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Factors affecting seed germination of *Heliopsis helianthoides* (L.) Sweet

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Summary

Low and erratic seed germination of *Heliopsis helianthoides (L.)* Sweet have hindered breeding efforts. The objectives of this study were to characterize seed dormancy and to investigate factors (cold stratification, ethylene, gibberellic acid, priming, and red light) as potential germination enhancers. Physiological, but not physical nor morphological seed dormancy was found. Cold stratification (1-6 weeks), imbibing seeds in ethephon (1-5 mM), and red light exposure (1.5 h) all contributed favourably to cumulative germination at 2 weeks, and cold stratification and ethephon also promoted quicker germination. Germination enhancement for any of the factors was variable and depended on seed lot and duration of seed storage prior to experiments. As storage time increased, seed dormancy decreased and seeds germinated more readily with less sensitivity to germination cues. Gibberellic acid (500 mgl^{-1}) treatments were not significantly different than water controls. Priming seeds in water, KNO_3 (0.5%), and ethephon (1 mM) for 12 h, drying, and reimbibing in water resulted in reduced germination and increased pathogen attack. Imbibing dormant seeds of *H. helianthoides* with ethephon (1-5 mM), followed by exposure to red light (1.5 h), and at least one week of cold stratification (4°C) is recommended in order to promote both fast and maximum germination.

Introduction

Heliopsis helianthoides (L.) Sweet (common names include false sunflower, sunflower heliopsis, and smooth oxeye) is an herbaceous perennial in the Asteraceae family (Fisher, 1957). It is native to the prairies of Canada and the Eastern and Central United States and is cultivated widely as an ornamental (Fisher, 1958). Its combination of drought-tolerance, pest resistance, and long bloom season (several weeks in summer) makes it an attractive choice for wildflower seed mixes and perennial gardens.

Heliopsis helianthoides is propagated primarily by seed (achenes) for naturalization and restoration purposes, while cultivars with improved ornamental traits are propagated either vegetatively or by seed (Nau, 1996; Jelitto Staudensamen, 2004; North Creek Nurseries, 2004). Germination recommendations for this species are sparse, and propagators typically advocate light and/or cold stratification (Nau, 1993; Pyle, 2003; Stokes, 2003; Oak Prairie Farm, 2004; Thompson and Morgan, 2004). In closely-related genera such as *Coreopsis, Echinacea, Helianthus,* and *Zinnia,* multiple factors have been identified which have increased overall germination percentage and/or hastened the rate of germination. These factors include cold stratification (Baskin *et al.,* 1992; Feghahati

and Reese, 1994; Wartidiningsih *et al.,* 1994; Macchia *et al.,* 2001), ethylene supplied as ethephon (Feghahati and Reese, 1994; Macchia *et al.,* 2001; Sari *et al.,* 2001), oscillating temperatures (Macchia *et al.,* 2001), hydrogen peroxide (Ogawa and Iwabuchi, 2001), light (Feghahati and Reese, 1994; Macchia *et al., 2001),* osmotic priming (Kathiresan and Gnanarethinam, 1985; Samfield *et al.,* 1990; Wartidiningsih *et al.,* 1994), and gibberellic acid (Cseresnyes, 1979; Chandler and Jan, 1985; Seiler, 1998).

Seed germination has generally been low (<50%) and erratic in the *H. helianthoides* breeding program I initiated in 1997, especially so with attempts to germinate seed soon after harvest. Germination challenges as well as limitations in seed numbers from controlled pollinations have resulted in small seedling populations and germination at inopportune times of the year. The objectives of this research were to characterize the type(s) of seed dormancy and identify factors that promote both faster germination and a higher final germination percentage in *H. helianthoides.*

Materials and methods

Plant material

Open-pollinated *H. helianthoides* seed was collected, cleaned, and bulked on October 24, 2003 from 50 open-pollinated, first-year seedlings of selection BN4 growing at the University of Minnesota, St. Paul campus (lat. 45°00'N; long. 93°10'W), and was used for year-one experiments (seed lot one). BN4 is an open-pollinated seedling of selection E2, which is a cross of 'Ballerina' x 'Helhan' (Loraine Sunshine). Open-pollinated seed was collected, cleaned, and bulked October 16, 2004 from 50 open-pollinated, first-year seedlings of *H. helianthoides* `Prairie Sunset' growing at the same location and used for year-two experiments (seed lot two). Seed was cleaned using air columns to separate empty or light seeds/chaff and retain filled seed. Seed was stored dry at 4°C until the commencement of experiments. Seed lot was, thus, confounded with year.

Heliopsis helianthoides is a strongly self-incompatible tetraploid species (Fisher, 1957), which may enhance heterozygosity of individuals and heterogeneity within populations. Numerous advanced selections (>200 tracing back to seven founding parents) and cultivars ('Bressingham Doubloon', 'Summer Sun', and 'Venus') were growing adjacent to the open-pollinated families used in this study. Therefore, considerable genetic variability within these seed lots is expected, although each seed lot traced back to a single maternal grandmother ('Ballerina'- seed lot one; 'Prairie Sunset'- seed lot two).

