A Continued Study of the Invasive Potential and Competitiveness of the Invasive Plant *Centaurea stoebe* as Compared to the Native plant *Lespedeza capitata*

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INTRODUCTION

An invasive species is an organism that, for a variety of reasons, is able to out-compete endemic species and therefore better survive in a given environment. A species is classified as invader if it is not native to the environment that it begins to dominate. As invasive is a negative term, it indicates that the invasive species have adverse effects on the environments that they invade ecologically, environmentally or economically. Invasive species are recognized as one of the greatest current threats to biodiversity (1). By nature, invasive species exploit their environment to become completely dominant in an area, outcompeting endemic populations. This then reduces the biodiversity of the ecosystem and can have detrimental effects, such as extirpating species from an area and even causing rare or endangered species to go extinct.

Looking at an invasive species' ability to successfully invade shows some interesting characteristics of these species. Invasive species' characteristics often include fast growth, quick reproduction and an ability to adapt to a very wide range of environmental conditions. However, each invader is different so it is difficult to generalize their characteristics to pinpoint exact mechanisms that can be attributed to their invasive abilities. Many invaders are known as environmental engineers, meaning that they are able to adapt to the environment quickly, or in some cases even change the environment around them to better suit their needs. An important class of environmental engineers, particularly in plants, includes plants that are allelopathic. Allelopathy means that plants with this characteristic are able to produce chemicals that aid in their growth by inhibiting the growth of possible competitors. Allelopathic invasive species are dangerous to the environment they invade because they can have long lasting effects even if the invasive species is extirpated from the area.

Centaurea stoebe ssp. *micranthos* (Asteracea), commonly referred to as spotted knapweed, is an invasive species in the Asteraceae family native to Europe. It is found extensively throughout North America, and recently has been increasing in frequency in the eastern U.S. following broad patterns of infestation in western states. It grows quickly, is difficult to get rid of, and has been shown to competitively exclude native species from locations through a variety of competitive mechanisms. In its North American invasive range, the basal rosette of leaves may send up a flowering stalk over successive seasons for an indefinite period and may grow above a meter or more in height. *C. stoebe* produces seeds annually, the invaders also clonally reproduce, even budding from the flowering stem, allowing it to take over entire fields to the exclusion of native competitors very quickly allowing it to take over entire fields. *C. stoebe* is believed to be able to produce the allelochemical (±)catechin. This chemical is exuded through the roots and into the substrate and works to inhibit the growth of potential competitors (7).

Current research in plant genomics suggests that plants of the *Centaurea* genus are native to Europe, although the species in Europe are diploid while the species in the United States are polyploid (4). These findings suggest that *C. stoebe* in the United States is better adapted to multiple types of environments that would aid to its invasive potential.

Lespedeza capitata (Fabaceae) is a native species found to grow in very close proximity to *C. stoebe* in very similar circumstances. *L. capitata* has a mutualistic relationship with *Rhizobia* bacteria that fix nitrogen for nutrition for the plant. *L. capitata* makes use of a taproot that can be as much as two times larger than the shoot of the plant. The leaves are arranged into leaflets and are coated in silvery hairs, as is the rest of the plant. It produces white flowers with a purple spot that are formed in clusters in top of each stem of the plant (5). *L. capitata* was found along the High Bridge Trail growing in similar conditions to where *C. stoebe* so it seemed to be a suitable choice for a competition experiment.

The goal of our research was to investigate the invasive potential and threat posed by invasive species in a local Virginian system. We hypothesized that, given its nearly nationwide dispersal and invasive nature, and the results we collected from previous competition experiments in the summer of 2013, *Centaurea stoebe* would outcompete *Lespedeza capitata* in competition experiments and therefore pose a threat to wild populations of *Lespedeza capitata* along the High Bridge Trail.

Principal Approaches

To begin our experiments, we travelled out to the High Bridge Trail, a reconditioned railroad grade converted to managed state park lands in central Virginia, and located a nursery of *C. stoebe* seedlings as well as a nursery of *L. capitata* seedlings in the same area. We then carefully extracted seedling plants and transplanted them into growth tubes (4cm in diameter) filled with sand. Each growth tube was labeled with colored tape to assign it to an experimental group: Red group containing 1 *C. stoebe* plant, Orange group containing 2 *C. stoebe* plants and 1 *L. capitata* plant, White group containing 1 *C. stoebe* plant and 2 *L. capitata* plants, Blue group containing 1 *C. stoebe* plant and 1 *L. capitata* plant, and lastly, Green group containing 1 *L. capitata* plant. A total of 300 growth tubes were filled and transported back to the Hampden-Sydney Greenhouse.

Plants were allowed to grow for a total of 8 weeks. All plants in all treatment groups were watered daily, fertilized once a week with lab-made 10% Hoagland's solution, and rotated position in the greenhouse weekly to avoid block effect. After the allotted growth period, 250 of the growth tubes were disassembled. All living plants were removed from growth tubes, cut in half between the roots and shoots, then root length and shoot length were measured as well as leaf count. Severed plants were placed in individual envelopes labeled accordingly then placed in a drying oven at 60 degrees Celsius for a minimum of 24 hours. Dried plants were removed and weighed on an analytical balance.

