Dormancy Breaking Methods on Freshly Harvested Hybrid Oil Seed Sunflowers

Miranda Smidt, Lauren Shearer, Tim Gutormson

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Introduction:

The Association of Official Seed Analysts (AOSA) "Rules for Testing Seeds" lists multiple methods to break dormancy on freshly harvested seeds. One method cited in Chapter 6.9h is for two rates of ethephon: standard rate of 0.0029% (29 ppm) and a concentrated rate of 0.0145% (145 ppm). In Table 6A *Helianthus maximilliani* references section 6.2f (Light) and 6.9m (Viability testing of ungerminated seed) with its known dormancy but has no reference to dormancy breaking methods. For *Helianthus annuus*, no dormancy breaking method is specified in Table 6A; the objective of this study is to identify an effective and consistent method to overcome primary dormancy in freshly harvested hybrid oil seed sunflowers. Primary dormancy can lead to questionable preliminary viability evaluations and uncertainty that the seed viability will meet production contract specifications. Typically, within 2-3 months post-harvest, after ripening diminishes the remaining primary dormancy in the seed population, producing seed germinations that meet the production standards. Confirming a method that effectively breaks dormancy would provide a streamlined test that can forego 6.9m, and further allow processing, conditioning, and packaging a freshly harvested hybrid oil seed sunflower lot in a timely manner. Findings of this study could lead to a proposal on a *Helianthus annuus*, specifically hybrid oil seed sunflowers.

Literature Review:

Dormancy in seeds can be expressed in exogenous and endogenous types and are imposed morphologically, physically, or physiologically. Hybrid oil seed sunflowers can produce a normal plant from embryo twenty-one days after anthesis, and onset of dormancy happens thereafter, so it can be concluded that they do not experience morphological dormancy. To break the physical and physiological types of dormancies, methods such as growth regulators, pretreatments, mechanical scarification, and combinations thereof have been evaluated.

The physical barrier of a sunflower embryo includes its hardened pericarp and the integument/testa maternal layer. Pretreatments are a commonly used method in initiating germination for dormant species. In sunflowers, pretreatments have ranged from dry heat treatment (ranging: 60°C for 15 minutes to 100°C for 5 minutes) [6, 12], water soak (ranging: 25°C for 15 minutes to 100°C for 30 minutes) [1, 4, 7, 8, 10], pre-chill (ranging 5-10°C) [15], freeze/thaw cycling (-80°C to 20°C cycling) [8], microwaving (80% for 30 seconds to 100% for 60 seconds) [12], smoking (sambrani for 3 hours) [12], seed chipping [3, 4, 8], and complete removal of the pericarp and maternal layer [13].

Physiological inhibitors are a balance of the hormones within the sunflower seed. Seed chemicals, namely that of abscisic acid (ABA) and gibberellic acid (GA_3), are a delicate balance that trigger the seed when to initiate germination. Induction and maintenance of dormancy is regulated by ABA, while germination is enhanced by GA_3. Growth regulators are then sought out to offset the balance of ABA and initiate germination for dormant species. GA_3 applications (ranging from 0.01% -0.1% or 100ppm-1000ppm) have been evaluated for overcoming the ABA inhibition [3, 4, 12, 15]. Various other chemical treatments have also been investigated, such as the efficacy of thiourea (0.01%-0.5%) [10, 12], Ethrel, whose active ingredient is ethephon (0.0005%-0.3%) [6, 9, 10, 12], hydrogen peroxide (5 minutes) [3], ethanol (25% for 15-30 minutes) [9, 10], acetone

