Breeding and Testing Corn for Reduced Aflatoxin Contamination and Increased Drought Tolerance for Texas

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Abstract: Optimal corn production in Texas is greatly limited by aflatoxin and drought stress resulting in lost profits for producers. Breeding and improvement of Texas adapted corn will increase yields, decrease yield losses due to stress and decrease economic losses due to aflatoxin. A variety of related objectives for improving Texas corn were accomplished under this project. 1) We advanced and selected new Texas adapted inbred lines for testing within a summer nursery and a fall nursery. 2) We tested previously developed Texas AgriLife public inbred lines as hybrids for both yield and aflatoxin. This involved the development of new techniques for subsampling harvested material from the combine and near infrared spectroscopy (NIRS) for predicting aflatoxin. Importantly, we identified a number of lines that had yield comparable to or exceeding commercial checks. 3) We finished the development and testing of two new techniques for quickly screening drought tolerance, using seedlings in the greenhouse and leaf epicuticular wax in the field. We found that although these methods are of use in testing hybrids but not of use predicting which inbreds will be the most useful. 4) Finally, we tested a modern open-pollinated population, the Texas Argentine composite (TAC) and found that although it is lower yielding than elite commercial hybrids, the lower seed costs mean that it would be more profitable for many producers than current commercial hybrids and lowers crop investment risk in marginal years. Additional progress was made on training students, publicizing Texas corn and Texas corn research, and gaining a better understanding of Texas corn production. In conclusion there was a wealth of new findings and products that will ultimately result in better corn for Texas producers.

Procedures and Objectives:

Inbred lines need to be created to produce the hybrids that growers will ultimately use in commercial production. This generally involves crossing two or more cultivars with desired traits and then selfing these plants each generation until they are stable inbreds. The primary goal of the TAMU corn breeding program is to breed inbred lines that can produce hybrids with both high yield and aflatoxin resistance.

For inbred selection we generally self five plants within each plot planted; a plot is often derived from a single ear selected the previous year. We then select the best plants ears from these to be planted for further inbreeding and also crossing to a commercial tester line (a commercial inbred, preferably having Roundup Ready and Bt traits). This resulting hybrid is then tested for yield, aflatoxin resistance, and agronomic traits. Hybrids with superior performance then need to have their inbred seed further increased and advanced through self pollination in 'increase nurseries', crossed to other inbred lines for additional testing and then be released. Self pollination and crossing are performed in 'nurseries' two times per year; in the summer in College Station, TX and in the fall in Weslaco, TX.

***Objective 1:* Continued breeding of AgriLife improved inbred line development: Texas adapted by adapted crosses**

College Station

In College Station this year 799 inbred (F_3 to F_7's) breeding plots specifically supported under this project were planted on March 18th. Although some stands were good, many were only fair. Out of 25 seeds planted, five plants were self pollinated. We then used a new inoculation method, jointly spraying *A. flavus* spores and a nutrient solution, into our pollinating bags to maximize *A. flavus* exposure. We believe this allowed us to better evaluate *A. flavus* resistance of inbreds while we were performing selection. College Station had relatively minimal *A. flavus* this year to select against. In order of importance, we strongly selected against visual appearance of *A. flavus* and other ear-molds, selected for large ear size and good seed set, selected for larger and more robust plants, and selected for higher ear and plant height. A total of 584 individual plant ears were selected out of 19,975 kernels planted (3%).

Successful advanced lines and 'to be released' lines that were created with the assistance of previous Texas Corn Producers Board support needed to have seed increased. For seed increases, 145 inbred lines with up to six plots were planted (505 plots total). This was unfortunately in one of the poorest parts of the field, so these increases were not as successful as we had hoped.

Weslaco (Fall nursery)

Because of resource restrictions only 325 of these 584 ears (1.6% of what was planted in College Station) were sent to Weslaco for our fall nursery which was planted August 18th. There was a wide range of additional plots in Weslaco winter nursery for making new hybrids, USDA projects, graduate student projects, and other cooperative work. As of this final report, Weslaco has been harvested but not shelled so we do not know how exactly how many plots will be retained.

<u>Objective 2:</u> Evaluate AgriLife hybrids for aflatoxin accumulation and agronomic performance of across several environments.

