Keeping Up with the Investigation on Invasives

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INTRODUCTION

The natural world is something I never saw, nor is it something I ever will see. But this story begins much earlier than with myself. People tend to take things with them as they go— traveling on a global scale or even on a simple commute poses a risk to a living organism traveling along. It is not hard for such an occurrence to take place. Invasive species specialize in taking over natural populations and wreaking havoc in the areas in which they establish. They are usually trade-marked by a high reproductive output, a quick growth rate, and the unique advantage of having no natural predators in a new territory. Alien species pose the risk of harming stable environments through outcompeting native species.

In a recent Virginia Department of Conservation and Recreation study, more than 90 plants were labeled as invaders that posed a risk on the spectrum. They were labeled from high, medium, to low risk. *Centaurea stoebe*, or the spotted knapweed, received a high risk rating in the DCR's Virginia Invasive Species List. *C. stoebe*, which began in the Western United States and is now established in the East, poses a threat to both the natural and commercial world. It can live, or even thrive, in a plethora of habitats and conditions. The plant also reproduces seeds that possess the capability of remaining dormant a number of years after they are fertilized. The aim of my research was to use local *Centaurea* seeds to enhance our knowledge on how the species grows and survives under different stress levels and substrates. I also wanted to expand previous research to stay up to date on the growing problem along Virginia's High Bridge Trail.

Principal Approaches

My research was multi-faceted; I included both greenhouse and field components.

Part A: Greenhouse Experiments

In order to gain more knowledge on how *Centaurea* competes with native species, we needed to control multiple variables and tests. *C. stoebe* plants collected from High Bridge Trail State Park in 2014 provided seeds for our experiment. In order to have a working stock of *C. stoebe* plants, 300 seeds were separated from *Centaurea* flowers. Each flower contained roughly 2-7 seeds. Initially, we planted the seeds in two pots containing a substrate of combined soil and sand. These pots received water two times per day in the Hampden-Sydney greenhouse. The plants' first rosettes sprouted, within a week after planting, and they were then removed and relocated into plastic growth tubes. Each tube contained only sand. Scraps of paper towels placed in the bottom of each tube insured the sand would not leak from the tube. Any seedling thought to be mutated or unfit to survive was not placed in a growth tube for experimentation. Each seedling had its own tube in order to eliminate competition between seeds.

The plants continued to receive water two times per day for another week. To control watering treatments, Dr. Lowry and I set up an automated Aqua Timer watering system in the greenhouse. The timer was connected to the greenhouse spigot and mounted on a stable bar protruding from a table. From the timer, we connected three tubes for water to travel to the different watering regime areas. They were held in place with zip ties connected to a table. Furthermore, smaller sets of tubing were then connected to the original tubing. In order for this to be completed, holes were poked in the larger tubing where joints connected the newest tubing. After completion we poked holes in the growth tubes where joints were fitted into the hole for the plants to receive water. There were three possible watering treatments: low— one time/day, medium— two times/day, or high— three times/day. The plants were randomly assigned to one treatment for the duration of our experiment, which lasted 7 more weeks. From June 10 to July 15, the plants in each treatment group received 10% Hoagland's solution every Wednesday. At the end of the experimentation period, thirty plants from each watering treatment were randomly chosen for data collecting.

In order to remove the *Centaurea* from their growth tubes, experimenters immersed each tube in a container filled with water. Each plant was carefully removed from the substrate when the sand was wet. Every leaf per plant was counted and measured individually in millimeters on a Vernier Caliper. Roots were measured using the same procedure. After all the plants were measured and counted, they were then placed in a drying oven for twenty-four hours at 72 degrees Celsius. Afterword, leaves and roots were separated and weighed on an analytical balance.

In an effort to be thorough, we measured the water holding capacity of sand and soil in growth tubes. The data we recorded aided our study by investigating how much water was necessary for the *Centaurea* to grow by the retention of water in the differing substrates. Firstly, growth tubes were filled with the substrates as mentioned before. Thirty tubes were filled with sand and thirty tubes were filled with soil. Each growth tube was saturated in water and then weighed after most of the water dissipated. This gave us our wet weight of each tube. The tubes were then put into the drying oven at 90 degrees Celsius overnight. When the samples were completely dry, we weighed them, which gave us our dry weight. To measure the water holding capacity of each sample, the wet weight was subtracted by the dry weight, and then divided by the wet weight.

