

Lane Richter  
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## Germination and Growth of Native Understory Forbs in Relation to Shade Levels

### Introduction:

*Euonymus fortunei* (wintercreeper, Celastraceae) is a perennial evergreen woody vine native to China, Korea, and Japan (Hutchison, 2005) that is widely used in the landscape industry (Ningen et al., 2005) and is considered an invasive plant species (Hutchison, 2005). *E. fortunei* can spread vegetatively or through seed, and has aerial and trailing roots that are able to spread rapidly (Czarapata, 2005). *E. fortunei* can tolerate full sun as well as heavily shaded areas, and invades relatively undisturbed habitats (Czarapata, 2005). *E. fortunei* is present throughout the Litzsinger Road Ecology Center (LREC) site, and can form dense mats capable of displacing native species. One of the ways that *E. fortunei* could displace native species is by shading them out either at the germination stage (Chory et al., 1996) or early growth stage. Another way *E. fortunei* could impact seedling emergence is through physical obstruction. Physical obstruction may prevent seedling emergence or force seedlings to allocate more of the available energy to hypocotyl growth than to the cotyledons and radicle (Ellsworth et. al, 2004). A seedling draws initially on reserves stored in its cotyledons or endosperm, and then the plant builds on its rudimentary form through the activity of the root and shoot apical meristems (Taiz and Zeiger, 2006).

Seedling emergence of six native understory forbs (*Polemonium reptans*, *Phacelia purshii*, *Salvia lyrata*, *Campanula americana*, *Blephilia ciliata*, and *Iodanthus pinnatifidus*) were evaluated over four light levels. By conducting this experiment in a controlled setting using shade cloth, I was able to separate out the effects of the shade

cover on these species. Shade cover may be the variable that causes them not to compete well with *E. fortunei*. Light levels under the shade cloth and under *E. fortunei* were compared to determine which shade cloth best approximates the light conditions under *E. fortunei*. The data collected may be important in determining sites at LREC where the seedlings have the greatest likelihood of establishing. My hypotheses are: (1) Germination will decrease with increasing shade levels. (2) Seedlings will allocate more to hypocotyl growth with increasing shade levels.

### **Methods:**

Germination of six native understory forest species (*Polemonium reptans*, *Phacelia purshii*, *Salvia lyrata*, *Campanula americana*, *Blephilia ciliata*, *Iodanthus pinnatifidus*) were evaluated under four shade levels (90%, 60%, 30% , 0%) in the LREC Greenhouse. These species were chosen because they were either already present in the LREC woodland, or the LREC staff were interested establishing them on site. The experiment for *P. reptans*, *P. purshii*, and *S. lyrata* began on June 28, 2007, *C. americana* began on July 6, 2007, and *B. ciliata* and *I. pinnatifidus* began on July 11, 2007.

At each light level there were four replicated treatments for each plant species. Each of the four replications in a treatment for *P. reptans*, *P. purshii*, and *S. lyrata* consisted of a six plug molded plastic planting tray. Each cup of the molded plastic planting tray was filled to the top with Fafard Custom Mix<sup>TM</sup> germination growing media. Water was then added to allow the growing media to settle. Growth media was added until it was a couple centimeters from the top while moist. Three seeds from one of the plant species were then placed in each cup of the molded plastic planting tray. Seeds for *P. reptans*, *P. purshii*, *S. lyrata* were taken from seed collections taken at LREC and

Shaw Nature Reserve between May and June, and were not stratified. A 1/5 teaspoon of sand was spread evenly over the surface of each cup in the planting tray. Trays were kept well watered throughout the experiment to keep the growing mixture moist.

Each of the four replications in a treatment for *C. americana*, *B. ciliata*, and *I. pinnatifidus* consisted of a five plug molded plastic tray. Soil growth media was added and treated in the same way as described for the other three species. *C. americana*, *B. ciliata*, and *I. pinnatifidus* seeds were provided by Shaw Nature Reserve on July 5, 2007 and had been stratified. Due to the difficulty of separating the stratified seed from the storage mixture, a 1 mL measuring spoon was used to distribute the same amount to each plug in the molded plastic tray. The stratified seed storage mixture was mixed before distribution began, so that the mixture was uniform throughout.