Corn producers grow hybrid lines because of their far superior yield potential and agronomics over inbred lines. Inbred lines *per se* are difficult to evaluate for yield, and depending on the type of resistance, may also not be relevant for testing aflatoxin resistance. Therefore, we make testcrosses with testers, lines that predict the general combining ability of inbreds. This year, 394 hybrids were tested between Texas AgriLife inbred lines and one of three testers. Material tested for a second year was tested with either a commercial "Stiff Stalk" (SS) or a commercial "Non-Stiff Stalk" (NSS) inbred tester line, both Roundup Ready. A third tester, an older white TAMU inbred, was used to test new inbred lines for the first time.

1096 yield trial plots directly related to this project, were planted in Weslaco on February 18th and harvested on July 22nd. 368 of these were inoculated using ground kernel inoculum on May 10th. In College Station, 1223 yield trial plots directly related to this project, were planted on February 18th and harvested between August 13th - 27th. 368 of these were inoculated using ground kernel inoculum on June 3rd. 163 plots were planted in Thrall. 364 plots were planted in Corpus Christi were inoculated June 10th with ground kernel inoculum and harvested on August 1st. Additionally, hybrid trials led by Dr. Wenwei Xu, Dr. Tom Isakeit, and others were also harvested in Weslaco with the corn breeding programs assistance.

Agronomic data on plant height, ear height, silking date, anthesis date, stand count, lodging and any other notable observations were taken on all plants. Yield, bushel weight and grain moisture were taken with the combine at harvest. The results are presented for yield only with only the top 10% of all hybrids across all tests (early through advanced generation material) in each location shown.

Subsampling for aflatoxin and NIRS

We refined a new technique to subsample grain from all harvested plots. In the photo (below) you can see it was a three person operation to keep up with the combine. One person caught the samples, one found the correct barcode labeled bag and the third stapled and carried the samples. This was hot, dirty and loud work but resulted in samples that should be similar to a producers (all of the trash and light kernals were blown out of the back of the combine). This allowed us to simultaneously get accurate estimates of yield and have samples to test in near infrared spectroscopy (NIRS see supplemental 1) for aflatoxin. While aflatoxin predictions have now been made via NIRS, Vicam Aflatests have not yet been run to confirm these numbers. These tests will be completed before planting next year. For more information on the NIRS experiments please see the supplemental section at end.

Findings for next year

Many superior inbreds were identified with generic testers. The next step is to cross with additional testers to find the best combination and expand testing to four row four replicate tests in more locations.

Table 1: Top 10% performing hybrids across three locations in alphabetical order. Top 10% bolded in each location. Pedigrees in top 10% at two or more locations also are in bold.