(25% for 15 minutes) [6, 9, 10], 1-aminocyclopropane-1-carboxylic acid (ACC) (ranging 0.0000001%-0.01%) [11], and ethylene gas (20 hours at saturated atmosphere) [8].
The challenge proposed to the seed testing industry is finding a quick and effective method for breaking dormancy in freshly harvested hybrid oil seed sunflowers. We propose bringing a new angle to breaking dormancy with three new methods: 1.) Lengthening the preheat dry time to 3d @ 30°C, 2.) Lengthening preheat dry time to 7d @ 30°C, as well as 3.) Introducing a lengthened preconditioned stratification where seeds are exposed to -20°C conditions for 7d and further submerged for 18 hours in a solution of 0.05% (500 ppm) and 0.025% (250 ppm) of GA_3 and ethephon respectively prior to roll towel planting. Then comparing this to previous tried methods: 1.) 18-hour 0.05% GA_3 soak [4] 2.) 18-hour 0.025% Ethrel soak 3.) Utilizing 0.05% GA_3 as a blotter moistening agent, and 4.) Soaking the seeds in tap water for 18 hours and clipping the cotyledons prior to planting. These will be compared against a roll towel test with tap water as the blotter moistening agent.

Materials and Methods:
SoDak Labs, Inc. received 10 seed lots of freshly harvested (September 2022) hybrid oil seed sunflowers *Helianthus annuus* (contributed by RemSun Inc. and Syngenta Seeds Inc., Arbuckle and Glen, California, respectively). Upon receival, all seed lots were subjected to an electronic seed moisture reading using a Steinlite SL95 Moisture Meter. Seed was then screened to remove inert matter and sized over three round screens (16/64", 14/64", and 11/64") to create two working seed sizes. Any seed over the 16/64" and under the 11/64" screen was discarded and not included in this study. Seed below the 16/64" and above the 14/64" was classified as 'size 2,' while seed below the 14/64" and above the 11/64" was classified as 'size 3.'
A Tetrazolium (TZ) test of two (2) replicates comprised of 100 seed per size per lot was conducted according to the prescribed methods in the Tetrazolium Testing Handbook published by AOSA and SCST (2010 edition) to establish maximum viability of each respective seed lot and size. Germination and dormancy breaking treatments were determined using four (4) replicates of 50 seeds per size per seed lot. All germination tests were conducted using the rolled towel method on 38#, 12x24 cm brown paper towels (Anchor Paper Company, St. Paul, MN) and completed within 30-45 days of harvest. These towels were positioned upright and enclosed in plastic bags to prevent towel drying and were incubated at 20°C for 7 days. Evaluation occurred on day 7 and classified the seedlings as normal seedlings, abnormal seedlings, dead and firm seedlings in accordance with the AOSA Seedling Evaluation Handbook 'Asteraceae, Sunflower Family II – Kinds other than lettuce' [1]. 'Dead seeds' were ungerminated seeds that exhibited flaccid embryo tissue. 'Firm seeds' were classified as ungerminated seeds with firm embryo tissue at the conclusion of the test duration. (These seeds were not further investigated for viability at the termination of the test so they cannot be classified as either dead or dormant.)
Germination studies include 1.) a water check (no dormancy breaking) and the following seven seed dormancy breaking methods: 2.) heat drying pretreatment for 3d @ 30°C, 3.) heat drying pretreatment for 7d @ 30°C, 4.) 18-hour pretreatment soak in 0.05% gibberellic acid ≥90% (Product # G7645, Sigma Aldrich) (GA_3) solution 5.) 0.05% GA_3 as media moistening agent, 6.) 18-hour pretreatment soak in 0.025% ethephon/Ethrel (21.7%, Product # 5P95, HGI Worldwide, Inc.), 7.) stratify seed by placing on a screen for 7d @-20°C followed by a preplant treatment of an 18-hour soak in a 0.05% GA_3 + 0.025% Ethrel solution, and 8.) 18-hour water soak followed by clipping cotyledons. All treatments were planted with the same methodology as the germination test: using water as the blotter moistening agent unless otherwise stated. Data was analyzed using

R Studio Version 1.4.1103. An ANOVA model was used to calculate treatment means significant differences with LSD at ($P<0.05$).

Results and Discussion:

Seed moisture levels of the 10 composite seed lots ranged from 3.51% to 5.73% (Table 1.). These are considered low seed moistures as the target range for hybrid oil seed sunflowers is 6-8% post-harvest (personal communication, Dan Howe, RemSun Inc., Arbuckle, CA). The seed moisture levels of lots 5 and 6 were 3.51% and 4.21% respectively and exhibited the highest seed dormancy levels: 53% and 51% respectively.

