

Rule Change Proposal No. 2

PURPOSE: To add wheat and field beans to the crops included in **3.7 Seed count test for soybean (*Glycine max*) and corn (*Zea mays*)** in the AOSA Rules.

PRESENT RULE and PROPOSED RULE: (New wording is underlined, deleted wording is stroked out.)

3.7 Seed count test for soybean (*Glycine max*) ~~and~~, corn (*Zea mays*), wheat (*Triticum aestivum*) and field bean (*Phaseolus vulgaris*) --The following method shall be employed when using a mechanical seed counter to determine the number of seeds contained in a sample.

- a. *Samples*--Samples for testing shall be of at least 500 grams for soybean, corn and field beans and 100 grams for wheat and received in moisture proof containers. Samples shall be retained in moisture proof containers until the weight of the sample prepared for purity analysis is recorded.
- b. *Seed counter calibration*--The seed counter shall be calibrated daily prior to use.
 - (1) Prepare a calibration sample by counting 10 sets of 100 seeds. Visually examine each set to insure that it contains whole seeds. Combine the 10 sets of seeds to make a 1,000 seed calibration sample. The seeds of the calibration sample should be approximately the same size and shape as the seeds in a sample being tested. If the seeds in a sample being tested are noticeably different in size or shape from those in the calibration sample, prepare another calibration sample with seeds of the appropriate size and shape. Periodically re-examine the calibration samples to insure that no seeds have been lost or added.
 - (2) Carefully pour the 1,000 seed calibration sample into the seed counter. Start the counter and run it until all the seeds have been counted. The seeds should not touch as they run through the counter. Record the number of seeds as displayed on the counter read out. The seed count should not vary more than + or - 2 seeds from 1,000. If the count is not within this tolerance, clean the mirrors, adjust the feed rate and/or reading sensitivity. Rerun the calibration sample until it is within the + or - 2 seed tolerance. If the seed counter continues to fail the calibration procedure and the calibration sample has been checked to ensure that it contains 1,000 seeds, do not use the counter until it has been repaired.
- c. *Sample preparation*--Immediately after opening the moisture proof container, mix and divide the submitted sample to obtain a sample for purity analysis and record the weight of this sample in grams to four significant figures. Conduct the purity analysis to obtain pure seed for the seed count test.
- d. *Conducting the test*--After the seed counter has been calibrated, test the pure seed portion from the purity test and record the number of seeds in the sample.
- e. *Calculation of results*--Calculate the number of seeds per pound to the nearest whole number for using the following formula:

$$\text{Number of seeds per pound} = \frac{453.6 \text{ g/lb} \times \text{no. of seeds counted in d}}{\text{weight (g) of sample analysed for purity}}$$

- f. *Tolerances for results from different laboratories*--Multiply the labeled seed count or first seed count test result by 4% for soybean samples ~~and~~ 2% for corn (round, flat or plateless) samples, 5% for field bean samples and 3% for wheat samples. Express the tolerance (the number of seeds) to the nearest whole number. Consider the results of 2 tests in tolerance if the difference, expressed as the number of seeds, is equal to or less than the tolerance.

SUPPORTING EVIDENCE

Wheat Seed Count Referee Tests:

Seed count referee tests were conducted on two samples of soft red winter (SRW) wheat (tables 1&2), one sample of soft white winter wheat (SWW) wheat (table 3), and one sample of hard red spring (HRS) wheat (table 4) following the seed count testing procedure in the AOSA Rules for corn and soybeans. Large samples of seed provided for each of these referee tests were mixed and divided to obtain samples for testing that were slightly in excess of 800 grams. Each of the 13 participating laboratories received a sample which they mixed and divided to

obtain eight samples of 100 grams each. One sample provided seed for calibrating the seed counter and six of the samples were used for seed counting.

An Analysis of Variance of the data obtained from each of the four referee tests was conducted and 95% confidence intervals calculated. The confidence intervals can be used as tolerances and take into account both the variation in seed counts among laboratories, as well as the variation within laboratories. The tolerances were 1.2% and 1.4% for the SRW samples, 1.3% for the SWW sample and 1.1% for the HRS sample.