Pedigree	Tester	WE10 (lbs/ac)	WE10 Rank	CS10 (lbs/ac)	CS10 Rank	CC10 Yield	CC10 Rank
ArgentineFlintyComposite-C(1)-15-B1-B	NSS	5714	248	7865	**14**	3801	84
ArgentineFlintyComposite-C(1)-16-B-B	NSS	6143	94	7823	**18**	.	.
ArgentineFlintyComposite-C(1)-23-B-B	SS	6338	**23**	7503	84	3703	101
ArgentineFlintyComposite-C(1)-24-B-B	NSS	6076	128	7057	240	4364	**9**
ArgentineFlintyComposite-C(1)-24-B-B	SS	6034	151	7368	139	4295	**11**
ArgentineFlintyComposite-C(1)-26-B-B	NSS	6285	**29**	6971	262	4006	37
(((B104/NC300)x(CML285/B104))-2-3-BBB/LAMA2002-22-3-BB1)-B*5-1	NSS	.	.	7894	**11**	.	.
(((B104/NC300)x(CML415/B104))-4-2-B-B/Tx760-B-B-B)-B-B-1-B-B-B	NSS	6213	53	7257	183	4193	**13**
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-1-B-B-B	NSS	6159	80	7400	125	4426	**5**
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1	NSS	6208	58	7464	99	4643	**3**
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B1	**SS**	6134	98	7850	**15**	4276	**12**
((B104-1xTx714-B-B)-1-4-B-B-B-B/CML161)-B-B-2-B-B-B2	**NSS**	6391	**15**	7705	**29**	4422	**6**
((B104-1xTx714-B-B)-23-1-B-B-B-B/(CML288/NC300)-B-9-B1-B-B-B)-B-B-1-1-B-B-B	TAMU	6456	**9**	7187	205	.	.
((B104-1xTx714-B-B)-23-1-B-B-B-B/(CML288/NC300)-B-9-B1-B-B-B)-B-B-2-3-B-B-B	TAMU	.	.	8117	**4**	.	.
BS13(S)C8-15-1-B-B-B-B-B-B-B-B-B	TAMU	6291	**27**	6818	291	.	.
CML269/TX114-B-B-B-1-1-B-B-B-B-B-B	TAMU	6409	**14**	7586	58	.	.
CML269/TX130-B-B-B-1-3-B-B-B-B-B-B	TAMU	5903	215	7776	**21**	.	.
CML269/TX130-B-B-B-1-3-B-B-B-B-B-B	TAMU	6298	**26**	7121	217	.	.
((CML269/Tx110)/(CML311/Tx110)-1-B-B-B-B/DTPWC8F31-1-1-2-2-BBBB-B)-B-B-3-1-B	NSS	6427	**12**	7370	137	.	.
((CML269/Tx114)-B-B-B-B/Tx114/CML78-B-1-B-B-B)-B-B-1-3-B-B-B	TAMU	6245	43	7713	**28**	.	.
((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B-B-B)-B-1-B-1-B	NSS	6480	**8**	7111	220	3880	62
((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B-B-B)-B-1-B-2-B	NSS	6390	**17**	7352	142	3778	88
CML451/TX760-B-B-1-2-B	SS	.	.	8279	**2**	.	.
((Tx114(B73w)-BxCML343/Tx110xPop24)-B-B-B-9-B-B-B/CML269/TX130-B-B-B-1-1-B-B-B)-B-B-1-1-B-B-B	TAMU	6535	**6**	7677	38	.	.
((Tx114(B73w)-BxCML343/Tx110xPop24)-B-B-B-9-B-B-B-B/CML78)-B-2-B-2-B	NSS	6348	**22**	7286	165	3865	66
(Bs13(S)C8-26-1-BxNC380)-B-B-B-B-B	SS	6092	126	7889	**12**	3932	49
(BS13(S)C8-33-1-B-B-BxTx745)-B-B-B-B-B	SS	6235	46	6868	284	4339	**10**
(CML269/Tx114)-B-B-B-B-B-B-B-B	TAMU	5570	269	7843	**17**	.	.
(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-1-B	NSS	6246	41	7683	**33**	3690	102
(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-1-B	SS	6303	**24**	7392	128	.	.
(CML379/CML311-B-1-B-B-B-B/Tx110)-B-2-B-4-B	NSS	6243	44	7524	75	4783	**2**