Before the substrate was discarded, 5 tubes from each treatment group were randomly selected for catechin extraction in order to approximate the concentration of the allelochemical in accordance with the protocol written by Callaway et al in their paper "Dual Role for an Allelochemical: (±)-catechin from Centaurea Maculosa Root Exudates Regulates Conspecific Seedling Establishment" (7). Approximately one gram of soil was measured and extracted from each grow tube and placed into a 2.5 mL eppendorf tube. One mL of HPLC grade methanol was added to each tube to extract catechin. All tubes were vortexed for 30 seconds, then centrifuged for 1 minute to separate soil particulates. The remaining clear liquid was pipetted off the top into new eppendorf tubes. The samples were labeled and stored under dry ice for transport. A standard solution of Catechin was made using pure catechin obtained from University of Richmond's chemistry department in order to serve as a comparison. Samples were analyzed at VCU's Medicinal Chemistry Lab using a Perkin Elmer Flexar UHPLC with PDA detector and a Brownlee Carbon 18 column. We were able to find a peak in the spectra at 254 nm that matched the molecular weight of catechin, as determined on the VCU Perkin Elmer AxION 2 Time-of-flight Mass Spectrometer, but were unable to obtain a linear calibration curve using a (±) catechin standard and so discontinued these particular trials, but they may be resumed at a later date.

We then prepared larger extracts by randomly selecting 3 tubes from each treatment group and then measuring approximately 10 grams of soil per tube and then adding 10 mL of methanol to each. We started with newly prepared catechin standards at University of Richmond. From there, we were able to construct a linear calibration curve for the (±)catechin standards using a Jasco 550 UV-Vis spectrometer. The peak for the catechin standards was at 280 nm. The results were puzzling and inconclusive. While we did observe a molecular ion that corresponds to the exact mass of catechin, there was no correlation in the peak position observed in the UV-Vis spectra. Further investigation is required and is underway.

Present Knowledge

Initial data taken was reported through survival rates (percentage of plants still alive at the end of the growing period per treatment group).

Using the root and shoot length measurements, leaf count and root and shoot dried weights, we conducted ANOVAS on each group to determine if there was any significance to our findings. Below is a table showing the ANOVA results per treatment group for *C. stoebe* plants. Significance is reported in bold and was deemed to be "p-value < 0.05".

Group	*L. capitata* Survival rate	*C. stoebe* Survival rate
Orange (2 CS, 1 LC)	97.222%	CS1 – 63.889% CS2 – 38.889%
Blue (1 CS, 1 LC)	92.683%	43.9024%
White (1 CS, 2 LC)	LC1- 94.231% LC2- 88.462%	50%
Red (1 CS)	n/a	61.702%
Green (1 LC)	91.379%	n/a

Group (*C. stoebe)*	F-statistic	P-value	Degrees of Freedom
Total plant mass	1.348342	0.26296	3, 237
Root mass by treatment	1.297174	0.276527	3, 237
Shoot mass by treatment	0.993262	0.397003	3, 237
Root to shoot ratio by treatment	1.449621	0.22966	3, 237
Leaf number by treatment	2.396915	0.069187*	3, 237
Shoot length by treatment	0.772211	0.510725	3, 237
Root length by treatment	1.604657	0.189378	3, 237

We then conducted ANOVAS for *L. capitata* to see if there was any significance to that data operating within the same parameters as before. The results are shown in the table below.

Group (*L. capitata*)	**F-statistic**	**P-value**	**Degrees of Freedom**
Total plant mass	2.367818	0.071495	3, 237
Root mass by treatment	1.794681	0.148888	3, 237
Shoot mass by treatment	2.169621	0.092316	3, 237
Root to shoot ratio by treatment	**3.848616**	**0.010238**	**3, 237**
Leaf number by treatment	**5.481136**	**0.001175**	**3, 237**
Shoot length by treatment	**2.680255**	**0.047641**	**3, 237**
Root length by treatment	1.839306	0.140718	3, 237

Conclusion

The results show significance within three of the *L. capitata* treatment groups. Significant findings suggest that *L. capitata* grown with a single *C. stoebe* competitor had, on average, a higher number of leaves than those grown with more competitors or alone (Figure 1). It is difficult to explain this result definitively though it could be that, due to its nitrogen fixing capabilities, *L. capitata* plants allocated more growth energy to growing leaves (vital to photosynthesis) and proportionally less to roots. These data also suggest that *L. capitata* grown with more competitors have, on average, proportionally larger roots as shown by the root to shoot ratio (Figure 3). This could be explained by *L. capitata* allocating more of its growth to roots because they are energetically cheaper to grow (chlorophyll required for leaf growth is energy-expensive) and extremely important in nutrient uptake. With increasing number of competitors in a confined space, the total amount of available nutrients drops. Therefore, it would be a good competitive strategy for a plant to maximize root growth in order to capitalize on the limited availability of nutrients.