Each Monday, Wednesday, and Friday all planting trays were evaluated for seed germination. Planting trays were watered every morning with a mister nozzle to keep the growing medium moist. The mister nozzle was used to minimize disturbance at the growing medium surface. On each measuring date the number of seedlings in each tray was reduced to one by trimming extra seedlings with a scissors. The seedling that was left standing was the largest seedling present in the tray. Percent germination for *P. reptans*, *P. purshii*, and *S. lyrata* was calculated as the number of seeds that germinated divided by the total number of seeds in a planting tray, and then multiplied by a hundred. Germination for *C. americana*, *B. ciliata*, and *I. pinnatifidus* was evaluated by using ratios, because exact number of seeds added was not known in the 1mL measuring spoon. To calculate percent germination for a species, I took the number of seeds that germinated in a cup and divided that by the number of germinated seeds in the cup with

the highest germination number for that species. The decimal number was then multiplied by a hundred to achieve a percent value. Seedling emergence was evaluated to observe differences among light levels. Seedling energy allocation due to light levels was evaluated by measuring stem height. This method was chosen because it was nondestructive.

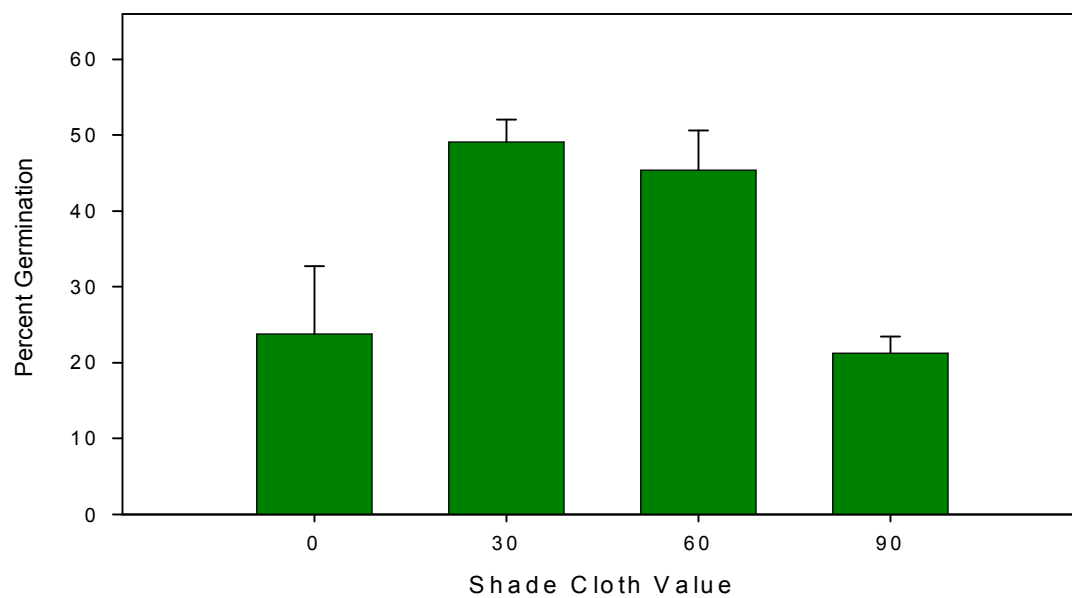
Light levels under the various light treatments were evaluated by placing a HOBO UA-002-08 Pendant Temp/Light meter under the shade cloth on two clear sunny days (July 23, 2007 and July 31, 2007) and comparing them to the light levels above the shade cloth and outside the LREC greenhouse on the same days. A relative shade value was then calculated, so that shade conditions could be compared to shade values in the understory. Light values were taken at the soil surface above and below *E. fortunei*, under closed canopy, and in canopy gaps. The average light value for a specific condition was calculated (Ex. Above *E. fortunei*). The average light value was divided by the average direct sunlight value for the day and then multiplied by a hundred. The product represents a percent of the direct sun value.

All data was analyzed using an ANOVA test to evaluate differences among germination rates, and energy allocation across the four shade treatments for a given species. Treatments were compared for significant differences (alpha 0.05).

