Supplementary Materials: Effects of different shelling methods on data variability during field screening for reduced aflatoxin contamination in maize

Table S1. Shelled Kernel Mass Measurements by Shelling Method

Line	Trait				CV	Q1	Q3	IQR
Method 1 - Whole Ear Shelling (WE)								
Inbreds								
B73	56.8	±	17.3	d	30.35	47.0	66.0	19.0
SynAM1 P43	105.0	±	21.9	c	20.90	91.5	116.8	25.3
Mp719	53.5	±	9.4	d	17.52	48.0	58.8	10.8
Hybrids								
SynAM1 P43 x B73	142.2	±	20.9	b	14.69	128.0	151.0	23.0
DKC 67-44	183.6	±	33.0	a	17.92	170.0	202.8	32.8
Method 2 - Ear End Removal Shelling (EER)								
Inbreds								
B73	23.3	±	5.8	f	24.72	19.2	27.1	8.0
SynAM1 P43	35.5	±	9.3	e	26.09	30.5	40.5	10.0
Mp719	20.6	±	4.5	f	21.91	18.5	23.5	5.0
Hybrids								
SynAM1 P43 x B73	35.9	±	6.3	e	17.46	32.3	40.9	8.5
DKC 67-44	50.1	±	7.5	d	14.91	45.6	54.8	9.2
Method 3 - Inoculation Site-Surrounding Shelling (ISS)								
Inbreds								
B73	3.2	±	1.3	g	41.49	2.2	3.8	1.6
SynAM1 P43	4.3	±	1.0	g	22.45	3.8	4.8	1.0
Mp719	2.9	±	0.7	g	25.09	2.4	3.3	0.9
Hybrids								
SynAM1 P43 x B73	4.3	±	1.0	g	22.38	3.7	5.0	1.3
DKC 67-44	6.1	±	1.3	g	20.80	5.3	6.8	1.6

Shelled kernel masses are average measurements of five pooled ears from ten replicate plots in grams (g) ± standard deviation. CV - Coefficient of Variance (100x(standard deviation / mean for all reps for a given line)), Q1 - Lower Quartile, Q3 - Upper Quartile, IQR - Interquartile Range. Measurements within a trait possessing the same letter labels were not significantly different based on Tukey's Post-hoc Analysis (α = 0.05). The shelled kernel mass analysis was based on two-way factorial ANOVA with the assumption of normal data distribution.

Table S2. Analysis of Average Shelled Kernel Mass

Kruskal-Wallis Rank Sum Test[1]				
Model	**Df**	**X²**	**P-Value**	
Mass ~ Treatment	2	127.32	< 2.2E-16	***
Mass ~ Line	4	18.52	< 2.2E-16	***
Mass ~ Rep	4	0.044	0.9998	
Mass ~ Row	1	0.02	0.8887	

