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The Effect of Gibberellic Acid Concentration on *Gentiana andrewsii* Germination

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THE EFFECT OF GIBBERELIC ACID CONCENTRATION ON *GENTIANA ANDREWSII* GERMINATION

A Thesis Submitted
in Partial Fulfillment
of the Requirements for the Designation
University Honors

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May 2023

This Study by: Laura Spies

Entitled: The Effect of Gibberellic Acid Concentration on *Gentiana andrewsii* Germination

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Abstract

Closed Bottle Gentian, *Gentiana andrewsii*, is an herbaceous flowering plant found in wetland prairies. The seed is difficult to germinate, so it is not on the native seed market. This study determines the optimal concentration of gibberellic acid for this species. The results were assessed based on the morphology changes, germination rates, and survivorship after transplanting. The 50 ppm concentration of gibberellic acid resulted in the highest rate of *Gentiana andrewsii* germination. The number of seedlings in the experimental group exhibited only slight differences between concentrations. Transplant data shows more significant advantages in determining an optimum concentration. The 50 ppm treatment produced significantly more vigorous seedlings that were able to survive a greenhouse environment after being transplanted from a more environmentally controlled germination chamber. The 50 ppm treatment also caused the most significant stem etiolation, making it susceptible to air flow damage in the greenhouse. Gibberellic acid affected the morphology of the seedlings, causing not only stem etiolation, but root size reduction, and leaf shape changes. Since the number of seedlings in the experimental group exhibited only slight differences, only transplant data shows more significant advantages. Given the morphological changes caused by treatment, using gibberellic acid in agar may not be an effective methodology.

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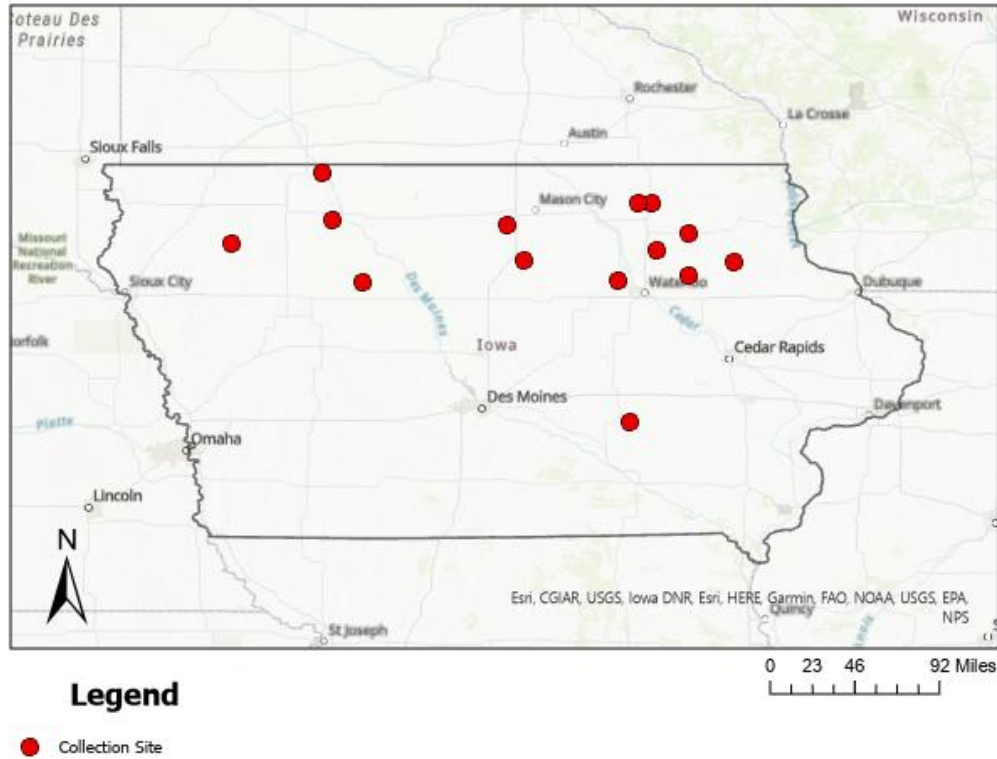
Introduction