**Results:** Figures 1-8: Error bars represent 1 standard error.

Figure 1:

*Campanula americana* Germination in Relation to Shade



$P < 0.001$

Figure 2:

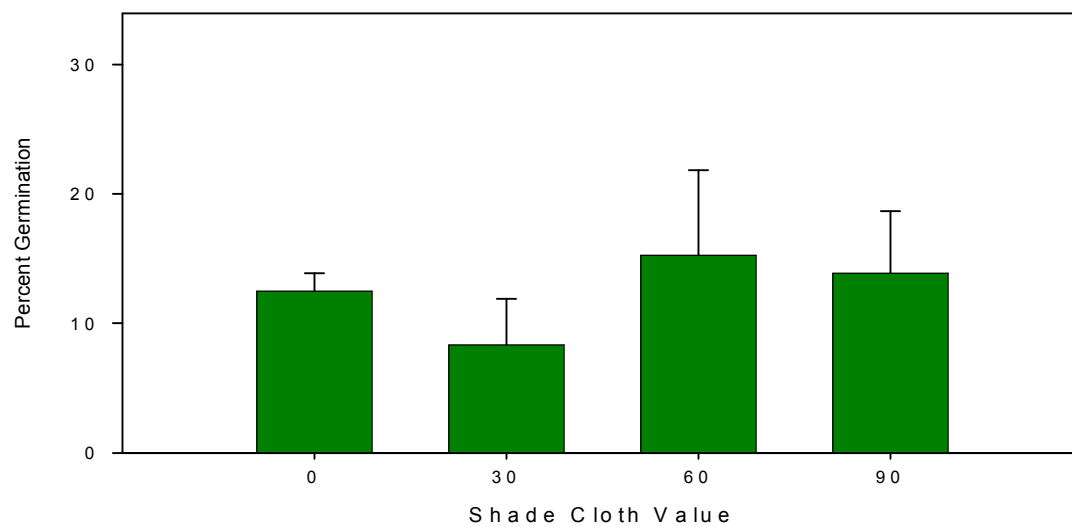
*Salvia lyrata* Germination in Relation to Shade $P < 0.01$ 

Figure 3:

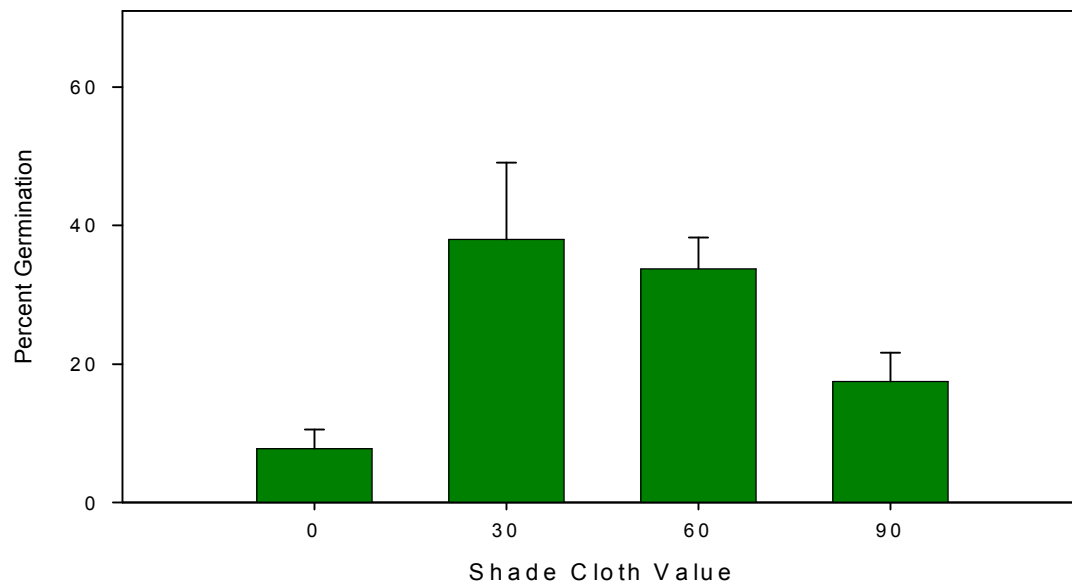
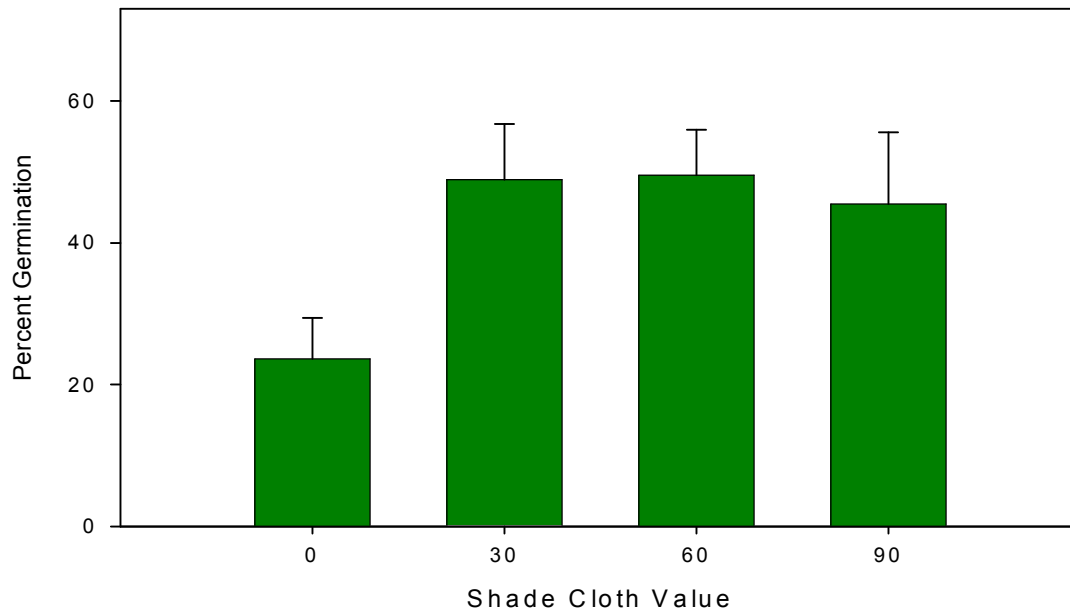
*Blephilia ciliata* Germination in Relation to Shade $P < 0.001$ 

