Proceedings of the 10th World Congress on New Technologies (NewTech'24)
Barcelona, Spain - August 25-27, 2024
Paper No. ICBB 129
DOI: 10.11159/icbb24.129

Environmental Factors Affecting the Growth and Phenology of Horseweed

Ishwari Bhatt[1]
[1]Kalamazoo Area Math and Science Center
600 W Vine St #400, Kalamazoo, United States
03Ishwari.Bhatt@Kamsc.org

Abstract **-** Canadian horseweed [Conyza canadensis] is a winter annual plant, native to North America, that typically emerges in the fall and overwinters as a rosette; however, throughout the Midwest region recent observations have been recorded of the plant shifting to emerging in the spring as an upright growth type, skipping the rosette stage. As the best way to prevent horseweed infestations (a common issue in agriculture) is to treat the weed at the rosette stage, this phenological shift could have significant implications for crop management. This experiment was conducted as an attempt to determine the cause of the phenotypic plasticity in the growth type of horseweed. Horseweed plants were grown from seed for nine weeks and the effects of seed source, stratification of the seeds, gibberellic acid treatment (GA), and variations in lighting were studied. Plant height and leaf width and roundness were recorded, and the growth type was visually determined (the rosette plant is smaller in height with wider and rounder leaves than the upright growth type). From the analysed data, it was determined that the combination of GA and light exposure most increased plant height, stratification significantly decreased leaf width, and GA most decreased leaf width and roundness. Stratification and GA were the most significant factors affecting visually recorded growth type. It was concluded that gibberellic acid and stratification both significantly increase the probability of the plant developing into an upright growth type, suggesting that these factors could be potentially increasing in the environment resulting in the phenological shift.

Keywords**:** Horseweed, Gibberellic acid, Stratification, Crop biotechnology

1. Introduction

Research on plant phenology, the study of recurring events in plant life cycles, has greatly expanded over the past few decades due to the increase in phenological shifts caused by the impact of climate change on environmental factors [1]. Plant phenology traits respond to changes in the environment to synchronise the reproduction of individuals within a population, which increases outcrossing opportunities, and to avoid seasons with unfavourable growing conditions. Such trait responses can occur within a plant's lifetime through plastic adjustments to growth or across generations through evolved genetic change. Both abiotic and biotic factors can cause phenological shifts. These include temperature, photoperiod, overwintering conditions, competition and resource availability. Phenological shifts can have both negative and positive implications on ecosystems, affecting productivity, carbon cycling, food webs, and competition between other species. Therefore, advancing the field of plant phenology is essential to understanding the ecological consequences of environmental changes.

Canadian horseweed [Conyza canadensis] is a plant native to North America [2]. It is historically a winter annual that emerges in the fall and overwinters as a rosette; however, in Michigan it has recently been observed shifting to emerging in the spring as an upright growth type, skipping the rosette stage. The rosette growth type tends to grow closer to the ground, with darker, more spread out leaves, while the upright growth type typically grows taller and its leaves have a lighter hue (Figure 1).

Fig. 1: Upright growth type (on the left) and rosette growth type (on the right) emerging simultaneously in a field in midsummer [2]. Upright growth types tend to be lighter in shade and have longer stems with narrower leaves. Rosettes are closer to ground, darker, and have rounder leaves.

Horseweed infestation is a common issue for Michigan farmers, as approximately 200,000 seeds are produced per plant, which germinate upon release and are dispersed quickly by the wind, aggressively competing with crops, resulting in reduced yield [3]. Figure 2 shows an image of a horseweed seed. Although it can be detrimental to agricultural crops, horseweed is effectively controlled through crop rotation, planting crops in high populations that limit lighting, tilling later in the spring, and applying chemical herbicides at the rosette stage, before they begin to grow upright. However, growing resistance to herbicides and phenological shifts have made it extremely hard for farmers to manage.

Fig. 2: Horseweed seeds are approximately 3 mm in length not including the pappus of bristly hairs attached to the end. [3].

Schramski et al. [2] conducted an experiment in an attempt to determine the cause of the shift in horseweed growth types and accompanying phenology. They found that both growth types can emerge from one parent, meaning they aren't separate biotypes and the change is most likely a result of some within-generation response to one or more environmental factors (i.e. phenotypic plasticity). Using this information, the researchers then tested various environmental factors: temperature and photoperiod, competition, shading, soil moisture, and stratification. They found that cold-stratification of the seeds was the only factor tested that caused the plant to grow upright, rather than the rosette growth type.

