

**Wildlife Preservation Fund Grant #02-022W**  
**Seed Vigor of Virginia Bunchflower (*Melanthium virginicum*) from Illinois**  
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**Final Report (7/16/02)**

**Introduction**

Virginia Bunchflower (*Melanthium virginicum* L.) is a member of the Liliaceae family, and a native species of the eastern United States. It occurs in predominantly wet-mesic prairie habitats and can still be located in moist meadows, savannas, and along streams. A recent study of the plant's status in Illinois indicated that although it was found in 15 counties as late as 1960, it is now found in only three (Moorehouse *et al.*, 2002). The species is currently listed by the Illinois Endangered Species Board as threatened. Seed collected from five sites in the three Illinois county area in the fall of 2000 was stored in a cooler at a temperature of 4.0 C and a relative humidity of 50%, and then used for this experiment.

Samples of seeds from the five sites were earlier tested with tetrazolium to determine their viability. The overall viability of the seeds was approximately 94% (see appendix). It was also determined by a germination trial in a germinator at 23 C and 24 hours of light, with no attempt to break dormancy, that only 4% germination occurred. This low rate suggests the presence of some type of dormancy. The dormancy trials performed on the seed were designed to determine which of three possible dormancies or combinations might be present. The three types of dormancy tested were, scarification, stratification, and stratification plus scarification. The effect of seed age on germination after scarification was also examined.

**Materials and Methods**

The seeds tested came from the following 5 sites: McDonough Co.: NW1/4 S31 T7NR1W, 2 miles W. of Bushnell (MH1), MH2 (location not known), McDonough Co.: SW1/4 S30 T7N R3W, 2 miles W. of Sciota (MH3), Hancock Co.: NE1/4 S1& T5N R7W, 3 miles NE of Elvaston (29182, and Hancock Co.: SW ¼ S17 T5N R7W, 1.5 miles NE of Elvaston (29183).

**Stratification design:**

1. 120 seeds from each of 5 sites were dipped in a slurry of Captan and deionized water, mixed at 1 tsp. Captan per pint of water. The seeds were divided into two lots of 60 seeds per site each.
2. Each group of 60 seeds was wrapped in two layers of cheesecloth, secured with a rubber band, and labeled.
3. The seeds were placed in a bin of moistened half peat and half coarse sand. The bin was covered, and stored in a cold room.
4. One lot was stratified for 5 weeks, and the second lot for 7 weeks.
5. When the seeds were removed, 30 seeds of each 60 seed lot were used to test stratification dormancy. They were divided into 3 groups of 10 seeds and placed into petri dishes for the germination part of the experiment. This was done at 5 weeks, and repeated at 7 weeks.

**Stratification and scarification design:**

1. When the stratified seeds were removed from the bin, each 60 seed group was divided into two 30 seed parts. One part was not scarified, and used for the test of just stratification

above. The second part was scarified with a razor blade, making a slight nick in the center of each seed.

2. Each scarified 30 seed group was divided into 10 seed lots and placed in petrie dishes for the germination part of the experiment. This provided 3 repetitions for each dormancy test and site.

#### Scarification design:

1. 60 seeds per site, which had not been stratified, were dusted with Thiram, and divided into two lots of 30 each.
2. One set of 30 seeds was scarified as performed above. They were then divided into 3 groups of ten seeds each and placed in petrie dishes for the germination part of the experiment.
3. The second set of 30 seeds was divided into 3 groups of ten seeds each and placed into petrie dishes for germination. These seeds served as the control, since no dormancy technique was applied.

#### Germination procedure:

1. The petri dishes used for germination contained 2 pieces of filter paper, 5 ml of deionized water, and 10 seeds each. They were each sealed with parafilm, and labeled.
2. The dishes were randomly distributed in clear bins, and placed in a germinator at 23 C, with 24 hours of light.
3. The number of germinated seeds for each dish was recorded every two days from the start of the test for a 20-day period.

## Results and Discussion

### Germination Means

Tables 1 + 2 indicate the germination means and standard deviations for 5 week stratified seed, including significant differences based on Duncan's Multiple Range test at 5%. Tables 3 + 4 provide similar information for the 7-week stratification test.

The germination means were considerably higher for the 7-week stratified seed for most sites. If one considered only the 5-week stratified seed, it would appear that scarification improves germination percentages. However, seed stratified for 7 weeks germinated with percentages close to that of seed scarified as well as stratified. Further study would be needed with longer stratification periods, to determine whether scarification is truly beneficial. Some sites germinated more poorly when scarified, although only one site, #29183, had poorer germination with stratification and scarification together for both the 5-week stratification periods and the 7-week.