Dunn's Test for Multiple Comparisons[2]				
Comparison	**Z**	**Unadjusted P-Value**	**Adjusted P-Value**	
By Treatment				
EER v. ISS	5.80	6.55E-09	1.96E-08	***
EER v. WE	-5.54	3.06E-08	9.18E-08	***
ISS v. WE	-11.3	1.59E-29	4.76E-29	***
Pairwise Comparisons[3]				
B73 ISS v. B73 WE	-4.87	1.09E-06	1.15E-04	***
B73 ISS v. DKC 67-44 EER	-4.47	8.00E-06	8.40E-04	***
B73 EER v. DKC 67-44 WE	-4.29	1.78E-05	1.87E-03	**
B73 ISS v. DKC 67-44 WE	-6.77	1.28E-11	1.35E-09	***
DKC 67-44 ISS v. DKC 67-44 WE	-4.89	9.92E-07	1.04E-04	***
DKC 67-44 WE v. Mp719 EER	4.55	5.34E-06	5.60E-04	***
B73 WE v. Mp719 ISS	5.09	3.56E-07	3.74E-05	***
DKC 67-44 EER v. Mp719 ISS	4.68	2.85E-06	2.99E-04	***
DKC 67-44 WE v. Mp719 ISS	6.99	2.81E-12	2.95E-10	***
B73 ISS v. Mp719 WE	-4.69	2.78E-06	2.91E-04	***
Mp719 ISS v. Mp719 WE	-4.9	9.43E-07	9.90E-05	***
Mp719 ISS v. P43 EER	-3.59	3.29E-04	3.45E-02	*
B73 WE v. P43 ISS	4.07	4.73E-05	4.97E-03	**
DKC 67-44 EER v. P43 ISS	3.66	2.53E-04	2.66E-02	*
DKC 67-44 WE v. P43 ISS	5.96	2.45E-09	2.57E-07	***
Mp719 WE v. P43 ISS	3.88	1.04E-04	1.09E-02	*
B73 ISS v. P43 WE	-5.73	1.02E-08	1.07E-06	***
DKC 67-44 ISS v. P43 WE	-3.91	9.24E-05	9.70E-03	**
Mp719 EER v. P43 WE	-3.51	4.52E-04	4.74E-02	*
Mp719 ISS v. P43 WE	-5.94	2.78E-09	2.92E-07	***
P43 ISS v. P43 WE	-4.92	8.59E-07	9.02E-05	***
Mp719 ISS v. P43 x B73 EER	-3.6	3.16E-04	3.32E-02	*
B73 WE v. P43 x B73 ISS	4.02	5.71E-05	6.00E-03	**
DKC 67-44 EER v. P43 x B73 ISS	3.61	3.01E-04	3.16E-02	*
DKC 67-44 WE v. P43 x B73 ISS	5.92	3.21E-09	3.37E-07	***

Mp719 WE v. P43 x B73 ISS	3.84	1.25E-04	1.31E-02	*
P43 WE v. P43 x B73 ISS	4.88	1.08E-06	1.13E-04	***
B73 EER v. P43 x B73 WE	-3.77	1.64E-04	1.72E-02	***
B73 ISS v. P43 x B73 WE	-6.25	4.13E-10	4.34E-08	***
DKC 67-44 ISS v. P43 x B73 WE	-4.4	1.07E-05	1.13E-03	**
Mp719 EER v. P43 x B73 WE	-4.03	5.59E-05	5.87E-03	**
Mp719 ISS v. P43 x B73 WE	-6.47	1.01E-10	1.06E-08	***
P43 ISS v. P43 x B73 WE	-5.44	5.24E-08	5.50E-06	***
P43 x B73 ISS v. P43 x B73 WE	-5.4	6.71E-08	7.04E-06	***

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Two-Way Analysis of Variance[4]

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Factor	Df	Sums of Squares	Mean Sums of Squares	F-Value	P-Value	
Line	4	70,758	17,689	449.057	< 2.0E-16	***
Treatment	2	279,133	139,566	3542.979	< 2.0E-16	***
Rep	4	33	8	0.208	0.933	
Row	1	35	35	0.899	0.346	
Line:Treatment	8	65,781	8223	208.736	< 2.0E-16	***
Line:Treatment:Rep	55	2227	40	1.028	0.452	
Residuals	73	2876	39			

[1]Kruskal-Wallis test allows for analysis of variance without the assumption of a normal data distribution. [2]Dunn's test allows for multiple comparisons. It also does not have the assumption of normal data distributions. P-values were adjusted using the Bonferroni method. [3]Of the 105 possible pairwise comparisons, only the pairs with adjusted p-values < 0.05 are shown. The "SynAM1" of P43 was removed for simplicity. [4]Two-way ANOVA performed on raw data. Results may overestimate significance due to non-normal distribution. However, results generally agree with the Kruskal-Wallis and Dunn tests. Significant at $P < 0.05$ *, $P < 0.01$ **, and $P < 0.001$ ***.