Characterization of dormancy

In order to better understand seed dormancy in *H. helianthoides,* embryo size relative to the amount of endosperm and rate of water imbibition were investigated to help ascertain if morphological or physical seed dormancy are present. Embryo size relative to endosperm was determined by cutting open and observing 25 mature seeds of each seed. lot after imbibing with distilled water for eight hours. In order to determine the imbibition rate, only seed lot two was used: four replicates of 25 seeds were used for each of 17 imbibition durations: 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 4.0, 6.0, 8.0, 12.0, 16.0,

20.0, 24.0, and 28.0 h. Each replicate was weighed and placed on a single 90 mm sheet of Whatman® #1 filter paper (Whatman International Ltd., Maidstone, England) within a plastic Petri dish (20 x 100 mm). Distilled water (5 ml) was added, and the dish was covered and then sealed within a 16.8 x 14.9 cm polyethylene bag (Glad® zipper bags, Oakland, CA). After the allotted time, seeds were removed from the dish, blotted dry, and weighed. Percent weight increase was calculated by:

Percent weight increase= [(final weight-initial weight)/initial weight] * [100]

General germination protocols and data calculations

Four replicates of 100 seeds were each placed on a single 90 mm sheet of Whatman ® #1 filter paper. Each replicate of seeds and filter paper disk were placed within a plastic Petri dish (20 x 100 mm) and imbibed with 5 ml of distilled water or treatment solutions as described below. Seeds were imbibed in a dark room and maintained in darkness except for exposure to dim green light to allow handling (initiation of experiments and data collection), and red light for the treatments which included red light. Except for cold stratification (4°C), imbibed seeds were kept at a constant 25°C in a dark room.

Seeds were imbibed in covered Petri dishes for one hour before red light was administered. Red -light was provided using red plastic filters over cool white fluorescent bulbs and seeds were exposed for 1.5 h. Subsequently, Petri dishes were placed in resealable, 16.8 x 14.9 cm polyethylene bags to conserve moisture and allow for easy access and resealing between readings. No additional moisture was added throughout the duration of the experiment. Cold stratification was administered after red light exposure. Germination was monitored daily for 14 days post cold stratification, at which time the number of seeds with an emerged radicle were scored as germinated and removed.

Data calculated for each replication included total germination percentage after 14 days (germination), days to 50% of total germination (T_{50}), and area under the germination progress curve (AUGPC). The two days (X and X. $_1$) bracketing the 50% total germination value and their cumulative germination values (C, and C_2, respectively) were used to calculate T_{50} with the following formula: $T_{50}=X + [(50\% \text{ total germination-}C_1)/(C_2-C_1)]$. Earlier and/or a greater germination percentage increases the AUGPC. The formula for AUGPC was calculated modifying the formula for area under disease progress curve, substituting percentage infected tissue (Y). with germination percentage, and time was measured in days (X) (Shaner and Finney, 1977).

$$AUGPC=\sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2] [X_{i+1} - X_i]$$

Ethephon/ red light/ cold stratification

The effects of imbibition with ethephon (2-chloroethylphosphonic acid, $C_2H_6ClO_3P$ 1 mM), red light (darkness and red light), and cold stratification (0, 1, 2, and 3 weeks) were investigated using a completely randomised design (ERS) and repeated over two years, once with each seed lot. An additional cold stratification treatment of 6 weeks was included for seed lot two. The experiment was initiated on January 14, 2004 for seed lot one and on October 28, 2004 for seed lot two. At the end of this experiment using seed lot

two, ungerminated seeds from one replication (Petri plate) per treatment were cut in half longitudinally, plates were flooded with 1% tetrazolium chloride until seeds were covered, and 12 h later the colour of the embryos were observed.

Ethephon concentration

The effects of imbibition with ethephon at varying concentrations (0, 0.1, 0.5, 1, 3, and 5 mM) were investigated. For seed lot one, all treatments received one week of cold stratification and red light. Using seed lot two the experiment was repeated with the addition of all ethephon concentrations with red light, but without cold stratification. The experiment was initiated on March 18, 2004 for seed lot one and on January 6, 2005 for seed lot two.

Gibberellic acid

The effects of imbibition with gibberellic acid (GA_3) and varying cold stratification or red light were investigated. The effects of imbibition in GA_3 (0 and 500 mgl^{-1}) and cold stratification (0 and 1 week) were investigated in a completely randomised design with seed lot one. Seeds which were imbibed in ethephon (1mM), given red light, and cold stratified (0 and 1 week) were also included as reference treatments. All treatments received red light and the experiment was initiated on July 21, 2004. Using seed lot two the effects of imbibition in GA_3 (0 and 500 mgl^{-1}) and red light (darkness and red light) without cold stratification were investigated in a completely randomised design. This GA_3 experiment was initiated on the same date (October 28, 2004) as the seed lot two ERS, and they shared common treatments.