Figure 4:

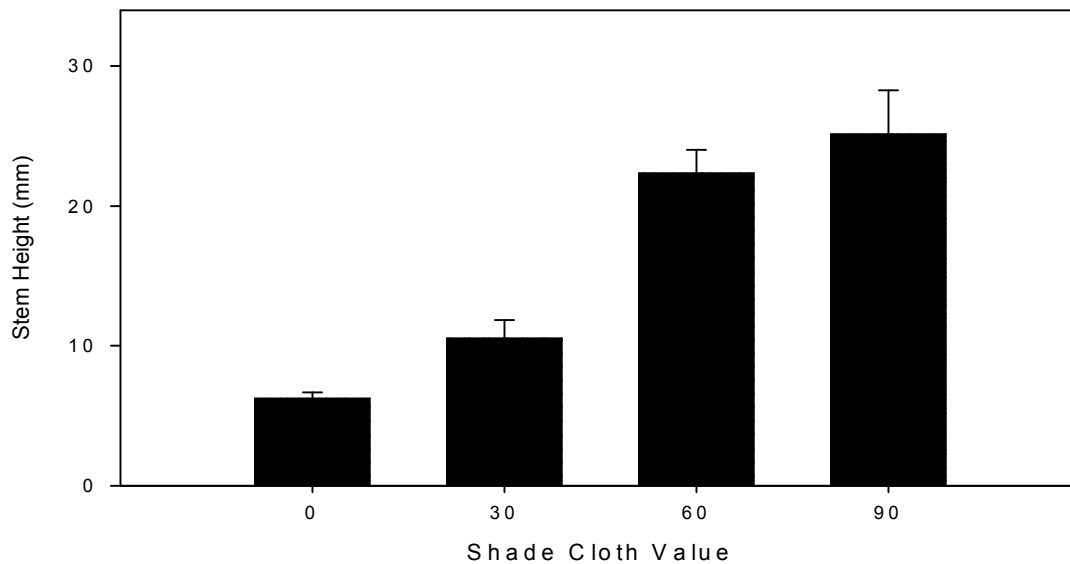
*Iodanthus pinnatifidus* Germination in Relation to Shade



$P > 0.10$

Figure 5:

