

Rule Change Proposal – 3

PURPOSE OF PROPOSAL: To add pleated paper (PP) to the substrata used for planting onion (*Allium cepa* L.).

PRESENT AND PROPOSED RULE:

Kind of Seed	Substrata ^a	Temperature (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
<i>Allium cepa</i> onion Alternate methods Pelleted onion	B, T, <u>PP</u>	20	6	10		
	S	20	6	12		
		20		10	See sec. 6.8r	

HARMONIZATION AND IMPACT: FSA does not have PP as a recommended medium for the germination test of onion (*Allium cepa*). Current ISTA Rules allow for pleated paper.

SUPPORTING EVIDENCE: Pleated paper is used in the onion seed industry to conduct standard germination tests. It is thought that pleated paper may enhance germination compared to paper towels or blotters, especially for pelleted seeds, therefore should be included in the AOSA Rules. A national referee study was designed to verify this claim. If the results showed that using pleated paper helps improving germination, or gives similar results to the paper towel/blotter, then it may be recommended to add pleated paper as an alternative media in the AOSA Rules for germinating onion.

The results of the referee (attached appendix) did not show a superior pattern for using paper toweling over pleated paper or vice versa. This suggests that either the paper towels/blotters or the pleated paper can be used alternatively without expecting a significant difference in germination results of onion seed. The preference of using one medium over the other will depend on the availability of substrate, convenience, and economy. The difference in germination results detected among laboratories was greater than those due to difference in the media used in the germination tests regardless of the quality of onion seeds used.

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APPENDIX to Proposal 3

National Referee

A national referee was conducted in 2009 to compare germination of various onion seed lots using paper towels or blotter versus pleated paper.

Materials and Methods

- **Seed Lots:** Six onion seed lots from the 2007 crop year with different initial qualities (96-84% germination), two raw seed (samples 1 & 2), two film coated seed (samples 3 & 4) and two pelleted seed (samples 5 & 6), were subdivided into 16 sister samples to be distributed among participating laboratories. The study was conducted in 2008.
- **Substrate**
 - Choice of paper towels (**T**) or between blotters (**B**), according to the AOSA Rules.
 - Pleated paper - "paper strips", placed beneath the pleated paper (**PP**).
- **Moisture:** Moisture was provided for blotters and paper towels according to the guideline of AOSA Rules; and 40 ml of water for pleated paper.
- **Temperature:** 20°C
- **Replicates:** 4 replications of 100 seeds each were used for each treatment.
- **Counts:** Laboratories recorded the first count after 6 days and the final count after 10 days.
- **Evaluation:** Normal seedlings with hypocotyl and radical length of ½" from knee (1.25 cm) or more were removed at 6 days from planting (first count) to minimize secondary fungal infection. Final count was recorded 10 days after planting. The germination tolerance Table 13J (AOSA Rules, 2008) was used to determine the actual difference in germination results between the two media used in this study.

Results

Fourteen laboratories completed the referee. Some laboratories used paper towels others used blotters as a standard substrate according to the AOSA Rules for Testing Seeds. Pleated paper was donated by some companies and was distributed among the participating laboratories. Six-day germination count, final germination, abnormal seedlings and dead seeds data was collected. Only the final germination data is included in this report.

The analysis of variance (ANOVA) showed no significant difference in the final germination results between the use of the standard media (i.e., paper towels/blotters) or pleated paper. The ANOVA also indicated significant differences in germination results among seed lots and laboratories at P=0.05 (Table 1). Although all laboratories used the same germination procedures, i.e., AOSA Rules, significant difference in the final germination results was detected among laboratories, even when they used the same substratum.

Table 1. Analysis of variance of six onion seeds lots germinated at fourteen laboratories using paper towel and pleated paper.

Source	Degrees of Freedom (df)	Sum of Squares (SS)	Mean Square (MS)	F Value	Prob
Replication	3	55.70	18.57	1.58	0.19 ns
Media (M) T vs. PP	1	2.88	2.88	0.25	ns
Seed lots (SL)	5	15277.35	3055.60	260.80	0.000***
M x SL	5	157.35	31.47	2.69	0.02*
Laboratories (L)	13	2791.69	214.75	18.33	0.000***
M x L	13	875.58	67.35	5.75	0.000***
SL x L	65	4733.55	72.82	6.22	0.000***
M x SL x L	65	1085.94	16.71	1.43	0.02*
Error	501	5869.80	11.72		
Total	671	30850.48			

Coefficient of Variation: 3.83%

For seed lot one, germination results of the fourteen participating laboratories were within tolerance according to the AOSA germination tolerance Table 13J, regardless of paper towels or pleated paper was used (Fig. 1). For seed lots three, four, and five, the germination results of thirteen out of the fourteen laboratories who participated in the study were within tolerance regardless of the germination media used (Figs. 3, 4 and 5). Eight out of the fourteen laboratories who participated in the referee had germination results within tolerance for seed lot 'six' (Fig. 6), and eleven out of the fourteen had similar results for seed lot 'two' (Table 2 and Fig. 2).