Viable seed percentages of the 10 seed lots averaged across the two sizes ranged from 93 to 100% indicating high freshly harvested seed quality. Post germination firm seed percentages ranged from 1 to 53%. Total viability established in the tetrazolium test, paired with the normal seedlings and firm seeds of each lot provided a basis to measure efficacy of treatment without detrimental effect on readily germinating lots; furthermore, this provided a realistic set of seed lots for the study objectives.

Normal seedling, abnormal seedling, and dead seed percentages of the seed lot set were as to be expected in freshly harvested hybrid oil sunflower seed. The abnormal percentage of seed lot 6 was 13%, significantly higher than all other seed lots. Since seed lot 6 had a considerably high level of seed dormancy, some of these abnormal seedlings may be in transition from primary dormancy to a normal seedling but had insufficient development at the time of normal seedling evaluation.

Table 1. Comparison of 20°C germination normal, abnormal seedlings, dead, firm seed and viable seeds from the TZ results of ten sunflower seed lots averaged across eight treatments and two sizes.

Lot	Normal Seedlings	Abnormal Seedlings	Dead Seeds	Firm Seeds	Viable Seeds	Seed Moisture
	----------%----------					
1	86 c	4 de	6 a	4 f	99	5.34
2	70 f	5 cd	8 a	17 c	93	4.24
3	80 e	4 de	4 b	12 d	98	4.6
4	90 b	2 e	1 d	7 e	99	5.21
5	40 g	5 c	2 bcd	53 a	99	3.51
6	35 h	13 a	1 d	51 a	98	4.21
7	84 cd	6 c	3 bc	7 ef	94	4.16
8	94 a	3 e	2 cd	1 g	99	5.73
9	69 f	8 b	2 cd	21 b	100	4.64
10	83 de	5 cd	2 cd	10 d	99	4.19
LSD	3	1.7	1.6	2.5	5.5	4.2

Results of eight treatments are presented in Table 2. Treatments 6 and 7 were significantly different and higher than that of other treatments for normal seedling percentages: 91% and 89% normal seedlings, respectively. The water check was statistically significantly lower than all treatments except treatment two. Both preheating treatments (2 and 3) had minimal impact on reducing seed dormancy levels. Treatments utilizing GA_3 and Ethrel (4, 5, 6, and 7) had the most significant

impact on breaking sunflower seed dormancy ranging from 76% to 91% normal seedlings and 1 to 17% firm seeds. It appears that a slight synergistic effect occurs with a combination of Ethrel and GA_3 (7). It is unclear whether the synergistic effect is equivalent to a presoak or a blotter moistening agent: further comparative studies of these methods could focus on the synergistic reaction of these two growth regulators.

Abnormal seedling percentage was significantly higher in treatment 8. Clipping did reduce seed dormancy significantly compared to the water check (1) but did induce more abnormal seedlings at the time of evaluation. These seedlings were considered abnormal due to their top-down fashion of growth. Seedling cotyledons would begin to develop active chlorophyll and start photosynthesizing, while the radicle tip had shown little to no sign of developing: remaining intact and firm, with little to no elongation and dividing (Figure 1).

Figure 1. Developing cotyledons at day 7 of clipped cotyledons treatment. Left: seedlings stuck in seed coat. Right: seedling seed coat removed.

Dead seed percentages were statistically different but only varied 4% between the highest (3) and lowest (8) treatments' dead seed percentages.

Table 2. Comparison of 20°C germination of normal, abnormal seedlings, dead, firm seeds for eight treatments averaged across ten seed lots and two seed sizes using freshly harvested hybrid oilseed sunflower seeds.