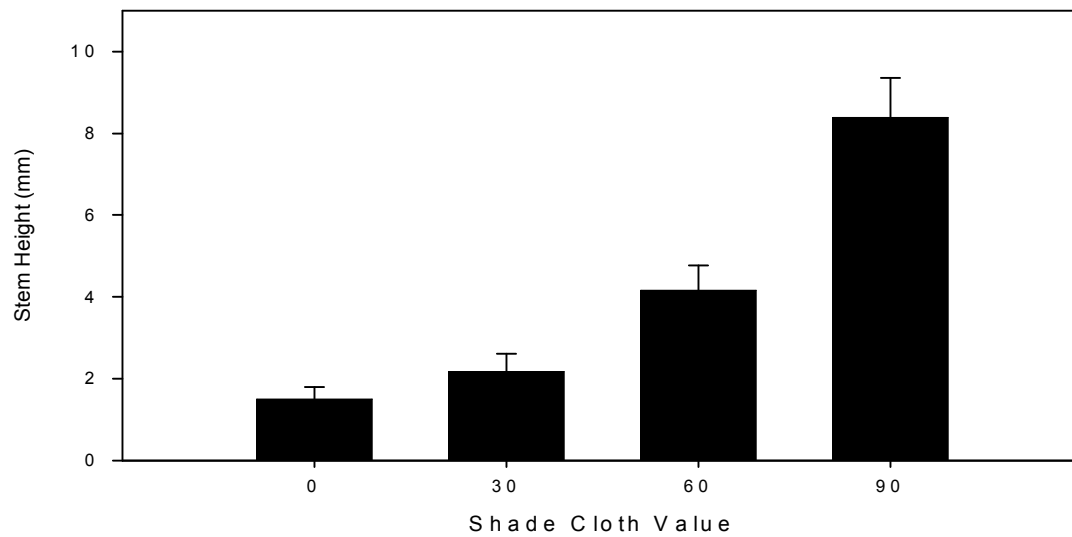
*Campanula americana* Stem Height in Relation to Shade



$P < 0.001$

Figure 6:

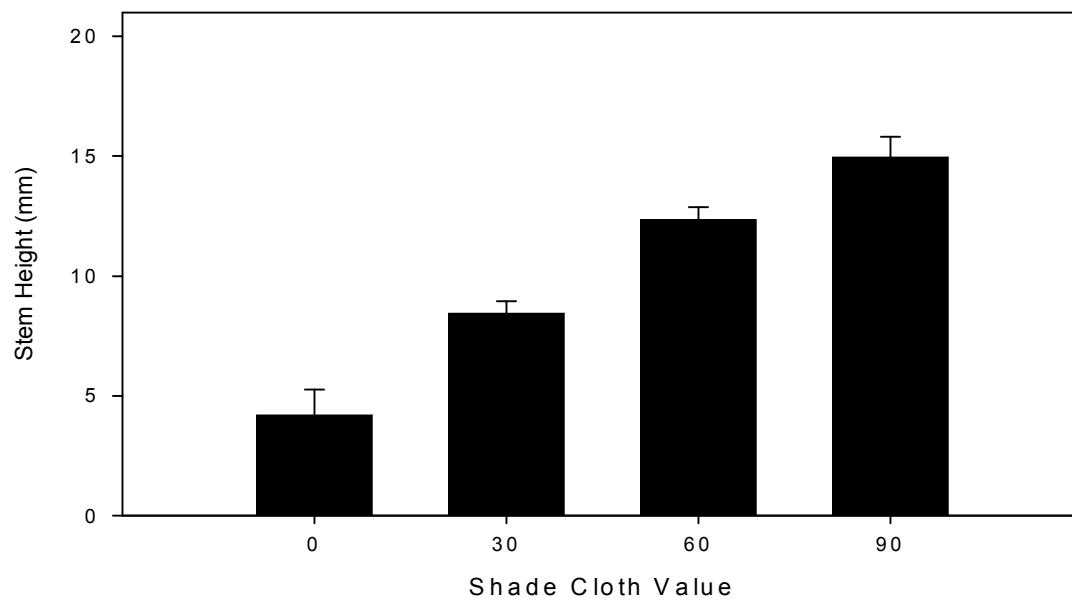
*Salvia lyrata* Stem Height in Relation to Shade



$P < 0.001$

Figure 7:

