**Investigation into the potential invasiveness of the exotic Narrow-leaved Bittercress,**
**(*Cardamine impatiens* L.), Brassicaceae**


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ABSTRACT

Exotic species often invade new areas and displace native species. The problems associated with such invasions are well known, but for many exotic species, experimental work has not yet been done to predict which, and under what conditions they may become a problem. Two greenhouse experiments were devised to investigate the plasticity, shade tolerance, and phenotypic differences of full-siblings from 3 populations of *Cardamine impatiens*, a Eurasian species potentially invasive in North America. Potted plants were subjected to 0, 54, 76, or 91% shade created by neutral density shade cloth application. In addition, the impact of a cold pre-treatment of seedlings on the growth and reproductive output of *C. impatiens* plants was examined.

In our first experiment, we subjected *Cardamine impatiens* to non-shaded cages, 54%, or 76% shade intensity. Plants died very quickly, so LD50 data were used as a relative measure of fitness, and relative growth indices were calculated over time. Other relative measures of fitness included canopy area, leaf area, number of leaves, number of leaves per canopy area, and final plant weight. Plants in cages with no shade treatment grew faster than those in cages with shade cloth and final plant weight decreased as shade treatment percentage increased. In each population, the number of leaves increased over time and the number of leaves per canopy area decreased over time under shade treatments.

Our second experiment involved the application of 54%, 76%, and 91% shade intensity. The additional shade treatment of 91% was applied to determine the extent of plant tolerance and plasticity in response to light reduction. Due to high plant mortality in our first experiment, we treated *Cardamine impatiens* with a 4 week cold period prior to treatment, which simulates its biennial growth form in its natural western Virginia region habitat. Since this second experiment took place later in the year, day length was extended to more accurately duplicate the conditions during the first experiment. LD50 calculations were not

necessary, and 7 of the 135 plants produced seed. Relative measures of fitness included canopy area, leaf area, number of leaves, number of leaves per canopy area, and final plant weights. As in experiment one, the number of leaves per plant increased over time, final plant weight decreased as shade treatment increased, and the number of leaves per canopy area decreased as shade treatment increased.

From these two experiments, we determined that *Cardamine impatiens* is a species that exhibits phenotypic plasticity and therefore may pose a threat as an invasive species. *C. impatiens* is able to grow and exhibit plasticity of plant architecture under the conditions of very low light. The number of leaves per canopy area decreased as shade increased, suggesting that *C. impatiens* is highly adaptable to low light conditions, and therefore may be exhibiting phenotypic plasticity by reallocating its resources by producing fewer leaves while maintaining canopy area. This data along with other *C. impatiens* traits such as high levels needed for seed production, its persistence in seed banks, along with a lack of known major enemies, indicates that they have a great capacity to invade a wide variety of habitats. We also determined that a cold treatment is necessary in order for *C. impatiens* to obtain optimal growth and reproduction.

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**1.00   Introduction**

*1.10    Introduction: Invasive Plant Species*

Exotic species that inhabit relatively intact habitats and dominate, displace, or otherwise alter such ecosystems are termed "invaders" (Bazzaz 1986).  Rates of invading exotic plant species surpass the rates of invading animals (Vitousek *et al.* 1996).  As of 1996 censuses, anywhere between 5 % and 25% of all vascular plant species in the United States' natural reserves were non-native species (Vitousek *et al.* 1996).  The increasing rate at which exotic organisms are spreading and becoming naturalized is an increasing global threat to native biodiversity, second only to habitat loss (Vitousek *et al.* 1996; Wilcove *et al*. 1998; Ewel *et al*. 1999).

*1.11    Definitions, Dispersal Mechanisms, and History*

Invasive species are introduced both unintentionally and purposefully, and introductions have taken place throughout history without human intervention (di Castri 1989).  Natural vectors for plant introductions to new communities include wind, water, and animals, but humans have played a big part in the introduction of exotic organisms due to the high number of ships, railroads, and airplanes traversing the world.  Upon arrival to non-native ecosystems, exotic species may not have any natural predators or competitors in their new habitat.  Often with little resistance, these species can establish themselves among natives and increase their populations.  Once such a species is well established it is very difficult to eradicate (Ewel *et al*. 1999), and species that successfully propagate without the aid of humans are deemed 'naturalized'.  If a naturalized species is proven to cause ecological change, it is considered "invasive".