The prairie ecosystem is the most decimated ecosystem in North America; only 2-3 percent remains, and what remains is fragmented. This decimation began with the Louisiana Purchase of 1803, followed by the Homestead act, which allowed anyone to claim a parcel of land after five years of living on it. This legislation contributed to the removal of indigenous communities who lived in the Midwest to the Great Plains. This spurred a conversion of North American prairies into cropland. After plowing over the prairie ecosystem, the settlers found black fertile soil that could nurture the production of corn and soy. Today, efforts to restore Iowa's lost ecosystem are pioneered by the Tallgrass Prairie Center at the University of Northern Iowa (Figure 1). The Plant Materials Program works toward this mission through a process of collecting Iowa remnant prairie seed, increasing the seed amount through production, and providing source-identified Iowa Ecotype seed to native seed growers.

Many species of prairie plants are not available commercially because they are difficult to cultivate. Closed Bottle Gentian, *Gentiana andrewsii*, is an herbaceous flowering plant found in wetland prairies. The seed is difficult to germinate, so it is not on the native seed market. The purpose of my experiment was to test the effect of concentration of gibberellic acid (GA-3) on germination, seed vigor, and morphology of bottle gentian. I wished to determine an optimal concentration of gibberellic acid for both dormancy-breaking and seedling vigor. I also wished to determine which method would produce seedlings that can survive transplant.

The seedlings generated from my experiments will ultimately be used for new seed production plots at the UNI Tallgrass Prairie Center. In order to gain comprehensive results, this study included seeds from 10 unique prairies across the state of Iowa (Map 1). This set of

accessions will provide a diverse set of traits that make up the Iowa Ecotype. The results of this study will be shared with native seed growers.



Map 1. Iowa collection sites for *Gentiana andrewsii* that make up the accessions used in this study.

Background

Gentiana andrewsii is an herbaceous perennial species commonly known as closed bottle gentian. It belongs to the Gentianaceae (Gentian) family, which has 99 genera and approximately 1,736 species (Struwe, 2014). *Gentiana andrewsii* is native to both the Midwestern and Northeastern United States as well as Eastern Canada. This wetland prairie species can be found in moist to wet prairies, flood plains, thickets, and fens (Pringle, 1967); (Nemec, 2023). Since it thrives in wetland areas, it would be an advantageous species for restoration in roadside ditches

and areas vulnerable to flooding. The bottle gentian begins flowering from early to late September, and begins fruiting in mid-September. In Iowa, it is frequently found in remnant prairies in the Iowan Surface and the Southern Iowa Drift Plain (Eilers and Roosa, 1994). The closed bottle gentian is a threatened species in several states due to the loss of habitat caused by clearing land for agriculture (USDA, NRCS, 2021).

It is characterized by tight clusters of tubular or bottle-shaped, deep blue to indigo flowers (Figure 1). These flower clusters appear at the upper leaf axils around mid autumn in Iowa. The flowers are about 3 - 4.5 cm in length and never open (Pringle, 1967). The distinctly closed corolla makes it a unique flower to pollinate. It is almost exclusively pollinated by bumble bees because they are one of the few pollinators strong enough to pry open the closed flowers and reach the nectar (Chayka, 2008); (Macior, 1969). The process of prying open the flowers covers the pollinator in excess pollen, making it an effective pollination method.



Figure 1. Flowering *Gentiana andrewsii* at the UNI Tallgrass Prairie Center (L. Spies photo)

The seeds of *G. andrewsii* are very small, winged, light seeds that are difficult to germinate (Figure 2). In difficult-to-germinate plants such as *G. andrewsii*, gibberellic acid can aid in producing higher germination rates. Gibberellic acid is a plant hormone that stimulates seed germination, transitions from meristem to shoot growth, and grain development (Gupta 2013). Gibberellins (GAs) are plant growth regulators that have tetracyclic, diterpenoid compounds (Hedin, 2012). Gibberellic acid has been shown to significantly enhance seed germination rate and plant growth (Ma et al., 2018). Therefore, I chose gibberellic acid to germinate this species that has difficulty in breaking dormancy.



