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Investigating the Effects of Temperature on Lesser Celandine

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Introduction

According to the United States Department of Agriculture (2016), an invasive species is any species that is not native to an ecosystem where its existence is causing or will probably cause harm economically, environmentally or to humans. Invasive species have several major qualities that enable this type of organism to establish itself and have a strong effect on the affected environment. Some of these features include, but are not limited to, rapid growth and reproduction rates, strong adaptability and high rates of dispersal allowing for overwhelming colonization (CSU BIO 301 lab). Due to these characteristics, alien species can become threats to natural wildlife in affected areas. It can be determined from a variety of sources and studies that invasive and alien species prove to be detrimental to ecosystem biodiversity through competition and environmental or community alterations. Carlson and Gorchov (2004) state the possibility for invasive plants to have adverse effects on the native plant species, encompassing reduced biodiversity, changes in community components and modifications in dynamics . Loomis et. al. (2015) examines how alien plants introduced into environments can decrease native species' fitness as well as initiate shifts in nutrients in the soil. These alien species are known to be tenacious contenders in competing for resources, affecting the survival and success of the natural wildlife (Muñoz and Cavieres 2008).

Evert et al. (2013) discusses how invasive species can prevent growth of native species. For example, studies have found that species like *Alliaria petiolata* (garlic mustard) and *Lonicera maackii* (amur honeysuckle) cause reductions in the growth of understory plants or sensitive tree saplings like sugar maples in woodland areas (Carlson and Gorchov, 2004, and Loomis et. al., 2015). Muñoz and Cavieres (2008) look into the negative effects that brightly colored invasive species have on the pollination of native organisms. They found that in high densities, invasive species disrupted alpine native species' pollinator activity and seed output. While much of the information available from studies conducted concerning the effects of invasive plants show negative effects for certain native species, it is important to note that in some cases, the presence of such a species can actually benefit natural wildlife. For example, in the same Muñoz and Cavieres (2008) study, they also found that the exotic common dandelion (*Taraxacum officinale*) had a positive effect for native plant pollination and seed output, when the alien species was present in low densities (2008). A small amount of brightly colored flowers would attract "unusual" pollinators to the area, making up for the loss of "usual" pollinators (Muñoz and Cavieres 2008).

With the knowledge that invasive species are capable of being a threat to biodiversity in ecosystems, some efforts have been made in attempt of removal projects for various species yielding mixed results. Studies involving the growth of sugar maple seedlings and an its invasive competitor honeysuckle have shown that the sugar maples are still negatively affected by the remnants of honeysuckle, even if the plant itself has been removed from the plot (Loomis et. al., 2015). Carlson and Gorchov (2004) found that garlic mustard response to herbicide treatments was somewhat effective for the target species but also decreased the growth of native species. For farmers, many invasive weed species do not respond well to pesticides and chemical sprays or even tilling the land, which sometimes causes an increase in the presence of the unwanted weed (Kertabad et. al., 2013). Further, Axtell et al.(2010) review a number of different methods of control for invasive species, by chemical, mechanical, and biological means, specifically for the species of interest, the weed Lesser celandine (*Ficaria verna*, *Ranunculaceae*).

Lesser celandine is an invasive species officially affecting at least twenty-one states in the United States, including Ohio where this experiment takes place, as well as four Canadian provinces (Axtell et al., 2010). *Ficaria verna* is native to northern Africa, eastern Asia and Europe where the soil is wetter in spring and drier in summer (Axtell et al., 2010; Kertabad et. al., 2013). The plant has a preference for temperate environments with plentiful sources of water as it is often found by riverbanks, marshes and streams. It is an herbaceous ground-cover weed in the Ranunculaceae family (Kertabad et. al., 2013). The plant appears very similar to American marsh marigolds, as both produce yellow floral blooms; however, marsh marigold flowers have between five and nine sepals and do not produce tubers or bulbils (Axtell et al., 2010). Lesser celandine itself has leaves that are typically heart-shaped, sprouting green in midwinter (end of January/beginning of February) and then blossoming with bright yellow flowers starting in March and ending in May (Klooss et. al., 2016). Its flowers are nyctinastic, meaning blooms will open during the day and close during the evening, as well as when struck by rainfall (Prokop and Fedor, 2016). Lesser celandine begins its senescence and dormancy period as the temperate summer season begins at the end of May and beginning of June (Kertabad et. al., 2013). Most of its subspecies typically reproduce by means of starchy tubers and bulbils rather than seed dispersal (Klooss et. al., 2016). As Kertabad et. al. mention, root tubers and bulbils are easily transported by human shoes and lawn mowers, animals, moving water sources (such as flooding streams and riverbanks) as well as other equipment (2013). Due to its ability to easily spread, Lesser celandine is highly effective at colonizing and taking over large areas forming dense carpets of the plant (Axtell et al., 2010).