Many exotic species are hand selected for use in biological control programs to aid with existing ecological problems.  In Appalachia, Kudzu, *Pueraria montana* var. *lobata* (Willd.)  Maesen & S. M. Almeida, was imported from Japan in 1876 to aid in soil stabilization efforts along slopes of road cuts (Forseth & Innis 2004).  This vine now covers all things in its path and hinders or altogether inhibits the ability of vegetation underneath it to undergo photosynthesis. Other species are purchased and brought to the United States for

food production or aesthetic value.  The invasive Asian shrub, *Rosa multiflora* Thunberg, for example, was introduced as an ornamental plant for landscaping purposes in the 1880s (Department of Conservation and Recreation 1999).  Today, *R. multiflora* is such an aggressive invader that in some states it has become illegal to buy, sell, or distribute (Yates *et al*. 2004).

Many invasive species exhibit plasticity under different environmental conditions. For example, *Schinus terebinthefolius* Raddi is considered to have a very high level of plasticity due to its high level of adaptation to drought stress (Nilsen & Muller 1980).  High plasticity allows invasive plants to proliferate in many different habitat conditions. Such proliferation results in a higher probability that a naturalized species will become invasive.

Exotic plants are often subject to escape into nearby wild, unmanaged habitats, as is evident from the escape of roadside erosion control species such as *Pueraria montana* and *Cytisus scoparius* Link into adjacent wild lands (Forseth & Innis 2004). In some cases naturalized species that escape into adjacent habitat hybridize with native species. The result of hybridization can be a new taxon with highly competitive traits that will displace the native species that hybridized with the naturalized species (Mack *et al*., 2000).

### 1.12  Factors Contributing to Invasive Species Success

Phenotypic plasticity levels are important when studying invasive organisms, because evidence of plasticity may mean that a plant can acclimate more easily and rapidly to changing environments than those species with a low level of plasticity (Baker & Stebbins 1965; Bazzaz 1986; Orians 1986). Species that achieve plasticity in response to changing resources could have a competitive advantage over those that do not. Interactions between exotic species and native communities are key to whether or not an exotic species can become naturalized and invasive.  Therefore, competition for sunlight, water, and nutrients become major factors of invasive species success.

One characteristic known to be a strong predictor of whether a plant will become invasive is how it has behaved in other countries that it has previously invaded (Ewel *et al*. 1999).  The traits that enable some introduced species to spread widely across their native habitats make them better able to invade new continents (Roy *et al*. 1991).  Therefore, a

plant's native latitudinal range is often seen as the best predictor of its invasive potential (Forcella *et al*. 1986; Rejmanek 1995).

Most invasive species prefer cultivated or otherwise disturbed areas (Baker 1974; Orians 1986).  Land altered by events such as fire, clear cuts, new roads, and other development is highly susceptible to invasive immigration. However, it is false to assume that invasions are limited only to disturbed habitats.  Forests, which were once assumed to be immune to the threat of invasive species, have been proven to be at risk (Webb *et al*. 2000). These invaders of more pristine habitats, which include garlic mustard (*Alliaria petiolata* (M. Bieb.) (Cavara & Grande) and tree-of-heaven (*Ailanthus altissima* (Miller) Swingle), are becoming common residents of deciduous forests in northeastern North America.

One important aspect of species invasiveness to consider is the lag time that is known to exist between species introduction and species invasion (Ewel *et al*.1999). Introduced species go through an adaptive or acclimating stage upon arrival to their new habitat (Bazzaz 1986), and only 5%-25% will become invasive (Vitousek *et al*. 1996; Williamson & Fitter 1996).  Invaders are known to go through morphological changes as they adapt or acclimate to new surroundings (D'Antonio *et al*. 1998). However, hybridization or other genetic mixtures may also be important to invasive success.  *Rhododendron ponticum* L., for example, has shown evidence of a genetic shift in invasive populations towards an increased investment in growth relative to native populations (Erfmeier & Bruelheide 2004;  Zou *et al*. 2007).

### 1.13  *Invasive Species Research History*

Definitions of critical terms in the field of invasion biology vary broadly from researcher to researcher. Most floras only vaguely attempt to make the distinction between native, exotic, and invasive; furthermore, the botanists who author most floras fail to reveal the criteria they use to make such distinctions (Ewel *et al*. 1999). The differences between terms such as casual, established (naturalized), exotic, alien, and introduced species need to be more clearly defined (Heywood 1989).  Results of experiments involving invasive species are sometimes difficult to interpret because two independent researchers often come up with two different answers to the same question, due to differences in 'expert opinion' (Ewel *et al*.1999) and confusion among terms.  Some level of consistency among ecological

interpretation of invasive events and standardation of terms must be achieved if we are ever to be able to delineate such events properly. We define 'exotic' species as those that come from other countries and have never been considered native to existing communities, 'naturalized' species as those exotic species that are able to survive and reproduce without the aid of man, and 'invasive' species as the naturalized species that displace native species form a community.