Priming

The effects of priming seeds with water, ethephon (1 mM), or KNO_3 (0.5%) in combination with cold stratification (0 and 1 week) were investigated. Replications of 100 seeds were placed within pouches made from folded 4 x 4 cm square pieces of sheer nylon cloth (bridal toule) secured with wire (22 gauge) and placed in capped, one-litre glass bottles filled with the designated priming solution which was agitated on a stir plate (about 200rpm) for 12h (Kathiresan and Gnanarethinam, 1985). After imbibition, seeds were allowed to air dry for 12 h before they were removed from the cloth pouch and prepared for germination. Primed seeds were reimbibed with water, exposed to red light, and then given the designated cold stratification treatment. In addition, unprimed seeds imbibed in water or ethephon (1 mM) and exposed to red light were included as reference treatments. This experiment was conducted using seed lot one only and was initiated May 18, 2004.

Statistical analyses

Analysis of variance (ANOVA) was used to compare the effects of germination factors and individual treatments. Tukey's Honestly Significant Difference (HSD) ($P<0.05$) was used for mean separation. Calculations were made using SPSS software (SPSS 11.0 for Windows, Chicago, IL).

Results

Characterization of dormancy

Mature, imbibed seeds contained a well-developed embryo which filled the seed cavity and visible endosperm could not be detected (figure 1). Seeds readily imbibed water during the first 12h at which point a plateau of about 70% water weight increase calculated on a fresh mass basis was reached.

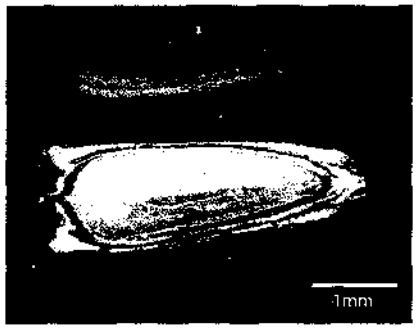

Figure 1. Longitudinally cut achene of *Heliopsis helianthoides* with embryo (above) removed from pericarp (below).

Ethephon/red light/cold stratification

Ethephon, red light, and cold stratification were significant factors contributing positively to all three dependent variables; germination, T_{50}, and AUGPC, with the exception of red light not significantly affecting T_{50} (table 1). Seedlot had a significant effect, with seed lot two showing a reduction in germination and AUGPC and a longer T. For germination and AUGPC, the ethephon x stratification interaction was significant and codirectional for all three variables, except for T_{50} for seed lot two, which was not significant. The light x stratification interaction was not significant, but the light x ethephon interaction was significant only when considering the two seed lots combined. There was a trend for increased germination and AUGPC as the duration of cold stratification increased (table 2). There was a general decrease in T_{50} as cold stratification increased among the non-ethephon treated seeds in both seed lots one and two. For seed lot one, 1 mM ethephon compensated for multiple weeks of cold stratification for treatments without ethephon, while ethephon just partially compensated for cold stratification in seed lot two (table 2).

Ethephon concentration

Various ethephon concentrations with one week of cold stratification and red light resulted in no significant differences in germination or AUGPC for seed lot one. Ethephon did, however, reduce T_{50} relative to non-ethephon treatment (tables 1 and 3). In comparison, there was a significant increase in germination and AUGPC as the ethephon concentration increased with seed lot two, but there were no significant differences among treatments for T_{50}. Cold stratification increased germination and AUGPC, but did not significantly affect T_{50}. Additionally, the ethephon x stratification interaction was not significant for all three dependent variables.

Table 1. The F-test significance of various factors using ANOVA on germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) for *Heliopsis helianthoides*.

Experiment	Germination percentage	T_{50}	AUGPC
Ethephon/red light/cold stratification			
Seed lots one and two combined			
Seed lot (T)	<0.01[z]	-[y]	<0.01[z]
Ethephon (E)	<0.01	-	<0.01
Light (L)	<0.01	-	<0.01
Stratification (S)	<0.01	-	<0.01
T × E	<0.01	-	<0.01
T × L	0.31	-	0.25
T × S	<0.01	-	<0.01
E × L	0.04	-	0.02
E × S	<0.01	-	<0.01
L × S	0.44	-	0.47
T × E × L	0.59	-	0.37
T × L × S	0.62	-	0.85
T × E × S	0.01	-	0.01
E × L × S	0.12	-	0.39
T × E × L × S	0.09	-	0.28
Seed lot one			
E	<0.01[x]	<0.01[x]	<0.01[w]
L	<0.01	0.43	<0.01
S	<0.01	<0.01	<0.01
E × L	0.63	0.16	0.55
E × S	0.02	0.02	<0.01
L × S	0.45	0.44	0.51
E × L × S	0.43	0.24	0.79
Seed lot two			
E	<0.01[w]	<0.01[v]	<0.01[w]
L	<0.01	0.62	<0.01
S	<0.01	<0.01	<0.01
E × L	0.18	0.14	0.09
E × S	<0.01	0.12	<0.01
L × S	0.35	0.34	0.27
E × L × S	0.22	0.46	0.61

Table 1. *continued*

Experiment	Germination percentage	T_{50}	AUGPC
Ethephon concentration			
Seed lot one			
E	0.70	<0.01	0.41
Seed lot two			
E	<0.01	0.61	<0.01
S	<0.01	0.09	<0.01
E × S	0.37	0.11	0.17
Priming			
Priming (P)	<0.01	<0.01	<0.01
S	0.04	0.07	0.03
P × S	0.28	0.56	0.30
Gibberellic acid experiments			
Seed lot one			
Gibberellic acid (GA_3)	0.16	0.45	0.18
S	0.06	<0.01	<0.01
GA_3 × S	0.93	0.50	0.75
Seed lot two			
GA_3	0.49	0.77	0.71
L	0.49	0.07	0.13
GA_3 × L	0.26	0.95	0.53

[z] Data within dependent variable and experiment were square root transformed.
[y] A suitable transformation could not be found.
[x] Data within dependent variable and experiment were transformed with x^4.
[w] Data within dependent variable and experiment were transformed with one over the square root.
[v] Data within dependent variable experiment were transformed with $x^{-0.75}$.

