

2020 Sunflower Referee

Southwest Region 4
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Introduction

- In the AOSA Rules Table 6A (Volume 1) there is a discrepancy for the germination testing method for Sunflower (*Helianthus* spp.) – specifically, the temperature regime:

Kind of seed	Substrata	Temperature (°C)	First count (days)	Final count (days)	Specific requirements and notes	Dormant seed
<i>Helianthus annuus</i> Sunflower	T, B, S,	20	4	7		
Alternate method	TCS	25	4	7		
<i>Helianthus</i> spp. sunflower: large and small seeded	B, T	20-30	3 ^b	7 ^c		

Background

- The AOSA Rules list 20°C but not 20-30°C for *Helianthus annuus* and 20-30°C but not 20°C for *Helianthus* spp. “Sunflower” falls under both of these, and a clear discrepancy exists.
- In the past the AOSA Rules germination methods were presented in 4 different tables: Agricultural; Vegetable and Herb; Flower; and Tree and Shrub seeds. In 1997, these four tables were combined into one (now Table 6A). This discrepancy is likely a relic of the merging of the four tables.

Purpose

- The purpose of this referee was to compare the two temperature regimes (20°C and 20-30°C) in the AOSA Rules for sunflower, *Helianthus annuus*.
- The results might provide useful data in order to remove the current discrepancy in the AOSA Rules regarding the germination temperatures for sunflower.

NOTE: A third temperature of 25°C for *Helianthus annuus* is listed in the Alternate method using TCS (top of creped cellulose covered with sand).

Organization

- The organizers of this referee acquired 6 lots of sunflower seeds (*Helianthus annuus*) from three different companies. These included ornamental, oil seed, and confectionary varieties.
- Due to the difficulty in obtaining large quantities of seed, the number of participants was limited to eight laboratories.
- A survey was sent out to the AOSA and SCST membership, with the purpose of finding volunteers for the survey with experience in testing sunflower seed for laboratory germination.

Survey of Participants

The following survey was sent out to the membership looking for referee participants:

1. How many years' experience do you have in seed testing?
2. How many samples of sunflower (*Helianthus annuus*) do you evaluate for germination each year?
 - a. Ornamental varieties?
 - b. Oil seed varieties?
 - c. Confectionary varieties?
 - d. Other sunflower varieties?
3. Are you interested in participating in a referee comparing the germination temperatures for sunflower using AOSA Rules?

Results of Survey

More than eight laboratories / individuals responded to the survey, but since the participation was limited, the eight respondents with the most experience, particularly with sunflowers, were chosen. Here is a summary of the results:

1. How many years' experience do you have in seed testing?

Range 8 to 42 years, average of 24 years' experience

2. How many samples of sunflower (*Helianthus annuus*) do you evaluate for germination each year?

a. Ornamental varieties? Average 14

b. Oil seed varieties? Average 737

c. Confectionary varieties? Average 81

d. Other sunflower varieties? Average 10

Overall average of all varieties: 842 samples per year, per laboratory

Methods

In August 2020, samples were prepared and sent out. The following are the specific directions given to the referee participants:

- Enclosed are six sunflower (*Helianthus annuus*) samples. Please test each sample using two different temperature regimes: test 400 seeds at 20-30°C and an additional 400 seeds at 20°C, using towels as the germination media. Please do a 4 day first count and a 7 day final count, using the AOSA Seedling Evaluation as described in AOSA Rules Volume 4, pp. 27-29, Asteraceae II. You may plant the replicates in 4 replicates of 100 seeds or in 8 replicates of 50 seeds – if you use 8/50 please record the data as 4/100 (add two 50 seed replicates together).

