How well does an aggressive invasive plant species, *Centaurea stoebe ssp. Micranthos* (Asteraceae), perform when placed in different substrates

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INTRODUCTION

Invasive species have been singled out as one of the greatest current threats to biodiversity on the planet (1). Invasive species are generally able to outcompete the current local species for resources, and are therefore have the potential to oust the native species. *Centaurea stoebe* is suspected to be allelopathic, meaning it is capable of putting certain biological chemicals into the soil to deter the growth of native plants. Such traits make invasive species exceptionally dangerous and harmful to local systems. To better understand invasive species’ ability to out compete native species, research was conducted focusing on the early growth of *C. stoebe* – specifically how would it would perform in substrates with varying particle sizes, and by extension, varying water holding capacities. We planned to take valuable information on the growth patterns of this invasive species as it grew in a controlled environment.

C. stoebe is an aggressive plant that rapidly invades pastures, rangeland, dry meadows, flood plains, roadsides and any other dry, gravelly or sandy sites. The seeds are also produced prolifically and remain viable for a number of years. (2) *C. stoebe* was accidentally introduced into North America in the late 1800s in contaminated alfalfa and clover seed and in soil used for ship ballast. In North America, plants generally live 3 to 7 years but can live up to nine years or longer and regrow from buds on the root crown. (3) *C. stoebe* is suspected to be allelopathic, meaning it can chemically alter the soil it grows in to prevent other plants from growing. However, some recent studies suggest that the original eastern *C. stoebe* from Eurasia had no allelopathic properties. (4) It is therefore possible that western *C. stoebe* developed allelopathic abilities to better compete in areas such as North America.

The plant was easily accessible and we were able to collect our own samples of young *C. stoebe* plants from a nursery located on the High Bridge Trail. About 200 plants were collected in total, and separated into groups that were then placed in different soil types. The goal of this experiment was to observe the growth patterns on *C. stoebe* when placed in substrates of differing particle size. Each substrate was homogenous mix of a single particle size. Our hypothesis was: if *C. stoebe* is grown in different substrates, then it will exhibit the most substantial growth (measured in biomass, root length and shoot length) in the finest particle size soil.

Principal Approaches

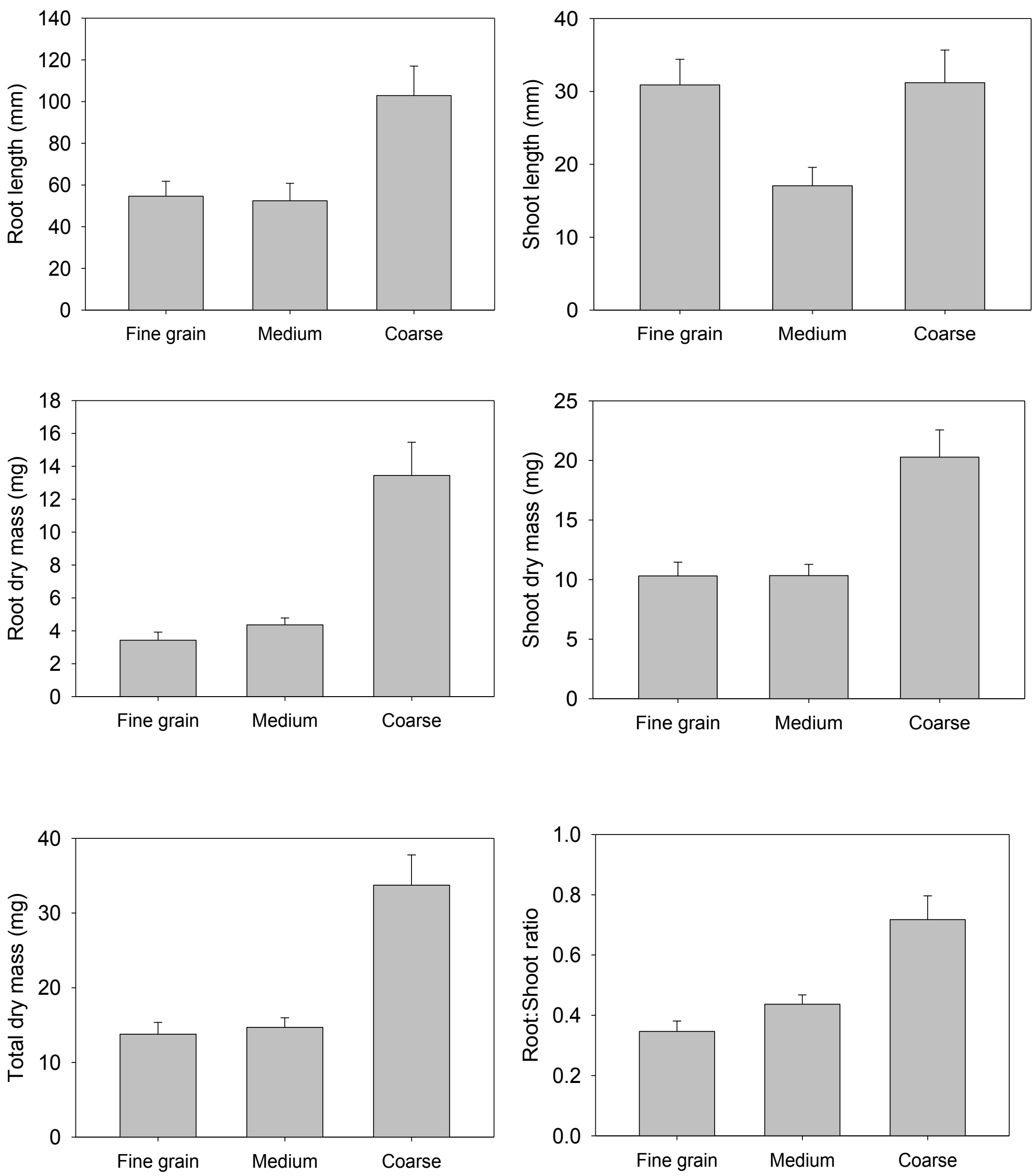
To begin this experiment, we first had to collect about 200 very young seedlings of *Centaurea stoebe*. All specimens were collected from the same “nursery” on the High Bridge Trail, and were placed immediately into their respective growing tubes directly after being picked. Each tube consisted of a single Kimtech wipe in the bottom to prevent the soil from spilling out of the holes in the bottoms of the tubes, and filled bout $3/4^{th}$’s of the way up with soil. We had three separate treatments: fine particle sized soil, medium grain, and coarse grain.