Pedigree	Tester	WE10 (lbs/ac)	WE10 Rank	CS10 (lbs/ac)	CS10 Rank	CC10 Yield	CC10 Rank
(CML442-B/CML343-B-B-B-B-B-B)-B-B-1-1-B	SS	6456	**10**	6959	265	3447	119
(CML442-B/CML343-B-B-B-B-B-B)-B-B-1-1-B-B-B	TAMU	5415	280	8223	**3**	.	.
(CML450-B/(Tx106-Tx714)-1-1-714-1-1-1-B-B-B-B-B)-B-2-B-3-B	NSS	6384	**18**	7370	138	3582	110
(CML450-B/Tx110)-B-3-B-3-B	NSS	6390	**16**	7151	211	3944	46
(Ko326yxTx806)-2-2-1-1-B-B-B-B-B-B-B-B-B-B-1	TAMU	5632	258	7737	**26**	.	.
(LAMA2002-12-1-B/(CML325/B104)-B-1-B-B-B-B)-B-B2-3-2-B-B-B	TAMU	6030	155	7785	**20**	.	.
(LAMA2002-12-1-B/(CML325/B104)-B-1-B-B-B-B)-B-B2-4-1-B-B-B	TAMU	5985	182	7901	**10**	.	.
(LAMA2002-23-1-BB/LAMA2002-11-1-BB)-B*5-1	SS	6283	30	7726	**27**	.	.
(LAMA2002-23-3-B/SCR82-B)-B-B-1-1-B	NSS	6290	**28**	6678	310	.	.
(LAMA2002-25-5-B/LAMA2002-2-3-B)-B-B-1-1-B	SS	6559	**5**	7418	118	3956	45
(LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-1-1-B-B-B	TAMU	6525	**7**	7796	**19**	.	.
(LAMA2002-2-5-B/(CML285/B104)-B-4-B-B-B-B)-B-B2-2-1-B-B-B	TAMU	5478	276	7845	**16**	.	.
(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B	**NSS**	6302	**25**	7740	**25**	3887	60
(LAMA2002-6-5-B/(CML326/B104)-B-9-B-B-B-B)-B-B-1-2-B-B-B	TAMU	6055	141	7755	**23**	.	.
RedEar2-2-2-1-1-B	TAMU	.	.	7703	**30**	.	.
RedHybridEar-B-1-2-2-1-B	TAMU	6412	**13**	7012	253	.	.
(Temp.NSSLateB-105-B-B-B-B/CML161)-B-B-B-B-B1	NSS	6362	**21**	.	.	.	.
Temp.SSLate(B37,B73,B84)B-62-B-B-B-B-B-B-B-1	TAMU	6723	**1**	6881	281	.	.
(Tx601xB104-B/FR2128-BxBord)-2-2-B-B-B-B-B-B-B	**TAMU**	6382	**19**	7741	**24**	.	.
(Tx745-B-B/CML161)-B-B-1-B-B-B	SS	5722	246	7757	**22**	3659	103
Tx770/CML288-B-3-B-B-B-B-B-B-B	TAMU	6153	81	7687	**32**	.	.
Tx811-B-B-B-B-B-B-B	TAMU	6375	**20**	6700	308	.	.
Commercial Testers							
BH9014VT3		6651	**4**	6915	276	4876	**1**
DK697		.	.	8104	**5**	.	.
DKC66-08		6433	**11**	6793	297	4365	**8**
DKC67-23		6703	**3**	8085	**6**	4573	**4**
P31G66		.	.	7876	**13**	4151	15
P31G66(2007)		6718	**2**	6999	254	.	.
W4700		6147	89	7562	63	4420	**7**
Location mean yield (lbs/ac)		**6000**		**7256**		**3867**	
Number of hybrids in final analysis			**293**		**331**		**130**

<u>Objective 3:</u> Evaluate drought resistance by comparing well watered and water limited conditions in hybrids created with 52 AgriLife finished inbred lines.

Drought tolerance is important to maintain corn grain yields and aflatoxin resistance under drought stress. However, drought tolerance is hard to identify and predict, especially in inbred lines. In this objective we tested two high-throughput methods to measure drought tolerance: 1) epicuticular wax extractions, leaf wax is believed to protect the plant against excess water loss and we wished to test this hypothesis; and 2) seedling drought tolerance screens, if seedling drought tolerance predicted adult plant drought tolerance this would be a very easy screen for breeding program selection. In 2009, 62 finished inbred lines (40 from AgriLife College Station, 12 from AgriLife Lubbock, 10 publicly available) were evaluated as inbred lines *per se.* These inbred lines were crossed to a hybrid commercial tester and evaluated as hybrids in 2010. Evaluation of plants in the field in both 2009 (inbreds) and 2010 (hybrids) were compared in two well watered replicates, and two reduced water replicates in both College Station and Weslaco. Agronomic traits (height, flowering time, etc.), were also evaluated in the field. Leaf epicuticular wax (a potential predictor of drought tolerance) was extracted from the flag leafs of three plants using chloroform. Yield was measured by hand harvesting and shelling each plot. For the second study, seedlings were evaluated for drought tolerance in a highly replicated greenhouse study.