Priming

Priming seeds significantly reduced germination and AUGPC while increasing T_{50} (tables 1 and 4), while cold stratification, consistent with other experiments, generally had the opposite effect. The priming x stratification interaction was not significant. Seeds from primed treatments showed visible fungal infection by the end of the experiment, and none were significantly different from each other in terms of germination and AUGPC. Ethephon primed seeds however, did have a significantly lower T (table 4). Among non-primed seeds, those receiving cold stratification and/or ethephon had significantly higher germination and AUGPC relative to the water and non-stratified treatment (table 4).

Table 2. Germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) for *Heliopsis helianthoides* seeds exposed to varying durations of cold stratification (4°C), red light, and ethephon.

		Ethephon (0 mM)					Ethephon (1mM)					
Weeks strat		0	1	2	3	6	0	1	2	3	6	x̄ light trts
Germination percentage												
Seed lot one												
Red light	+	43.3ef[z]	66.3cd	71.0abc	75.3abc		75.3abc	82.5ab	84.5a	83.0ab		72.4
	−	36.0f	54.5de	65.3cd	69.5bc		74.0abc	81.8ab	76.3abc	76.5abc		66.7
x̄ strat trts		39.6C	60.4B	68.1AB	71.5A		74.6A	82.1A	80.4A	79.8A		
		x̄ ethephon (0 mM) = 59.9					x̄ ethephon (1 mM) = 79.2					
Seed lot two												
Red light	+	1.5g	11.0ef	15.3e	19.8de	31.3cd	36.0e	59.5ab	59.3ab	66.3ab	71.0a	37.1
	−	1.8g	3.5fg	10.8ef	12.5e	28.3cd	30.0cd	55.3ab	55.0b	64.5ab	73.0a	33.5
x̄ strat trts		1.6D	7.3CD	13.0BC	16.1B	29.8A	33.0C	57.4B	57.1B	65.4AB	72.0A	
		x̄ ethephon (0 mM) = 13.6					x̄ ethephon (1 mM) = 57.0					
T_{50}												
Seed lot one												
Red light	+	3.15f	2.77ef	2.51def	2.38bcdef		2.56cdef	1.95ab	2.06abcd	1.96abc		2.42
	−	3.34f	3.01f	2.40bcdef	2.37abcdef		2.14abcde	1.89a	1.87a	2.08abcd		2.39
x̄ strat trts		3.25C	2.89BC	2.46AB	2.37A		2.35B[y]	1.92A	1.96AB	2.02AB		
		x̄ ethephon (0 mM) = 2.74					x̄ ethephon (1 mM) = 2.06					

Table 2. continued

		Ethephon (0 mM)					Ethephon (1mM)					
Weeks strat		0	1	2	3	6	0	1	2	3	6	$\bar{x}$ light trts
T_{50}												
Seed lot two												
Red light	+	4.08ab	4.42ab	3.50ab	2.71a	3.26ab	3.37ab	3.40ab	3.49ab	2.75a	2.69a	3.35
	–	7.17b	4.88ab	3.06a	2.99a	2.76a	2.95a	2.91a	3.08a	2.67a	2.48a	3.40
$\bar{x}$ strat trts		5.62B	4.65AB	3.28A	2.86A	3.01A	3.16A	3.15A	3.28A	2.71A	2.59A	
		$\bar{x}$ ethephon (0 mM) = 3.8					$\bar{x}$ ethephon (1 mM) = 3.0					
AUGPC												
Seed lot one												
Red light	+	429.4ef	672.8cd	758.9abc	804.9abc		805.6abc	917.6a	909.1ab	923.4a		777.7
	–	361.5f	564.3de	724.3bcd	764.5abc		814.4abc	897.5ab	853.8abc	855.3abc		729.4
$\bar{x}$ strat trts		395.4C	618.5B	741.6A	784.7A		810.0A	907.6A	881.4A	889.3A		
		$\bar{x}$ ethephon (0 mM) = 635.0					$\bar{x}$ ethephon (1mM) = 872.1					
Seed lot two												
Red light	+	13.3h	101.6fg	154.8ef	214.4de	324.5d	341.8d	595.5bc	561.1c	688.1abc	736.4ab	373.1
	–	9.1h	32.5gh	115.5efg	135.6ef	305.6d	305.8d	566.6c	541.4c	657.1abc	781.8a	345.1
$\bar{x}$ strat trts		11.2C	67.1C	135.1B	175.0B	315.1A	323.8D	581.1C	551.3BC	672.6AB	759.1A	
		$\bar{x}$ ethephon (0 mM) = 140.7					$\bar{x}$ ethephon (1 mM) = 577.6					

[z] Treatment means for the ethylene × stratification × light interactions followed by the same lower case letter and ethylene x stratification interactons followed by the same upper case letter within seed lot and dependant variable do not differ significantly (HSD, $P \leq 0.05$).