### 1.14 *Importance of Early Detection*

The factors influencing invading species tolerance to new habitat are often hard to determine, because invasions are only studied after a species has invaded (D'Antonio *et al*, 1998). Invasive plants can cause habitats to change drastically, which makes it difficult to obtain accurate natural history data of the invasion event. When recognized early, extirpation of potential invasive species from their new habitat by chemical or mechanical means would be much easier. More research is needed to determine which exotic species could potentially become invasive. Invasive potential could be assessed by investigating naturalized, exotic species and their response to changing environments.

### 1.20 *Cardamine impatiens*, **Linnaeus**

### 1.21 *Species Distribution and Historical Significance*

*Cardamine impatiens* was first recorded as a native annual or biennial in the European flora. Irish botanists reported in 1983 (Breen *et al*. 1984) that *C. impatiens* should be included in the Scottish flora as a native plant based on the observation that it occurs in base-rich woodland. Therefore, although *C. impatiens* is considered native to Scotland, its status is still confusing (Cormer 1988). *Cardamine impatiens* has also been proliferating in Finland since around 1990 (von Numers 1987, 1991; Pykala 1991).

Williams (2000) monitored 34 populations of *Cardamine impatiens* in Middlesex, England, for 10 years. *C. impatiens*' average spread was determined to be 17 meters per year, moving in a southeasterly direction and population sizes changed dramatically from year to year. Cyclic variation, the weather, and ground disturbance were the presumed mechanisms of these changes.

The full distribution of *Cardamine impatiens* in the United States to date is as follows. It was first recorded in the U.S. in 1919 at the edge of a New Hampshire lawn (per herbarium data). To date it is mostly confined to the northeastern areas of the United States, but it has moved down into the Mid-Atlantic region as far south as North Carolina (USDA website 2004). Current information available from the USDA indicates that the species has been naturalized in 12 states (USDA website 2004). According to data we collected from 159 herbarium sheets of *C. impatiens* collected across the East Coast of the USA (Table 1), this species is currently most prevalent in Pennsylvania (49 specimens collected in 21 counties) and West Virginia (36 specimens collected in 14 counties).

## 1.22   Life History

*Cardamine impatiens* is a biennial in the U.S.A. Glenn and Berringer (2004) conducted a population survey of this species in New Jersey. According to their observations, the plant is biennial, and seeds germinate from May through August as soon as they are ejected from the plant's mechanically explosive siliques. A basal rosette is formed, only to die back over the winter season and emerge again the following spring to produce seed. Kimata (1983) found that this species is 99.5% self-compatible and that seeds of *C. impatiens,* because of the species tall infructescences, were found to have a greater seed dispersal distance than *C. flexuosa* and *C. scutata. Cardamine impatiens* at the age of reproduction is reported to reach one meter tall.

## 1.23   Habitat and dispersal mechanisms

In Central Europe, *Cardamine impatiens* grows in moist, shady woodlands, at the base of ravines, by streams, along forest roads, and on rock walls (Pykala 1991). In the US, it has been found in riparian zones and on rocky outcrops in the northeastern US since at least 1950 (Fernald 1950; Pykala 1991). Pykala (1991) noted the growth of *C. impatiens* in habitats that contained much sandier soil than where the plant is usually found, which demonstrates its adaptability to different substrates and nutrient levels. Additionally, it was determined that this plant may not be capable of long distance dispersal without the aid of water (Pykala 1991).

Williams (2000) found that this plant was most prevalent in hedgerow edge habitats in Europe. He also observed that it prefers shade and cannot tolerate competition from other

plants. However, Pedersen (1958) observed that *C. impatiens* does not seem to occur regularly and is prone to go missing for years at a time (Pedersen 1958; Pykala 1991). Based on the fact that plants were located in sites where they had not been previously observed for a year or more, Williams (2000) determined that dormant seeds buried in the soil must remain viable for many years. However, this type of boom and bust life cycle could also be indicative of herbivory or evidence that the species is adapting to new habitats (Williamson & Fitter 1996).

**1.30    Objectives**

The purpose of my thesis is to explore traits of *Cardamine impatiens* that may influence its potential to become an invasive species. Specifically, I examined growth and plasticity of key morphological characteristics in response to shade treatments. To examine plasticity, I investigated responses to 4 different shade treatments over the course of 2 reaction-norm experiments involving full siblings from 3 populations. If *C. impatiens* had an adaptive response to shade treatment, it would be considered to have phenotypic plasticity and could therefore flourish upon introduction to many different environments. Also, it is possible that levels of plasticity could vary from population to population, indicating that the genetic makeup of each population determines its capacity to adapt or acclimate in response to various shade regimes. Variation in response among populations may suggest microevolution in response to different habitats.