Many of the habitats affected by Lesser celandine are wetlands and marshlands, areas which are often home to already endangered biodiversity. On account of this species usually growing near water sources, its spread can be enhanced by perennial flooding and moving water in streams and rivers, leading to less reliance on animals for dispersal and further limitation of native species growth (Axtell et al., 2010). The easy spread of the plant can cause major disruptions in biodiversity, especially in wetland areas that are already threatened by other external factors. The feature that enables Lesser celandine to grow in expansive areas not only imposes limitations on native species growth, it also has an effect on animal populations. Axtell et. al. (2010) mentions that loss of food and shelter are some of the negative impacts that Lesser celandine overgrowth can have on animal wildlife. Additionally, its leaves are toxic to most mammals, causing sickness. Increasing populations of Lesser celandine without the growth of native species that wildlife populations rely on for food will lead to many animal species resorting to eating the weed and becoming sick.

Kertabad et al.(2013) examined the effects of temperature on tuber germination, finding that tubers have the highest sprouting-germination rates between 5 and 12o Celsius. However, the study also recognizes the plant's dormancy period as a means of drought survival, as well as avoiding some herbicides. In some cases, if tubers or bulbils are too deep under the surface, they are less likely to germinate, so Kertabad et al. (2013) recommend farmers to plow at least fifteen centimeters rather than a more shallow turning of soil. On the other hand, Masters and Emery (2015) found in their leaf litter study that *Ficaria verna* still produced bulbils and flowered when under twenty centimeters leaf debris. Axtell et al. (2010) discuss some chemical control methods with specific timing and type of herbicides with negligible success rates. Some experiments with cutting the Lesser celandine plant stems very short seems to stop growth for that particular season, followed by abundant repopulation the next growing season (Axtell et al., 2010). Prokop and Fedor's study regarding flower closing had some interesting findings: Lesser celandine flowers forced to stay open at night were more likely to be eaten by slugs or deer (2016). This finding suggests that the nyctinastic flower opening and closing is an effective self-preserving adaptation, enabling Lesser celandine to be a competitive invader.

Kertabad et al. (2013) discovered some of the more optimal temperatures for tuber germinations, finding that the weed tubers had the highest rates of success when stored in cold temperatures (ranging from 4–14oC depending on size). With this information, we are experimenting with varying temperatures to determine their influence on growth of Lesser celandine bulbils. Will there be a significant difference in growth of Lesser celandine height, leaf diameter or biomass between ambient temperatures of 13oC and 20oC? It is expected that the cold treatment 13oC temperature will initiate more germination of bulbils, yielding more successful plant growth for testing. Its adaptation for growing earlier than most temperate climate plant species enables the ground-cover plant to have less competition for resources, effectively out-competing the later germinating plants with already established root and shoot systems. The cold treatment temperatures better reflect the natural growing conditions of the winter months January and February, when *Ficaria verna* begins its seasonal life cycle in the wild. It is predicted that the ambient 20oC temperature will not be ideal for the invasive species to grow for this same reason.

Methods

1.) Bulbils were collected Dec 8, 2016 near the maintenance shed at Rocky River Reservation Metropark and stored in tubs with soil refrigerated at 2.7$_{o}$C until used in the experiment (official start Feb. 15, 2017).
2.) Each group obtained two pots and labeled each with group name and treatment (either ambient temperature or 13$_{o}$C).
3.) Each group added a pre-soaked wick (3–4” in length) to each pot, with 1” hanging out of the bottom.
4.) 240g of Sunshine redi-earth professional growing soil was massed using a standard laboratory scale and transferred into each pot.
5.) Two germinated bulbils were carefully placed in the soil of each pot so that the top of the plant was visible above the soil.
6.) Groups of pots were placed in bins and then placed in their respective growth chambers.
7.) The plants were watered every week by pouring water into the collective bin, which could then be soaked up by the wick-system.
8.) Light levels (in micromoles per square meter per second) were at 148 on the right side, 173 in the center, and 160 on the left side in the 13$_{o}$C growth chamber. The ambient growth chamber light levels were at 60 (for section 3 lab) and 57 (for sections 1, 2).
9.) The growing plants were monitored like this until harvest on March 29, 2017. **It should be noted that initially, the 13$_{o}$C growth chambers were three times the strength of the ambient lights for the first couple weeks.
10.) Plants were harvested on March 29, 2017 by manually extracting the plants and bulbils from the soil and removing as much of the soil from the roots as possible without damaging the roots by shaking and distilled water spray.
11.) The plants were then massed and allowed to dry on paper towels.
12.) All remaining plant materials or contaminated soils were properly disposed of to contain invasive species.
13.) Collected data from each group was submitted for the entire class as a whole.
14.) Data calculations and statistical analysis was performed.