To monitor the survival of *Centaurea* in differing substrates and environments, we initiated another experiment. On June 7, two hundred seeds were separated from their flowers. They were then further subdivided into groups of fifty to receive treatments. Group 1 was planted in soil and placed in the greenhouse. Group 2 was planted in sand and was also placed in the greenhouse. Group 3 was planted in in soil and placed in the growth chamber. Group 4 was planted in sand and also placed in the growth chamber. Each group was watered every day, but did not received 10 % Hoagland's solution. For the remainder of the experiment, the plants were monitored for survival rate.

Part B: Field Components

Experimenters also wanted to observe the *Centaurea* population growing close to Hampden-Sydney College. Due to this, we used the High Bridge Trail for observation. Every *C. stoebe* plant found along one kilometer of the HBT was scored. For the purposes of comparison, one researcher counted along the left side of the trail and another researcher counted along the right side. The area of census was from the trail to a ditch on either side. This was done by laying down a 30 m tape measure along the trail. Counting was accomplished in subsets of 10m. Researchers documented how many *Centaurea* were found on each of the 10 m stretches along of the trail, and correlated by their section of the 1 km area.

We also used the same kilometer measured in the previously shown procedure to survey how much light each side of the trail received. On two separate days, researchers went to the High Bridge

Trail in order to analyze this. On the first day, we surveyed 600 m and on the second day, we measured 400m. Weather conditions were roughly the same, although cloud movement was a possible confounding variable, as well as the time of day in which experimenters took data. Each of the experimenters downloaded a Lux Light Meter application onto our phones in order to measure incoming light. The Apps were calibrated to be similar, and then measurements of light were taken on both sides of the trail in 10 m section. Each section was correlated to the respective measurement of light.

Present Knowledge

Part A: Greenhouse Experiment

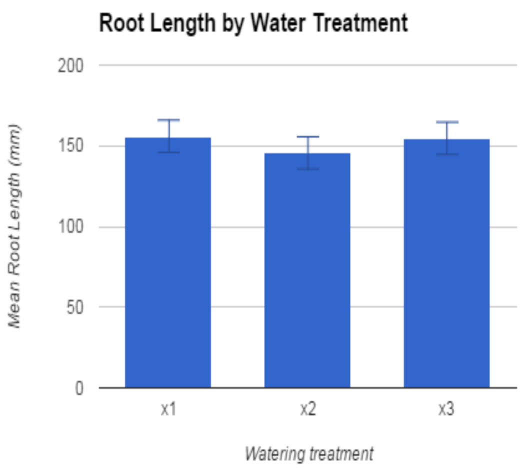
After 8 weeks of *Centaurea* growth, the 30 plants from each treatment group were uprooted for statistical analysis. Each plant's roots and leaves were measured to the nearest millimeter. Roots and leaves were then separated and weighed individually. Data was labeled to distinguish one population from the other. The mean values for the measured traits are reported in chart 1 and in graphs 1 through 5 shown below.

Chart 1. The mean values in each treatment group for the measured traits. Values are means ± the standard error.
Column 1, Graph 1: Root Length
Column 1, Graph 2: Sum of all leaf lengths
Column 1, Graph 3: Root to shoot mass ratio
Column 2, Graph 4: Leaf mass
Column 2, Graph 5: Root mass

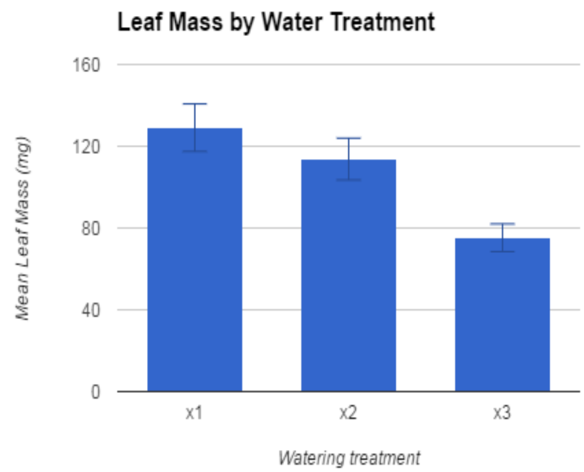
Chart 1.