To obtain these soils we first had to construct a shifter box. After the shifter box was constructed, we sifted bags of play-box sand through the two separate levels to obtain our appropriate division of particle-sized soil. After these soils were added to the tubes, they were taken out to the High Bridge Trail to the location of the nursery. All seedlings were obtained from the same nursery under a much larger *C. stoebe* plant. The seedlings were plucked from the ground and placed immediately into the tubes. Seedlings planted earlier were systematically watered while the remaining seedlings were extracted to prevent any unnecessary agitation. Once all the tubes were filled (totaling 53 in the fine grain, 84 in the medium grain, and 55 in the coarse grain). The tubes were transported back to the greenhouse and randomly distributed in two separate tube holders.

After about two days in the greenhouse, all of the seedlings planted in the coarse treatment died despite daily watering and a single treatment with Hoagland’s solution. We then went back out to the High Bridge Trail and gathered slightly larger seedlings to plant in the coarse treatment. The seedlings were small enough that they could technically be considered zeros on the biomass scale, but they had longer roots allowing for more efficient access to water (to prevent drying out). “Water reservoirs” were also added to both of the tube holders. These reservoirs were simply storage bins filled with water and then the tube holders were placed in the bins. The water level came up about half way up the tube. A couple drops of dish soap were added to the water to prevent mosquitoes from using the reservoir to reproduce.

The experiment was then left to run for 8 weeks; with daily watering and a treatment of 10% Hoagland’s solution added every Friday. At the end of the 8-week period, the tubes were removed from the greenhouse for data collection. The plants were removed from the tubes by rinsing the soil away from the roots and soaking the plants in water to remove any more debris from the substrates. The root length (total, not taproot) was then measured along with the longest leaf. The plants were then cut in half at the hypocotyl and placed in envelopes, with one envelope for the roots and one envelope for the longest leaf for each plant. The envelopes were then placed in a drying oven at 60 degrees Celsius for two days. At the end of the two days, samples were removed and weighed in grams to collect a biomass measurement.

Present Knowledge



Graphs from left to right:

Row 1: Graph 1 – *Centaurea* root length; Graph 2 – *Centaurea* shoot length

Row 2: Graph 1 – *Centaurea* root dry mass; Graph 2 – *Centaurea* shoot dry mass

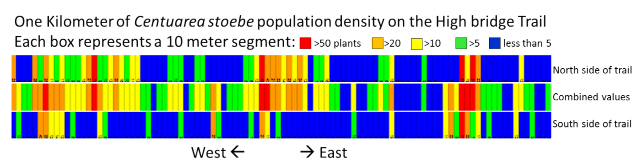
Row 3: Graph 1 – *Centaurea* whole plant dry mass; Graph 2 – *Centaurea* R:S ratio (dry mass)

After the growth period was over, all *C. stoebe* were uprooted and root and shoot length were measured to the nearest millimeter. Total change for both root lengths and longest leaf length were recorded along with the biomass of the roots and shoots. There are no initial values to compare to, as all initial values were presumed to be zero. A series of ANOVAs were performed on all data collected, and the graphs shown below were created.

We performed a series of ANOVAs to test for statistical difference between the results. All tests were performed using a 95% confidence interval, meaning that in order for a statistical difference to be present, the p-value must be less than .05. If the p-value is greater than .05, then we can be 95% confident that there is no statistical difference between the two variables. All p-values collected from these experiments were well below 0.05, ranging from 0.002218 to 8.51E-08. This proves statistical significance in the results collected. Even the graphs provide a fine visual as to the significance of the tests. The plants placed in coarse soil outperformed the plants placed in other soils by a large margin, with the other two treatments being about equal in growth and biomass, apart from a spike in the longest leaf growth of the plants placed in fine grain soil.