Table S3. Total and Filled Ear Lengths Measured for Each Line

Line	Trait				CV	Q1	Q3	IQR
Total Ear Length (mm)								
Inbreds								
B73	135.4	±	11.5	e	8.46	129.0	142.0	13.0
SynAM1 P43	162.7	±	16.1	c	9.89	151.0	172.0	21.0
Mp719	144.1	±	13.8	d	9.61	137.0	152.0	15.0
Hybrids								
SynAM1 P43 x B73	179.8	±	14.1	b	7.86	170.0	189.5	19.5
DKC 67-44	190.7	±	13.1	a	6.85	183.0	200.5	17.5
Kernel Filled Ear Length (mm)								
Inbreds								
B73	122.4	±	13.1	e	10.71	115.0	132.0	17.0
SynAM1 P43	149.6	±	17.2	c	11.49	138.0	160.0	22.0
Mp719	132.3	±	14.0	d	10.60	126.0	140.0	14.0
Hybrids								
SynAM1 P43 x B73	169.2	±	15.5	b	9.16	161.0	180.0	19.0
DKC 67-44	181.3	±	15.5	a	8.54	175.0	190.5	15.5
Percent Ear Fill (%)								
Inbreds								
B73	90.4	±	6.2	b	6.90	86.2	95.0	8.8
SynAM1 P43	92.0	±	5.5	b	5.92	89.5	95.6	6.1
Mp719	91.9	±	6.0	b	6.50	89.2	95.8	6.7
Hybrids								
SynAM1 P43 x B73	94.2	±	5.2	a	5.55	92.6	97.0	4.4
DKC 67-44	95.1	±	5.3	a	5.61	93.4	98.9	5.5

Each line had a max of 150 ears. Measurements within a trait possessing the same letter labels were not significantly different based on Tukey's Post-hoc Analysis (α = 0.05). CV - Coefficient of Variance (100x(standard deviation / mean for all reps for a given line)), Q1 - Lower Quartile, Q3 - Upper Quartile, IQR - Interquartile Range.

Table S4. Two-Way Factorial Analyses of Variance of Ear Length and Filling

Factor	Df	Sums of Squares	Mean Sums of Squares	F-Value	P-Value	
Ear Length						
Line	4	306,876	76,719	422.46	<2.00E-16	***
Treatment	2	3,017	1,508	8.31	0.0003	***
Rep	4	1,096	274	1.51	0.2	
Row	1	1	1	0.004	0.95	
Line:Treatment	8	1,936	242	1.33	0.22	
Line:Treatment:Rep	54	13,699	254	1.40	0.04	*
Residuals	648	117,676	182			
Ear Fill Length						
Line	4	342,193	85,548	379.44	<2.00E-16	***
Treatment	2	150	75	0.33	0.72	
Rep	4	934	234	1.04	0.39	
Row	1	10	10	0.04	0.84	
Line:Treatment	8	1,239	155	0.69	0.7	
Line:Treatment:Rep	54	16,124	299	1.32	0.07	
Residuals	648	146,096	225			

Significant at $P < 0.05$ *, $P < 0.01$ **, and $P < 0.001$ ***.