The results show little significance within the *C. stoebe* groups, though Figure 4 shows a possibly significant trend within the leaf number count of *C. stoebe* within the different treatment groups. The graph shows that *C. stoebe* plants grown alone (no competition) had a higher average leaf count than those that were growing in competition. This could be due to the lack of competition for nutrients in the soil when a plant is growing alone, which would allow it to allocate more growth to leaf development.

The survival rates of the different species of plants in the different treatment groups clearly show that *L. capitata* had a much higher survival rate than *C. stoebe* in all relevant treatment groups.

There are many possible explanations to why *L. capitata* was able to outcompete *C. stoebe* when both were grown together. It is likely that, because *L. capitata* is a legume and capable of fixing its own nitrogen (a vital nutrient in early plant growth and development), it was able to meet its own nutritional needs better than the *C. stoebe* was. Results may have differed significantly had we used a more concentrated Hoagland's solution. Adding potting soil to the sand we used as growth medium (a nutrient poor substrate with low cation exchange capacity) may also have encouraged *C. stoebe* growth by providing more essential nutrients. It is also important to note that our water schedule may have been too rigorous and impeded the growth of *C. stoebe,* which tends to favor warm dry climates.

Because our results conflicted heavily with the results we collected last summer, it is difficult to definitively state whether or not *L. capitata* is under threat of *C. stoebe* invasion and potentially facing competitive displacement. It is also difficult to state whether or not the biodiversity of the High Bridge Trail is threatened. Further monitoring and continued surveys of the flora along the trail is required to answer these questions.

Figure 1

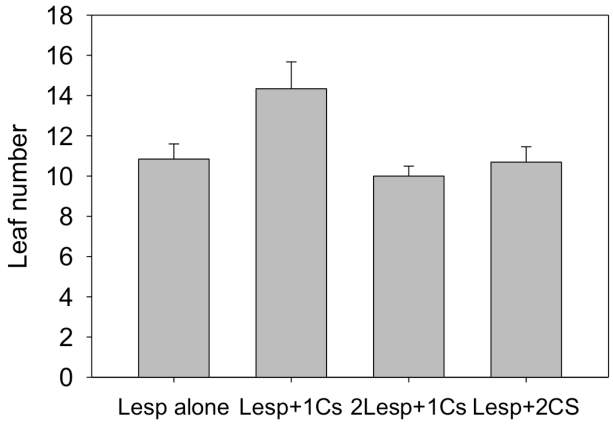


Figure 2

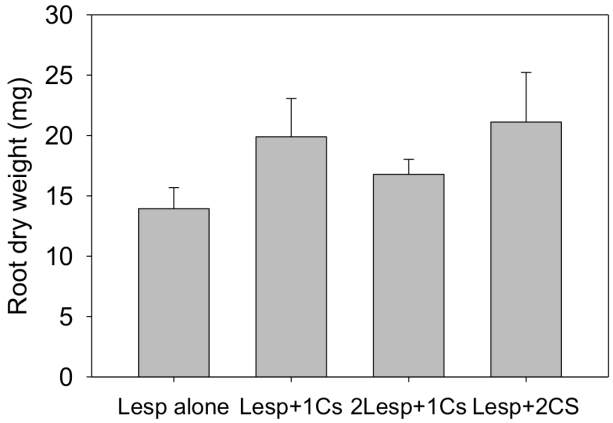


Figure 3

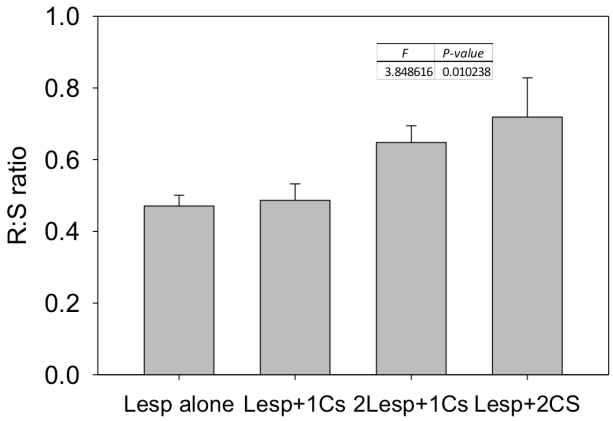
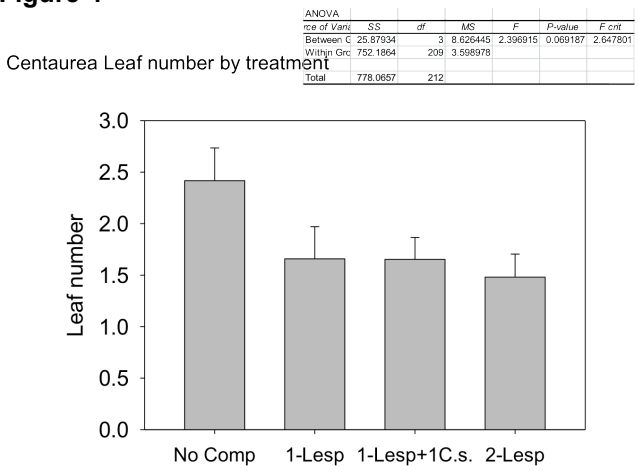


Figure 4



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