### Germination Rates between Dormancies

Germination rates over time can also be compared, either between dormancy treatments, or between seed sites. Tables 5, 6, and 7 contain the average number of seeds germinated over a 20-day period for the three dormancy tests on 5-week stratified seed. For each entry, the total number of seeds germinated for three repetitions was averaged. Even though seed that was both stratified and scarified had somewhat higher rates of germination than just the stratified, the rates are very uneven between the sites. This could be due to site differences, or incomplete removal of dormancy. In all tests seed that was scarified only had extremely low germination rates. It appears that some length of stratification is necessary for high germination rates.

Turning to the results of the 7-week stratified seed, Tables 8, 9 and 10 contain the average number of seeds germinated over a 20-day period for these seeds. These seeds had a much more even

germination rate between sites for the stratification test. However, the stratification plus scarification treatment showed poor germination at two sites, MH2 and 29183. The other three sites with that treatment had much higher rates. Most likely further testing on these seeds with longer periods of stratification would indicate whether scarification is needed. The uneven results when stratification and scarification were combined could also be due to the difficulty of scarifying each seed identically. The seeds are quite small, and it takes care with a razor to prevent damaging the seed. There was also a noticeable differences in the two stratification periods relating to when germination began, and how quickly the bulk of the seeds germinated. Both groups had some germination beginning on the 6<sup>th</sup> day after commencing the germination treatment. The 7-week stratified seed also had two sites begin germination 4 days after the start of treatment, and on the whole, had more seeds germinate earlier.

#### Germination Rates between Sites

Each site can be examined to see how the seed germinated relative to the three dormancy tests. For the 5-week seed, all the sites had relatively close rates between stratification and stratification plus scarification except for MH3. Tables 11-15 show the percent germination averages over a 20-day period by site. In all cases scarification alone has very low rates of germination.

For 7-week seed, Tables 16-20 list germination averages by percent. In this case MH2, MH3, and 29183 show large differences between the average for stratification, and that for stratification plus scarification. In two cases, stratification is much higher, but in the third, it is lower. The remaining two sites show high rates for both dormancy tests.

It is impossible to draw any definite conclusions about the relative vigor of the seed between these sites. Looking at the germination means, all sites exhibited relatively good germination with at least one of the two best dormancy tests, stratification, and stratification plus scarification.

#### Germination Rates for between Years

To compare the vigor of seeds collected in 2000 versus 2001, seed from three locations (Elvaston, Bushnell, and Sciota) collected in 2000 (year-old seed) and 2001 (freshly harvested seed) were stratified for 5 and 7 weeks as previously described. Germination percentages between 5-week stratification treatments and 7-week stratification treatments showed an increased germination percentage after 7 weeks of stratification, as observed previously. In addition, germination percentages were similar between year-old and fresh seed for each location (Table 21).

**Table 1**  
**Melanthium virginicum. Percent Germination Means and Standard Deviations. 5-week Stratified Seed.**

<u>Dormancy</u>	<u>Site:</u>	<u>MH1</u>	<u>MH2</u>	<u>MH3</u>	<u>29182</u>	<u>29183</u>
Stratification		56.7 ± 5.8 a	30 ± 20 a	23.3 ± 20.8 b <sup>z</sup>	20 ± 10 ab <sup>z</sup>	33.3 ± 11.5 a
Stratification and Scarification		70 ± 17.3 a	36.7 ± 5.8 a	56.7 ± 5.8 a	30 ± 5.8 a	26.7 ± 15.3 a
Scarification		10 ± 10 b <sup>z</sup>	3.3 ± 5.8 b <sup>z</sup>	0 b <sup>z</sup>	3.3 ± 5.8 bc <sup>z</sup>	0 b <sup>z</sup>
Control		0 b <sup>z</sup>	0 b <sup>z</sup>	0 b <sup>z</sup>	0 c <sup>z</sup>	0 b <sup>z</sup>

<sup>z</sup>=mean followed by different letters within a column are significant differences based on Duncan's Multiple Range test at the 5% level.

**Table 2**  
**Melanthium virginicum. Percent Germination Means and Standard Deviations. 7-week Stratified Seed.**

<u>Dormancy</u>	<u>Site:</u>	<u>MH1</u>	<u>MH2</u>	<u>MH3</u>	<u>29182</u>	<u>29183</u>
Stratification		70.0 ± 0 a	63.3 ± 15.3 a	50.0 ± 10.0 b <sup>z</sup>	70 ± 20 a	70.0 ± 17.3 a
Stratification and Scarification		73.3 ± 20.8 a	36.7 ± 11.5 b <sup>z</sup>	86.7 ± 15.3 a	66.7 ± 11.5 a	33.3 ± 11.5 b <sup>z</sup>
Scarification		3.3 ± 5.8 b <sup>z</sup>	0 c	3.3 ± 5.8 c	0 b <sup>z</sup>	0 c
Control		0 b	0 c	0 c	0 b	0 c

<sup>z</sup>=Mean followed by different letters within a column are significant differences based on Duncan's Multiple Range test at the 5% level.