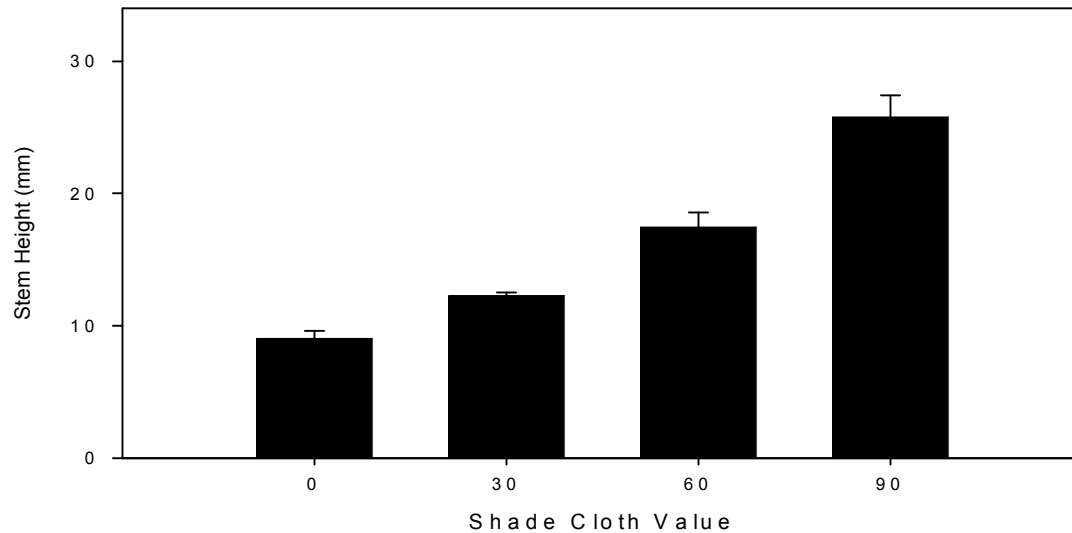
*Blephilia cilata* Stem Height in Relation to Shade



$P < 0.001$



Figure 8:

*Iodanthus pinnatifidus* Stem Height in Relation to Shade

$P < 0.001$

Table 1: Light Levels By Location

Location	% Light
Direct Sun	100
0 Shade Treatment	11.38
30 Shade Treatment	5.77
60 Shade Treatment	2.42
90 Shade Treatment	0.51
Above <i>E. fortunei</i> (closed canopy)	6.22
Above <i>E. fortunei</i> (canopy with gaps)	7.57
Below <i>E. fortunei</i> (closed canopy)	2.26
Below <i>E. fortunei</i> (canopy with gaps)	1.19

*P. reptans* or *P. purshii* did not germinate in any of the treatments over the duration of the experiment. They may have required a stratification period for after-ripening to become physiologically mature (Raven et al., 2003), or needed some other environmental condition to break dormancy.

*C. americana* displayed higher germination rates at intermediate shade cloth treatments (30 and 60) and were not significantly different (Fig. 1). The highest and lowest shade cloth treatments (0 and 90) displayed the lowest germination rates and were not significantly different. Germination rates in the 30 and 60 treatments were both significantly different from the 0 and 90 treatments.

*S. lyrata* displayed the greatest germination rate at the 60 shade cloth treatment but this was not significantly different from the other treatment. Germination rate at the 30 shade cloth treatment was significantly lower than the 0 shade cloth value, but all other differences among treatments were not significant (Figure 2).

*B. ciliata* displayed the greatest germination rate at intermediate shade cloth treatments (30 and 60), and the differences between the two treatments were not significantly different (Figure 3). Germination rates under the 30 and 60 shade cloth value treatments were both significantly different from the 0 shade cloth value treatment. Germination rate under the 90 shade cloth value treatment was not significantly different from any of the other treatments.

*I. pinnatifidus* displayed the greatest germination under 30, 60, and 90 shade cloth treatments, but these were not significantly different from the 0 shade cloth treatment (Figure 4). Differences between the 30, 60, and 90 shade cloth treatments were also not significant.

*C. americana* stem height increased with decreasing light levels. Stem height differences of seedlings were not significantly different between 0 and 30 shade cloth treatments as well as between the 60 and 90 shade cloth treatments. However the means

for the 0 and 30 shade cloth treatments were significantly different from the means of the 60 and 90 shade cloth treatments (Figure 5).

Stem height increased with decreasing light levels for *S. lyrata* as well. The 0 and 30 shade cloth treatments were not significantly different from one another. The 30 and 60 shade cloth treatments were also not significantly different. The 60 shade cloth treatment was significantly taller than the 0 shade cloth treatment. The 90 shade cloth treatment was significantly taller than all other treatments (Figure 6).

*B. ciliata* also displayed an increase in stem height with decreasing light levels. Stem height in the 0 shade cloth treatment was significantly lower than all other treatments. Stem height under the 30 shade cloth treatment was significantly lower than stem height under both 60 and 90 shade cloth treatments. Differences between stem height under the 60 and 90 shade cloth treatments were not significantly different (Figure 7).