Epicuticular wax and drought tolerance: We evaluated sixty-two maize inbred lines and their hybrid testcross progeny for epicuticular wax accumulation on flag leaves at flowering under full and limited irrigation regimes. Extracted wax was measured as a percentage of wax weight to leaf weight (%wxlfwt) and leaf area (%wxwta). Eleven genotypes had above average %wxlfwt as both inbred lines and hybrid testcrosses. Thirteen genotypes had above average %wxwta as either inbred lines or hybrid testcrosses. Irrigation treatment was not significant ($P > 0.05$) for epicuticular wax or additional traits. Heritability of %wxlfwt was 0.17 (inbred lines) and 0.58 (hybrid testcrosses). Heritability of %wxwta was 0.41 (inbred lines) and 0.59 (hybrid testcrosses). Correlations (R^2) between inbred lines and their testcross progeny were 0.19 and 0.03 for %wxlfwt and %wxwta respectively. Heritability of grain weight per ear and plot yield was highest in hybrid testcrosses, with no correlation between inbred and hybrid germplasm. We concluded from this work that epicuticular wax is not an ideal primary trait to screen in inbreds or for total yield; however epicuticular wax is a good secondary trait to screen for in hybrids already known to be high-yielding.

Seedling drought tolerance: In 2009 and 2010, sixty-two maize inbred lines and their hybrid testcross progeny were evaluated in greenhouse environments for germination ability, seedling survival and recovery percentages after a series of drought cycles. For germination percentages, significant ($P < 0.05$) inbred lines were identified lower than the mean estimate of 83%, but no hybrid testcrosses were significantly different ($P > 0.05$) from the mean estimate 87%. Genotypic differences among inbred lines and hybrid testcrosses were best explained at approximately 13 and 18 days after planting, respectively. Heritability of inbred and hybrid genotypes were moderate. However, no genotypes performed well as both an inbred line and hybrid testcross. Poor

correlation over the sample set (R2 = 0.029) indicated seedling stress response from our germplasm is not a heritable trait and was not inherited in testcross progeny using our tester. Although this method allowed for easy visualization of seedling shoot response to water stress, seedling drought tolerance should be used as a secondary trait selection variable used amongst elite hybrid germplasm.

Resulting publications:

Meeks M., S. Murray, S. Hague, D. Hays, A. Ibrahim (submitted) Genetic Variation for Maize Epicuticular Wax Response to Drought Stress at Flowering. Journal of Agronomy and Crop Science.

Meeks M., S. Murray, S. Hague, D. Hays (submitted) Measuring Maize Seedling Drought Response in Search of Tolerant Germplasm. Maydica.

Meeks M, Two Approaches to Evaluate Drought Tolerance in Maize: Seedling Stress Response and Epicuticular Wax Accumulation. MS Dissertation.

***Objective 4*: Evaluate and improve a composite population across multiple locations.**

The College Station corn breeding program has developed a unique and modern composite population by crossing nine elite Argentinean hybrids among and between each other and selecting the best plants for eight cycles. In this project, different generations and derivatives of this Texas Argentine Composite (TAC) population were formally tested for yield and aflatoxin resistance, as they had been previously selected to maximize both. The ears of this population are very large, it is prolific (multiple ears) with high row number, it is very uniform in height and importantly *A. flavus* contamination and other fungi are rarely observed.

In cooperation with the Crop Testing Program (Dennis Peitch, Steve Labar, Dr. Jurg Blumenthal). Eight different generations of this population were planted in 11 locations along with the elite commercial hybrids entered into the crop testing program (Table 3). Samples from three of these locations (College Station, Corpus Christi and Thrall) were caught from the combine for aflatoxin testing (Table 2) and a fourth location (Leonard) was harvested by hand. Two generations were also placed in cooperative SERAT trials (data not shown).

Results were outstanding considering that this is an open pollinated population, and showed high yield potential. Overall the results were much better than expected in some locations such as the high plains and worse in others. Visual observations at poor performing sites suggest that the main barrier to yield was a variable flowering time under stress, resulting in some ears not being pollinated and others harvested barren without grain. Future selections will be made for increased consistency of flowering.

The TAC population continues to remain of interest for many reasons:

1) This population can be used as a source for deriving new inbreds. Some of the inbreds previously derived from this population had among the highest yields in hybrid trials across multiple locations and testers (Table 1, previous name of Argentine Flinty Composite). We expect higher levels of genetic diversity in these inbreds than found in current popular commercial inbreds including traits which will help to resist stress. Private industry and other breeders may be interested in

this population as a source for deriving new elite lines with increased genetic diversity.

