REGION IV FORAGE KOCHIA REFEREE 2014

This referee is on Bassia prostrata, Forage Kochia. It is an introduced species that is drought and salt tolerant. It occurs in several Western states. It is excellent forage for wildlife and livestock. It can also be used as a firebreak by planting in strips to create a green barrier that won't burn on a range wildfire. Seed is sometimes grown as a crop and many times harvested from wildland stands. An average cost is about $15/lbs. For firebreaks it is seeded at about 2.5 to 3 pounds per acre.

Seed companies and growers continue to comment on the disparity in test results on this high value crop.

Objectives

We wanted to review testing protocols by testing viability, both germination test and Tz test, and doing a purity analysis. Also we wanted to review the need for a chill period in the germination test method. We wanted to find possible sources of variability in any of these tests.

Methods

We had nine participants which included some labs outside of the Southwest Region.

We tested two samples, one of better quality than the other. The samples were mixed, divided and weighed at our lab, then were sent out.

The following instructions were given.

PART A. Determine the pure seed, inert matter and other seed for 2 different forage kochia lots.

1. Screen the working sample as described under PSU 38 in the AOSA Rules.

   "For *Bassia prostrata,* seed units that are retained on a 1-mm opening square-hole sieve, when shaken for 30 seconds shall be considered pure seed units. Seed units that pass through the 1-mm sieve shall be classified as inert matter." (AOSA Rules, pg. 3-25, 2013)

2. Separate pure seed units retained on top of the screen from weed seed, other crop seed and inert matter (e.g. rocks, leaves etc.). Record weight of pure seed units.
3. Separate out weed and other crop seeds that have passed through the screen, combine with weed and crop from #2, record weight ("other").
4. Combine inert matter (e.g. rocks, leaves etc.) from top of screen and seed units and other inert matter that has passed through the screen). Record weight of inert matter.
5. Record the working sample weight for each sample on answer sheet.
6. Calculate purity % and record.

PART B. Determine germination percentages for each sample; with pre-chill and no pre-chill.

1. Plant 400 seeds (from each sample) in P. Prechill at 5°C for 14 days, then germinate at 20°C for 4/7 day counts. Evaluate the germination, determine abnormals, record results. Test ungerminated seeds for dormancy using TZ; record results.
2. Plant 400 seeds (from each sample) in P. Germinate at 20°C for 4/7 day counts. Evaluate the germination, record results. Test ungerminated seeds for dormancy using TZ; record results.

PART C. Determine stand alone TZ test results for each sample.

1. Plant and imbibe overnight, 2 replicates of 100 seeds from each sample.
2. Refer to the AOSA/SCST Tetrazolium Testing Handbook, 2010 edition, Family *Chenopodiaceae* page. Follow Preparation and Staining Method (1) and "Notes" to prepare the seed to imbibe TZ and stain. ("Cut between tips of radicle and cotyledons; … Or pierce at center and drag needle between radicle and cotyledons"). Place seeds in TZ solution; Stain for time/temp recommended.
3. Evaluate embryos according to Evaluation instructions in the AOSA/SCST Tetrazolium Testing Handbook, 2010 edition (Family *Chenopodiaceae)*. Record results.

We wanted to have analysts review the current testing "procedures" for Kochia. We gave instructions for doing a germination test and determining any dormant seeds by using Tz on ungerminated seeds at the end of germ test to get Total Viable Percentage.

We also wanted to see if not using a chill period in the germ test would give the same total viable percentage as a germ test with chill. So we did this second test.

We did a stand alone Tz test.

We did a purity analysis to determine the pure seed percentage component. Purity analysis includes screening or sieving the seed. As you can see, PSU 38 given in the Rules describes this procedure:

Special consideration: For Bassia prostate, seeds that are retained on a 1-mm opening square-hole sieve, when shaken for 30 seconds shall be considered pure seed units. Seed units that pass through the 1-mm sieve shall be classified as inert matter. (AOSA Rules, pg. 3-25, 2013)

We also asked three questions related to doing a purity analysis. These will be given later in this report.

