± 0.14h
ATx635	6.4 ± 0.81ef	2.3 ± 0.15f-h	13.8 ± 0.12gh
Malisor84-7-476	7.4 ± 1.11de	2.5 ± 0.20fg	15.2 ± 0.16g
Kuyuma	7.3 ± 1.75de	2.7 ± 0.24ef	16.8 ± 0.24g
Tx2882	7.5 ± 1.35de	2.7 ± 0.21d-f	17.4 ± 0.18fg
Segaolane	8.6 ± 0.94de	2.8 ± 0.27c-f	21.8 ± 0.21f
B1	10.6 ± 1.11b-d	3.1 ± 0.18c-e	27.2 ± 0.16e-g
RTx430-5451	10.1 ± 0.81b-d	3.3 ± 0.12b-d	27.4 ± 0.17d-f
Tx2737	10.0 ± 2.08b-d	3.2 ± 0.37b-e	30.2 ± 0.31d-f
RTx430-5362	10.5 ± 1.31b-d	3.4 ± 0.24a-c	32.0 ± 0.25c-e
ATx623	10.4 ± 1.31b-d	3.2 ± 0.20b-e	32.2 ± 0.23b-e
Tx2911	9.5 ± 1.39c-e	3.4 ± 0.33a-c	33.8 ± 0.29a-d
SRN39	12.9 ± 1.49ab	3.7 ± 0.24ab	35.8 ± 0.22a-d
ATx631	12.0 ± 1.50a-c	3.9 ± 0.21a	37.2 ± 0.25a-c
CE151	14.2 ± 0.95a	4.0 ± 0.15a	43.4 ± 0.14ab
SC630-11^{E}11	12.1 ± 1.01a-c	3.9 ± 0.21a	46.8 ± 0.21a
LSD (<0.0001)	3.351	0.615	0.547

bugs. The females were fed greenbugs from the original sorghum from which they previously were fed. Daily numbers of eggs laid and hatched per female were recorded until the beetle died or 90 days passed after emergence. Lady beetle larvae and adults consumed the same numbers of greenbugs from resistant or susceptible sorghum. But, larvae ate 1.7 and adults ate 2.0-3.1 times more greenbugs at 23 than 30°C. Each adult ate 17,124.0 and 16,324.5 greenbugs from resistant and susceptible sorghum at 23°C. Lady beetle eggs hatched in 3.0 versus 2.0 days, larvae required three times longer to develop, and the pupal stage lasted 6.0 and 3.0 days at 23 and 30°C, respectively. Almost 8.5 times more eggs were produced per lady beetle fed greenbugs from susceptible sorghum at 23°C than by beetles fed greenbugs from resistant sorghum at 30°C (Table 4). Numbers of eggs were greatest from beetles fed greenbugs from susceptible sorghum. At 23°C, 91.0% hatched of the 2,893.1 eggs produced by beetles fed greenbugs from susceptible sorghum. At 30°C, more eggs hatched, 75.4%, per female when beetles were fed greenbugs from susceptible sorghum. Only 21.8-39.4% of eggs hatched from other combinations of beetles. Greenbugs from resistant sorghum negatively affected the number and viability of eggs produced by convergent lady beetles.

Table 4. Effect of temperature and sorghum source of greenbugs on eggs laid and hatched per convergent lady beetle female.

Pair (female x male)[a]	Number of eggs laid	% of eggs hatched
	23°C	
SxS	2,893.1 ± 183.0aA	91.0 ± 5.2aA
SxR	1,814.3 ± 224.1bA	64.4 ± 6.4bcA
RxS	1,242.0 ± 317.0bA	44.2 ± 9.0cA
RxR	1,586.0 ± 388.2bA	81.3 ± 11.1abA
	30°C	
SxS	651.0 ± 191.3aB	75.4 ± 15.2aB
SxR	498.0 ± 135.2aB	34.8 ± 10.7bB
RxS	343.0 ± 110.4aB	39.4 ± 8.8abA
RxR	342.0 ± 156.2aA	21.8 ± 12.4bA

For each temperature, means ± SE followed by the same lower-case letter in a column are not significantly different (LSD, $P = 0.05$). Between temperatures, means ± SE followed by the same upper-case letter in a column are not significantly different (LSD, $P = 0.05$). [a]S = lady beetle fed greenbugs from RTx430 sorghum; R = greenbugs from PI550607 sorghum.