**Chapter 2.00**

**2.00  Greenhouse Study, Experiment 1: Comparison of growth and acclimation among different populations of *Cardamine impatiens* under varying shade regimes.**

**2.10  Introduction**

Few plants introduced to new environments successfully establish a viable population (Williamson & Fitter 1996). This low success rate is the basis for the 'tens rule', which states that each transition stage (escaping, establishing, and becoming a pest) of invasion has a 10% probability of success, such that any particular non native species has only a 0.1% chance of successfully invading (Vitousek *et al.* 1996). Based on this theory, 10% of the non native species that have established an introduced population have the potential to become invasive species. A critical management issue is how to determine which 10% of the naturalized species will be the invaders. Complicating this task of identifying potential invading species is the time that passes between establishing a naturalized population and becoming an invader. This time-lag is common among invasive species and may relate to processes of adaptation of the invader, genetic hybridization with native taxa or other invaders, the establishment of needed propagule pressure, or other factors (Ewel *et al.,* 1999). The low rate of invasion success and the unknown lag-time before invasion makes it very difficult to identify likely invaders among the many naturalized exotic species. Consequently, management of species invasions is initiated after the invasion has occurred, which makes control of the invasion much harder than if the invader had been identified before the invasion.

Phenotypic plasticity levels are important when studying invasive organisms, because a high level of plasticity may mean that a plant can acclimate more easily and rapidly to changing environments than those species with a low level of plasticity (Baker & Stebbins 1965; Bazzaz 1986; Orians 1986). A fundamental hypothesis about the evolution of phenotypic plasticity is that it provides a mechanism for adaptation to spatially or temporally variable environments (Dudley & Schmitt 1996). Many documented invasive species exhibit significant plasticity compared with indigenous species. For example, *Schinus terebinthefolius*, an invasive species in Florida, is considered to have a very high level of plasticity. Relative to native species, *S. terebinthefolius* exhibited a greater adaptation to

drought conditions than another species in the same genus (Nilsen & Muller, 1980). Another example of a highly plastic invasive plant is *Pennisetum setaceum* (Forssk.) Chiov., a noxious invader of Hawaii, which is known to attain higher stomatal conductance and lower leaf areas in mid-altitudinal sites when compared to other sites in different altitudes (Williams *et al*. 1995). Also, plants that inhabit diverse habitats in their home ranges may enable those species to spread widely across their native habitats, making them more likely to invade novel habitats (Roy *et al*. 1991). Plasticity in morphological and physiological traits may allow plants to utilize resources when they are available, and store these resources to avoid stressful conditions during periods of scarcity (Stratton & Goldstein 2001). Therefore, species that achieve the highest level of plasticity may be more likely to become invasive than species with low plasticity. Screening for phenotypic plasticity of introduced species may provide important evidence for distinguishing likely invaders among the multitude of naturalized species.

*Cardamine impatiens* L. was first established in the United States in 1919 (per herbaria records) and has successfully escaped and established naturalized populations that are expanding among states in the Eastern United States. *Cardamine impatiens* is known to have different ecotypes. For example, *C. impatiens* has been reported as an annual in parts of Asia (Ihsan Al-Shehbaz, Curator and Head of the department of Asian botany, Missouri Botanical Garden, personal communication), and a biennial in the United States (Glenn & Berringer 2004). Also, *C. impatiens* is 99.5% self –compatible (Kimata 1983), which would allow small numbers of individuals to establish a population. The "founder effect" will potentially lead to genetic variation among populations. An introduced species can experience a substantial loss of genetic diversity during the colonization process because of its origins as a small founder population and often experiences consequences of a severe founder effect and genetic drift (Sakai *et al* 2001; Yonekura *et al* 2007).

Individual plants of *C. impatiens* make large numbers of seed and seeds of *C. impatiens* were found to have greater seed dispersal distances than other species in *Cardamine*, because of its tall infructescences (Kimata 1983). Based on these fundamental characteristics of *C. impatiens*, the limited ecological knowledge of this species, and the current range expansion of *C. impatiens* in the United States, this species has the potential to

be an invader. Therefore, it is important to understand the extent of phenotypic plasticity of *C. impatiens.*

       *Cardamine impatiens* produces leaves and completes its growth at about the same time as the tree canopy, indicating that shade tolerance is important to the species. Per herbarium data, *C. impatiens* is found as a basal rosette from early April until mid November. Flowering plants were found from mid-May through late June, and fruit were produced from late May through late August.