In a study similar to that conducted by Schramski et al. [2], Catlett et al. [4] further tested the effect of cold-stratification on horseweed seeds to confirm that that is the factor causing the phenological shift in growth type. However, their results did not align with those found by Schramski et al. [2], and they concluded that stratification did not impact the growth type. The seeds that were tested by the two studies did not originate from the same region, which could be a potential reason for the researchers' different results.

Catlett et al. [4] also studied the effects of gibberellic acid on horseweed growth type and concluded that horseweed plants grew taller (a key indicator of the upright growth type) when seeds and juveniles were treated with this compound compared to control plants, but the treatment wasn't continued into adulthood, so it was unclear whether the acid had a lasting influence.

According to Gupta and Chakrabarty [5], gibberellic acids (GAs) are plant hormones that stimulate plant growth and development such as seed germination. Also known as gibberellins, they contain tetracyclic, diterpenoid compounds, which are molecules that give them the ability to regulate plant cell progressions. One main function of GAs is the regulation of flower initiation as it promotes pollen germination and pollen tube growth; plants that are GA-deficient mutants are not able to produce viable pollen. Seed germination and stem elongation are other factors that are dependent on GAs, making them essential for plant survival. However, the exact process of GA biosynthesis is still unknown to researchers.

In this study the effects of stratification were examined on horseweed seeds originating from the same populations used by both Schramski et al. [2] and Catlett et al. [4] as an attempt to explain discrepancies between their results and more conclusively determine whether stratification affects growth type. As another possible environmental cue affecting the growth type, the seeds were grown with different amounts of light. Schramski et al. [2] tested light levels in a growth chamber and found that varying light levels had little to no impact on growth type, however they didn't test light levels in a greenhouse or interactions with stratification and GA. For this reason, it was decided to test shading in a greenhouse to confirm their results. Finally, the growth of seeds repeatedly treated with or without gibberellic acid was examined due to previously observed correlation between gibberellic acid and taller growth types.

This study is significant as the results further current research on horseweed and expand the scientific understanding of plant phenology and how it is being affected by changes in the environment. Other than academic interest, the increased understanding of horseweed growth types gained from this study could also allow for improvements in the management of crops that compete with horseweed infestations.

Based on previous research it was hypothesised that stratification and gibberellic acid would produce upright growth types. The usage of light would most likely result in higher plant production and overall better growth and development. Also, different seed origins could have a potential impact on both growth type and size, and were expected to help explain differences in the results of previous studies.

2. Materials and Methods

2.1. Seed Sample

Seeds were sourced from either new 2023 collections (MSU Agronomy Farm), or existing collections from 2021 (MSU Horticulture Teaching and Research Center Farm and Lansing River Trail). Five families from each collection year were used (with i.d. numbers 443, 455, 470, 582, 596 for the 2021 families and 1, 2, 3, 4, and 5 for those collected in 2023). Families were used regardless of growth type, since it had previously been determined that both rosette and upright types can come from one parent and the difference is not a result of separate biotypes. 8 microcentrifuge tubes per family were filled with 5 seeds (2 collection years x 5 families x 8 tubes x 5 seeds = 400 total seeds).

2.2. Experimental Groups

For each microcentrifuge tube a 7 x 7 cm pot was labelled with the i.d. number corresponding to the family from which the seeds originated and the variables tested on those five seeds: s or n representing whether the group was stratified or not, g or w representing gibberellic acid treatment or water control, and l or d representing growth in lighting or shading (e.g. pot 1sgl had seeds from family 1, and was stratified, treated with GA, and grown in the light). Each group of seeds from any given vial had a unique combination of variables so that all combinations of stratification, lighting, seed origin and collection year, and gibberellic acid were tested.

2.3. Stratification

The 40 pots labelled for stratification were filled with potting soil that had been saturated with water. Then the seeds from the corresponding microcentrifuge tubes were placed in the pots, on the surface of the soil (one in each corner and the fifth in the centre). The pots were then randomised on trays and placed in a growth chamber set to 4°C with full lighting. The soil was kept moist and monitored to ensure that no early germination occurred. The pots were removed from the growth chamber after four weeks. Once the stratified seeds were removed from the growth chamber, the remaining 40 pots were filled with saturated potting soil and the seeds corresponding to the labels on the pots were placed on top of the soil in the same manner as the stratified seeds.