Figure 2. *G. andrewsii* seed on agar medium

Other species in the genus have responded with higher germination rates when treated with gibberellic acid, such as *Gentiana lutea*. For this species the best treatments to break dormancy were 100, 500, and 1000 ppm (González-López & Casquero, 2014). These results influenced my choice of gibberellic acid concentrations treatments. Another study on *G. lutea* found that cold stratification, a common germination technique, was not enough to break dormancy (Cuenca-Lombrana et al., 2016). This study supported the results of the 2014 study, that seed germination of all accessions was promoted by GA₃. Another species native to Korea, *Gentiana triflora* var. *Japonica*, used 100 ppm treatment yielded 90% germination and 1000 mg/L GA₃ yielded 84% germination (Kim et al., 2021). This study concluded alternating day/night temperatures of 25°C and 15°C were suitable for growth (Kim et al., 2021).

Research Questions

1. Which concentration of gibberellic acid results in the highest rate of *Gentiana andrewsii* germination?
2. Which concentration of gibberellic acid treatment produces the most viable transplants?
3. Does gibberellic acid affect the morphology of seedlings?
4. Is an agar and gibberellic acid growing medium a suitable method for germinating *Gentiana andrewsii*?

Methodology

A stock solution was created by adding 100 mg of GA-3 powder and 5 ml of isopropanol was added to a 100-ml container and swirled to dissolve. Different amounts of distilled water were added to dilute the solution dependent on desired concentrations of treatment. The desired concentrations for three trials were as follows; 0 ppm, 50 ppm, 150 ppm, 200 ppm.

A 1% agar solution was prepared by dissolving 1g of agar powder in 100 ml of warm distilled water. The solution boiled until the agar was completely dissolved, then it was cooled slightly to 50°C. Clear 4x4 inch germination boxes were labeled with treatment and accession information according to each treatment level (0 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm). Agar was poured into germination boxes. Then, before adding the seeds, I allowed for 24 hours for the agar to fully set up in the germination boxes (Figure 2).

I added 50 seeds into each box equidistant on the surface of the agar. The germination boxes were covered and placed into a germination chamber. The chamber was programmed for temperature light at 25°C for 12 hours, and 15°C for 12 hours, alternating. Seeds were

monitored for about a month, ensuring proper temperature and light and dark cycles. While in the germination chamber, germinated seeds were counted and recorded every few days, over the course of one month.

After about a month of growth in the germination chamber, the seedlings were transplanted into plug flats with soil. The plug flats were first filled with $\frac{3}{4}$ of potting soil (Including perlite, vermiculite, and fertilizer amendments) and then with $\frac{1}{4}$ of the plug with a germination mix. This specific layering and proportion of soil was chosen to allow the seeding a looser, less nutritious and less wood debris-laden top layer, that would allow the seedlings to grow into the coarser more nutritious material. To ensure root integrity, seedlings were removed from the plates while still contained in the agar layer. Moreover, the root structure remained intact because the area of agar surrounding the seeding was not removed from its connection to the seedling. The plug flats hold 73 seedlings. There were 2 plug flats for each of the 10 accessions. In order for the seedlings to be used in production at the UNI Tallgrass Prairie Center after the completion of this study, the number of seedlings used in the transplant section of this experiment was reduced. The plug flats were divided; accordingly, 31 seedling plugs for each treatment group for each accession, and 22 for each control group. This decision affected the N values of the transplant data as shown in Table 1.

Results

A seed was recorded as germinated if I was able to see the hypocotyl. The initial results of this assessment are exhibited in Table 4. Some seedlings that displayed a hypocotyl also experienced mold growth and did not produce further growth. Mold was not manually removed from seeds. These moldy seeds were recorded for the germination count, but not recorded in

the next measure; seed vigor. Seed vigor was assessed by which seedlings can survive a transplant into soil plug in a greenhouse environment after producing a stem and cotyledons.