       The purpose of this study is to determine the effect of population source and light availability on growth and acclimation traits for *C. impatiens* in the Western Virginia region. A reaction norm experiment was performed in the greenhouse on *C. impatiens* collected from three different populations. Plant growth of *C. impatiens* in response to shade was assessed by counting numbers of leaves per plant over time. Survivorship was calculated from LD50 data (number of days associated with the death of 50% of a population). Acclimation to shade was estimated by measuring the number of leaves per canopy area and specific leaf area under each shade treatment and leaf area.

       We hypothesized that *C. impatiens* would grow and exhibit different phenotypes in different shade treatments, and may therefore have potential to be invasive. We considered that if the number of leaves per canopy area decreased as shade levels increased, *C. impatiens* was exhibiting a phenotypic response. Also, we predicted a decrease in growth and survivorship as shade treatment increased. Furthermore, we predicted that different populations would respond differently to shade, due to genetic differences among populations.

## 2.20    Materials and Methods

### 2.21    *Species Description*

       *Cardamine impatiens* L. (Brassicaceae) is an exotic, naturalized biennial plant in the Western Virginia region that begins its growth as a basal rosette in early spring. Leaves arise from the center of the plant and form into elongated and heavily toothed leaves. The plants go dormant over the winter and basal rosette leaves die off. The following spring, plants re-sprout to form flowering stalks that grow up to 1 meter tall. During the second season of growth, shorter leaves form and are characteristically preceded by small auricles. As the plant

grows taller, simple flowers form in clusters, at first at the apex of the plant and eventually at the base of each leaf.  Plants then self-pollinate and form sets of siliques, each containing eight to twenty seeds.  Glenn and Berringer (2004) documented that seeds germinate from May through August as soon as they're ejected from the plant's mechanically explosive siliques. *Cardamine impatiens'* range extends from Eurasia to North America, where it has proliferated from Maine southward to North Carolina and as far west as Kentucky.  The preferred habitat of *C. impatiens* is boggy, forested areas and riparian zones (Ihsan Al-Shehbaz, personal communication). The native habitat of *C. impatiens* suggests that it will proliferate in moist areas and riparian zones of the eastern deciduous forest, and may  be intolerant of drought.

## *2.22    Population Selection and Seed Collection*

Seeds were collected from May-July 2004 and 2005 in 3 locations in the Southern Appalachian Mountains. The Bluestone Wildlife population (N 37°38'25", W 80°53'9") was collected at Bluestone Lake, located in the town of Hinton, in Summers County, West Virginia. The Old Wolf population (N 37°18'34", W 80°50'53") was collected along the banks of Wolf Creek, in the town of Shumate, in Giles County, VA.  The Spruce Run population (N 37°15'58", W 80°36'5") was collected along the Spruce Run, a New River tributary in the historical area of Goodwins Ferry, in Giles County, VA. Individuals from each population were chosen at random and seeds were collected. Seeds from each individual and population were kept separate throughout the study.  The possibilities of genetic variation within a sample collection were decreased by using 3 full-sibling collections from each population.

## *2.23    Plant Cultivation, Treatment, and Statistical Procedure*

To induce germination and insure similar germination times, *C. impatiens* seeds were treated with 500ppm gibberellic acid. The seeds germinated quickly (3-7 days) on moist sand, and seedlings were transplanted into 2-inch pots.  Fifteen seedlings from each full-sib seed collection for each of 3 different populations, for a total of 135 plants, were transplanted into 2.5-gallon pots after they had grown 7-10 leaves.  A mixture of Metro-Mix 360™ and perlite was used to insure adequate drainage.  All plants were arranged on benches in a greenhouse and assigned a shade treatment of 0% (cage only with no shade cloth), cage plus one layer of

30% shade cloth, or cage plus one layer of 73% shade cloth according to a stratified random design. Using 15 full-siblings from each population, one third of plants were assigned the 0% shade treatment, one third was assigned the 54% treatment, and one third was assigned the 76% treatment. All plants and their assignments were combined and chosen randomly for placement in the greenhouse. Plants were started in late May of 2005 as rosettes, in hopes that they would go dormant over the winter in the greenhouse and come back the following spring, as seen by Glenn and Berringer (2004).

Shade cages were constructed from metal poultry netting. Each cage measured 32 cm in diameter and 1 meter in height. Five plants of each full-sib were covered with an individual cage with no shade cloth, or neutral density shade cloth of 30% or 73% (n = 5 / full-sib / shade treatment). Quantification of irradiance received by plants in each treatment was determined by placing quantum sensors (LI-190s; LI-COR Inc, Lincoln, Nebraska) directly under each treatment cage. Diurnal cycles of photosynthetic photon flux density (PPFD) were recorded for 1 day in each light treatment. The influence of each shade treatment was determined as a % reduction of daily PPFD compared with the readings from the cage-only treatment sensor. Although the manufacturer rated the shade cloth at 30 and 73 percent light reduction respectively, the sensors revealed that the actual photosynthetic photon flux density (PPFD) was reduced by different percentages. Average maximum radiation in treatments was 186.77, 85.42, and 45.77 umol $m^{-2}s^{-1}$ respectively for the 0%, 30%, and 73% neutral density shade cloth. The treatments were redefined to 54% and 76% shade intensity, as the 30% shade cloth reduced light by 54% and the 73% shade cloth reduced light by 76%.