Figure S1. Example of kernel and ear size variation within a replicate plot inoculated with *Aspergillus flavus* strain AF13 for each of the shelling methods- whole ear shelling (WE, top), ear end removal shelling (EER, middle), and inoculation site-surrounding shelling (ISS, bottom). White box indicates example of typical ear damage (i.e., insect and improper filling).
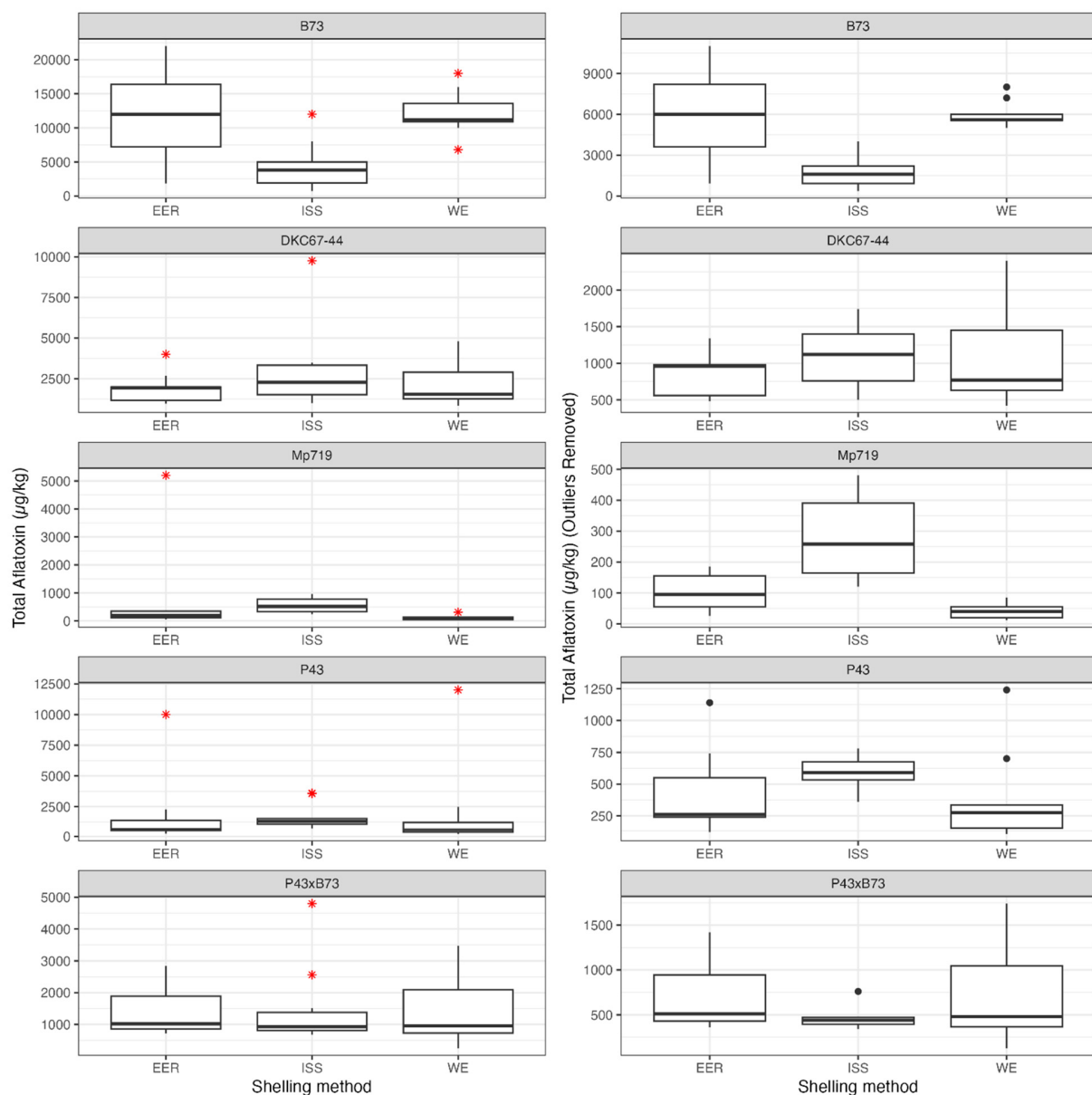
Figure S2. Boxplots of raw aflatoxin levels (µg/kg) for each line per each shelling method. Outliers identified by red asterisks (left) were removed from the