[y] T_{50} data for stratification means with ethephon treated seeds for seed lot one were transformed with x^{-2} for analysis; untransformed means are presented.

Gibberellic acid

Exposure to GA_3 (500 mgl^{-1}) did not significantly affect germination (tables 1, 5, and 6). There were no instances across the two GA_3 experiments where comparable treatments (same red light and stratification level) differing for only imbibition in water or GA_3 differed for any of the three dependent variables. In the GA_3 experiment where cold stratification duration was varied, cold stratification generally increased germination and AUGPC and reduced T_{50} (tables 1 and 5). Among non-stratified seeds ethephon significantly reduced T_{50} relative to water and GA3 and increased AUGPC relative to water, but the GA_3, water, and ethephon treatments did not differ significantly for any dependent variable for stratified seeds (table 5). In the GA_3 experiment where red light was varied, red light did not significantly affect germination (table 1 and 6).

Table 3. Germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) for *Heliopsis helianthoides* seeds exposed to red light, zero or one week of cold stratificatiobn (4°C), and differing concentrations of ethephon.

		Ethephon(mM)					
	Stratified	0	0.1	0.5	1.0	3.0	5.0
Germination %							
Seed lot one	+	73.3a[z]	79.0a	79.8a	74.5a	77.3a	77.8a
Seed lot two	+	39.5e	63.3bc	75.0ab	73.3ab	82.5a	84.5a
	–	11.5f	44.0de	48.3de	54.5cd	58.0cd	66.0bc
x̄ ethephon trts		25.5C[y]	53.6B	61.6AB	63.9AB	70.3AB	75.1A
T_{50}							
Seed lot one	+	2.0b	1.7a	1.7a	1.7a	1.8a	1.8a
Seed lot two	+	4.0a	4.6a	3.7a	4.0a	3.8a	3.8a
	–	4.0a	2.9a	3.1a	3.9a	3.9a	4.1a
x̄ ethephon trts		4.0A	3.8A	3.4A	3.9A	3.9A	3.9A
AUGPC							
Seed lot one	+	820.5a	931.8a	937.4a	880.9a	900.3a	905.4a
Seed lot two	+	349.6d	558.5bc	689.6ab	675.3ab	768.1a	793.6a
	–	103.8e	448.6cd	454.9cd	493.8cd	488.9cd	565.4bc
x̄ ethephon treatments		226.7B	503.6A	572.3A	584.5A	628.5A	679.5A

[z] Treatment means for the ethtylene or ethylene × light interaction within seed lot and dependant variable followed by the same lower case letter do not differ significantly (HSD, $P \leq 0.05$).

[y] Ethphon treatment means pooled over red light treatments in seed lot two within dependant variable followed by the same upper case letter do no differ significantly (HSD, $P \leq 0.05$).

Table 4. Germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) for cold stratified (1 week, 4°C) and non-stratified *Heliopsis helianthoides* seeds primed with water, ethephon (1mM), and potassium nitrate (0.5%) and unprimed seeds imbibed with water or ethephon (1mM).

		Primed			Unprimed		
		H_2O	Ethephon	KNO_3	H_2O	Ethephon	x̄ strat trts
Germination %							
Stratified	+	53.8b[z]	47.3b	58.0ab	79.5a	80.0a	63.7
	–	39.3b	52.3b	56.8b	57.0b	79.5a	57.0
T_{50} (days)							
Stratified	+	4.0d	1.8ab	3.6cd	1.9ab	1.4a	2.5
	–	4.6d	2.1ab	3.9cd	2.7bc	1.6ab	3.0
AUGPC							
Stratified	+	518.4b	567.6b	571.8b	902.9a	946.3a	701.4
	–	345.5b	600.5b	534.1b	606.3b	932.9	603.9

[z] Treatment means for priming × stratification interactions within dependant vaiable followed by the same lower case letter do not differ significantly (HSD, $P \leq 0.05$).

Table 5. Germination percentage, days to 50% germintaion (T_{50}), and area under the germination progress curve (AUGPC) for cold stratified (1 week, 4°C) and non stratified *Heliopsis helianthoides* seeds imbibed in water, gibberelic acid (GA_3, 500 mgl^{-1}), and ethephon (1mM).

	No stratification			Stratification (1week, 4°C)		
	GA_3	H_2O	Ethephon	GA_3	H_2O	Ethephon
Germination %	63.3ab[z]	56.8b	69.5ab	71.3ab	65.5ab	74.0a
T_{50} (days)	2.81b	2.96b	1.90a	1.99a	2.00a	1.82a
AUGPC	654.4ab	577.6b	795.8a	774.4ab	726.0ab	824.8a

[z] Treatment means for stratification × imbibition solution interactions within dependant variable followed by the same lower case letter do not differ significantly (HSD, $P \leq 0.05$).