DATA AND RESULTS

Figure 1.
Averaged results and standard errors

Treatment	**Ambient**	**13C**
Height (cm)	5.14	11.66
Standard error	0.62	1.17
Leaf diameter (cm)	2.54	2.80
Standard error	0.20	0.15
Biomass (g)	0.65	1.03
Standard error	0.10	0.15

A two tailed T-test statistical analysis was performed to determine if the obtained means were significant (not due to random chance) meaning that the probability value was less than .05. The p values obtained for height, leaf diameter, and biomass were calculated to be 0.000011, 0.323, and 0.047 respectively.

DISCUSSION

In the p-values obtained, the differences in height and biomass can be considered significant values that are not due to random chance, as they are less than 0.05. These results are consistent with the data presented in Figure 1 where the 13$_{o}$C cold treatment values are consistently higher than the ambient treatment values. Figure 1 also shows that height has the largest difference in averages measured. During the growing period of this experiment, of all the group trials, the ambient treatment had one pot that seemed to flower while the cold treatment had two plants that flowered within the timeframe of February 15–March 29, 2017. Generally, the ambient treatment plants seemed to be growing much more rapidly and therefore began to die sooner than the cold treatment plants. As a result, which can be seen in the averaged values for height, biomass and leaf diameter, the 13$_{o}$C cold treatment plants generally had greater heights, leaf diameters and biomass.

One potential error in this experiment includes the fact that the lighting strength in the cold treatment growth chamber was three times the level of the ambient growth chambers for the first couple weeks, even though the cold treatment plants seemed to grow at a slower rate. A repeat of the trial–experiment with matched initial lighting that stays consistent may yield different growth results. Additionally, issues with obtaining the most precise and accurate height and biomass measurements could provide a source for error. In removing the bulbils and full *Ficaria verna* plants from the soil, the delicate roots could easily have been separated and/or damaged in the process without the students fully realizing, the event of which could cause minor changes in the mass as well as height measurements. Other factors that may have affected the mass are remaining water or soil debris still attached to the roots during weighing. Due to the delicate nature of the roots, it was difficult to fully remove all leftover soil components even with the use of gentle water spray. This water spray additionally may not have fully dried with the paper-towel drying method, again affecting the mass of the plants. The plant biomasses were wet biomasses rather than dry which could affect the measured masses as some plants may be more likely to retain different amounts of water compared to others. Lastly, the scales used may have produced minor errors or disjoint readings due to pseudo-par equipment. For future experimentation, better equipment, more time and higher caution can be taken to ensure the most accurate measurements.

CONCLUSION

In consideration of the question asking whether the different temperature treatments will have an effect on the growth of Lesser celandine, this experiment provides an affirmative answer. The cold 13oC treatment did have an overall positive growth effect on *Ficaria verna* in comparison to the ambient temperatures. The average values for each growth component of height, leaf diameter and biomass were consistently larger for the cold temperature treatment than the ambient. Additionally, statistical analysis of these values showed that for height and biomass, the p-values accounting for random chance were below .05 (0.0000111 and 0.047 respectively), indicating that these results are significant. In this respect, the experiment was successful. Based on the information that the cold temperature generally had higher growth rate success, the proposed hypothesis is supported. Logically, the colder temperature would produce better growing results, as it is closer to natural growing conditions in the wild. Further experimentation is still needed to determine if temperature can be responsible for significant changes in leaf diameter as well as to affirm that light intensity or any other errors did not affect growth or measurement. Seeing the effects of temperature on growing *Ficaria verna* could prove to be extremely beneficial for growing the plant in laboratory settings in order to perform further testing in effort to contain the invasive species in outdoor ecosystems. Repeat trials of this particular study could lead to more solid information regarding the influence of temperature on the species' growth.

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