*I. pinnatifidus* stem height increased with decreasing light levels. Stem height between 0 and 30 shade cloth treatments were not significantly different, and stem height between 30 and 60 shade cloth treatments also were not significantly different. Stem height under the 60 and 90 shade cloth treatments were significantly greater than in the 0 shade cloth treatment. Stem height under the 90 shade cloth treatment was significantly taller than under the 60 shade cloth treatment (Figure 8).

The 0 shade cloth treatment had higher levels of light than the sites sampled in the understory on site (Table 1). The 30 shade cloth treatment had slightly lower light levels than measurements taken above *E. fortunei* in both closed canopy and canopy with gap sites (Table 1). Light levels taken under the 60 shade cloth treatment were similar to light

levels below *E. fortunei* in closed canopy sites. Light levels below *E. fortunei* (closed canopy) were lower in the open canopy sites (Table 1). Light levels below *E. fortunei* (canopy with gaps) were most similar to the 90 shade cloth treatment.

**Discussion** *C. americana* displayed a greater germination rate at intermediate shade cloth values and stem height increased with decreasing light (Figure 1 and 5). Some seedlings under the 60 and 90 shade cloth treatments were not able to support themselves and therefore did not survive. It may be advantageous to increase stem height in the understory, so that more light is available for photosynthesis. Increasing stem growth may result in a greater chance that the stem will collapse, and that photosynthetic capacity will be reduced though. There may be costs in shifting allocation to the hypocotyls (stem) such as reduced initial photosynthetic area and greater susceptibility to physical damage (Ellsworth et. al, 2004)

*S. lyrata* displayed an increase in stem height with decreasing light levels, and had similar germination rates over all treatments (Figure 2 and 6). Light may not be as important as other factors such as moisture level or temperature (Raven et al., 2003) in *S. lyrata* germination rates. Although the species displayed plasticity in stem length none of the individuals collapsed for the duration of the experiment. All other species had some hypocotyl collapse under higher shade treatments. *S. lyrata* had low germination rates but this may have been because the seeds had not been stratified prior to the experiment.

*B. ciliata* displayed an increase in stem height and higher germination rates at the two intermediate shade level treatments (Figure 3 and 7). Light values above *E. fortunei* in the forest understory were similar to light values in the 30 shade level treatment, and light values below *E. fortunei* were slightly lower than the 60 shade level treatment

(Table 1). Light may play an important role in germination of this species because it does not tolerate relatively high levels of light (0 shade treatment). At higher levels of shade *B. ciliata* showed hypocotyl collapse (60 and 90 shade treatments). .

*I. pinnatifidus* germination was not significantly impacted by shade level, but stem height did display an increase with decreasing light levels. Light may not play as important a role in seed germination as some other factors in this species, but does impact initial energy allocation. Only one seedling collapsed in the 90 shade treatment, and there were none that collapsed in the other treatments. It appeared that although there was an increase in stem height with decreasing light, *I. pinnatifidus* was still able to support itself well.

The 30 and 60 shade cloth treatments were similar to the light levels found above and below *E. fortunei* (closed canopy). The 90 shade cloth treatment was most similar to the below *E. fortunei* (canopy with gaps) values. The reason light levels were lower below *E. fortunei* in the canopy with gaps area I believe may be caused by the differences in *E. fortunei* height in the respective measuring sites.

### **Conclusion:**

Germination rates were either not significantly affected by different shade levels, or displayed higher germination rates at the two intermediate shade levels. All species displayed stem height plasticity that increased with decreasing light levels. *S. lyrata* was the only species whose stem didn't collapse under higher light levels. This may have been due to lower germination rates and therefore a smaller sample. *I. pinnatifidus* was also able to support itself well despite increasing stem height. At the end of the

experiment some seedlings in the 0, 30, and 60 shade treatments had leaves, and all seedlings in the 90 shade treatment had only their cotyledons. The initial reallocation of energy to stem growth in higher shade treatments, or low light levels may reduce the photosynthetic capacity of these seedlings. The results in this experiment compare to what others have found about seedling development under different light conditions. Dark-grown seedlings have hypocotyl elongation and small cotyledons, while light inhibits hypocotyl elongation and induces leaf expansion (Chory et al., 1996). Light levels play an important role in seedling development, and it appears that this is one of the ways that *E. fortunei* is able to prevent seedling establishment in the understory. If seeds are able to germinate under *E. fortunei* they will likely allocate more energy to the hypocotyl, and stability and further survival will be reduced.

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