**Table 3**  
**Melanthium virginicum. Percent Germination Means and Standard Deviations. 5-week Stratified Seed.**

<u>Site</u>	<u>Stratification</u>	<u>Stratification and Scarification</u>	<u>Scarification</u>	<u>Control</u>
MH1	56.7 ± 5.8 a	70.0 ± 17.3 a	10.0 ± 10.0 a	0
MH2	30.0 ± 2.0 a	36.7 ± 5.8 bc <sup>z</sup>	3.3 ± 5.8 a	0
MH3	23.3 ± 20.8 a	56.7 ± 5.8 ab	0 a	0
29182	20.0 ± 10.0 a	30.0 ± 10.0 c	3.3 ± 5.8 a	0
29183	33.3 ± 11.5 a	26.7 ± 15.3 c	0 a	0

<sup>z</sup>= see note on previous tables.

**Table 4**  
**Melanthium virginicum. Percent Germination Means and Standard Deviations. 7-week Stratified Seed.**

<u>Site</u>	<u>Stratification</u>	<u>Stratification and Scarification</u>	<u>Scarification</u>	<u>Control</u>
MH1	70.0 ± 0 a	73.3 ± 20.8 a	3.3 ± 5.8 a	0
MH2	63.3 ± 15.3 a	36.7 ± 11.5 bc <sup>z</sup>	0 a	0
MH3	50.0 ± 10.0 a	86.7 ± 15.3 a	3.3 ± 5.8 a	0
29182	70.0 ± 20.0 a	66.7 ± 11.5 ab	0 a	0
29183	70.0 ± 20.8 a	33.3 ± 11.5 c	0 a	0

<sup>z</sup>= see note on previous tables.

**Table 5**  
**Melanthium virginicum. Stratification Percent Germination Rate Averages. 5-week Seed**

Site	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
MH1	0	0	36.7	43.3	50	56.7		56.7	56.7	56.7
MH2	0	0	16.7	23.3	23.3	33.3		33.3	30*	30
MH3	0	0	0	6.7	10	20		23.3	23.3	23.3
29182	0	0	0	0	06.7	13.3		20	20	20
29183	0	0	0	0	10	20		30	33.3	33.3

\* fungus covered previously germinated seed.

**Table 6**  
**Melanthium virginicum. Stratification and Scarification Percent Germination Rate Averages. 5-week Seed.**

Site	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
MH1	0	0	36.7	43.3	63.3	63.3		66.7	70	70
MH2	0	0	6.7	10	13.3	13.3		36.7	36.7	36.7
MH3	0	0	16.7	36.7	43.3	50		56.7	56.7	56.7
29182	0	0	3.3	6.7	10	13.3		26.6	30	30
29183	0	0	3.3	13.3	20	23.3		26.7	26.7	26.7

**Table 7**  
**Melanthium virginicum. Scarification Percent Germination Rate Averages. 5-week Seed.**

Site	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
MH1	0	0	0	0	0	6.7		10	10	10
MH2	0	0	3.3	3.3	3.3	3.3		3.3	3.3	3.3
MH3	0	0	0	0	0	0		0	0	0
29182	0	0	0	0	0	0		3.3	3.3	3.3
29183	0	0	0	0	0	0		0	0	0

**Table 8**  
**Melanthium virginicum. Stratification Percent Germination Rate Averages. 7-week Seed.**

Site	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
MH1	0	10	40	50	56.7	70	70	70	70	70
MH2	0	0	6.7	33.3	30	56.7	63.3	63.3	63.3	63.3
MH3	0	0	1	33.3	33.3	46.7	50	50	50	50
29182	0	0	0	16.7	36.7	53.3	63.3	66.7	70	70
29183	0	0	13.3	23.3	30	63.3	70	70	70	70

**Table 9**  
**Melanthium virginicum. Stratification and Scarification Percent Germination Rate Averages. 7-week Seed.**

Site	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
MH1	0	16.7	30	60	63.3	73.3	73.3	73.3	73.3	73.3
MH2	0	3.3	16.7	23.3	23.3	30	36.7	36.7	36.7	36.7
MH3	0	0	20	53.3	56.7	80	86.7	86.7	86.7	86.7
29182	0	0	3.3	23.3	33.3	46.7	60	63.3	66.7	66.7
29183	0	0	3.3	20	23.3	30	33.3	33.3	33.3	33.3

**Table 10**  
**Melanthium virginicum. Scarification Percent Germination Rate Averages. 7-week Seed.**

Site	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
MH1	0	0	0	0	3.3	3.3	3.3	3.3	3.3	3.3
MH2	0	0	0	0	0	0	0	0	0	0
MH3	0	0	0	0	0	0	3.3	3.3	3.3	3.3
29182	0	0	0	0	0	0	0	0	0	0
29183	0	0	0	0	0	0	0	0	0	0