Data Results

Forage Kochia Referee Analysis Results

Sample A						Sample B			
Lab #	Germ with Pre-Chill (Total Viable %)	Germ with-out Chill (Total Viable %)	Tz % Sample A	Pure Seed % Sample A		Germ with Pre-Chill	Germ with-out Chill	Tz % Sample B	Pure Seed % Sample B
1	28	20	29	73.20		79	72	76	90.92
3	18	19	19	73.86		66	76	66	85.75
4	21	22	15	68.23		76	73	63	87.47
6	27	22	30	73.20		78	75	84	85.57
7	15	15	28	78.94		76	71	86	88.43
8	21	20	16	72.10		71	75	76	86.51
9	21	34	14	71.10		77	72	64	85.39
10	10	10	10	78.41		61	52	65	89.65
14	22	20	19	69.91		73	77	87	87.61
Avg	20	20	20	73		73	71	74	87
Max	28	34	30	78.94		79	77	87	90.92
Min	10	10	10	68.23		61	52	63	85.39
Sd	5.6	6.4	7.3	3.6		6	7.6	9.9	1.9

Another look at the results is in these bar graphs.

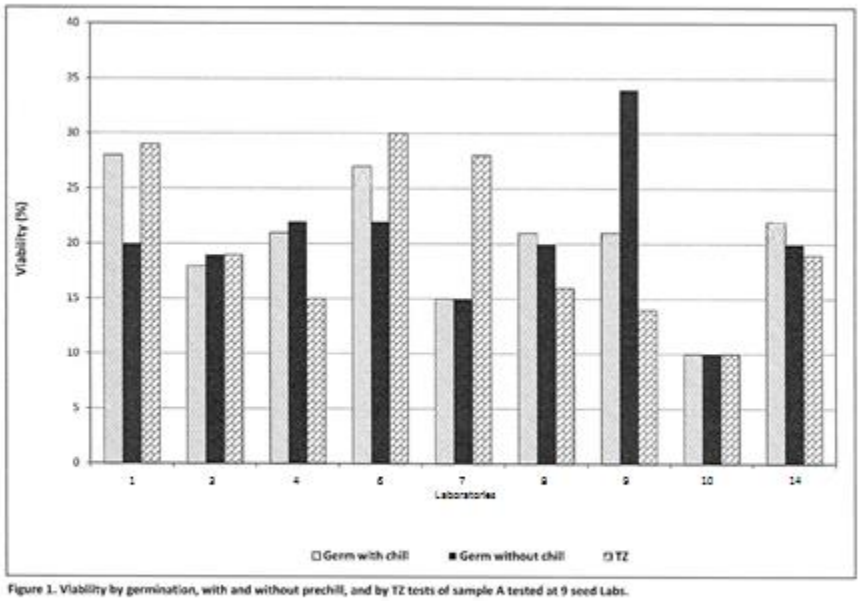

Figure 1. Viability by germination, with and without prechill, and by TZ tests of sample A tested at 9 seed Labs.

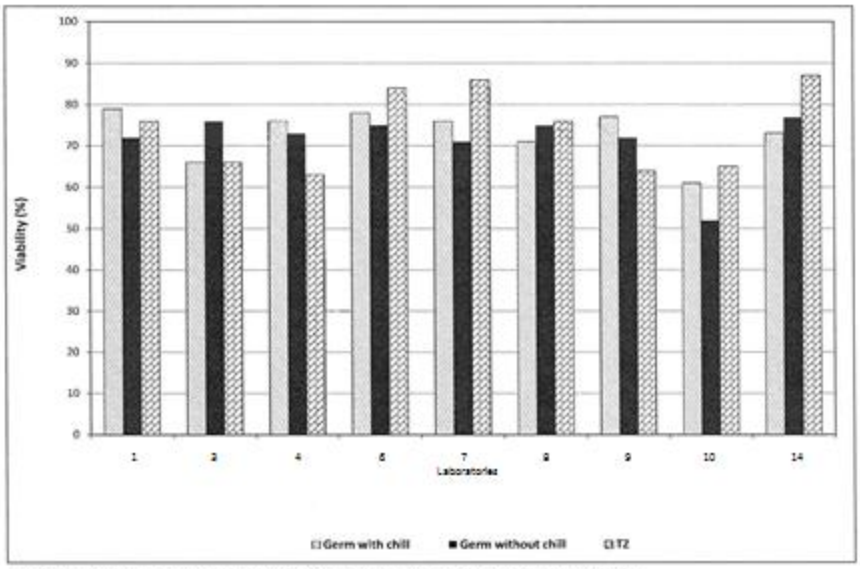

Figure 3. Viability by germination, with and without prechill, and by TZ tests of sample B tested at 9 seed Labs.

