How well does an aggressive invasive plant species, *Centaurea stoebe* (*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. Both *Centaurea stoebe* and *Vitex rotundifolia* are suspected to be allelopathic, meaning they are 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 better understand invasive species' ability to out compete native species, research was conducted focusing on the early growth of *C. stoebe* and *V. rotundifolia*. We planned to take valuable information on the growth patterns of these invasive species as they grew in a controlled environment. The original plan was to take *V. rotundifolia* and grow it in different soil types as well as changing its access to water. The supplier sent us fruits instead of seeds and we were unable to extract the seeds efficiently. The seeds were very difficult to extract from the fruits, as the fruits were very tough and held a lot of fibrous material that was difficult to cut through. The seeds were buried towards the center of the fruits and were very small. With limited time, it was impossible to extract the seeds and attention had to be diverted to another local invasive species.

Focus was then shifted to *C. stoebe*, an invasive species that has affected many areas across Northern America. *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. 150 plants were collected in total, and separated into groups of 50 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 vastly different soil types. The 100% sand soil could serve as the control group, as *C. stoebe* grows naturally in sandier soil. Our hypothesis was: if *C. stoebe* is grown in different substrates, then it will exhibit the most substantial growth in sandier soil.

METHODS

In the early stages of this specific research project, the main goal was to study the early growth patterns of *V. rotundifolia*. 100 seeds of *V. rotundifolia* were initially purchased, along with 320 seeds of other assorted species of Vitex. Once the seeds arrived, it was discovered that they were not in fact seeds as were ordered, but instead fruits. Seeds could not be safely extracted. The fruits were then taken and placed in sauce containers that held a piece of sterilized filter paper soaked in water at the bottom. Between five and eight fruits were placed per container. The containers were capped and placed in a growth chamber to germinate. The growth chamber cycled between light from an UV lamp and total darkness. All seeds experienced the same exposure and treatment. Seeds remained in the growth chamber and were monitored, and were only removed once they began to germinate. Only about six of the *V. rotundifolia* sprouted and could be potted. Due to low germination count, no adequate data could be pulled.

For the next portion of this research, attention was shifted to *C. stoebe*. First, 150 individual *C. stoebe* plants were collected from the High Bridge Trail area (Prince Edward County, Virginia). These were taken by first locating a cluster of larger *C. stoebe* plants, and then searching the immediate surrounding area for small *C. stoebe* plants (usually no more than 5 leaves). Once 150 were collected, the plants were placed in Ziploc bags with water added and transported. 50 of the *C. stoebe* were placed in 6 ounce plastic cups filled with sand, 50 in 6 ounce cups filled with Metro Mix, and 50 in 6 ounce cups filled with a half-and-half mixture of sand and Metro Mix. Left over plants were placed in a refrigerator at ~40 degrees Fahrenheit.

Before the plants were potted, measurements were taken on their root length and their longest leaf length. This was achieved by first dipping the plants in water, and then measuring the root length and longest leaf on a meter stick. Measurements were taken in millimeters. The plants were left to grow for around 30 days. At the end of the growing period, the plants were pulled up, measured for their new longest leaf/root length, cut in half, and dried in a drying oven set at around 65 degreed Celsius. The plants were left in the drying oven for around 15 hours before being removed. After they were removed, the plants were weighed in grams.

During the four-week period where the *C. stoebe* were growing in the greenhouse, a survey was conducted on the High Bridge Trail, in which all individual *C. stoebe* plants that had more than 4 leaves were counted along 1 kilometer of the HBT. The survey consisted of laying out a 30m measuring tape, and hammering down a stake every 10m. Flagging tape was tied to each stake to improve visibility. After stakes were laid out every 10m for 1km, every individual *C. stoebe* plant that had more than 4 leaves were counted on both the left and right sides of the trail. This survey is intended to be an ongoing project and will be continued.

RESULTS AND DISCUSSION

***Vitex rotundifolia* experiment**: We were not able to get seeds to germinate in sufficient numbers to run an experiment, so this experiment has been planned to run in the future given adequate seed germination.

***Centaurea stoebe* experiment**: After the growth period was over, all *C. stoebe* were uprooted and root and shoot length were measured to the nearest millimeter and then entered into an excel spreadsheet. Total change for both root lengths and longest leaf length were recorded and the average change was calculated. The average change was used alongside the degrees of freedom and standard error to calculate the t-value and p-value for each comparison. The following graphs show the average changes for root lengths and longest leaf lengths. Plants that died during the experiment were not included in the analysis. The averages on the following graphs are:

Sand root change average: 90.4
Metromix root change average: 50.4
Half/Half root change average: 57.6

Sand leaf change average: 28.4
Metromix leaf change average: 47.7
Half/Half leaf change average: 46.4

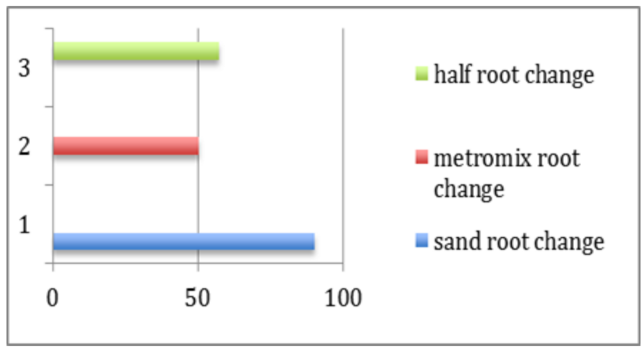

Figure 1. Average Root Change

We performed a T-test 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.