Master's student Kishan Sambaraju finished assessing fitness of biotype I greenbugs on wild grasses and resistant and susceptible sorghum and wheat. Seeds of susceptible RTx430 sorghum; resistant LG-35 sorghum; susceptible Custer wheat; resistant GRS1201 wheat; barnyardgrass, *Echinocloa crus-galli*; Johnsongrass, *Sorghum halepense*; jointed goatgrass, *Aegilops cylindricum*; and Arriba western wheatgrass, *Agropyron smithii*; were sown in a greenhouse. Eighteen to 21, 2.5-cm^3 plastic cages containing a greenbug were clipped onto leaves of each kind of grass in three replications. The greenbug was removed after it produced a nymph. The nymph was retained until it produced offspring, which were counted and removed daily. The number of days the greenbug lived was recorded. Fecundity was 13.9 nymphs per greenbug on barnyardgrass, but 62.2 and 61.5 nymphs on susceptible wheat and sorghum. Each greenbug lived 14.8 days on barnyardgrass, but longest on grasses of the genus *Sorghum* (29.4, 28.8, and 27.5 days on Johnsongrass, RTx430, and LG35, respectively). The resistance mechanism in the sorghum and wheat probably is antixenosis or tolerance, rather than antibiosis.

Master's student Suresh Veerabomma finished assessing effects of soil water potential and nitrogen on abundance and longevity of biotype I greenbugs. A greenbug enclosed in a clip cage was attached to a leaf of each of 90 sorghum plants, 10 per treatment combination. Soil water potential, but not nitrogen, affected greenbug fecundity, with almost twice as many nymphs produced per greenbug on sorghum in soil with -33 kPa (44.5 nymphs) as with -300 kPa of water potential (28.5 nymphs). Greenbug longevity was affected by soil water potential but not nitrogen. Longevity was 22.2 and 28.6 days on sorghum in soil with -300 and -33 kPa of water.

Two hundred one sorghum lines were evaluated for TAM 223 for resistance to greenbug biotypes C and E. Two hundred sorghum lines developed by Pioneer Hi-Bred International, Inc. were evaluated for resistance to greenbug biotype I, with one line being very resistant.

Networking Activities

Workshops

The PI organized and participated in an African Sorghum and Millet Entomology Workshop (21-22 April 2004, Ouagadougou, Burkina Faso) after the West Africa INTSORMIL meeting. During the Workshop, entomologists from Botswana, Burkina Faso, Eritrea, Ghana, Mali, Niger, Senegal, South Africa, and the United States prioritized insect pests by region of Africa, reported on pests from their countries, presented results of current research, planned collaborative research projects, and wrote a grant proposal on "Management of Panicle Bugs to Prevent Grain Mold and Improve Sorghum Grain Quality in Africa." The PI was program co-chair, organized a sorghum symposium, and co-authored five presentations for the 52nd Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists in conjunction with the Annual Meeting of the High Plains Association of Crop Consultants (23-26 February 2004, Lubbock, Texas). The PI gave two presentations on greenbugs at the Entomology Science Conference (11-13 November 2003, College Station, Texas).

Research Investigator Exchanges

From 8-19 October 2003, the PI traveled and discussed and reviewed research and needs with scientists and administrators from SARI in Ghana and IER in Mali. Entomological emphasis was on managing panicle-infesting bugs, sorghum midge, stalk borers, and storage beetles. From 4-14 March 2004, the PI traveled, discussed and reviewed research with scientists and administrators, and met prospective graduate students from Botswana College of Agriculture, North West University in South Africa, and ARC in South Africa.