Plants were allowed to grow under the shade treatments for 1 month, before weekly measurements of plant morphology were recorded. In this way we could minimize effects of pretreatment before we took measurements. We measured plant canopy diameter in 2 locations perpendicular to each other (to calculate canopy area = mean radius squared x pi) and counted the number of leaves per plant. We removed one recently mature leaf from each plant on the fourth week of treatment to measure leaf area with a leaf area meter (Model LI 1800; Licor inc., Lincoln Nebraska). Final above ground dry weights were measured to obtain plant biomass at the end of the experiment under each shade treatment and within each population.

We kept track of the number of live plants in each full-sibling, population, and treatment in order to calculate an LD50 (date at which 50% of the sample had died). Over the course of this experiment, many plants died, so we utilized LD50 as a measure of relative success under each treatment. Data were taken for 10 weeks, and the number of plants left alive weekly from each population (3) under each treatment (3) were calculated. The relative growth of all plants was calculated as the change in canopy area per time during the experimental growth period: (final canopy area - initial canopy area / ten weeks). These calculations could only be done on the few individuals that remained alive at the end of the experiment. Other data were analyzed at weeks 4 and 6 due to rapid plant mortality thereafter.

Two-way ANOVA (SAS, version 2005) were used to test the effects of shade treatment, full-sibling, and shade x full-sibling interactions. Significant ANOVA's were followed by multiple comparison tests (pdiff) to test for significant differences between treatment groups. Data not meeting the assumptions of normality were transformed before analysis. We considered that each individual was an experimental unit and we tested canopy area, leaf area, number of leaves, number of leaves per canopy area for the effects of full-sibling (df = 2), treatment (df = 2), and their interactions (df = 4). Canopy area data were used to analyze the number of leaves per canopy. Overall, there were very few full-sibling effects in each population (Appendix A), so we combined all 3 full-sibling groups into single populations for final analysis. All variables were then re-analyzed at week 6, at the peak of their growth in this experiment (Figure 1), with exception to leaf area, which was re-analyzed at week 4, due to plant mortality.

## 2.30    Results

The mean number of leaves increased throughout the growth period for all populations until week 7 (Figure 1). Each population grew to approximately the same size within treatment groups. There was no significant effect of population on number of leaves. Two-way ANOVA revealed a significant effect (p<.0001) of shade-treatment on number of leaves (Table 2). Multiple comparison tests of the C. *impatiens* populations' response to shade treatment revealed that both the 54% and 76% shade treatments were different from those under no shade cloth (pdiff, p<.0001). *Cardamine impatiens* populations also

responded significantly differently (pdiff, p<.0001) between the 54% shade treatment and the 76% shade treatment groups. As shade intensity increased, number of leaves decreased.

The total number of leaves per canopy area increased or remained the same for plants subjected to 0% and 54% shade intensity and decreased for plants subjected to the 76% shade treatments for all populations (Figure 2). After week 8 of the growth period, the number of leaves per canopy area became more variable for all populations, except those subjected to 76% shade intensity (Figure 2). Two-way ANOVA revealed no significant effect of population on number of leaves per canopy area. There was a significant effect (p<.0001) of shade treatment on number of leaves per canopy area (Table 3). Multiple comparison tests of C. *impatiens* populations' response to shade treatment effects showed that the number of leaves per canopy area was significantly less in the 54% and 76% treatments than under no shade (pdiff, p<.0001). The Bluestone Wildlife populations response to shade treatment effects showed that the number of leaves per canopy area was greatest (pdiff, p<.0001) within the 54% shade treatment and the 76% shade treatment groups. Plants in the 54% treatment group significantly differed (pdiff, p<.0028) from plants with no shade. There was a significant interaction (p<.0268) between shade and population (Table 3). Trends in our multiple comparison analyses of the shade x population interaction indicate that the most significant interactions (pdiff, p<.0005 to p<.0001) occurred between no shade treatment and the 76% shade treatment groups among all populations.

There was no effect of shade treatment, population, or shade treatment x population on C. *impatiens*' leaf area.