An additional referee test, involving four laboratories was conducted to obtain an estimate of the variation in seed counts among composite samples from the same seed lot. Three composite samples (Samples 1, 2, and 3) were obtained from each of three SRW wheat seed lots (Lot MD, Lot MI, and Lot IL). Each composite sample was mixed and divided to provide samples of 200 grams which were sent to each of the four laboratories.

The participating laboratories mixed and divided each 200 gram sample to obtain a 100 gram sample for testing and a second 100 gram sample for use in seed counter calibration. The laboratories counted the 100 gram sample they prepared for testing three times. There was little, if any, variation among the replicate counts of the same sample by a laboratory.

The average variation in seed counts among the three composite samples from the same seed lot was 0.58% for Lot MD, 0.66% for Lot MI, and 0.64% for Lot IL (table 5).

				SAMPLE					
Lab. #	Average	Rang	R/A (%)	1	2	3	4	5	6
1	13,929	113	0.81	13,871	13,898	13,912	13,971	13,935	13,984
2	14,091	325	2.31	13,925	14,083	13,957	14,214	14,250	14,119
3	14,033	218	1.55	14,121	14,124	14,072	14,038	13,906	13,938
4	14,047	155	1.10	14,010	14,059	14,040	14,031	13,993	14,148
5	14,039	233	1.66	14,040	14,181	13,948	14,001	14,062	14,004
6	14,125	173	1.22	14,090	14,136	14,135	14,216	14,127	14,043
7	14,024	204	1.45	13,903	13,758*	14,107	14,075	14,052	13,981
8	14,046	218	1.55	14,041	14,049	14,008	14,060	13,965	14,183
9	14,043	159	1.13	14,057	14,134	14,053	13,975	13,789*	13,994
10	14,025	197	1.40	14,076	14,113	13,916	13,919	14,038	14,085
11	14,087	158	1.12	14,129	14,034	13,994	14,065	14,148	14,152
12	14,036	133	0.95	14,015	13,990	14,047	14,108	13,975	14,082
13	14,050	190	1.35	14,139	14,037	14,035	14,082	13,949	14,058
AVG.	14,045	190	1.35						

Results expressed as seeds per pound. * considered an outlier and not used in calculations. Range – The difference between the highest and lowest replicate.

Table 2. SEED COUNT REFEREE TESTS RESULTS (SEEDS/LB) FOR SOFT RED WINTER WHEAT

Table 2. SEED COUNT REFEREE TESTS RESULTS (SEEDS/LB) FOR SOFT RED WINTER WHEAT									
				SAMPLE					
Lab. #	Average	Rang	R/A (%)	1	2	3	4	5	6
1	11,902	197	1.66	11,885	12,003	11,880	11,977	11,806	11,863
2	11,831	227	1.92	11,966	11,756	12,116*	11,839	11,739	11,856
3	11,912	135	1.13	11,871	11,896	12,006	11,881	11,906	11,786*
4	11,844	165	1.39	11,800	11,831	11,785	11,812	11,888	11,950
5	11,967	123	1.03	11,989	11,990	11,940	11,915	11,929	12,038
6	11,922	168	1.41	11,957	11,981	11,813	11,922	11,890	11,967
7	11,936	154	1.29	11,917	11,898	11,890	11,929	12,044	11,938
8	11,954	136	1.14	12,016	11,971	11,931	12,015	11,880	11,909
9	11,865	149	1.26	11,866	11,884	11,794	11,825	11,943	11,875
10	11,943	89	0.75	11,985	11,896	11,925	11,970	11,917	11,965
11	12,057	157	1.30	11,996	12,061	12,034	11,997	12,102	12,153
12	11,883	170	1.43	11,818	11,814	11,818	11,932	11,984	11,932
13	11,939	145	1.21	11,999	11,952	11,854	11,976	11,875	11,976
AVG.	11,920	155	1.30						

Results expressed as seeds per pound. * Considered an outlier and not used in calculations. Range - The difference between the highest and lowest replicate.