Variation in germination results can be attributed to two main sources: random sampling variation, and systematic variation among laboratories.

Table 2. Mean germination of six onion seed lots tested at fourteen laboratories using paper towel and pleated paper.

Seed Lots	La b	% Germination			Difference	Tolerance	Within tolerance?
		T/B ¹	PP ²	Average*			
1	1	84	83	83	1	7	yes
	2	92	93	92	1	5	yes
	3	87	89	88	2	6	yes
	4	83	81	82	2	7	yes
	5	85	90	87	5	6	yes
	6	86	92	89	6	6	yes
	7	83	86	85	3	7	yes
	8	88	87	87	1	6	yes
	9	82	84	83	2	7	yes
	10	88	89	88	1	6	yes
	11	83	85	84	2	7	yes
	12	81	80	81	1	8	yes
	13	84	85	85	1	7	yes
	14	83	86	85	3	7	yes

Mean		85	86				
	1	93	98	96	5	4	no
	2	92	94	93	2	5	yes
	3	95	98	96	3	4	yes
	4	99	99	99	0	2	yes
	5	98	98	98	0	3	yes
	6	99	98	98	1	3	yes
2	7	94	99	96	5	4	no
	8	91	84	88	7	6	no
	9	81	85	83	4	7	yes
	10	98	97	97	1	3	yes
	11	97	98	98	1	3	yes
	12	89	82	86	7	7	yes
	13	88	81	85	7	7	yes
	14	98	98	98	0	3	yes
Mean		94	93				
	1	94	96	95	2	4	yes
	2	95	95	95	0	4	yes
	3	96	98	97	2	3	yes
	4	95	96	95	1	4	yes
	5	97	98	97	1	3	yes
	6	96	99	98	3	3	yes
3	7	94	98	96	4	4	yes
	8	97	96	96	1	4	yes
	9	96	91	94	5	4	no
	10	94	97	96	3	4	yes
	11	96	96	96	0	4	yes
	12	99	96	98	3	3	yes
	13	96	97	97	1	3	yes
	14	95	98	97	3	3	yes
Mean		96	96				
	1	82	83	83	1	7	yes
	2	91	91	91	0	5	yes
	3	84	85	85	1	7	yes
	4	81	87	84	6	7	yes
	5	86	87	86	1	7	yes
	6	95	86	91	9	5	no
4	7	77	85	81	8	8	yes
	8	87	80	83	7	7	yes
	9	79	80	80	1	8	yes
	10	79	79	79	0	8	yes
	11	82	84	83	2	7	yes
	12	87	85	86	2	7	yes
	13	81	84	83	3	7	yes
	14	81	87	84	6	7	yes
Mean		84	85				
	1	79	82	81	3	8	yes
5	2	93	92	93	1	5	yes
	3	87	82	85	5	7	yes
	4	87	90	89	3	6	yes

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	5	83	88	85	5	7	yes
	6	84	84	84	0	7	yes
	7	88	87	87	1	6	yes
	8	85	74	79	11	8	no
	9	81	79	80	2	8	yes
	10	82	84	83	2	7	yes
	11	86	87	87	1	6	yes
	12	86	82	84	4	7	yes
	13	86	83	85	3	7	yes
	14	83	80	82	3	7	yes
Mean		85	84				
	1	91	92	91	1	5	yes
	2	93	95	94	2	4	yes
	3	92	93	92	1	5	yes
	4	93	93	93	0	5	yes
	5	88	94	91	8	5	no
	6	96	91	93	5	5	yes
	7	95	94	95	1	4	yes
6	8	96	88	92	8	5	no
	9	92	89	90	3	6	yes
	10	92	92	92	0	5	yes
	11	85	92	89	7	6	no
	12	80	90	85	10	7	no
	13	85	92	89	7	6	no
	14	86	94	90	8	6	no
Mean		90	92				

Method 1 = Paper towel or top of blotter paper

Method 2 = Pleated paper

Means are average of 4 replications of 100 seeds each

The results of this referee did not show a superior pattern for using the paper towel/blotters over the pleated paper or vice versa. This suggests that either the paper towels, blotters or the pleated paper can be used alternatively without expecting significant difference in germination results of onion seed. The preference of using one medium over the other will depend on the availability of substrate, convenience, and economy. The difference in germination results detected among laboratories was greater than those due to difference in the media used in the germination tests regardless of the quality of onion seeds used (Figs 1-6).