**Table 11**  
**Melanthium virginicum. MH1 Percent Germination Rate Averages. 5-week Seed.**

Dormancy	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
Stratification	0	0	36.7	43.3	50	56.7		56.7	56.7	56.7
Stratification + scarification	0	0	36.7	43.3	63.3	63.3		66.7	70	70
Scarification	0	0	0	0	0	6.7		10	10	10
Control	0	0	0	0	0	0		0	0	0

**Table 12**  
**Melanthium virginicum. MH2 Percent Germination Rate Averages. 5-week Seed.**

Dormancy	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
Stratification	0	0	16.7	23.3	23.3	33.3		33.3	30	30
Stratification + scarification	0	0	6.7	10	13.3	13.3		36.7	36.7	36.7
Scarification	0	0	3.3	3.3	3.3	3.3		3.3	3.3	3.3
Control	0	0	0	0	0	0		0	0	0

**Table 13**  
**Melanthium virginicum. MH3 Percent Germination Rate Averages. 5-week Seed.**

Dormancy	Days after start of germination									
	2	4	6	8	10	12	14	16	18	20
Stratification	0	0	0	6.7	10	20		23.3	23.3	23.3
Stratification + scarification	0	0	16.7	36.7	43.3	50		56.7	56.7	56.7
Scarification	0	0	0	0	0	0		0	0	0
Control	0	0	0	0	0	0		0	0	0



**Table 19**  
**Melanthium virginicum. 29182 Percent Germination Rate Averages. 7-week Seed.**

<u>Dormancy</u>	Days after start of germination									
	<u>2</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>10</u>	<u>12</u>	<u>14</u>	<u>16</u>	<u>18</u>	<u>20</u>
Stratification	0	0	0	16.7	36.7	53.3	63.3	66.7	70	70
Stratification + scarification	0	0	3.3	23.3	26.7	46.7	60	63.3	66.7	66.7
Scarification	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

**Table 20**  
**Melanthium virginicum. 29183 Percent Germination Rate Averages. 7-week Seed.**

<u>Dormancy</u>	Days after start of germination									
	<u>2</u>	<u>4</u>	<u>6</u>	<u>8</u>	<u>10</u>	<u>12</u>	<u>14</u>	<u>16</u>	<u>18</u>	<u>20</u>
Stratification	0	0	13.3	23.3	30	63.3	70	70	70	70
Stratification + scarification	0	0	3.3	20	23.3	30	33.3	33.3	33.3	33.3
Scarification	0	0	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0	0	0

**Table 21**  
**Melanthium virginicum Percent Germination of Stratified Seed in 2001.**

<u>Seed Collected in 2001</u>	Stratification Period	
	<u>5 weeks</u>	<u>7 weeks</u>
Elvaston	46.7 ± 16.3	95.0 ± 5.5
Bushnell	83.3 ± 13.7	98.3 ± 4.1
Sciota	73.3 ± 18.6	95.0 ± 8.4

  

<u>Seed Collected in 2000</u>	Stratification Period	
	<u>5 weeks</u>	<u>7 weeks</u>
Elvaston	50.0 ± 14.1	76.7 ± 28.8
Bushnell	80.0 ± 11.0	91.7 ± 16.0
Sciota	70.0 ± 17.9	95.0 ± 5.5



## Summary

This set of experiments indicates that a type of dormancy is present, and that stratification and possibly stratification plus scarification breaks the dormancy. The control seeds received no treatment of any kind, other than the same germination conditions, and no meaningful germination occurred. This would then rule out a simple after-ripening need, since the seed had been stored for at least 6 months. Further experiments might include germination under different light exposure. This experiment used 24-hour light in the germination chamber. Changing the light exposure might influence germination. Also, trying longer stratification periods, to determine the optimum length of stratification. Perhaps a different scarification technique might also be tried, to see if germination results could be more even across replications. Results also indicate that germination percentages after stratification of freshly-harvested seed and seed stored for one year are similar.

## Literature Cited

Moorehouse, A., A. Mankowski, W.E. McClain, and J.E. Ebinger. 2002. Status of the known populations of the Virginia Bunchflower (*Melantherium virginicum*) in Illinois. *Castanea* 67(2):188-192.

## Appendix

**Tetrazolium Test** Structure: 10 seeds from each site, which had been scarified and placed in a germination chamber for 2 weeks, were then tested with a fresh solution of 2% Tetrazolium for viability. The tetrazolium was applied at noon.

Site	Number of seeds showing pink coloration (viable)	
	<u>after 3 hours</u>	<u>after 26 hours</u>
MH1	8	8
MH2	7	7
MH3	10	10
29182	10	10
29183	10	10