Table 3. SEED COUNT REFEREE TESTS RESULTS (SEEDS/LB) FOR SOFT WHITE WINTER WHEAT									
				SAMPLE					
Lab. #	Average	Range	R/A (%)	1	2	3	4	5	6
1	12,527	90	0.72	12,524	12,587	12,501	12,497	12,279*	12,374*
2	12,464	202	1.62	12,445	12,557	12,535	12,355	12,512	12,382
3	12,415	199	1.60	12,307	12,506	12,399	12,451	12,375	12,452
4	12,402	106	0.85	12,445	12,369	12,373	12,354	12,460	12,412
5	12,434	136	1.09	12,492	12,356	12,427	12,448	12,453	12,426
6	12,422	88	0.71	12,416	12,464	12,435	12,376	12,395	12,446
7	12,326	162	1.31	12,363	12,329	12,329	12,303	12,398	12,236
8	12,400	67	0.54	12,398	12,406	12,409	12,436	12,384	12,369
9	12,421	50	0.40	12,168*	12,445	12,395	12,421	12,423	12,421
10	12,379	136	1.10	12,324	12,320	12,424	12,451	12,442	12,315
11	12,342	204	1.65	12,283	12,243	12,447	12,442	12,302	12,333
12	12,309	212	1.72	12,356	12,202	12,317	12,414	12,359	12,204
13	12,441	134	1.08	12,431	12,423	12,485	12,449	12,361	12,495
AVG.	12,406	137	1.10						

Results expressed as seeds per pound. Range - The difference between the highest and lowest replicate. * The seed count data from replicates 5 & 6 from laboratory 1 and replicate 1 from laboratory 9 were considered outliers and not used when calculating the average, range, and % in the table, as well as when conducting the Analysis of Variance (ANOVA).

				SAMPLE					
Lab. #	Averag	Range	R/A	1	2	3	4	5	6
1	13,537	246	1.82	13,586	13,677	13,497	13,467	13,431	13,561
2	13,498	275	2.04	13,389	13,849	13,380	13,655	13,621	13,445
3	13,542	160	1.18	13,473	13,613	13,455	13,525	13,572	13,615
4	13,572	98	0.72	13,621	13,608	13,572	13,523	13,531	13,577
5	13,595	184	1.35	13,708	13,524	13,590	13,528	13,576	13,643
6	13,593	194	1.43	13,697	13,578	13,581	13,503	13,591	13,608
7	13,610	93	0.68	13,660	13,600	13,626	13,595	13,455	13,567
8	13,622	200	1.47	13,552	13,621	13,730	13,591	13,706	13,530
9	13,610	73	0.54	13,558	13,631	13,631	13,631	13,594	13,617
10	13,593	137	1.01	13,519	13,508	13,612	13,645	13,633	13,642
11	13,593	50	0.37	13,617	13,572	13,590	13,622	13,581	13,576
12	13,640	148	1.09	13,685	13,608	13,635	13,698	13,661	13,550
13	13,579	139	1.02	13,513	13,524	13,604	13,621	13,559	13,652
AVG.	13,583	154	1.13						

Results expressed as seeds per pound. Range - The difference between the highest and lowest replicate.

* The seed count data from replicate 2 from laboratory 2 and replicate 5 from laboratory 7 were considered outliers and not used when calculating the average, range, and % in the table, as well as when conducting the Analysis of Variance (ANOVA).

Lab.	Lot MD				Lot MI				Lot IL			
	Samples				Samples				Samples			
	1	2	3	Avg.	1	2	3	Avg.	1	2	3	Avg.
3	12,277	12,233	12,176	0.55%	9,127	9,114	9,080	0.34%	11,108	10,997	11,072	0.24%
5	12,423	12,440	12,301	0.75%	9,111	9,223	9,101	0.89%	11,031	11,027	11,054	0.16%
7	12,366	12,394	12,305	0.48%	9,103	9,179	9,066	0.82%	11,016	10,998	11,118	0.72%
10	12,461	12,429	12,364	0.52%	9,112	9,195	9,119	0.60%	11,214	10974	11,050	1.44%
Avg.				0.58%				0.66%				0.64%

Results expressed as seeds per pound. Avg. Var. = The average variation among seed counts from different samples obtained from the same seed lot. This figure was determined by calculating the average difference in seed counts from the three seed samples from the same lot and dividing this number by the average seed count of the three samples.