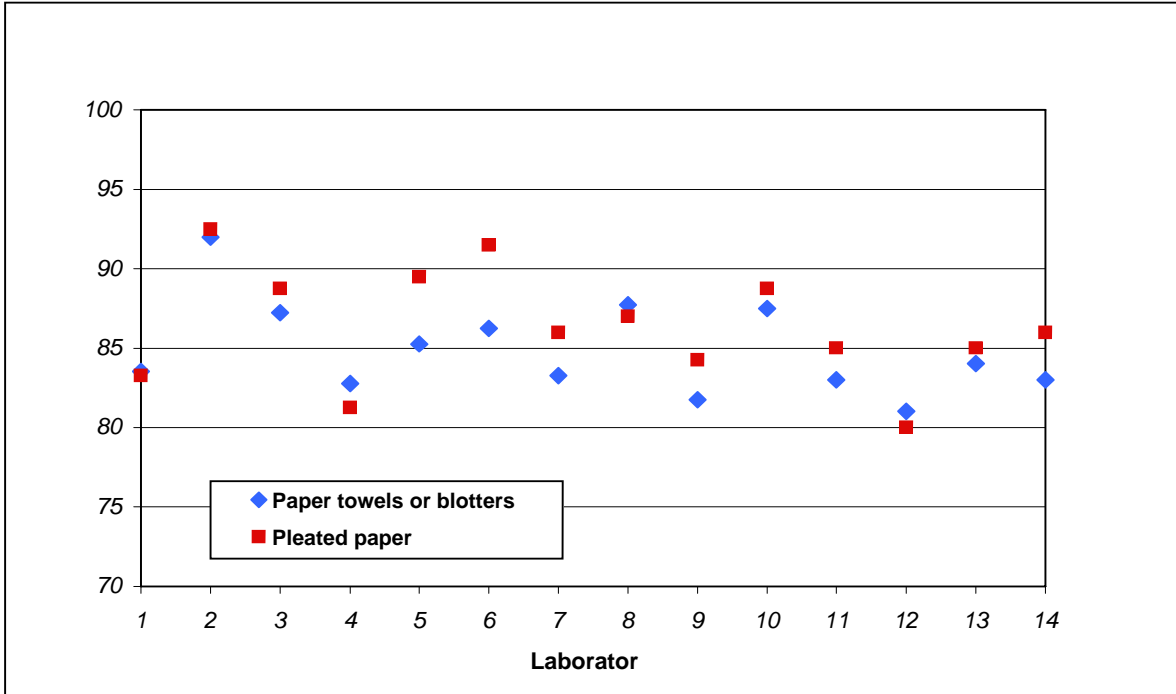


Figure 1. Mean germination of onion seed lot '1' using paper towels and pleated paper media tested at fourteen seed laboratories.