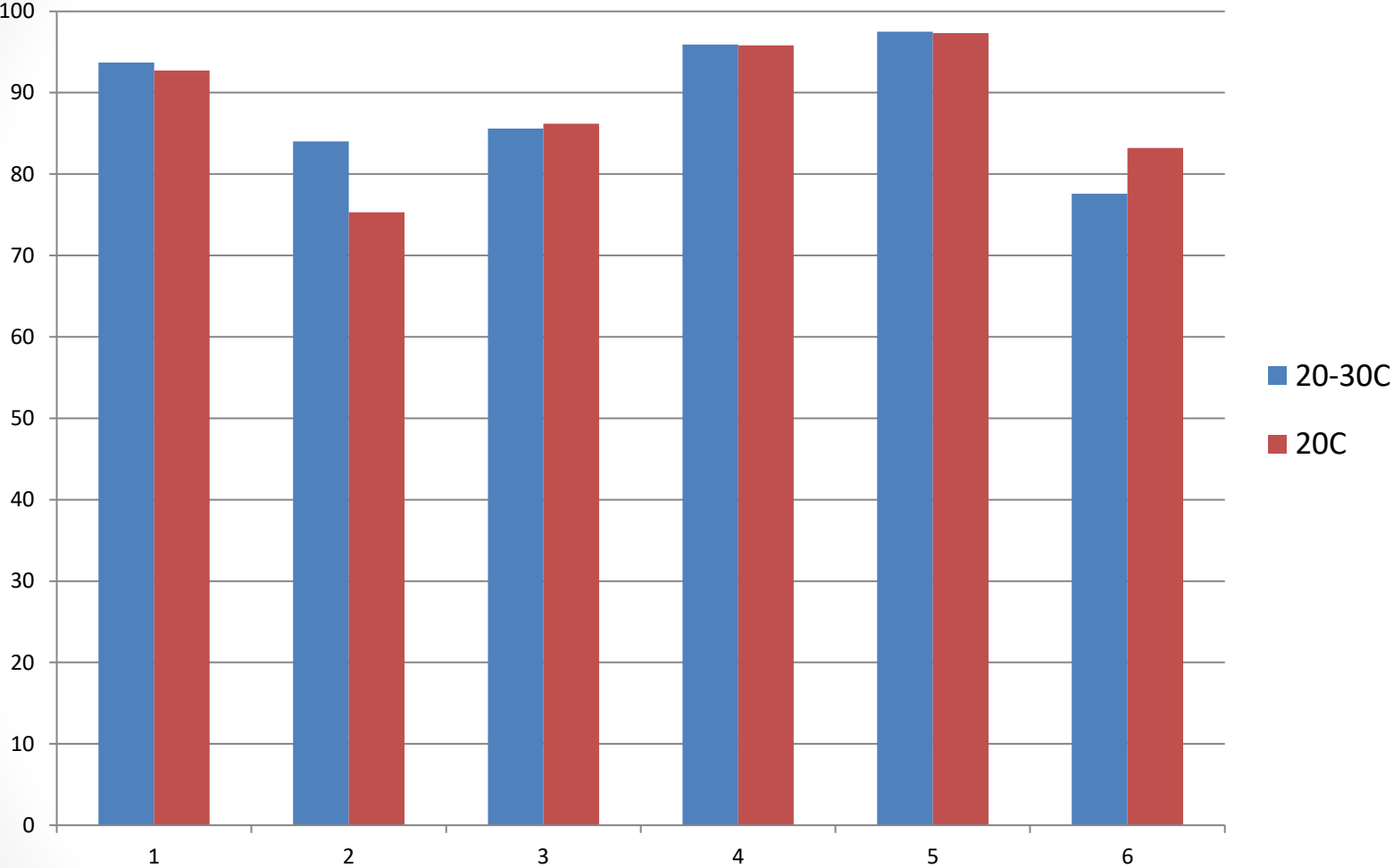
Results

- All eight laboratories completed the germination testing and sent in results by the first week of October 2020. All participants reported that they used rolled towels as the germination media.
- The results are summarized in the next two slides.

Average germination results for sunflower from 8 laboratories:

Sample	Tested at 20-30°C	Tested at 20°C
1	93.7	92.7
2	84.0	75.3
3	85.6	86.2
4	95.9	95.8
5	97.5	97.3
6	77.6	83.2
Overall Average	89.05%	88.42%

Percent germination



Sample number

Statistical Analysis

- The overall results show that there was little difference between the two temperature regimes. However, two of the samples showed greater variability. Sample 2 was higher in the 20-30°C regime while Sample 6 did better at the 20°C temperature.
- Further statistical analysis was performed on the data by Dr. Riad Baalbaki of the California State Seed Laboratory. His analysis is presented on the next few slides.