Watering treatment → Plant Trait ↓	Once Daily	Twice Daily	Three times Daily
Root length (mm)	156 ± 10	145 ± 11	155 ± 12
Sum of all leaf lengths	182 ± 13	146 ± 10	110 ± 6
Root mass (mg)	61 ± 5	69 ± 7	58 ± 7
Leaf mass (mg)	129 ± 10	114 ± 8	75 ± 7
Root to Shoot mass ratio	0.50 ± 0.04	0.62 ± 0.06	1.04 ± 0.14

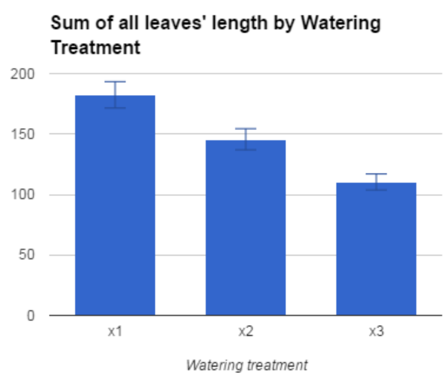
Graph 1



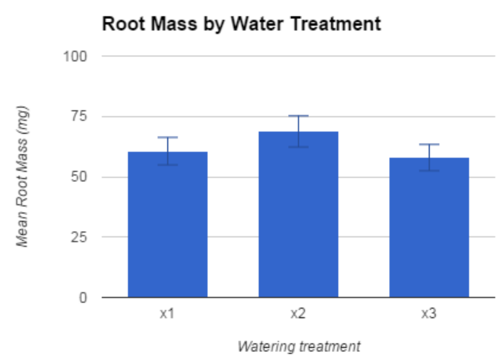
Graph 4



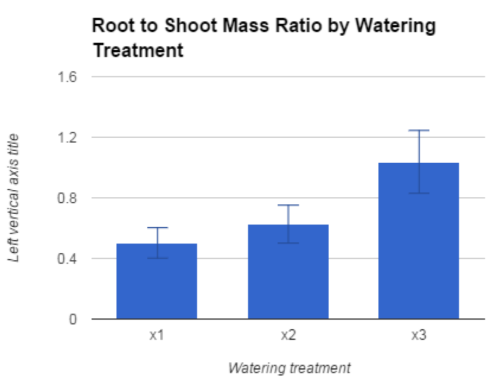
Graph 2