Root results:

Sand vs. metro mix:
T-critical: 2.02269092
P-value: 6.50806E-05

Metro mix vs. Half/Half:
T-critical: 2.085963447
P-value: 0.512983456

Sand vs. Half/Half:
T-critical: 2.085963447
P-value: 0.009566546

Longest leaf results:
Sand vs. metro mix:
T-critical: 2.02269092
P-value: 0.002155334

Metro mix vs. Half/Half:
T-critical: 2.085963447
P-value: 0.858378388

Sand vs. Half/Half:
T-critical: 2.085963447
P-value:0.00830975

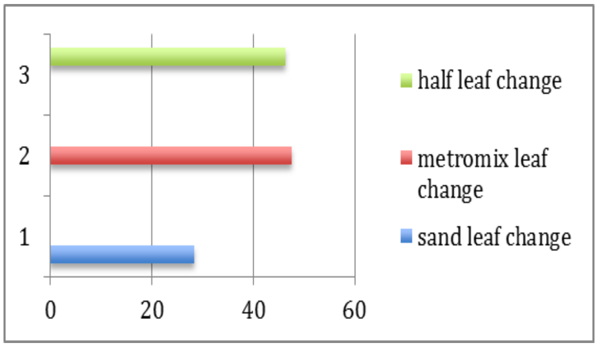

Figure 2. Longest Leaf Change

The results show that there is a statistical difference between sand and both metro mix and the half metro mix/half sand soil for both tests, as the p-value is less than .05. However, there is no statistical difference between the metro mix and half/half soil for both tests, as the p-values were all higher than .05. The most likely reason is that the *C. stoebe* placed in the sandier soil had use more of its nutrients and energy towards growing larger root systems. Therefore, the leaves showed considerably reduced growth in comparison to the *C. stoebe* that grew in the other two substrates. It is also probable that the metro mix contained far more nutrients than the *C. stoebe* needed, even in the half and half mixture of sand and metro mix. Therefore, the growth in both the metro mix and the half and half were fairly similar.

In the set of plants growing in sand, only one died. In the metro mix, nine died. In the half and half mixture, 29 *C. stoebe* died. It appeared as if some sort of fungus or mold infected the half and half mixture. *C. stoebe* is found naturally in sandier soil that drains well, so it is not moist enough for molds to grow. It is probable that *C. stoebe* has no real defense against molds. Metro mix retains much more moisture than the sand, so mold is much more likely to grow in the substrates with metro mix. This idea is further supported by the quick drainage of the sandier soil. In the samples of 100% soil, the sand drained very quickly and was often completely dry the following day, whereas the samples with metro mix in them (100% metro mix and 50% metro mix/50% sand) were often still very damp a day after being watered. It is also possible that the substrates with metro mix in them retained more heat overnight than the sand did, thus creating a more suitable environment for mold growth. The greenhouse is essentially a controlled environment; so all plants were tested in the same temperatures with the same access to water.

There is potential error involved in this experiment, as not all factors could be regulated. A good example of this is the mold that infested the *C. stoebe* placed in half and half substrate. We had no real control over whether or not the mold would infest, however *C. stoebe's* susceptibility to mold suggests that it is not genetically capable of thriving in moister areas as it appeared to have no defense against the mold. There were also lots of insects in the greenhouse that could have had some influence on individual *C. stoebe's* growth. Error also occurred in the measurements, as the roots of the plants are very tangled when extracted from the soil, but are also very delicate. It was very difficult to stretch the roots out to their full length for measurement without ripping off tiny pieces that could have had some impact on the overall length.

Errors in our censusing of the High Bridge Trail survey could occur fairly easily, as sometimes the young *C. stoebe* plants would be completely concealed by other plants and difficult to spot. It is more than likely that some individual *C. stoebe* plants were missed and not counted in the survey. The majority was counted and the image attached does provide a good visual for which portions of the High Bridge Trail are heavily infested with *C. stoebe*.

REFERENCES

1) Simberloff, D. (2000). Nonindigenous species: a global threat to biodiversity and stability. *Nature and human society: the quest for a sustainable world*. National Academy Press, Washington, DC, USA: 325-334.

2) "PMIS - Plant Information." (2011). *PMIS - Plant Information*. Engineer Research and Development Center, Apr. 2011. Accessed 27 Aug. 2013.

3) "Herbaceous Forbs – Spotted Knapweed (2010). *Nps.gov*. National Park Service. Accessed 28 Aug. 2013.

4) Reinhart, Kurt O., and Matt Rinella. (2011). Comparing susceptibility of Eastern And

Western U.S. grasslands to competition and allelopathy from spotted knapweed *[Centaurea stoebe* L. Subsp. Micranthos (Gugler) Hayek]." Plant Ecology 212.5: 821-828. Environment Complete. Accessed 30 Aug. 2013.

5) "*Centaurea Stoebe* L. Spotted Knapweed." *Natural Resources Conservation Services*. USDA, accessed 28 Aug. 2013.