Table 1. Analysis of variance of percentage germination results over all samples, labs and temperature regimes.

SV	df	MS	F	p-value	Sig.
Sample (S)	5	3899.8	316.7	9.2×10^{-15}	yes
Lab (L)	7	365.7	29.7	9.3×10^{-31}	yes
Temperature (T)	1	41.3	3.4	0.07	no
S x L	35	150.0	12.2	4.1×10^{-39}	yes
S x T	5	339.6	27.6	8.4×10^{-23}	yes
L x T	7	194.3	15.8	1.6×10^{-17}	yes
S x L x T	35	47.4	3.8	1.1×10^{-10}	yes
Error	288	12.3			
Total	383	93.0			

Explanation of results – Table 1:

- Analysis of variance (Table 1), combining all sample, lab and temperature results, indicated that, overall, use of either temperature regime will not lead to significant differences in final percentage germination (the 'no' associated with Temperature sig. effect in the table). However, the same analysis indicated that, when the variation due to temperature regimes and samples was excluded, labs significantly varied in their results (i.e., lab performance was non-uniform). As expected, sample differences, when other factors were accounted for, were significant (i.e., samples had a range of germination, as they should).

Table 2. Analysis of variance (mean squares) of percentage germination results per sample, over all labs and temperature regimes.

SV	df	MS					
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Temperature	1	16.0 NS	1216.3 **	5.1 NS	0.4 NS	0.8 NS	500.6 **
Labs	7	32.1 **	145.8 **	86.4 **	19.2 **	13.9 **	818.5 **
T x L	7	15.5 *	99.0 **	45.9 NS	20.8 **	11.9 **	238.1 **
Error	48	6.2	15.0	21.8	4.6	3.7	22.6
Total	63	10.3	57.9	31.4	8.0	5.7	142.5

NS, *, ** Not significant, significant at $p = 5\%$, and significant at $p \leq 1\%$, respectively.

Table 3. Average percentage germination (G) and variance (V) at each temperature regime (T1 and T2), for each tested sample.

Temp. regime	Sample											
	1		2		3		4		5		6	
	G	V	G	V	G	V	G	V	G	V	G	V
T1	93.7	7.3	84.0	30.4	85.6	30.1	95.9	8.5	97.5	1.7	77.6	84.6
T2	92.7	13.2	75.3	48.2	86.2	33.6	95.8	7.7	97.3	9.8	83.2	188.9
Sig. diff.	N		Y		N		N		N		Y	

T1 = 20-30 °C; T2 = 20 °C.

- **Explanation of results – Tables 2 and 3:**
- When each sample was analyzed separately (Table 2), most results indicated no difference in final germination between the two temperature regimes. However, use of two temperature regimes produced significantly different results for samples 2 and 6 (Tables 2 and 3). For sample 2, higher average germination over all labs was observed at 20-30 C compared to 20 C. While this trend was also observed for other samples, the effect was much larger for sample 2.
- In contrast, sample 6 results significantly differed, but in this case, 20 C resulted in the higher germination.
- It should be noted that the magnitude of variation (V in Table 3) was always lower at T1 compared to T2, implying that variation among labs was always lower when testing at 20-30 C, compared to a constant temperature of 20 C.

Discussion

- Riad's final conclusion is quoted here:

“I think you have a good case for claiming that both temperature regimes can be used.”

- A Rules Proposal for next year will be developed as a result of this referee. Hopefully the discrepancy in the AOSA Rules for *Helianthus annuus* will be eliminated.

Acknowledgements

- Thanks to Condor Seed Production of Yuma, Arizona for donating one of the black oil varieties used in this referee.
- Sue Alvarez purchased one of the microgreen varieties used in the referee online from True Leaf Market.
- Special thanks to Sakata Seed for providing four ornamental varieties and for preparing and sending out all of the samples used in the referee.
- Extra special thanks to Riad Baalbaki for the data analysis.
- And thanks to all of the participating laboratories:

Georgia Department of Agriculture, Indiana State Seed Lab, MD Seed Analysis, New Mexico State Seed Lab, Ransom Seed Lab, Sakata Seed Lab, SGS Brookings, and Wyoming State Seed Lab

Questions?

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THANK YOU!