There was a significant effect of population (p<.0364) on final dry weight (Table 4). Multiple comparison tests performed on the population effect showed that the Old Wolf population had achieved less mass (pdiff, p<.0124) than the Bluestone Wildlife and Spruce Run populations in response to shade treatments. Although there was a high mortality rate, we found a significant effect (p<.0001) of treatment on final weight (Table 4). Multiple comparison tests performed on the shade treatment effect revealed that all shade treatments were significantly different (pdiff, p<.0001) from each other. Plants had more biomass under the least to greatest shade intensities respectively. There were no significant interactions between shade treatment and population.

The LD50 value we calculated was highest, according to means and standard error, under the 76% light reduction treatment in each population, and the Bluestone Wildlife population lived longest, having the highest LD50 (date at which 50% of all plants died under treatment) value among all populations under the same irradiance treatments. LD50 of full-siblings of the Bluestone wildlife population under no light reduction was $11.5 \pm 0.026$ days, under the 54% light reduction it was $11.5 \pm 0.00$ days, and under the 76% light reduction it was $17 \pm 0.025$ days. LD50 of full siblings of the Old Wolf population under no light reduction was $9.17 \pm 0.42$ days, under the 54% light reduction it was $8.24 \pm 0.027$ days, and under the 76% light reduction treatment it was $12.3 \pm 0.046$ days. LD50 of all full siblings in the Spruce Run population under no light reduction was $7.39 \pm 0.077$ days, under 54% light reduction it was $7.09 \pm 0.077$ days, and under the 76% light reduction it was $13.5 \pm 0.00$ days. No statistical analysis could be done on relative growth of all plants because of a small and uneven data set. For all plants under the cage only treatment, the mean relative canopy area was $4.24 cm^2/$ week, under 54% light reduction treatments it was $3.38$ $cm^2/$ week, and under the 76% light reduction it was $2.03$ $cm^2/$ week (Figure 3).

## 2.40    Discussion

### 2.41    Experiment 1: Cardamine impatiens

Our results indicate that *Cardamine impatiens* can grow under forest shade conditions (76% shade intensity) and has the ability to express phenotypic plasticity under changing environmental conditions. Plants from all populations grew more slowly under shade treatments, but they did continue to grow until the end of the experiment (Figure 1). Among all populations, as shade increased, the plants compensated for lower light conditions by decreasing their number of leaves (Figure 2). Successful plant invaders have morphological and physiological traits that enable them to acquire substantial amounts of resources at low rates of carbon investment (Baruch & Goldstein 1985; Bazzaz 1986; Vitousek & Walker 1989; Stratton & Goldstein 2001). In our experiment, plants lived longest and acclimated even under the greatest shade conditions, so they may have reallocated their resources to the roots when fewer resources were available for photosynthesis. Other studies have included directly functional aspects of plasticity such as proportional allocation to different plant

tissues or assimilation rates (Schlichting 1986; Sultan 1987; Bradshaw & Hardwick 1989; Sultan 2000).

The type of plasticity an organism exhibits in response to reduced light can vary widely among species. Pattison *et al*. (1998) reported that invasive species in Hawaii adjust leaf area ratios and photosynthetic rates across a wider range of light environments than native species. Instead of adjusting leaf area ratios, *C. impatiens* reduces its number of leaves in response to increased shade. In herbaceous plants, shading can alter the plant's architecture as a result of effects on meristem initiation and fate as well as organ size and structure (Huber *et al*, 1999; Sultan 2000).

Acclimation to novel environments can cause a reduction in biomass. Phenotypic adjustments can partly compensate functionally for the inevitable reductions in total plant growth and biomass that occur under resource limitation (Sultan 2000). Environmental variation selects for plasticity by altering the relative benefits and costs of expressing an induced phenotype (Huber *et al*. 2004). The expression of a plastic trait may be associated with performance reductions due to correlated effects of plastic responses on other traits (DeWitt 1998; DeWitt *et al*, 1998; Huber *et al*. 2004).

Our LD50 data indicated that the Bluestone Wildlife population is better able to survive under shade conditions when compared to the other populations (Figure 3). Therefore, this population may be responding to resource variation due to decreased light environments more quickly than the other populations. Growth of all plants under treatment provided evidence that *C. impatiens* grew best when not subjected to shade treatments (Figure 4). Phenotypic adjustments can partly compensate functionally for the inevitable reductions in total plant growth and biomass that occur under resource limitation (Sultan 2000).