Trt No.	Treatment Name	Normal Seedlings	Abnormal Seedlings	Dead Seeds	Firm Seeds
		----------%----------			
1.	Water as Blotter Moistening Agent (BMA)	58 f	2 c	3 bcd	37 a
2.	3d @ 30°C pretreatment, water BMA	57 f	4 bc	3 bc	36 a
3.	7d @ 30°C pretreatment, water BMA	63 e	4 b	6 a	27 b
4.	0.05% GA_3 18h presoak treatment, water BMA	84 b	4 bc	2 cd	10 e
5.	0.05% GA_3 as BMA	76 c	4 b	3 bcd	17 c
6.	0.025% Ethrel 18h presoak treatment, water BMA	91 a	4 bc	4 b	1 g
7.	7d @ -20°C followed by 0.05% GA_3 + 0.025% Ethrel 18h presoak, water BMA	89 a	4 b	3 cd	4 f
8.	18h presoak, Clipped Cotyledons, water BMA	67 d	18 a	2 d	13 d
LSD		2.6	1.5	1.3	2.1

Seed size responses are presented in Table 3, as there was a 1% higher overall normal seedling count in size 2 than in size 3.

Table 3. Comparison of 20°C germination of normal, abnormal seedlings, dead, firm seeds of two seed sizes averaged across ten seed lots and eight treatments for sunflower seeds.				
Size	Normal Seedlings	Abnormal Seedlings	Dead Seeds	Firm Seeds
	----------%----------			
2	74 a	5 a	3 a	18 b
3	73 b	5 a	3 b	19 a
LSD	0.8	0.5	0.4	0.7

Seed sizes were compared across seed treatments in Table 4. With only three significant differences between size 2 and size 3 for normal seedling percentages. The water check had significant differences in dormancy between sizes for seed lot 2 and 7; however, lot 2 exhibited higher dormancy in size 3, while size 2 was more dormant in lot 7. Seed lot 4 had significantly higher dormancy in size 3 with the preheat 7d @ 30°C treatment. It is hard to draw a consistent conclusion on whether seed size and seed dormancy are closely related based on the findings of this study.

Table 4. Comparison of warm germination percent normal seedlings of eight treatments for ten seed lots with two seed sizes for sunflower seeds.																				
Treatment	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5		Lot 6		Lot 7		Lot 8		Lot 9		Lot 10	
	2	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**
1	85	95	62*	43*	67	71	93	77	5	2	1	3	70*	89*	91	82	40	40	74	71
2	82	80	22	24	34	50	76	67	26	30	13	14	89	83	93	97	59	54	82	72
3	75	92	57	55	62	67	89*	66*	28	21	6	14	84	91	94	96	49	50	84	75
4	84	81	93	90	93	94	100	98	67	56	55	62	88	82	99	98	90	88	86	75
5	74	89	89	84	92	92	96	98	38	32	16	31	91	87	97	97	71	80	90	85
6	80	94	84	80	93	93	97	97	92	91	92	94	88	93	97	97	94	97	93	95
7	91	90	93	85	95	96	100	98	74	68	73	69	95	91	97	98	96	91	97	98
8	94	96	94	79	93	94	98	93	2	8	13	7	73	61	93	90	60	44	80	74
LSD[1]	17																			
[1]Significant differences between seed sizes within lot noted by "*".																				

Conclusions can be made that stratifying seed and following with a treatment of GA_3 and Ethrel may have a synergistic value in reducing dormancy in freshly harvested hybrid oil seed sunflowers without negatively impacting the non-dormant seed population. Ethrel and prechilling may be the most practical solutions from a cost-effective standpoint. The Ethrel rates used in this study were significantly higher than that of which is stated in the AOSA Rules [1] – at a 0.025% study rate compared to that of a 0.0145% maximum. Ethrel at the rate of 0.025% and GA_3 at a rate of 0.05%

should be considered as an upper bound as it resulted in significant initial root curling and hypocotyl elongation, respectively. (Figure 2).

Figure 2. Left: root development of lot 10 with no treatment. Middle: Root development of Ethrel treated seed from lot 2. Right: Hypocotyl elongation from GA3 blotter moistening agent treatment in lot 8.

Based on the findings of this study, it is not clear whether the Ethrel must be used only as a presoak method at 0.0145% as inferred in section 6.9h.

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