2.4. Lighting

To test the variable of lighting on the growth of the plants, a shade tent was created by using pvc pipes to build a rectangular prism and then securing light blocking netting over the structure. On average the plants under shade experienced 75.8% less Photosynthetic Active Radiation compared to control plants that were not covered. The pots labelled "d" were placed under the shade tent, while the pots labelled with "l" were placed on the same bench but in open conditions. Pots were arranged in randomised positions within light treatments. This setup is shown in Figure 3.

Fig. 3: This photo of the setup was taken a few weeks into experimentation, so it is apparent that some plants have emerged.

2.5. Gibberellic Acid

A 0.035 g/L gibberellic acid solution was made with 0.028 g of GA3 added to 800 mL of water, and applied to corresponding seeds weekly through a spray bottle. The seeds that were not treated with the GA solution were sprayed with an equal amount of distilled water as a control.

2.6. Plant Management

Plants were grown at the Kellogg Biological Station Greenhouse in Gull Lake, Michigan. All pots were kept well-watered. Plant growth was observed for a nine-week growth period (November 29, 2023 to February 1, 2024).

2.7. Data Collection

Seedlings were defined as having emerged once a clear stem and both embryonic leaves (cotyledons) were visible. When this occurred, the seedling was marked with a certain coloured pin or toothpick to record the date of emergence. After emergence, height was also periodically recorded (days 21, 49, 64) as the length of the stem from the base of the soil to the last node. Data was recorded at the level of individual seeds; as they emerged, each seedling assigned a number based on their location in the pot to differentiate them from the other four seeds within that pot (1 - upper left, 2 - upper right, 3 - centre, 4 - bottom left, 5 - bottom right). Photosynthetically Active Radiation was also recorded using an Accupar LP-80 ceptometer, to measure the level of light received by the plants under the shade tent and in normal lighting depending on weather conditions. After the nine-week growth period, plants were assigned a growth type (rosette, upright, or intermediate) based on visual appearance of height, leaf shape, and colouring. Pictures were also taken of the largest leaf from one plant in each pot.

2.8. Statistical Analysis

ImageJ software was used to extract the following shape parameters from the leaf photos: area, perimeter, major and minor axis of the best-fitting ellipse, circularity, aspect ratio, roundness, and solidity. Downstream analyses focused on leaf roundness and width (minor axis) as those were the characteristics most different in rosette versus upright growth types. Additive and interaction linear regression models were produced with the response variables being the recorded plant heights, emergence dates, and leaf widths and roundnesses, and the explanatory variables being the various experimental groups of seed sources, stratification, GA, and light exposure. From the models the one with the lowest AIC was chosen to be plotted. Data were plotted as estimated marginal means, so they represent means adjusted for other variables in the model. For height over time and emergence time, linear mixed effect models were used with Plant ID as a random variable to account for repeated measures. All statistical models and figures were generated using R software [5].

3. Results

All explanatory variables tested: seed source, stratification of the seeds, gibberellic acid treatment, and variations in lighting, significantly affected the heights of the horseweed plants. Of these variables, gibberellic acid treatment was the strongest predictor of height over time, as seen in Figure 4. GA was also the strongest predictor of final height increasing it by an average of about 81 mm (263%) compared with the control (Figure 5). The interaction between GA and light is also significant, as GA resulted in a much bigger increase in height for plants growing in the light versus the plants grown in the shade.

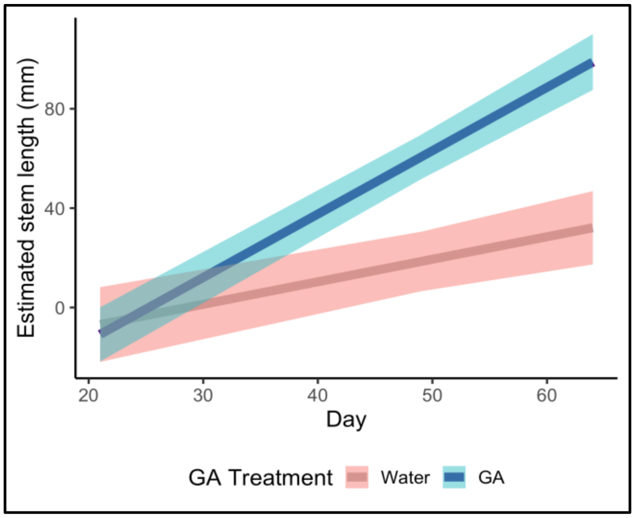

Fig. 4: The graph compares the heights of both plants treated with GA and those without GA throughout the experiment. Stem lengths of plants treated with GA are shown in blue while those not exposed to GA are represented in red. The shaded regions depict the standard deviation of the data points.