Research Information Exchange

The PI advised extension, National Grain Sorghum Producers, and commercial seed companies on management of sorghum insect pests. Two hundred sorghums developed for resistance to biotype I greenbug were evaluated for Pioneer Hi-Bred International, Inc. The PI is assisting Dr. John Burd, USDA-ARS, Stillwater, Oklahoma, with a multi-year, multistate study of greenbugs on wild and cultivated grasses. Reference materials and/or supplies were provided for entomological research for Mr. Abdou Kadi Kadi in Niger, Dr. Mamadou Baldé and Mr. Djibril Badiane in Senegal, Dr. Diarisso in Mali, Dr. Munthali in Botswana, Drs. Hannalene du Plessis and Johnnie van den Berg in South Africa, and Dr. Paul Tanzubil in Ghana.

Publications and Presentations

Publications

Sambaraju, K.R., B.B. Pendleton, C.A. Robinson and R.C. Thomason. 2003. Fecundity and longevity of greenbug on wild and cultivated grasses. International Sorghum and Millets Newsletter 44:131-132.

Ayyanath, M., B. Pendleton and G.J. Michels, Jr. 2004. Tritrophic interactions of resistant sorghum, greenbugs, and lady beetles. Pp. 2-3. In Proceedings of the 52nd Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists. Lubbock, TX. February 23-26, 2004.

Chitio, F., B. Pendleton and G.J. Michels, Jr. 2004. Resistance of stored cowpeas to cowpea weevil (Coleoptera: Bruchidae). Pp. 9-10. In Proceedings of the 52nd Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists. Lubbock, TX. February 23-26, 2004.

Sambaraju, K. and B. Pendleton. 2004. Fitness of greenbug (Homoptera: Aphididae) on wild and cultivated grasses. Pp. 20-21. In Proceedings of the 52nd Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists. Lubbock, TX. February 23-26, 2004.

Sambaraju, K.R. 2003. Fitness of greenbug (Homoptera: Aphididae) on wild and cultivated grasses. M.S. thesis. West Texas A&M University, Canyon, TX.

Veerabomma, S. 2003. Effects of different amounts of soil water and nitrogen on the fecundity and longevity of greenbug (Homoptera: Aphididae) on sorghum. M.S. thesis. West Texas A&M University, Canyon, TX.

Presentations

Entomology Science Conference, 11-13 November 2003, College Station, TX – *Greenbug review and update – biotyping, evaluating sorghums for resistance, and new insecticides* presented by Bonnie Pendleton; and *Using AFLP to distinguish sorghum greenbug biotypes* presented by Keyan Zhu-Salzman and Bonnie Pendleton.

52nd Annual Meeting of the Southwestern Branch of the Entomological Society of America and the Annual Meeting of the Society of Southwestern Entomologists in conjunction with the Annual Meeting of the High Plains Association of Crop Consultants, 23-26 February 2004, Lubbock, TX – *Tritrophic interactions of resistant sorghum, greenbugs, and lady beetles* by Muralimohan Ayyanath, Bonnie B. Pendleton, and G. J. Michels, Jr.; *Alternatives to organophosphates and carbamates for managing aphids in wheat and sorghum* by Roxanne Bowling, Bonnie Pendleton, Jerry

Michels, and Robert Bowling; *Resistance of stored cowpeas to cowpea weevil (Coleoptera: Bruchidae)* by Fernando M. Chitio, Bonnie B. Pendleton, and G. J. Michels, Jr.; *Fitness of greenbug (Homoptera: Aphididae) on wild and cultivated grasses* by Kishan Sambaraju and Bonnie B. Pendleton; and *Distinguishing sorghum greenbug biotypes by using AFLP fingerprinting* by Keyan Zhu-Salzman, Haiwen Li, and Bonnie Pendleton.

African Sorghum and Millet Entomology Workshop, 21-22 April 2004, Ouagadougou, Burkina Faso – *Current research on sorghum midge in Niger* by Hame Abdou Kadi Kadi, Bonnie Pendleton, and Issoufou Kapran; *Life table of millet head miner (Lepidoptera: Noctuidae) reared in a lab in Niger* by Hame Abdou Kadi Kadi, Frank Gilstrap, George Teetes, Ousmane Youm, and Bonnie Pendleton; *Head bug biology* by Niamoye Diarisso, Yacouba Doumbia, Mamadou N'Diaye, and Bonnie Pendleton; and *Sorghum midge* by Bonnie Pendleton.