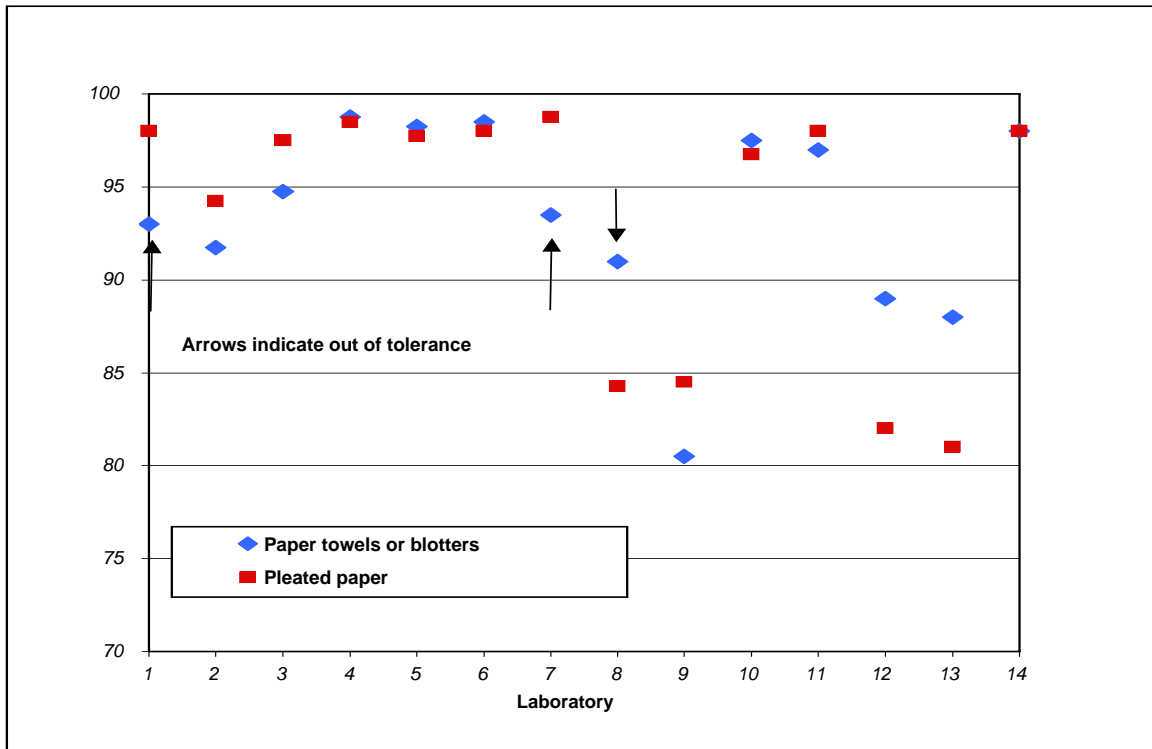


Figure 2. Mean germination of onion seed lot '2' using paper towels and pleated paper media tested at fourteen seed laboratories.

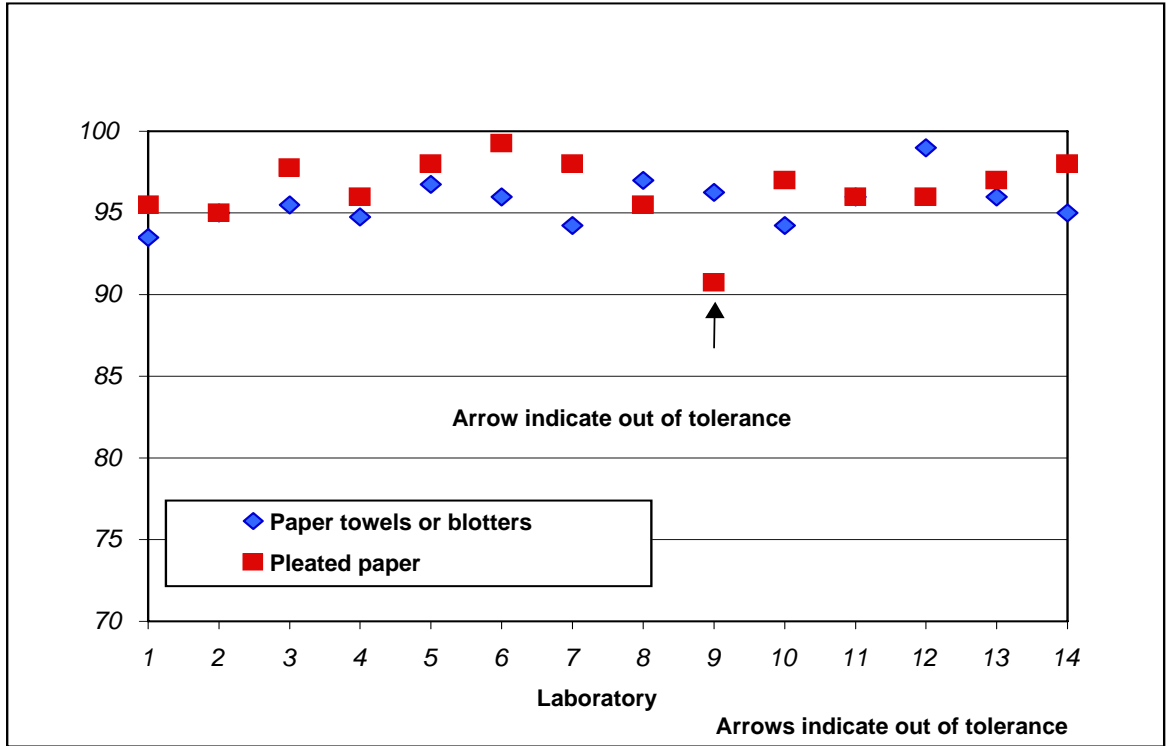


Figure 3. Mean germination of onion seed lot '3' using paper towels and pleated paper media tested at fourteen seed laboratories.