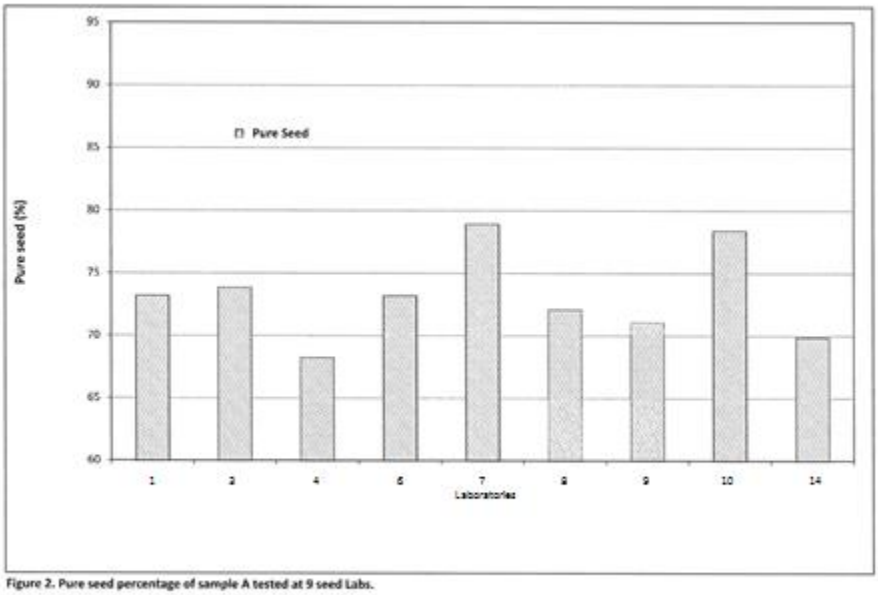

Figure 2. Pure seed percentage of sample A tested at 9 seed Labs.

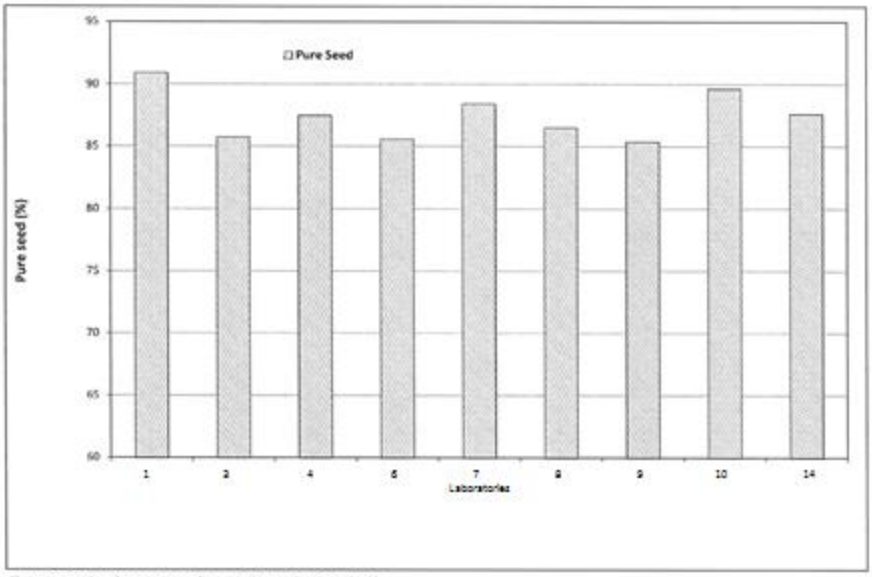

Figure 4. Pure seed percentage of sample B tested at 9 seed Labs.