2) This open-pollinated population may also be crossed to commercial Roundup Ready inbred lines to develop diverse hybrids all in one bag that might better minimize the risk of stress than a uniform hybrid. This will also make the flowering time more consistent which is expected to further increase yields. (This will be tested next year).
3) This population can be used as an inexpensive open pollinated alternative population without further deriving inbred lines or crossing. The advantages include a) cheap seed that no license would be required to grow and save seed from, and b) all organic production (transgene free). It is agreed that this is not a mainstream use but provides alternatives and can easily be put in the hands of growers. In Table 3 we estimated that if commercial seed is at $275 a bag and if we can produce this for $50 a bag, many producers would have made more money with less risk planting this open pollinated population. This is only one year of data and more testing is obviously needed, but it remains an interesting and potentially viable option for growers.

Table 2: Aflatoxin analysis of TAC at four locations each the mean of four replications. The rest of the commercial checks in the test were not included because of intellectual property restrictions.

	Aflatoxin (ppb)			
Population	LE	TH	CS	CC
TAC-1	1352.5	31.5	30.9	297.5
TAC-2	1122.5	76.1	22.3	258.5
TAC-3	2400.0	35.2	106.7	141.5
TAC-4	1260.0	40.1	30.8	186.8
TAC-5	378.0	16.0	64.7	233.5
TAC-6	1845.0	54.2	24.8	466.0
TAC-7	1392.5	17.5	78.2	705.0
TAC-8	1195.0	60.0	9.7	201.0

Objective 5: Host a field day. The field day was a success. Attendance by Scott Averhoff and Charles Ring of the Texas Corn Producers Board was greatly appreciated. A write-up can be found here: http://agnews.tamu.edu/showstory.php?id=2027 .

Table 3: Yield of eight derivatives of the Texas Argentine composite (TAC) across 11 locations and the return on investment (RTOI) that Texas Corn Producers would likely experience assuming $3.75 bu corn, $275 for a bag of commercial seed and $50 for TAC seed.

	CS (bu/ac)	BA (bu/ac)	DU (bu/ac)	WE (bu/ac)	LE (bu/ac)	WH (bu/ac)	DA (bu/ac)	TH (bu/ac)	CC (bu/ac)	HO (bu/ac)	TY (bu/ac)
TAC-1	116.3	72.7	208.6	117.2	48.1	100.3	226.2	52.3	68.1	124.6	141.3
TAC-2	126.7	67.9	205.0	113.9	51.4	98.8	224.6	55.2	68.2	125.5	139.7
TAC-3	112.7	76.0	210.6	111.0	52.1	102.5	215.3	48.0	62.6	122.7	133.5
TAC-4	115.6	78.8	205.9	116.4	46.1	100.7	207.6	54.2	74.4	121.2	140.9
TAC-5	116.2	71.5	235.4*	120.1	58.7*	91.8	262.4*	52.1	61.7	125.8	134.2
TAC-6	123.5	75.9	210.6	120.4	51.3	93.9	221.9	46.2	67.5	120.4	144.2
TAC-7	119.6	66.9	189.6	107.3	51.6	94.9	208.6	47.5	57.5	112.9	121.8
TAC-8	132.8	72.4	200.5	114.7	52.1	102.9	235.2	52.9	63.8	138.7	135.1
CTP Hybrid Test Mean	143.4	94.2	248.1	154.4	56.5	133.7	260.8	70.2	95.9	169.0	177.4
S.E. of CTP Test	6.8	2.5	5.7	3.3	9.1	4.6	5.1	2.6	4.6	3.9	4.3
Acre loss at $3.75 bu	-$39.52	-$57.97	-$47.64	-$127.60	$8.08	-$115.50	$6.12	-$56.51	-$80.74	-$113.68	-$124.29
Seed cost per acre											
Plant population	*22,438*	*23,734*	*32,031*	*24,502*	*20,832*	*22,545*	*31,899*	*20,832*	*18,818*	*26,100*	*27,588*
Commercial ($275 bag)	$77.13	$81.59	$110.11	$84.23	$71.61	$77.5	$109.65	$71.61	$64.69	$89.72	$94.83
TAC ($50 bag)	$14.02	$14.83	$20.02	$15.31	$13.02	$14.09	$19.94	$13.02	$11.76	$16.31	$17.24
Seed savings acre	$63	$67	$90	$69	$59	$63	$90	$59	$53	$73	$78
TAC RTOI per acre	**$23.59**	**$8.79**	**$42.45**	**-$58.68**	**$66.67**	**-$52.09**	**$95.83**	**$2.08**	**-$27.81**	**-$40.27**	**-$46.70**