The survey is intended to be an on going project, originally started in the summer of 2013. The goal is to complete a large portion of the High Bridge Trail by counting the total number of *C. stoebe* on both sides of the trail, and then measuring possible factors that promote *C. stoebe* growth in the more populated segments (sunlight, soil consistency, etc.). One kilometer was completed at the end of the research period. This specific graph shows 1km starting from mile marker 152 on the High Bridge Trail, progressing in the direction towards mile marker 153.

This image from the data collected in 2013 shows the abundance of *C. stoebe* over a distance of 1 kilometer, broken up into 10m intervals. Once data from 2014 has been analyzed, we will be able to see how the densities of the plant have changed over the course of just one year. The blue boxes indicate 10m segments where there were less than 5 *C. stoebe* plants growing, the green boxes indicate segments where 5-9 *C. stoebe* plants were growing, the yellow boxes indicate segments with 10-19 *C. stoebe* plants growing, the orange indicate 20-49, and the red boxes show where over 50 *C. stoebe* plants are growing. The top line of the graph is the north side of the trail, and the bottom line of the graph is the southern side of the trail. The middle row shows the combined values, or all of the *C. stoebe* within each 10m segment. Several lab groups as well as our team of researchers have collected more data for this project over the course of 2013 and 2014. Data has not yet been analyzed.



DISCUSSION

The results of the ANOVA tests show that there are statistical differences present in all sets of analysis, as all p-values calculated were less than 0.05. The young seedlings that we initially collected were tiny enough to be considered zeroes when conducting statistical analysis. However, the seedlings originally planted in the coarse substrate dried out and died rather quickly. We gathered slightly larger plants (that were about on par with the plants growing in the other two treatments) to grow in the tubes with coarse grain sand. This was simply because the coarse grain soil dried out incredibly quickly and the plants could not absorb enough nutriment to survive in such conditions. We then added a “water reservoir” which consisted of a plastic storage bin filled with water that the plants were then placed in while in their tubes. This ensured that the plants would always have access to sufficient amounts of water. This suggests that *C. stoebe* would have a difficult establishing itself in coarser grained soils, as it would dry out too quickly. This is potentially useful information, as one of the suspected dispersal methods for *C. stoebe* is by having seeds present in gravel used for construction that is shipped in to an area from elsewhere.

It was originally hypothesized that the plants grown in fine particle size soil would perform the best, however the results were not as expected. The plants grown in coarse soil were generally larger in both length and biomass produced, although the initial dying of the plants placed in this coarse soil suggests that C. stoebe has lower establishment potential in substrates of such consistency. The fine soil was much more efficient at holding water comparatively, with the water holding capacity of each soil type decreasing as particle size increased. The calculated water holding capacity for the coarse soil was about 10%, with the medium grain soil being about 30%, and the fine grain holding about 60% water by weight after being saturated.

The data collected points toward a fairly straightforward set of results. Once *C. stoebe* can access a reservoir of water, it will show the most significant growth in coarse-grained soil. The other two treatments had fairly similar results, suggesting that there is a possible maximum level of water holding capacity of a substrate that can be present to have an effect on *C. stoebe*. This research could further be useful, as it provides some insight into the invasiveness potential of *C. stoebe* in environments with coarse grain soil and large water reservoirs, such as along the bank of a river.

Potential sources of error in this experiment are primarily human error. It was difficult to extract the plants from the fine grain soil without damaging the roots at least a little bit. This would then influence the biomass measurements collected as some of the smaller roots would be ripped off and left in the substrate. We are currently planning further experiments involving larger plants in larger pots. These experiments would eliminate the establishment component of this research and instead explore the growth characteristics of larger plants in separate substrates.

CONCLUSION

C. stoebe is a growing threat in North America. It can be found in almost every state. (5) *C. stoebe* spreads very easily and can infest large areas in relatively short periods of time. Therefore, it is important to study this aggressive invader. As the data shows, *C. stoebe* has a difficult time establishing itself in very coarse grain soil, but once it reaches a ground water reservoir, or perhaps even substrate with higher water holding capacities, it will grow very rapidly.

The survey portion of this project is intended to be an on-going study. The results will show how aggressive *C. stoebe* is and how abundant it already is on the High Bridge Trail. This study hits close to home, as this aggressive invader is already taking over local species, such as Bush Clover. Future work will entail an entire survey of all *C. stoebe* found on the High Bridge Trail, with measurements such as sunlight intensity and soil consistency taken. A complete survey of the High Bridge Trail would be a very valuable resource and would provide great insight into how quickly and efficiently invasive species such as *C. stoebe* are able to take over native species. It would also provide fantastic data that could be used as a standard of comparison in following years. Same segments will also be measured multiple times in an attempt to track the spread of *C. stoebe*.

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