Treating the Data

We analyzed all the data by comparing results of each lab to the other labs to see if they were out of tolerance of each other. We applied tolerances from our AOSA tolerance charts:

For the germination tests we used Table 14J. Tolerances for the Tz tests are from Table 14N. For pure seed, tolerances came from Table 14A.

For each test there are 36 comparisons.

Each table shows the tolerances of each test. In comparisons where the two labs had the exact same result, then it was indicated by “ok”.

Tolerances for Germination Test with Chill, Sample A

Lab #	%	1	3	4	6	7	8	9	10	14
		28	**18**	**21**	**27**	**15**	**21**	**21**	**10**	**22**
1	**28**		8	8	9	8	8	8	7	8
3	**18**	8		8	8	7	8	8	6	8
4	**21**	8	8		8	7	ok	ok	7	8
6	**27**	9	8	8		8	8	8	7	8
7	**15**	8	7	7	8		7	7	6	7
8	**21**	8	8	ok	8	7		ok	7	8
9	**21**	8	8	ok	8	7	ok		7	8
10	**10**	7	6	7	7	6	7	7		7
14	**22**	8	8	8	8	7	8	8	7	

Tolerances For Germination Test with Chill, Sample B

Lab #	%	1	3	4	6	7	8	9	10	14
		79	**66**	**76**	**78**	**76**	**71**	**77**	**61**	**76**
1	**79**		9	8	8	8	9	8	9	8
3	**66**	9		9	9	9	10	9	10	9
4	**76**	8	9		8	8	9	8	10	9
6	**78**	8	9	8		8	9	8	9	8
7	**76**	8	9	8	8		8	8	10	8
8	**71**	8	8	ok	8	7		ok	7	8
9	**77**	8	9	8	8	8	9		10	9
10	**61**	9	10	10	9	10	10	10		10
14	**76**	8	9	9	8	8	9	9	10	

Tolerances For Germination Test without Chill, Sample A

Lab #	%	1	3	4	6	7	8	9	10	14
		20	**19**	**22**	**22**	**15**	**20**	**34**	**10**	**20**
1	**20**		8	8	8	7	ok	9	7	ok
3	**19**	8		8	8	7	8	9	7	8
4	**22**	8	8		ok	7	8	9	7	8
6	**22**	8	8	ok		7	8	9	7	8
7	**15**	7	7	7	7		7	8	6	7
8	**20**	ok	8	8	8	7		9	7	ok
9	**34**	9	9	9	9	8	9		8	9
10	**10**	7	7	7	7	6	7	8		9
14	**20**	ok	8	8	8	7	ok	9	7	

Tolerances For Germination Test Without Chill, Sample B

Lab #	%	1	3	4	6	7	8	9	10	14
		72	**76**	**73**	**75**	**71**	**75**	**72**	**52**	**77**
1	**72**		9	9	9	9	9	ok	10	9
3	**76**	9		9	8	9	8	9	10	8
4	**73**	9	9		9	9	9	9	10	9
6	**75**	9	8	9		9	9	9	10	8
7	**71**	9	9	9	9		9	9	10	9
8	**75**	9	8	9	9	9		9	10	8
9	**72**	ok	9	9	9	9	9		10	9
10	**52**	10	10	10	10	10	10	10		10
14	**77**	9	8	9	8	9	8	9	10	

Tolerances For Pure Seed, Sample A

Lab #	%	1	3	4	6	7	8	9	10	14
		73.20	**73.86**	**68.23**	**73.20**	**78.94**	**72.10**	**71.10**	**78.41**	**69.91**
1	**73.20**		3.26	3.33	ok	3.09	3.26	3.26	3.18	3.33
3	**73.86**	3.26		3.33	3.26	3.09	3.26	3.26	3.09	3.33
4	**68.23**	3.33	3.33		3.33	3.26	3.33	3.44	3.26	3.44
6	**73.20**	ok	3.26	3.33		3.09	3.26	3.26	3.18	3.33
7	**78.94**	3.09	3.09	3.26	3.09		3.18	3.18	2.99	3.18
8	**72.10**	3.26	3.26	3.33	3.26	3.18		3.33	3.18	3.33
9	**71.10**	3.26	3.26	3.44	3.26	3.18	3.33		3.18	3.33
10	**78.41**	3.18	3.09	3.26	3.18	2.99	3.18	3.18		3.18
14	**69.91**	3.33	3.33	3.44	3.33	3.18	3.33	3.33	3.18	