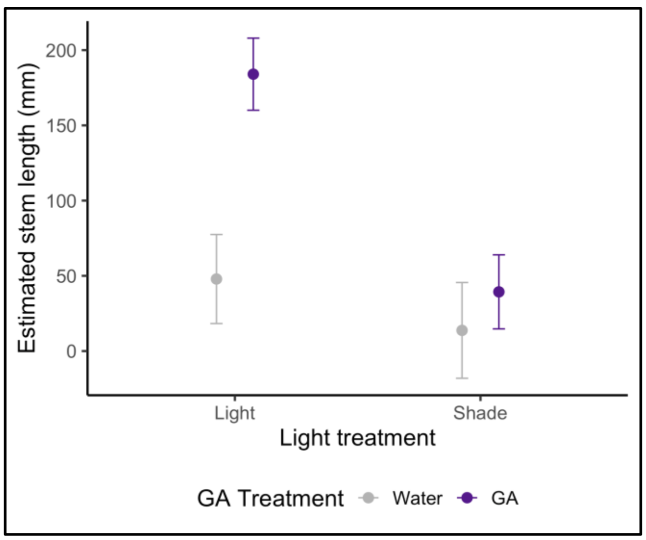

Fig. 5: In comparing the water and light treatment to the GA and light treatment, the difference in stem length was significant at the .01 level.

Light treatment was the only statistically significant variable affecting emergence date, causing seeds exposed to full light to emerge and begin growing before those grown in shade. On average, seeds exposed to light emerged 8 days earlier than those in the shade.

Leaf widths and roundnesses were used as measures to determine growth type, as rounder and wider leaves are characteristics of rosette growth types and narrower, more elongated leaves are typical of upright growth types. Seed stratification, gibberellic acid treatment, and light treatment each had an impact on leaf width, as seen in Figure 5. Gibberellic acid had the largest impact of all the variables, resulting in narrower leaf shapes by an average of 0.63 mm. Seeds that had undergone stratification were also more likely to produce leaves that were less wide compared to those that were not stratified. As for light treatment, leaves were likely to be wider when exposed to light. Like leaf width, leaf roundness was primarily affected by gibberellic acid, which was the only variable that significantly predicted roundness (Figure 6). For comparison, leaves sprayed with water were 60% rounder than those that had undergone gibberellic acid treatment.

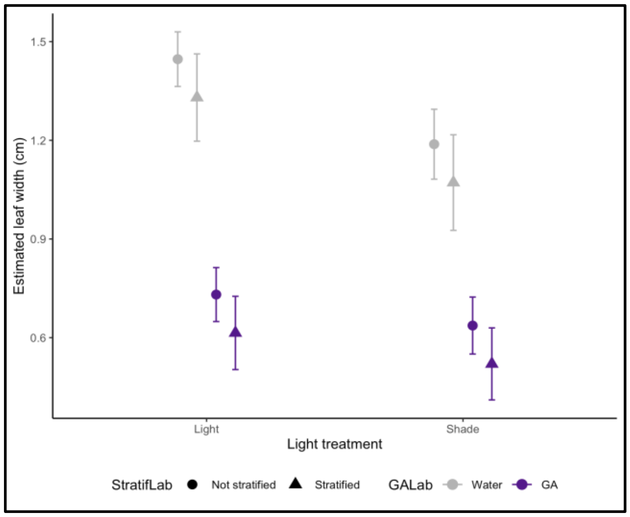

Fig. 6: All three variables: GA, light, and stratification, are significant at the .05 level.