* In three locations a TAC hybrid was used for TAC-5 because of a lack of seed.

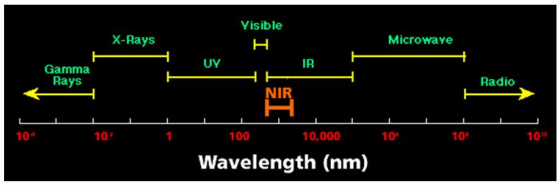
Supplemental 1: Use of near infrared spectroscopy for detecting aflatoxin in breeding programs.

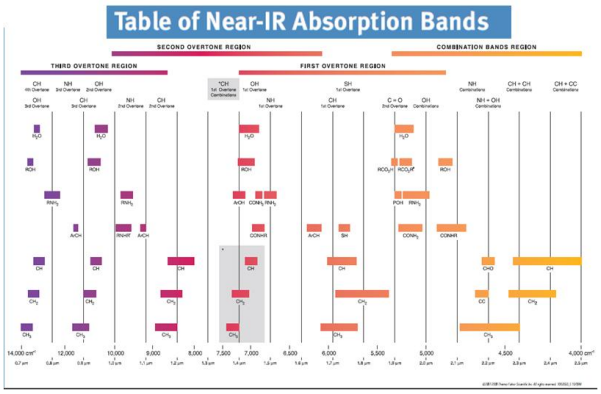
Problem: Measuring aflatoxin within a breeding program is extremely cost prohibitive. Multiple environments and replications are needed to test each cultivar, with high costs for labor and supplies. Therefore, if rare cultivars or genes that completely resist aflatoxin exist, they may not be found without expanding screening.

Potential Solution: Use near-infrared spectroscopy to test all corn samples collected in the breeding program. If successful this may be of interest for industry programs to easily incorporate for in house evaluation of a cultivars aflatoxin resistance.

Overview of NIRS: Near infrared (NIR) light is not visible to the human eye, just beyond the red part of the spectrum. Within the NIR spectrum, specific chemical bonds absorb NIR light at specific wavelengths. The amount of energy absorbed by a chemical compound is related to the amount in the sample (i.e. it is quantitative). Using a NIR spectrophotometer, an instrument that both emits a known amount of light and detects the reflected light, corn samples are scanned. Once a calibration to absorbed NIR light is developed for the trait(s) of interest (aflatoxin), the prediction of these traits will be immediate and nearly free.

NIRS calibrations can be *attempted* for any trait of interest (aflatoxin, protein, micronutrients, etc.) but a successful calibration depends on many things and it will not be known if it is successful until after completion. Based on multiple published articles, for instance Wicklow et al. (2006) and Fernández-Ibañez et al. (2009), we believe this technique would be useful. However, no previous studies have used corn of different genetic backgrounds or corn grown in different environments so we do not know if it will work in a breeding program.