Graph 5



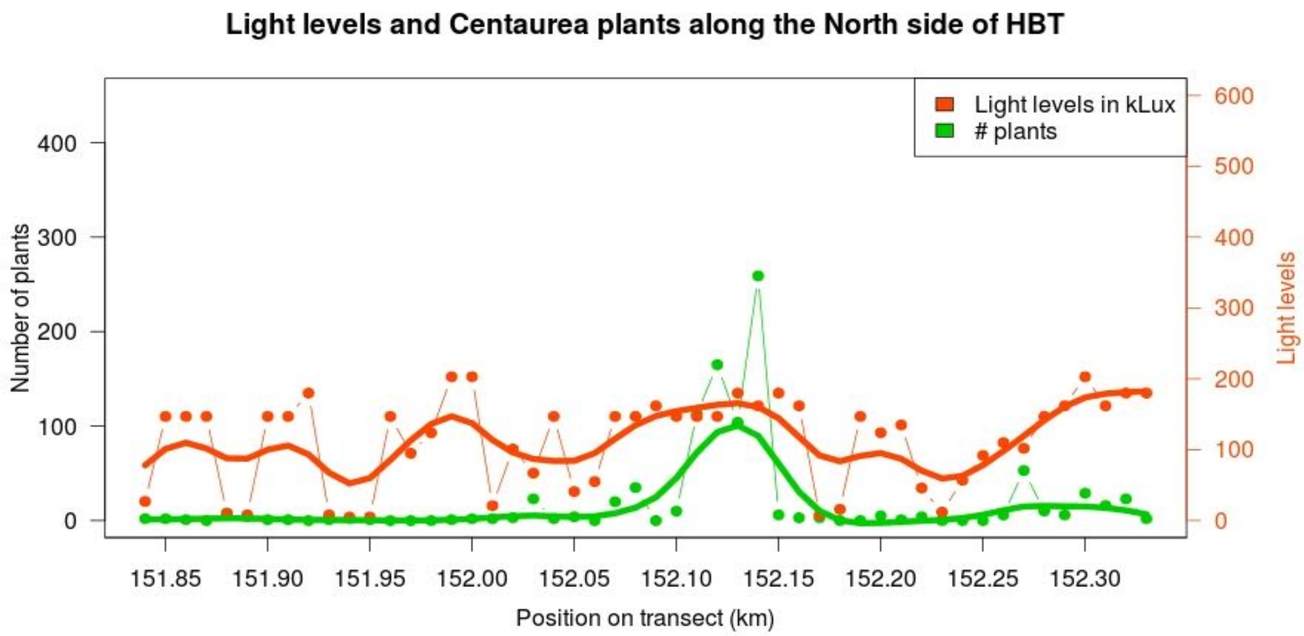
Graph 3



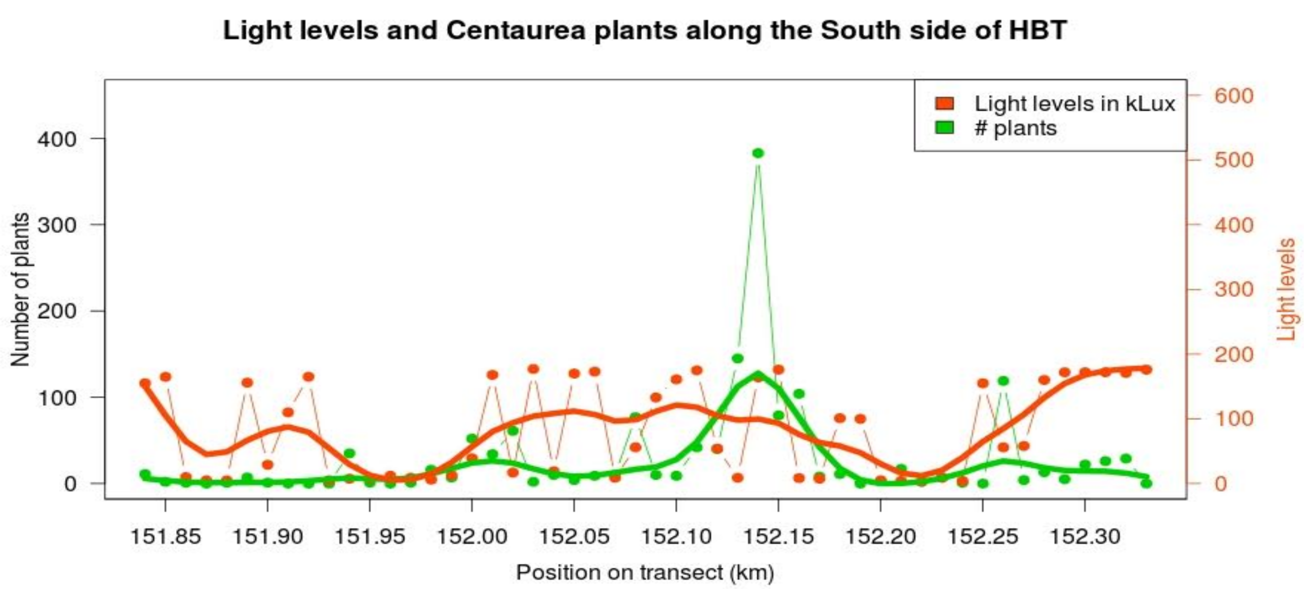
Part B: High Bridge Trail

In order to analyze the data, we used the freely available statistical package R and the interface RStudio. Graphs 6 and 7 show the distribution of *Centaurea* along the HBT, as well as their position on the HBT, and how much light each area receives. Graph 8 described the amount of incoming light in kLux in bar graph.