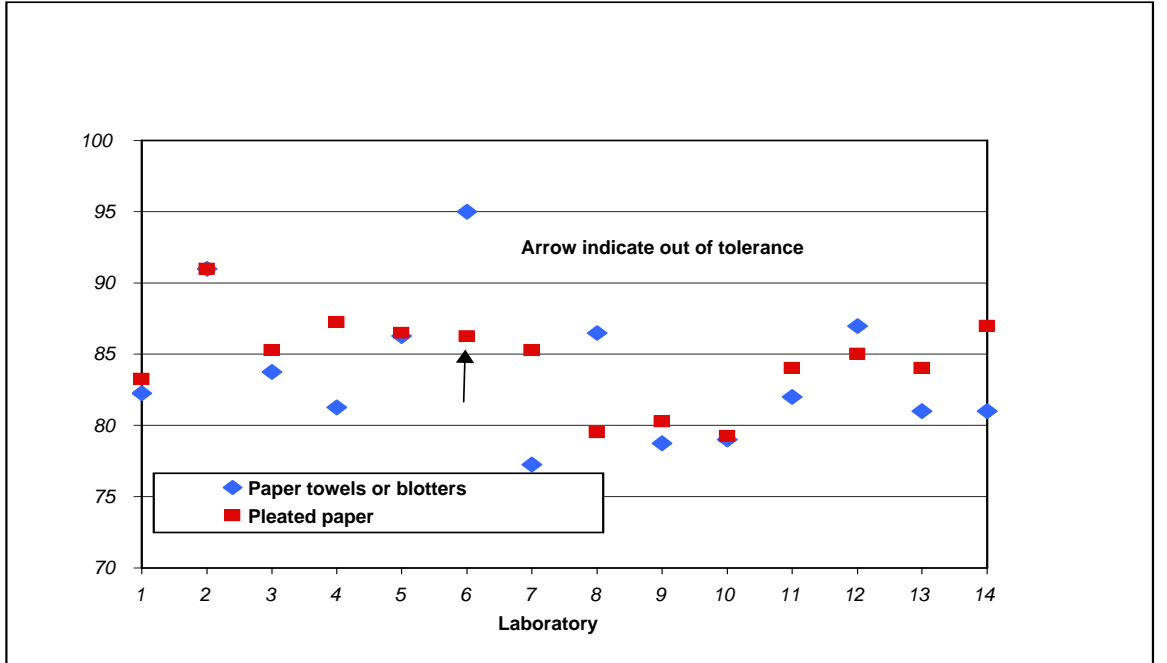


Figure 4. Mean germination of onion seed lot '4' using paper towels and pleated paper media tested at fourteen seed laboratories.