The results of seed vigor are skewed due to the greenhouse environmental conditions. The seedlings were transplanted in January, so the Tallgrass Prairie Center greenhouse environment was cold and dry. The first 4 accessions to be transplanted were placed in an area of the greenhouse that had a significant amount of air flow, causing some of the seedlings to lose stem integrity and perish. While the results of this metric have been slightly affected by uncontrolled environmental conditions, the results of seed vigor are an interesting examination of this methodology, because it suggests that extended time periods of exposure to gibberellic acid may affect viability and morphology overtime.



Figure 3. Morphology of each treatment level for accession 6 before transplant. Ruler provides measurement in centimeters. Treatments of GA-3 concentration are left to right as follows: 0 ppm (control), 50 ppm, 100 ppm, 150 ppm, and 200 ppm.

Based on visual observations, the level of gibberellic acid causes morphological changes such as leaf shape, and stem etiolation. Figure 3 shows an example seedling for concentration in accession 6. These seedlings were chosen for documentation because they represented the typical patterns of growth observed in the experiment. Figure 3 shows that the control treatment resulted in a short thick stem, and the longest root. The 50 ppm treatment resulted in an etiolated, slender stem, and a medium, thin root. The 100 ppm treatment resulted in a medium sized root and stem. The 150 ppm displayed similar stem morphology to the 100 ppm specimen, but had a shorter root. The 200 ppm treatment resulted in a shorter stem and the smallest root. The 200 ppm and 150 ppm treatments both caused smaller seedlings and resulted in the highest loss of seedlings after transplant (Figure 4).

The germination rate in the gibberellic acid treatment groups was greater than the control, so it was an effective method in increasing the rate of germination for this species. The control group had an average of 16.2 seedlings out of 50 seeds that were able to break dormancy without the input of gibberellic acid (figure 5). The results are close between the experimental groups, but the highest dormancy breaking concentration of gibberellic acid was the 50 ppm with an average of 42.5 seedlings out of 50 seeds (figure 5). The 50 ppm treatment germinated 85% of the seeds, while the control 0 ppm treatment germinated only 32%.

There was some variation between the accessions, indicating that different locations of seed collection can produce different results when using this method (Table 1). The control results between accessions were not consistent with each other. For example, the Joachim Prairie accession had 31 seedlings germinate, while Steele State Preserve had zero germination in the control group. This comparison demonstrates that breaking dormancy may be more difficult for some accessions, while others will break dormancy with no gibberellic acid

treatment.

Table 1. Number of germinated seedlings and surviving seedling transplants (out of 50 seeds planted) by accession and GA-3 concentration.

Accession	GA-3 Concentration									
	0 ppm		50 ppm		100 ppm		150 ppm		200 ppm	
	Germ. (%) (N=50)	Survival (%) (N=22)*	Germ. % (N=50)	Survival % (N=31)	Germ. % (N=50)	Survival % (N=31)	Germ. % (N=50)	Surviva % (N=31)	Germ. % (N=50)	Survival % (N=31)
Cedar Hills Sand Prairie	16	100	84	81	94	32	98	35	88	0
Daubendiek Prairie	58	68	72	39	78	8	76	0	80	0
Joachim Prairie	62	82	74	55	74	16	60	2	66	0
Soules Prairie	28	57* (N=14)	86	35	82	39	86	0	58	0
Fairbank Fen	38	42	90	58	84	13	82	0	78	0
Sweet Marsh	60	95	92	84	84	68	78	26	80	0
Kalsow Prairie	16	88* (N=8)	90	68	72	13	80	0	70	0
Longspur Prairie	16	63* (N=8)	86	42	84	10	90	0	82	0
Steele Prairie	0	0* (N=0)	86	68	90	45	88	0	94	0
Telford Prairie	30	47* (N=15)	90	48	82	32	94	0	74	0

*Indicates an N value different than stated in treatment column due to low germination counts