Tolerances For Pure Seed, Sample B

Lab #	%	1	3	4	6	7	8	9	10	14
		90.92	**85.75**	**87.47**	**85.57**	**88.43**	**86.51**	**85.39**	**89.65**	**87.61**
1	**90.92**		2.30	2.30	2.30	2.30	2.30	2.30	2.15	2.30
3	**85.75**	2.30		2.47	2.62	2.47	2.47	2.62	2.47	2.47
4	**87.47**	2.30	2.47		2.47	2.47	2.47	2.47	2.30	2.47
6	**85.57**	2.30	2.62	2.47		2.47	2.47	2.62	2.47	2.47
7	**88.43**	2.30	2.47	2.47	2.47		2.47	2.47	2.30	2.3
8	**86.51**	2.30	2.47	2.47	2.47	2.47		2.62	2.30	2.47
9	**85.39**	2.30	2.62	2.47	2.62	2.47	2.62		2.47	2.47
10	**89.65**	2.15	2.47	2.30	2.47	2.30	2.30	2.47		2.30
14	**87.61**	2.30	2.47	2.47	2.47	2.30	2.47	2.47	2.30	

Tolerance For Tz Test, Sample A

Lab #	%	1	3	4	6	7	8	9	10	14
		29	**19**	**15**	**30**	**28**	**16**	**14**	**10**	**19**
1	**29**		23	22	25	25	23	22	21	23
3	**19**	23		20	23	23	21	20	19	21
4	**15**	22	20		23	22	20	19	18	20
6	**30**	25	23	23		25	23	22	22	23
7	**28**	25	23	22	25		22	22	21	23
8	**16**	23	21	20	23	22		19	18	21
9	**14**	22	20	19	22	22	19		17	20
10	**10**	21	19	18	22	21	18	17		19
14	**19**	23	21	20	23	23	21	20	19	

Tolerances For Tz Test, Sample B

Lab #	%	1	3	4	6	7	8	9	10	14
		76	**66**	**63**	**84**	**86**	**76**	**64**	**65**	**87**
1	**76**		25	25	22	22	ok	25	25	21
3	**66**	25		26	24	23	25	26	26	23
4	**63**	25	26		24	24	25	26	26	24
6	**84**	22	24	24		20	22	24	24	19
7	**86**	22	23	24	20		22	24	23	19
8	**76**	ok	25	25	22	22		25	25	21
9	**64**	25	26	26	24	24	25		26	23
10	**65**	25	26	26	24	23	25	26		23
14	**87**	21	23	24	19	19	21	23	23	

For the germ tests with chill: Sample A results ranged from 10-28% Total Viable. When comparing results from two labs, 11/36 were out of tolerance (o.t.). Sample B ranged from 61-79% Total Viable and 11/36 were o.t. (Both had 31% of the tests o.t.)

For the germ tests without chill: Sample A results ranged from 10-34% Total Viable. When comparing results from two labs, 13/36 were o.t. (36% o.t.). Sample B results ranged from 52-77% Total Viable and 8/36 were o.t. (only 22% o.t.).

For the Tz tests: Sample A results ranged from 10-30% but no comparisons were o.t. Sample B results ranged from 63-87% and no tests were o.t. Even though the differences between tests were high, the tolerances were higher.

For the Pure Seed percentages, Sample A results ranged from 68.23-78.94%. When comparing results from two labs, 19/36 tests were o.t. (53%) Sample B results ranged from 85.32-90.92%. 13/36 were o.t. (36% o.t.).

Purity Questions

With this referee we asked the participants 3 purity related questions. 1. If you find a piece of broken utricle less than ½ the original size on top of the screen, do you consider it a pure seed unit or inert matter? The responses were: 67% would call them inert because of the ½ seed rule. 33% would call them pure seed because seeds retained on top of screen are all pure seed or pure seed units.