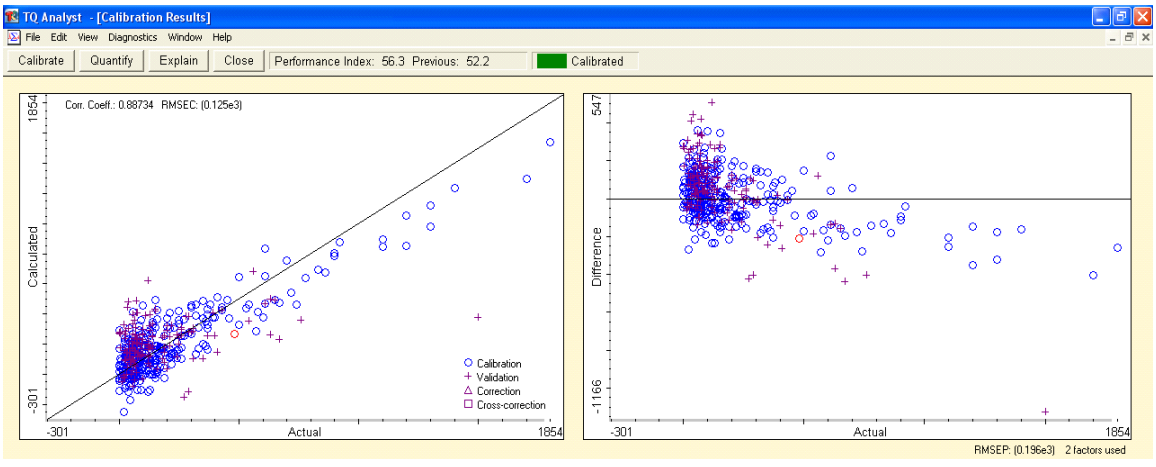


Steps to develop a NIRS calibration:

1) Scan all samples into machine using appropriate settings and under similar conditions.
2) Use integrated software features to select most informative samples for aflatoxin analyses and run Vicam Aflatest procedures on these samples.
3) Combine actual values from wet chemistry with spectra and run statistical analysis such as partial least-squares regression, principal component analysis (PCA) or other calibration techniques (This is important because you cannot tell what is contaminated just from looking at the spectra).
4) Use the best calibration to select new samples to include (return to step 3).
5) Include validation samples that are not used to develop the calibration to determine the "fit" and usefulness of the calibration.

Materials and Methods: Approximately 5000 different samples have been scanned as whole kernel corn. Approximately 2500 samples have been ground and scanned as ground kernel corn. These samples include TAMU breeding material, commercial lines, SERAT cooperative genetics tests with the USDA and others. Many of these samples we inoculated using silk channel, side needle, or spread innoculum procedures. The Vicam Aflatest procedures were then used to quantify the aflatoxin in a subset of samples (~1800 so far over 2 years). Ear ratings for visible *A. flavus* were taken on some samples (~1700). Finally, quantitative polymerase chain reaction (qPCR), was used by Dr. Marylin Warburton at the USDA to determine the percent of biomass from the fungus compared to the corn for samples from the 2009 cooperative USDA test samples (~800).

Results and discussion: Calibrations with decent fit to the Vicam Aflatest data and to ear rating data have been identified. The fit to *Aspergillus flavus* fungal biomass (measured by qPCR) has been poor so far. The calibrations within any one year or within any few cultivars were very good. Unfortunately as we added more complexity in the model with more cultivars and more environments, the predictions weakened. The manufacture of this NIRS equipment has never tried a project this large and complex before so they are continuing to work with us to improve the technique and calibration and we remain optimistic.



Limitations and Future uses of NIRS: The increased speed and decreased cost of NIRS will always require a tradeoff with lower accuracy compared to wet chemistry. ***Because of the limitation in accuracy NIRS will never be used for regulatory work.*** Importantly, however, NIRS allows us to test more samples and larger samples to see if they should be followed up with Vicam, or another test. The two main ways we envision it will be used instead are: 1. In breeding programs both public and private to screen material for ***preliminary*** resistance, which can later be followed up more extensively with official methods. 2. On producers combines, which will help to detect which fields or areas of fields have more *A. flavus* and potentially more toxin. This would give producers more information and might result in identifying areas that should be avoided, blended or treated more heavily with atoxigenic strains in the future.

References:

Fernández-Ibañez V, Soldado A, Martínez-Fernández A, De La Roza-Delgado B. 2009. Application of near-infrared spectroscopy for rapid detection of aflatoxin B1 in maize and barley as analytical quality assessment. Food Chem 113:629 - 34.

Wicklow, D.T., Pearson, T.C. 2006. Detection and removal of single mycotoxin contaminated maize grains following harvest. Proceedings of the 9th International Working Conference on Stored Product Protection. p. 109-119.