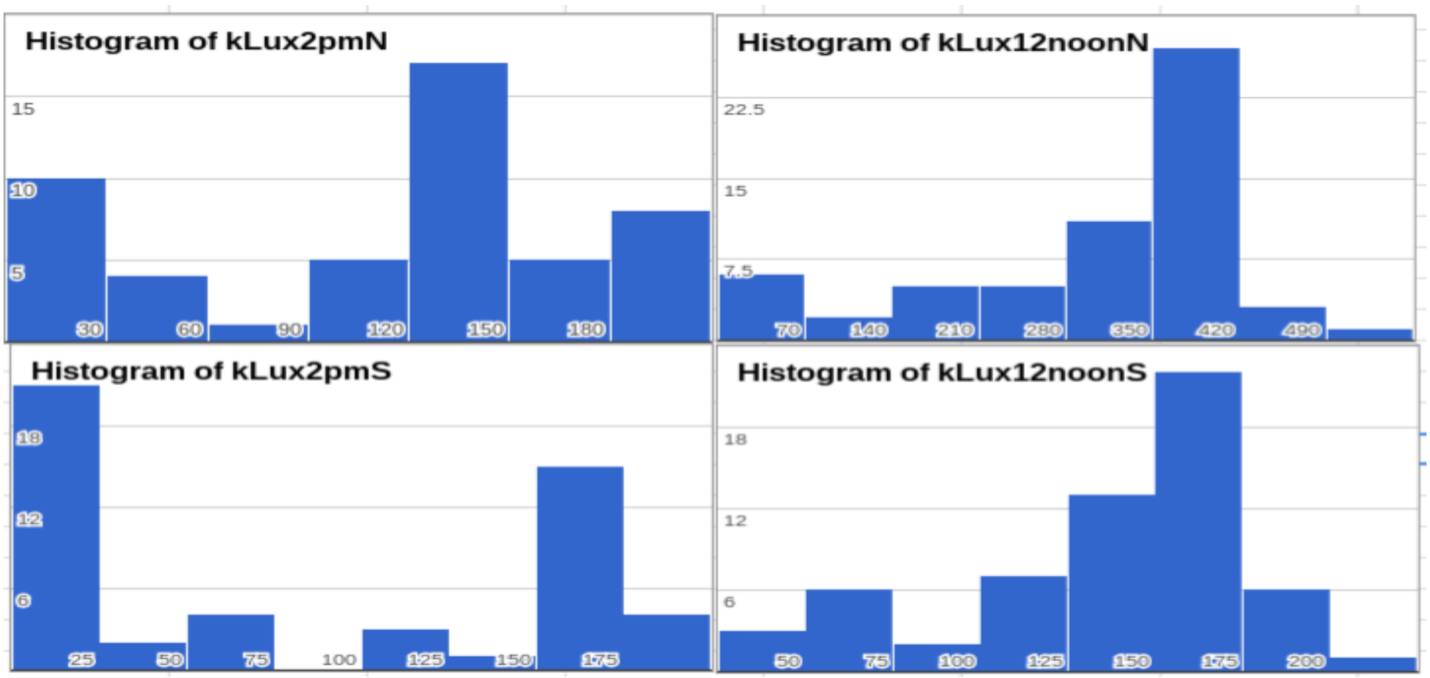
Graph 6



Graph 7



Graph 8



Part C: Water Holding Capacity and Survival

The average percent Water Holding Capacity from the thirty tubes with sand was 0.1391 with a standard error of 0.0076. The mean percent Water Holding Capacity for soil was 1.0886 with a standard error of 0.0273.

At the initiation of our germination and survival study, 50 *Centaurea* were planted in four different conditions. Of the fifty plants grown in soil in the growth chamber, 37 of the plants survived. From the fifty *Centaurea* planted in sand and placed in the growth, 39 of the plants survived. The greenhouse had very different results— only five of the plants survived in both sand and soil treatments.

DISCUSSION

The plants that received the one time per day watering treatment showed significantly more growth in their leaves than the other two treatment groups. This could speak to *Centaurea stoebe's* ability to withstand and grow in hostile conditions in which other competitors cannot.

The root to shoot ratio was closest in the first watering treatment, while the third treatment's ratios varied much more. The root mass ratio does not seem conclusive as no treatment group is significantly different than another.

The data we collected on the High Bridge Trail shows the correlation between incoming sunlight and the plants' ability to establish themselves in the area. Although the graph does not show exact positive and negative correlations occurring together, *Cenaturea* is a tenacious competitor with the ability to withstand the lack of, or excess, sunlight.

The Water Holding Capacity of sand and soil show significant differences. Sand holds much less water than soil, yet *C. stoebe* survived more prolifically in the sand. That is not to say that they grew better, however. Plants contained in the soil substrate grew larger than those in the sand. One explanation is that sand is easy to establish itself in, however soil with nutrients makes for a better environment as it pertains to longevity. Longer studies and experimentation would be need for any conclusive evidence.

CONCLUSION

Invasive species pose a threat to any natural environment. We cannot always control if a species establishes itself in a non-native area, however if it does, then it is up to us to control its outbreak. Invasive species are much like a disease, in that they only need one person or plant to carry it with them. Once it arrives at another destination, it can spread rapidly before we have time to slow its growth. This is why we need to act with haste to see what makes these flora and fauna so capable. *Centaurea* has already established itself across the United States, but it is not too late to learn and apply what we learn to future studies.

Acknowledgements

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