Change in dormancy over time and across seed lots

There were four treatments that were commonly used over time in different experiments. These four treatments received red light and differed in their combination of cold stratification (0 and lweek) and ethephon (0 and 1 mM). Comparing the results of these common treatments over time reveals changes in seed dormancy and viability (figures 2 and 3). For seed lot one, there was a general trend for an increase in germination and AUGPC and a decline in T_{50} from January to May, 2004. From May to July, 2004, however, this trend was reversed. Variability in seed dormancy was also observed relative to changes in significant differences among treatments within experiments over time. For instance, among seeds given one week cold stratification and red light in January

Table 6. Germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) for *Heliopsis helianthoides* seeds imbibed in water, gibberellic acid (GA_3, 500 mgl^{-1}) and ethephon (1 mM) and exposed to red light or darkness.

	Red light			Darkness		
	GA_3	H_2O	Ethephon	GA_3	H_2O	Ethephon
Germination %	1.8b[z]	1.5b	36.0a	0.8b	1.8b	30.0a
T_{50} (days)	4.62a	4.08a	3.37a	7.50a	7.17a	2.95a
AUGPC	14.4b	13.3b	341.8a	4.9b	9.1b	305.8a

[z]Treatment means for light × imibibition solution interactions within dependant variable followed by the same letter do not differ significantly (HSD, $P \leq 0.05$)

2004, the ethephon and non-ethephon treated seeds differed significantly for germination, T_{50}, and AUGPC (table 2). However, by March 2004 these two treatments no longer. differed significantly for germination and AUGPC (table 3), and by May and July 2004 these treatments did not differ for any of the three dependent variables (tables 4 and 5). Again, with seed lot two the trend for increased germination and AUGPC over time was seen regardless of treatments between October 2004 and January 2005. However, T" data points converged over time (figure 3). As seeds aged they germinated more readily regardless of ethephon and cold stratification treatments. In addition, by July 2004 seed viability may have begun to decline as suggested by declining germination and AUGPC and greater time to T (figures 2 and 3).

Comparing .the four common treatments in the seed lot one ERS experiment and seed lot two ethephon concentration experiment (all initiated about 12 weeks after harvest), reveals a significant effect due to seed lot for all three dependent variables (table 7). These four common treatments received red light and differed in their combination of cold stratification (0 and lweek) and ethephon (0 and 1 mM). Due to the relatively low germination for seed lot two compared to seed lot one, seed viability was checked within the seed lot two ethephon concentration experiment using tetrazolium chloride. All filled seeds turned pink and on average only 1.5 seeds per replication (range of 0 to 3 seeds out of the 100 seeds) were empty. Therefore, poor germination compared to the previous year (seed lot one) could not be attributed to a high rate of empty seeds with aborted embryos.

Discussion

Data from this study point to physiological dormancy as the primary type of dormancy affecting seed germination in *H. helianthoides.* Physiological dormancy generally declines during dry storage (Hartman *et al., 1990).* Decline of dormancy was observed in this study. since seeds given the same treatments over time tended to germinate more readily and became less sensitive to germination cues as duration of dry storage increased (figure 2). In addition, the seeds of many species with physiological dormancy respond to relatively

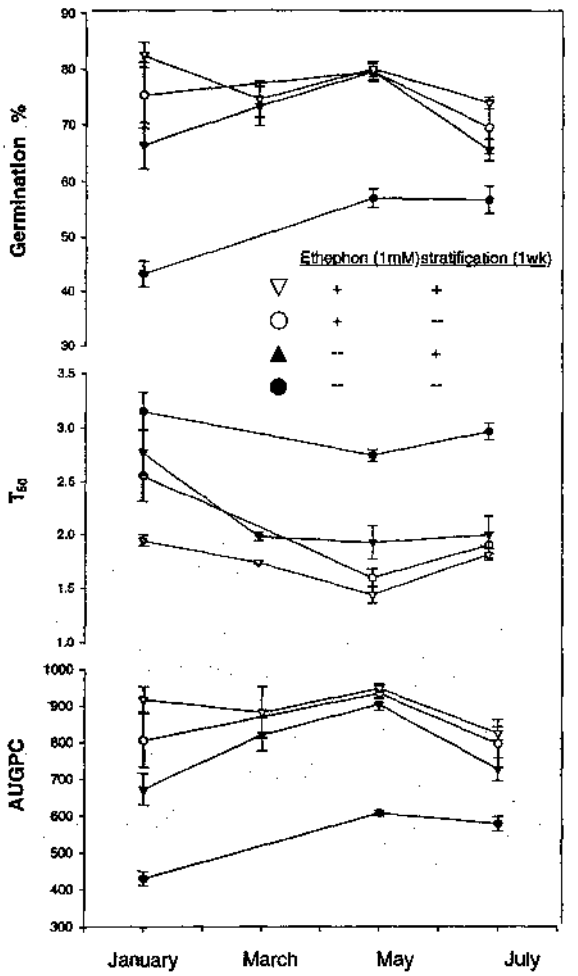

Figure 2. Germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) with standard errors of four treatment combinations of ethephon and stratification administered to *Heliopsis helianthoides* seeds (2003 seed collection) over time for seed lot one (2004).