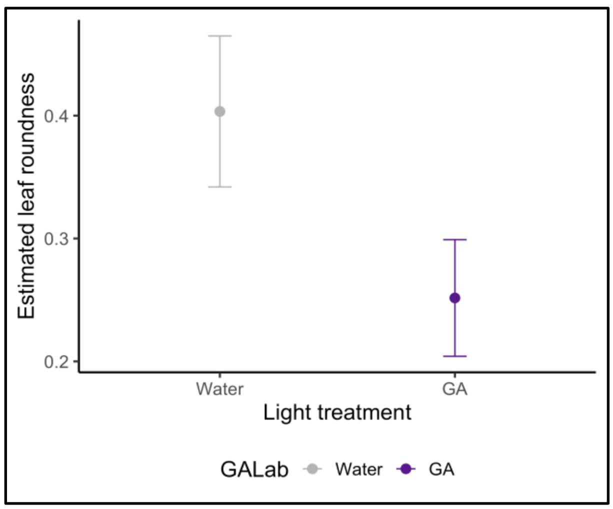

Fig. 7: A comparison of lead roundess for plants treated with both GA and light.

It is important to note, however, that gibberellic acid was not the only variable with a significant impact on growth type score. Seed stratification also affected both leaf width and growth type, especially in plants where both stratification and gibberellic acid were used. Plants were much more likely to be upright after seed stratification. The effect of these variables is shown in Figure 7.

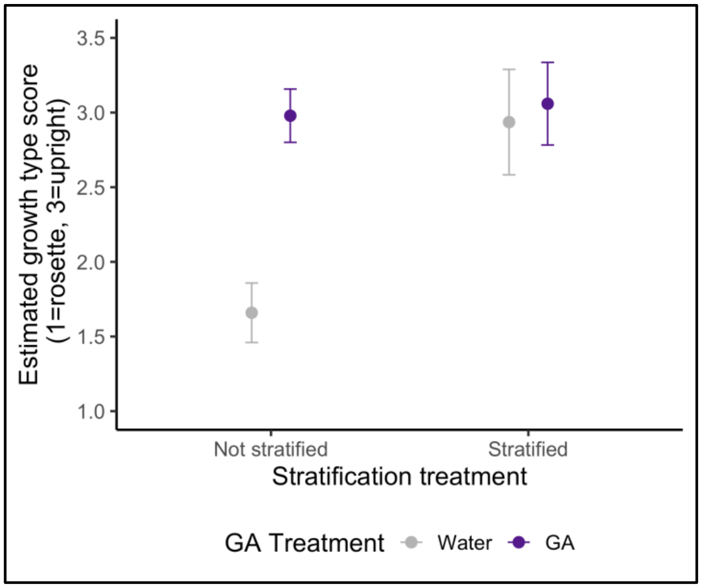

Fig. 8: The effects of both GA treatment and stratification on growth types of the plants are shown. Growth types were measured on a 1-3 scale, where 1 represents plants appearing to be rosettes and 3 describes upright plants, intermediate plants were given a 2 on this scale.

4. Discussion

Looking at the results, it is evident that gibberellic acid and stratification were the variables that had the most significant effect on the growth type and overall growth of the horseweed plants. This is shown in Figure 7 which demonstrates how they were most likely responsible for the upright plants grown in this experiment. Although the data suggest that there is a strong relationship between gibberellic acid and the phenological shift to the upright growth type it is unclear whether this is what is occurring naturally in the environment.

The increase in upright phenotypes in the environment could be due to global temperatures rising over the past few decades, as gibberellic acid responsiveness increases with higher temperatures [7]. Gibberellic acid is known to promote plant growth and cell differentiation, which is why it is often used in agricultural production around the world. A study conducted by Pinthus et al. [8] found that gibberellic acid performs best at warmer temperatures. During warmer conditions, gibberellic acid is more likely to promote the growth of upright plants, with longer and narrower stems and leaves. Therefore, the rise in average global temperatures may be contributing to the increase in upright horseweed plants in recent years.

Due to the uncertainty of this explanation future research is needed to support the validity of these findings. Experimentation on whether gibberellic acid concentrations are naturally increasing in the environment or are being biosynthesized by the plants themselves at a faster rate, is suggested.

5. Conclusion

There is a clear relationship between gibberellic acid and stratification, and the phenological shift of horseweed plants from rosette to upright emergence. Plants treated with GA were on average 263% taller than those left untreated. Furthermore, plants exposed to both GA and stratification were more likely to receive a growth score of 3, indicating upright growth types. Future research is needed to determine whether these environmental factors are the cause of the naturally occurring shift.

Acknowledgements

I would like to thank Ms. Robin Waterman for her mentorship throughout the entire study. Her expert advice and dedication to assisting us was greatly appreciated and was a major resource in our researching process.

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