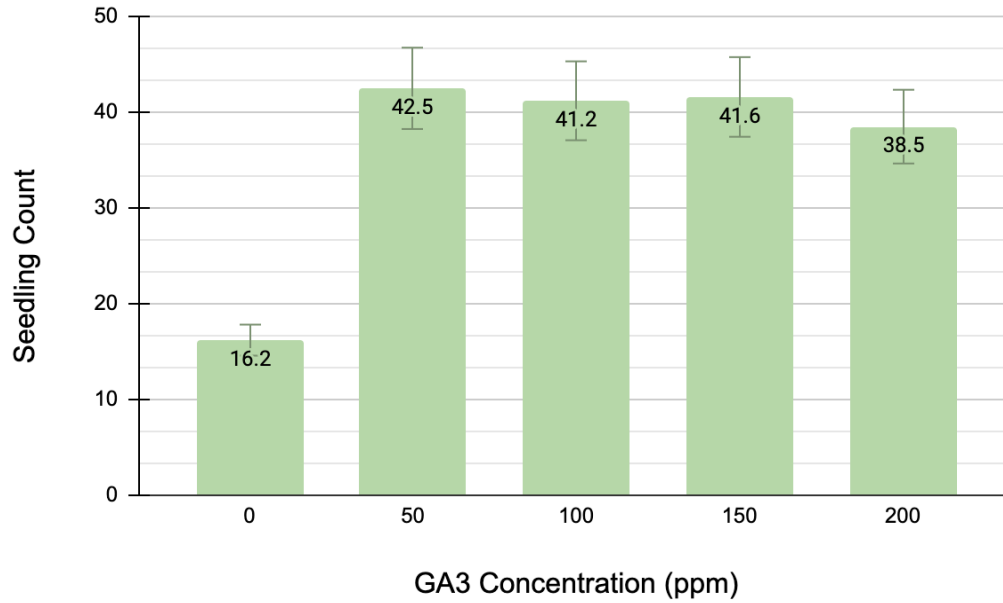


Figure 4. Average germination count for each concentration of GA-3 treatment mixed into the agar (N=10 sites).

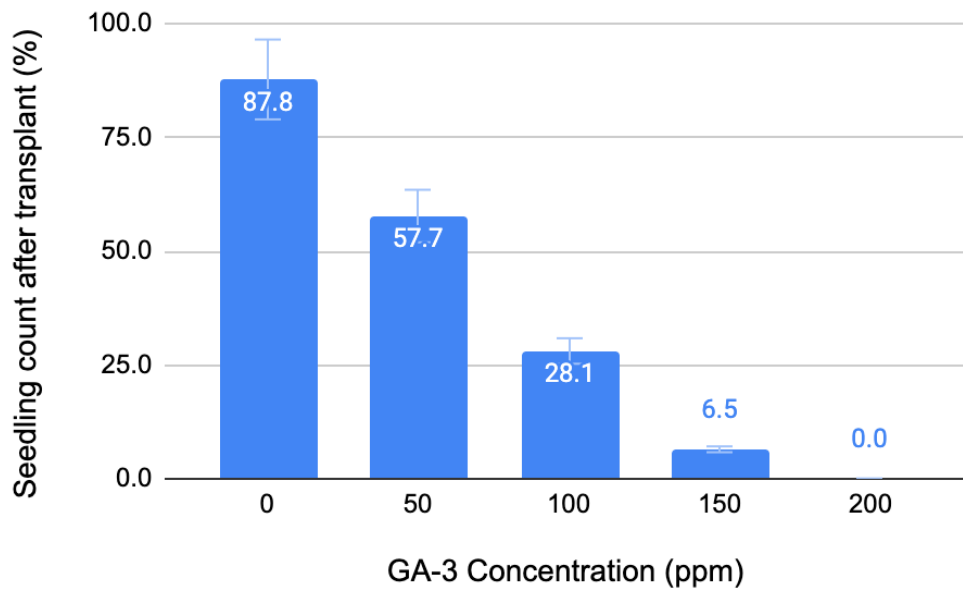


Figure 5. Average seedling count after transplanting by GA concentration (N=10 accessions).