Field Bean Seed Count Referee Tests:

Seed count referee tests were conducted on samples of kidney bean (tables 6), cranberry bean (table 7), and navy bean (table 8) following the seed count testing procedure in the AOSA Rules for corn and soybeans. Large

samples of seed provided for each of these referee tests were mixed and divided to obtain samples for testing that were slightly in excess of 1,000 grams. Each of the six participating laboratories received two samples of 1,000 grams of each type of bean. One 1,000 gram sample provided seed for calibrating the seed counter. The other 1,000 gram sample was mixed and divided to provide two 500 gram samples for counting. After the two 500 gram samples were counted, they were combined, mixed and divided to provide two additional samples for counting. This process was repeated until a total of six samples were counted.

An Analysis of Variance of the data obtained from each of the four referee tests was conducted and 95% confidence intervals calculated. The confidence intervals can be used as tolerances and take into account both the variation in seed counts among laboratories, as well as the variation within laboratories. The tolerances were 2.1% for the kidney bean sample, 0.9% for the cranberry bean sample and 1.3% for the navy bean sample.

An additional test, involving one laboratory was conducted to obtain an estimate of the variation in seed counts among composite samples from the same seed lot. Three composite samples (Samples 1, 2, and 3) were obtained from one lot kidney bean, cranberry bean, and navy bean were obtained. Each composite sample was mixed and divided to provide samples of 500 grams which were counted. The average variation in seed counts among the three composite samples from the same seed lot was 1.85% for kidney bean, 1.05% for cranberry bean, and 2.68% for navy bean (table 9).

Table 6. SEED COUNT REFEREE TESTS RESULTS (SEEDS/LB) FOR									
				SAMPLE					
Lab.	Aver	Rang	R/A	1	2	3	4	5	6
1	968	17	1.76	958	974	969	967	966	975
2	969	23	2.37	971	966	965	971	959	982
3	949	6	0.63	949	951	947	948	946	952
4	951	25	2.63	956	946	954	948	939	964
5	954	11	1.15	952	951	959	950	960	949
6	949	4	0.42	949	946	949	947	950	950
AVG	957	14.33	1.50						

Results expressed as seeds per pound.

Range - The difference between the highest and lowest replicate.

Table 7. SEED COUNT REFEREE TESTS RESULTS (SEEDS/LB) FOR									
				SAMPLE					
Lab.	Aver	Rang	R/A	1	2	3	4	5	6
1	1133	9	0.79	1129	1135	1133	1131	1138	1129
2	1136	7	0.62	1134	1138	1136	1134	1141	1134
3	1132	8	0.71	1127	1134	1134	1128	1135	1131
4	1129	15	1.33	1130	1126	1136	1121	1126	1132
5	1136	8	0.70	1138	1133	1133	1136	1133	1141
6	1131	23	2.03	1132	1129	1126	1135	1120	1143
AVG	1133	11.67	1.03						

Results expressed as seeds per pound.

Range - The difference between the highest and lowest replicate.

Table 8. SEED COUNT REFEREE TESTS RESULTS (SEEDS/LB) FOR NAVY									
				SAMPLE					
Lab.	Aver	Rang	R/A	1	2	3	4	5	6
1	2031	11	0.54	2035	2028	2029	2035	2036	2025
2	2020	5	0.25	2021	2018	2016	2021	2021	2020
3	2017	4	0.20	2018	2014	2019	2019	2015	2018
4	2050	17	0.83	2048	2050	2058	2041	2045	2056
5	2046	15	0.73	2043	2048	2051	2037	2045	2052
6	2039	22	1.08	2034	2038	2032	2043	2051	2029
AVG	2034	12.33	0.61						

Results expressed as seeds per pound.

Range - The difference between the highest and lowest replicate.

Table 9. FIELD BEAN LOT SAMPLE SEED COUNT TEST RESULTS (SEEDS/LB)												
	KIDNEY				CRANBERRY				NAVY			
	Samples				Samples				Samples			
	1	2	3	Avg.	1	2	3	Avg.	1	2	3	Avg.
	918	906	931	1.85%	1,144	1,136	1,154	1.05%	2,720	2,628	2,613	2.68%

Results expressed as seeds per pound. Avg. Var. = The average variation among seed counts from different samples obtained from the same seed lot. This figure was determined by calculating the average difference in seed counts from the three seed samples from the same lot and dividing this number by the average seed count of the three samples.

SUBMITTED BY

Steve McGuire
 Michigan Department of Agriculture Laboratory
 1615 South Harrison Road
 East Lansing, MI 48823
 (517) 337-5084

DATE OF PROPOSAL: July 24, 2001