2. If small sticks with several small utricles attached stay on top of the screen, how do you classify? 63% basically said separate stick from utricles before screening. 37%would not break off stick, and the utricles with the stick stay on top of screen, so use it as a pure seed unit.

3. If a cluster of small utricles is retained on top of the screen, how do you classify? 37% said before you screen your sample, separate the clusters then shake over screen. 63% do not separate the clusters, because they are pure seed.

Summary and Conclusions

Because of the out of tolerance comparisons there is evidence of testing disparity. In germination tests, evaluating normal and abnormal seedlings may not be uniform among analysts.

Even though Tz tolerances are very forgiving, there are problems if a seed lot has a relatively large range of Tz results on a lot. Seed companies selling Kochia are concerned when they lose revenue when there are such differences.

The “question survey” revealed that analysts were not in agreement as to how to classify certain components, especially in dirtier lots. This could contribute to testing inconsistency. Lower pure seed quality lots have more tests out of tolerance. High percentage of unfilled seeds may give more pure seed variability if different shaking techniques get different amounts of small unfilled seeds to fall through the sieve.

We looked at the test results of the germ with chill and germ without chill. Either way we did the germination test we still got variability. Therefore, we didn’t determine which test would give better results or if the two methods are comparable.

Want to thank all those who participated in this referee and helped in any way on this referee.

Referee Coordinators:

Stan Akagi and Terry Freeman, Utah Dept. of Agriculture and Food, Utah Seed Lab

Responses to the Purity Related Questions

The responses to the purity related questions are as follows.

1. If you find a piece of broken utricle less than ½ the original size on top of the screen do you consider it a pure seed unit or inert matter? Why?

Inert matter because less than ½ original size.

Inert. If it's less than1/2 and is right there with the other seeds to look through, pick it out and put in inert.

Inert matter. The less than ½ the original size rule.

PSU 38 states, Piece of broken utricle larger than one-half the original size. Therefore, less than half the original size would be inert.

Pure seed unit because of the 'special consideration' (on top of the screen) wording in PSU 38.

Pure seed. We're inclined to call less than ½ utricles inert matter, but Debbie Myer's interpretation of the PSU definition was to call it pure seed. It doesn't matter to me, but we all need to do it the same. Good discussion for purity committee.

Yes, it is consistent with majority of inert matter of crops to call this inert.

Pure seed. It is left on top of the screen.

Inert. ½ seed rule.

2. If small sticks with several small utricles attached stay on top of the screen, how do you classify? Why?

Would remove the utricles from the stick before screening because there is nothing about multiple units in PSU definition.

Leave as is, put in pure seed, stick and all.

Inert matter. If the small utricles were not on the stick they would pass through the screen.

Pure seed PSU 38, Intact utricle with or without perianth, enclosed or not enclosed by fruiting bracts (bracteoles), whether or not a seed is present. For Bassia prostrate, **seed units** that are retained on a 1mm opening square-hole sieve, when shaken for 30 seconds shall be considered pure seed units.

Pure seed units. Stay on top of the screen.

Separate utricles from stem before shaking over screen.

Break them apart, shake another 30 seconds. Classify the stick as inert.

Remove utricles from stick, put stick in inert, put tiny utricles in inert, plus bigger utricles go into pure seed.

3. If a cluster of small utricles is retained on top of the screen, how do you classify? Why?

Would separate the utricles apart from each other before screening. There is nothing about multiple units in PSU definition.

Pure seed. It's retained on top of screen.

Pure seed. The cluster is your seed unit.

Pure seed. PSU 38, Intact utricle with or without perianth, enclosed or not enclosed by fruiting bracts (bracteoles), whether or not a seed is present. For Bassia prostata, **seed units** that are retained on a 1mm opening square-hole sieve, when shaken for 30 seconds shall be considered pure seed units.

Pure seed unit. Retained on top of the screen.

Break up utricles before shaking over screen.

Pure seed. Simply because they stayed on top of the screen.

Separate multiples before screening.