The number of seedlings in the experimental groups exhibit only slight differences in

germination count (Figure 4). Transplant survivorship data shows that lower concentrations of gibberellic acid result in higher survivorship (Figure 5.) The highest concentration of gibberellic acid, 200 ppm, resulted in zero seedlings surviving after transplant, indicating the higher concentrations of gibberellic acid contribute to less vigorous seedlings. The average percentage of seedlings in the control group that survived transplant was 87.7%, demonstrating that seedlings not exposed to gibberellic acid are more vigorous seedlings (Figure 5).

Table 2. Variation among accessions in seed germination response to GA-3 treatment with the highest yielding concentration of seedlings based on treatment for each accession

#	Accession name	Number of seedlings in highest yielding treatment	GA-3 concentration (ppm)
1	CHSP	49	150
2	Daubendiek	40	200
3	Joachim	37	50, 150*
4	Soules	43	50, 200*
5	Fairbank Fen	45	50
6	Sweet Marsh	46	50
7	Kalsow	45	50
8	Longspur	45	150
9	Steele	47	200
10	Telford	47	150

*indicates a tie in highest yielding treatment

There is variation between the accessions and which treatment produced the highest yield of seedlings, and the greatest number of seedlings produced (Table 2). The highest number of seedlings produced for an accession was in group 1, Cedar Hills Sand Prairie, which

was 49, which means that 98% of the seeds germinated. In this accession, the 150 ppm concentration yielded these results. The 150 ppm treatment was also the highest yielding concentration for 4 out of the 10 accessions suggesting that it is the optimum, however, the 50 ppm concentration was the highest yielding for 5 out of the 10 accessions, which one of the accession receiving a tie in seedling count from both of concentration. This is an interesting result because the 100 ppm treatment, which is between the 50 and 150 concentration, yielded the highest germination for none of the accessions. These germination results indicate that the optimum concentration of gibberellic acid for this species is varied between accessions.

Discussion & Conclusion

This study intended to determine the optimum concentration of gibberellic acid to produce the highest germination rates and survivorship. From this methodology I was able to answer my original research questions. I found that the 50 ppm concentration of gibberellic acid resulted in the highest rate of *Gentiana andrewsii* germination. The 50 ppm treatment also produced more vigorous seedlings as shown in transplant data. The 50 ppm treatment also caused the most stem etiolation making it susceptible to air flow damage in the greenhouse. The transplanted seedlings in the 150 ppm and 200 ppm groups had very low survivorship (Table 1). This result may be due to morphological changes caused by the gibberellic acid exposure that reduced root length in the higher concentration groups (Figure 3). Since the number of seedlings in the experimental group exhibit only slight differences, only transplant data shows more significant advantages of the 50 ppm concentration, as it yielded 87.7 % of seedlings to survive transplant.

The seedling vigor results of this study may have been affected by greenhouse bench placement, given that the greenhouse environment has uneven air flow. Several of the

transplanted *Gentiana andrewsii* seedlings were placed in a higher air flow, and subsequently higher stress environment, making results for this metric of optimization difficult to conclude. The number of seeds placed in each germination box may also have affected results. Seeds were counted by hand, which may have incurred human error.

Given the morphological changes caused by treatment, using gibberellic acid in agar may not be an effective methodology. After the results of this study, we have begun a follow up experiment at the Tallgrass Prairie Center. This study employed a more typical use of gibberellic acid, soaking the seeds in the 62 ppm solution for 24 hours. Preliminary results of this trial suggest that this methodology did not result in the same morphological changes that the agar method did. The agar method allowed for the acid to continue affecting growth after 24 hours, and after transplanting. Part of this exposure may be due to the way that I included the surrounding agar with the transplanted seedling.

The results of this experiment are useful for informing future use of gibberellic acid to increase germination for *Gentiana andrewsii*. This information will allow native seed growers to increase their seedlings count, eventually facilitating increased commercial availability of this species. Hopefully, these results will guide native seed growers in their protocol regarding germination methodology. The potential for this species to be unique native pollinator food source is very encouraging. This species would be an advantageous wet prairie habitat addition. I hope to see more *Gentiana andrewsii* commercially available in Iowa, and beyond.

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