short durations of cold stratification and light (Hartmann *et al., 1990),* which is consistent with results from this study (tables 1 and 2). Morphological dormancy (seeds containing embryos which are not fully developed at time of seed dissemination) is not present since mature seeds contained embryos which filled the seed cavity and endosperm is depleted (figure 1). Physical dormancy is not present either since water was readily absorbed by seeds and embryos readily broke the pericarp longitudinally .during germination

Ethephon, which releases ethylene upon entering living plant tissue, consistently enhanced germination across experiments and seed lots (table 1). The degree of enhancement varied and may relate to the degree of dormancy of the seeds at the time of each experiment and potential genetic differences between the two seed lots. Different seed lots tracing back to different founding parents may account for inherent genetic differences in ethephon sensitivity as Sari *et al.* (2001) found among different populations of *Echinacea angustifolia* and *E. pallida.* In this study, variable germination was found

Table 7. The F-test significance of factors using ANOVA on germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) for common treatments across seed lots initiated after 12 weeks storage for *Heliopsis helianthoides*.

	Germination percentage	T_{50}	AUGPC
Seed lot (T)	<0.01	<0.01	<0.01
Ethephon (E)	<0.01	0.13	<0.01
Stratification (S)	<0.01	0.41	<0.01
T × E	0.01	0.23	0.45
T × S	0.11	0.27	0.56
E × S	0.02	0.84	0.12
T × E × S	0.52	0.80	0.59

across seed lots and across seed storage durations. Since seed lot was confounded with year, it is unclear whether the differences in germination observed are due to genetic differences across populations or different environmental conditions. With relatively dormant seed, as for the two ERSs which were the first experiments conducted with each seed lot, ethephon promoted greater germination compared to the water controls. However, as dormancy declined during storage, the stimulatory effect of ethephon over the water control also declined. After about 39 weeks in stroage for seed lot one, ethephon (1 mM) no longer made a difference (table 5). In addition, with relatively dormant seed as for the seed lot two ERS, ethephon (1 mM) could not compensate for cold stratification to the same extent it had for the seed lot one ERS; ethephon treatments for seed lot one were indistinguishable from each other for germination and AUGPC and were comparable for these dependent variables only to those water treatments receiving the longest durations of cold stratification.

The effect of a range in ethephon concentrations differed between seed lots. For seed lot one, there were no significant differences among treatments, except that the water control resulted in a significantly longer T_{50}. However, for seed lot two there was a trend for progressively enhanced germination and AUGPC as ethephon concentration increased. A stronger response to varied ethephon concentrations for seed lot two may be due to a greater degree of seed dormancy and sensitivity to ethephon. Some seedlings were grown to maturity from seed lot two and there were no visible, deleterious effects on growth and development of any seedlings, even those having been exposed to the highest ethephon concentrations (3 or 5 mM). Therefore, concentrations of ethephon at least up to 5 mM may be useful for heliopsis breeders and for commercial heliopsis seed propagation.

The mechanism by which ethylene can reduce or break seed dormancy in species where ethylene promotes germination is not well understood. Ethylene can be produced by both the seed itself and the surrounding environment (Baskin and Baskin, 1998). In apple seeds it was determined that endogenous ethylene levels and ability to germinate increased as cold stratification duration increased from 0 to 40 days (Sinska and Gladon, 1984). Germination was inhibited when culturing isolated apple embryos with $Hg(Cl0_4)_2$ (traps

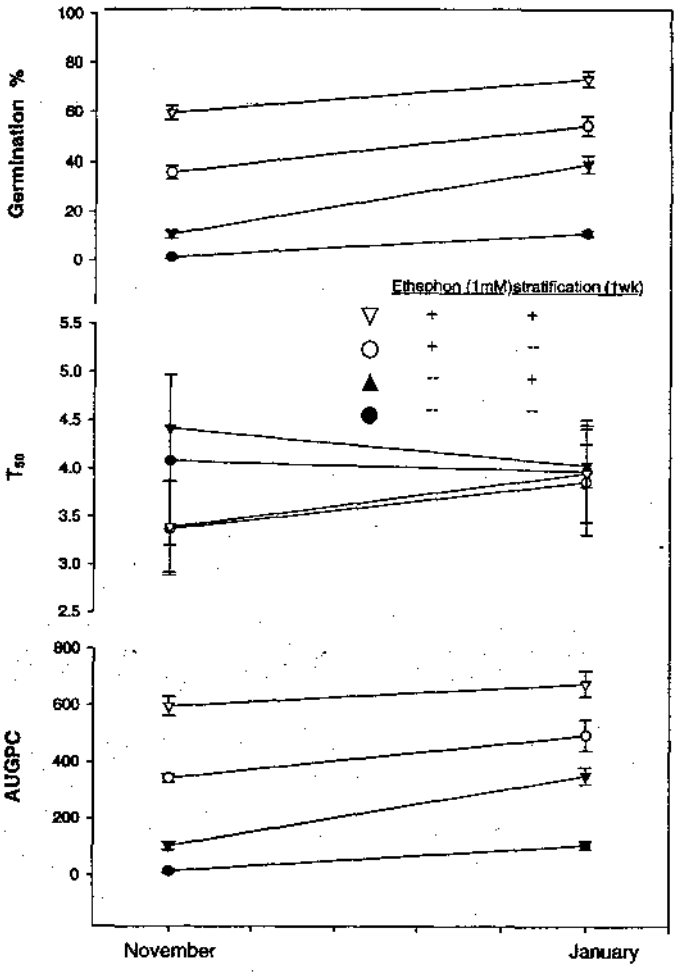

Figure 3. Germination percentage, days to 50% germination (T_{50}), and area under the germination progress curve (AUGPC) with standard errors of four treatment combinations of ethephon and stratification administered to *Heliopsis helianthoides* seeds (2004 seed collection) over time for seed lot two (2004-2005).