raw data (right) before $\log_2(x+1)$ transformed. WE is whole ear shelling, EER is ear end removal shelling, and ISS is inoculation site-surrounding shelling. The "SynAM1" of P43 was removed for simplicity.
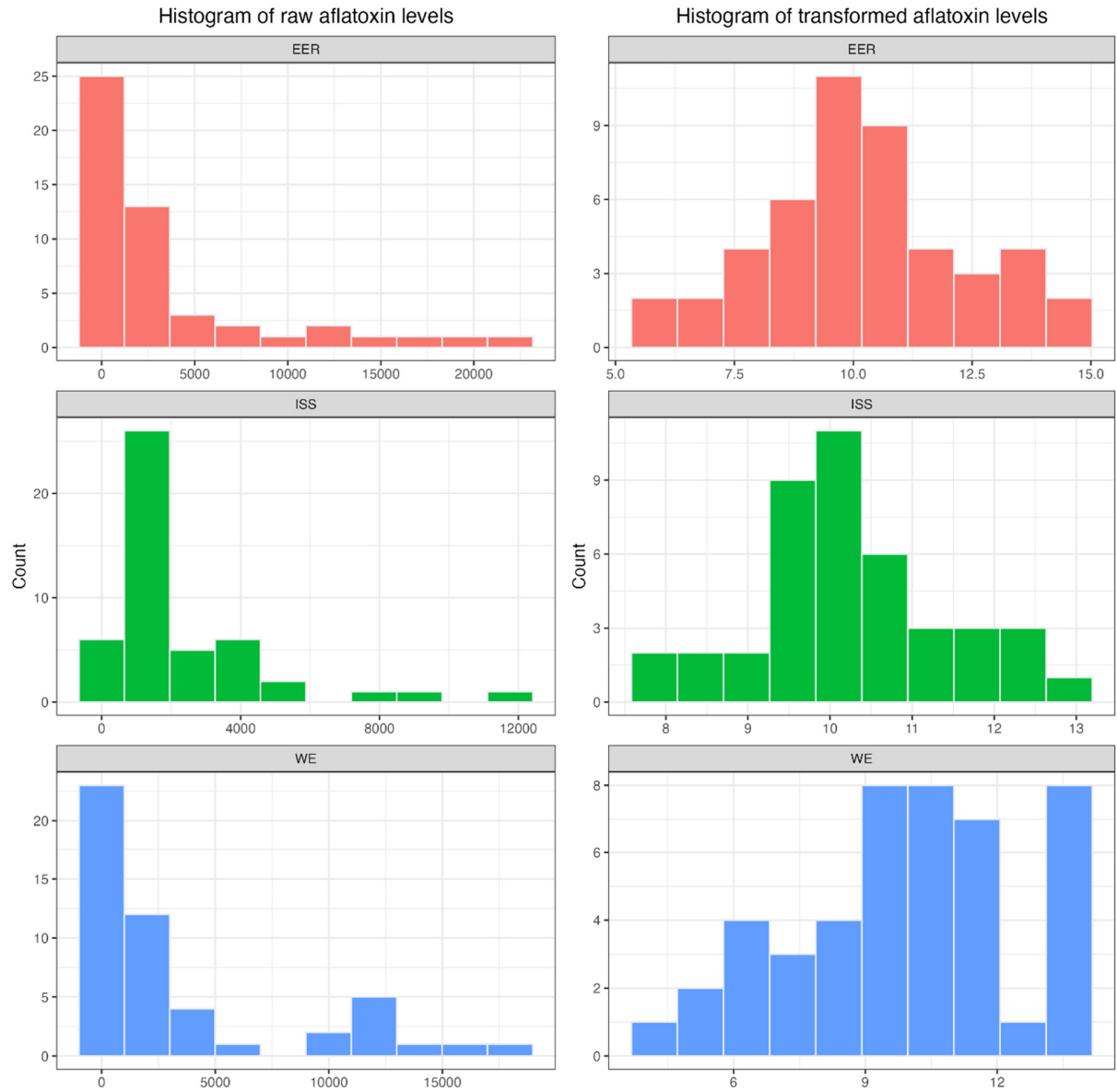
Figure S3. Histogram of raw and $\log_2(x+1)$ transformed aflatoxin levels (μg/kg) for each shelling method. Outliers from the raw data are excluded in the transformation and 10 bins were used for the histogram. WE is whole ear shelling, EER is ear end removal shelling, and ISS is inoculation site-surrounding shelling.