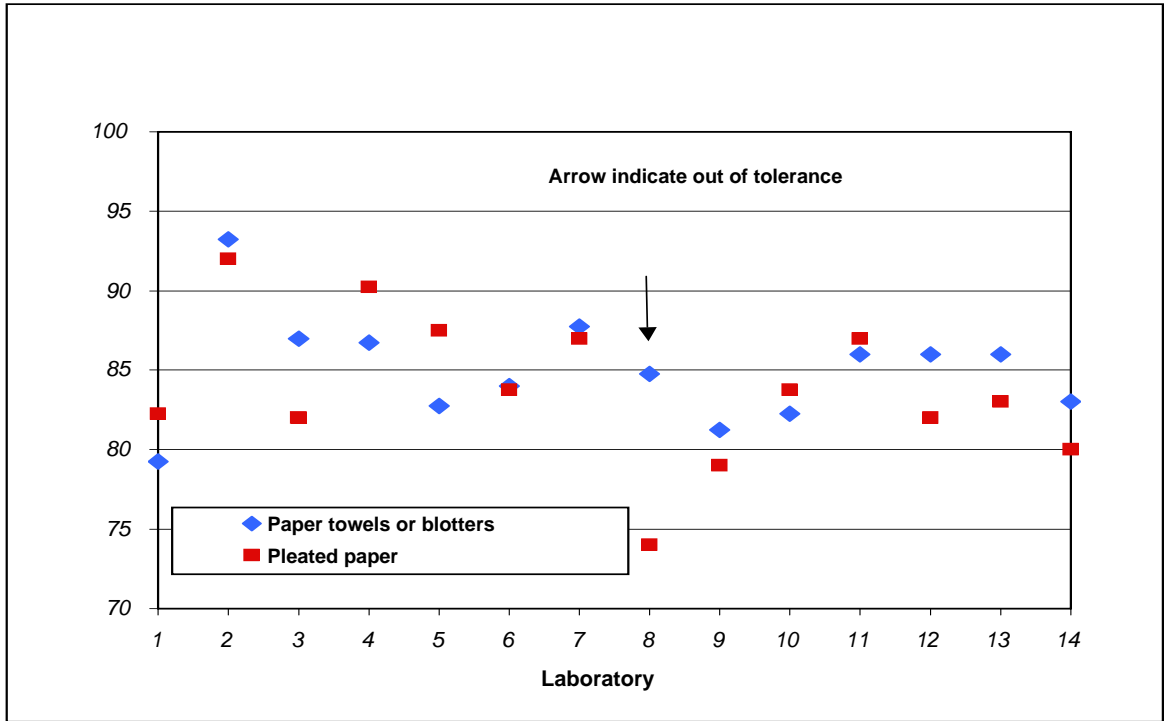


Figure 5. Mean germination of onion seed lot '5' using paper towels and pleated paper media tested at fourteen seed laboratories.

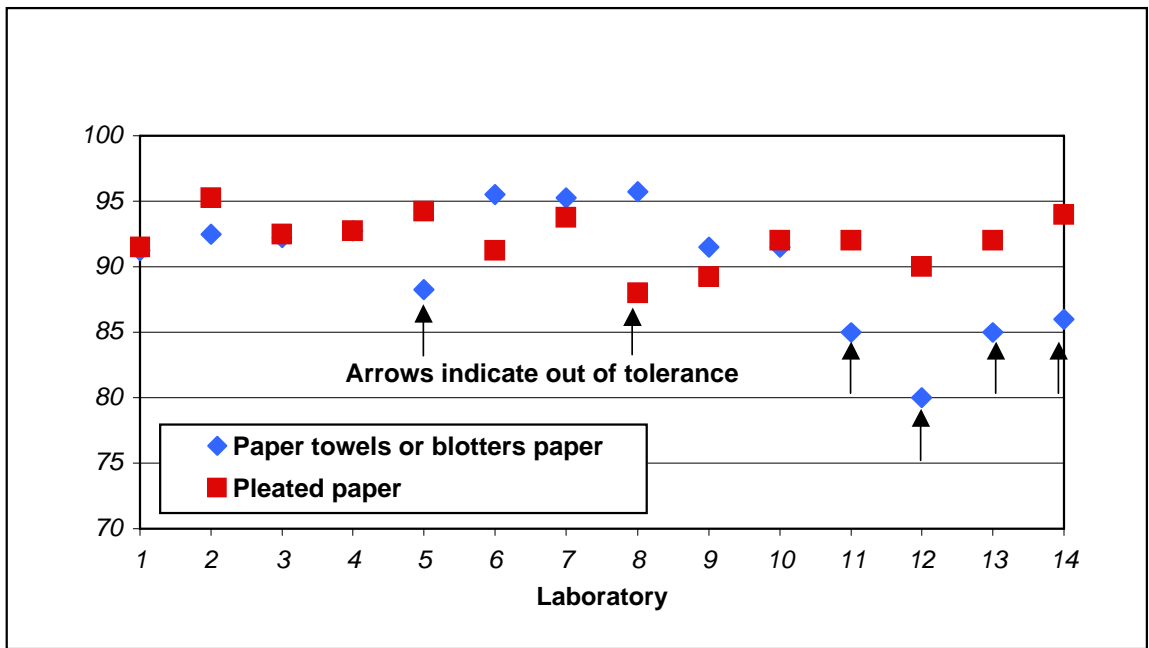


Figure 6. Mean germination of onion seed lot '6' using paper towels and pleated paper media tested at fourteen seed laboratories.

CONCLUSIONS

- 1) Paper towels and blotters perform similar to pleated paper when used as media in the standard germination tests of onion seeds used in this study regardless of the quality of the seeds.
- 2) The results suggest that adding pleated paper to the current media in the AOSA Rules is an option that will not affect the germination test results.

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