ethylene) having been stratified in water within in tact seed coats, however, inhibition was not observed when in tact seeds were stratified with $Hg(ClO_4)_2$ and then isolated embryos (seed coats removed) were cultured in water (Sinska and Gladon, 1984). In addition, ethylene is more soluble in air than water, and ethylene concentration may especially build in soils with high organic matter when temperature and moisture levels are high (Baskin and Baskin, 1998). High ethylene levels may be a favourable germination cue for *H. helianthoides* as this prairie species germinates in spring when moisture is generally abundant from snow melt and spring rains and temperatures are rising; prairie soils are also generally high in organic matter.

Cold stratification and red light also enhanced germination. This is in agreement with what has been previously suggested for H. *helianthoides* in trade magazines, popular books, and catalogues (Nau, 1993; Pyle, 2003; Stokes, 2003; Oak Prairie Farm, 2004; Thompson and Morgan, 2004). The duration of cold stratification needed to maximize

germination varied across seed lots and experiments and appears to relate to seed age and degree of dormancy. In the seed lot one ERS (initiated about 12 weeks after harvest) the benefit of cold stratification reached a plateau at about 2 weeks (table 2), while for the seed lot two ERS (initiated about 2 weeks after harvest) a plateau was not seen even after 6 weeks (table 2). Short-term exposure to red light enhanced germination to a small, yet significant, degree among the ERSs (table 1). Perhaps the reason why red light was not a significant factor in the seed lot two GA_3 experiment was due to it being a smaller experiment with less treatments and generally low germination. Further investigation of the potential benefit of red light on germination of *H. helianthoides* is warranted. Studies involving far-red light treatments in combination with red light treatments could help determine whether the positive effect due to red light is photoreversible and therefore phytochrome dependent. Further research could also explore light treatments for germination of *H. helianthoides* in commercial settings using standard commercial light sources and photoperiods. In addition, positive, significant ethephon interactions with light and also with cold stratification in the ERSs were present and codirectional (tables 1 and 2). Macchia *et al.* (2001) using similar ethephon and cold stratification levels also found significant interactions with ethephon and cold stratification and ethephon and light in *Echinacea,* although there were both co- and anti-directional interactions.

For many species, osmotic priming can reduce time to germination, allow germination to proceed at a faster rate and with greater uniformity, and increase overall germination percentage (Samfield *et al.,* 1991; Wartiginingsih *et al., 1994).* One possible explanation for decreased germination and pathogen attack in this study is that dormancy was inherently low when the experiment was initiated after about seven months of seed storage. During priming germination may have proceeded to a point where embryos were damaged upon subsequent drying. If true, damaged embryos may be more susceptible to pathogen attack which may have led to reduced germination, A positive effect on germination rate (T_{50}) due to priming with ethephon was observed in this study (table *4).* Similar results were found by Macchia *et al.* (2001) using *E. angustifolia* seeds that were dried for 24h after ethephon treatment and then reimbibed with water. Priming for less time, priming seed soon after harvest when dormancy may be greater, or altering factors such as temperature, osmoticum type and concentration, and aeration during priming are all potential possibilities which can be explored further.

Gibberellic acid did not alter germination for either GA_3 experiment. To determine if the lack of a response to GA_3 in seed lot one may have been due to reduced dormancy by the time the experiment was initiated (about 39 weeks after harvest), the seed lot two GA_3 experiment was initiated within 2 weeks of seed harvest, when dormancy would be expected to be high. As in seed lot one, GA_3 did not significantly alter germination. Insensitivity to GA_3 within some members of the Asteraceae family has been previously reported (Macchia *et al.,* 2001).

Many dynamic factors influence the degree of dormancy found across seed lots, as well as the degree of germination enhancement from factors known to stimulate germination. Such variability makes it very difficult to recommend a single treatment that is optimum for all seed lots. Variable dormancy and responses to germination cues can be due to factors such as year (Baskin *et al.,* 1992), population (Sari *et al., 2001),* seed maturity at

harvest (Miyajima, 1997), and seed storage environment and duration (Baskin and Baskin, 1998). Inbreeding can also influence germination in some species (Baskins and Baskins, 1998), and inbreeding is possible within the seed lots used for this study. Although the responses to germination cues were variable, improved germination of *H. helianthoides* seeds can generally be achieved by imbibing with ethephon (1 to 5 mM), exposure to red light (1.5 h), and providing at least one week of cold stratification (4°C). These conditions should generally improve final germination percentage and promote for quick and uniform germination of *H. helianthoides* seeds across seed lots and seed storage durations.

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