We accepted our hypothesis that *C. impatiens* would grow and exhibit different phenotypes in different shade treatments, and those different populations would respond differently to shade treatments. As we expected, the number of leaves per canopy area decreased as shade levels increased, indicating *C. impatiens* was exhibiting a phenotypic response. We also have evidence of a decrease in growth and an increase in survivorship under higher shade intensity (54 & 76%). Our evidence that different populations responded differently to shade was derived from our LD50 data, a significant interaction between shade

treatment x population in the number of leaves per canopy area (Table 2), and a significant effect of population on final biomass (Table 3). Differences between full-siblings from each population could have to do with the "founder effect". Although all plants likely came from a very small founder population, and have limits on their adaptability, recent studies have shown that some introduced species can still show an evolutionary adaptive response to a new environment (Reznick *et al*. 1997; Losos *et al*. 1997; Saccheri *et al*., 1998; Hendry *et al*. 2000; Huey *et al*. 2000; Radwin 2003; Reed and Frankham 2003).

The role of phenotypic plasticity in plant invasions has been explored by Richards *et al.* (2006). They suggest that successful invaders may benefit from plasticity by being better able to maintain fitness in unfavorable environments, increase fitness in favorable environments, or some level of both abilities. The results of our light reduction experiment confirms that forests, which were once assumed to be immune to the threat of invasive species, may be more at risk than previously thought (Webb *et al*. 2000).

**Chapter 3.00**

**3.00 Experiment 2:** *Cardamine impatiens* **treated with increased shade and cold treatment**

*3.10 Introduction*

In our first experiment, we found evidence for phenotypic plasticity and growth under high shade treatment. However, near the end of the experiment many plants died. We hypothesized that the high mortality rate was due to improper cold pre-treatment before the experiment started. Therefore, we repeated the experiment and included a simulated cold period for all seedlings before planting in the greenhouse. Moreover, we anticipated that the cold pre-treatment would induce flowering in many plants, which would allow a reproductive assay of fitness among shade treatments. We expected that a cold pre-treatment (a simulation of winter conditions) would decrease mortality during the experiment; based on the evidence that *C. impatiens* is a biennial in North America (Glenn & Berringer 2004). Also, we wanted to probe the extent of shade acclimation by *C. impatiens*. Therefore we utilized treatments of 30%, 73%, and 103% neutral density shade cloth.

We hypothesized that *C. impatiens* would grow and exhibit different phenotypes in different shade treatments. We considered that if the number of leaves per canopy area decreased as shade levels increased, *C. impatiens* was exhibiting a phenotypic response. Also, we predicted a decrease in growth and survivorship as shade treatment increased, and that different populations would respond differently to shade, due to genetic differences among populations.

*3.20 Materials and Methods*

*3.21 Plant material, growth protocol, and statistical procedure*

A new full-sibling collection was made from the Old Wolf population in May 2005 in order to obtain the same number of replicates as in the first experiment. Seeds from the Bluestone wildlife and Spruce Run populations were used from an existing, stored collection. *Cardamine impatiens* seeds were germinated in the same manner as in Experiment 1, and all seeds achieved successful germination and growth with the application of 500ppm gibberellic acid. After a 1 week acclimation in a fiberglass greenhouse at 23°C, transplantation to soil

and apparent establishment, seedlings were enclosed in a $4°C$ cold chamber under 12-hour light banks for 4 weeks. Following the cold acclimation, seedlings were transplanted into 1.5 gallon pots and moved to a glasshouse at $23°$ C.

Shade cages were constructed from metal poultry netting as in experiment 1. One third of the cages were covered by a 30, 73, or 103% neutral density shade cloth. The 103% shade treatment was achieved by wrapping cages with both 30% and 73% shade cloth. The actual irradiance environment experienced by the plants was different than the manufacturer's light reduction values for the shade cloth. Average maximum radiations in treatments were 85.42, 45.77, & 17.42 umol $m^{-2}s^{-1}$ respectively for the 30, 73, and 103% neutral density shade cloth. The treatments were redefined based on the direct measurements of PPFD to 54, 76, and 91% shade treatments respectively for the 30, 73, and 103% shade cloth treatments.

Each cage measured 1 foot in diameter and 1 meter in height. Five plants of each full-sibling were subjected to 54, 76, or 91% shade intensity (n = 5 / full-sib / shade treatment). Plants were then acclimated for just 2 weeks, because we thought that by starting measurements earlier we could have a longer experiment before mortality.

Canopy area, number of leaves, and leaf area measurements were then recorded weekly over a 9-week period, from October to December 2005. The area of 1 leaf was also recorded by measuring lengths and widths (area = length x width) of one basal leaf per plant. Plants were harvested on December 12th, and placed in a drying oven at $77°$ C for 2 months. Final dry weights were measured to obtain final biomass data.

In some cases, data were transformed to meet the assumptions of normality. All data were analyzed by SAS two-way ANOVA (SAS, version 2005) to test the effects of shade treatment, population, and shade treatment x population interactions. Significant ANOVAs were followed by multi-comparison tests (pdiff) to examine differences between treatment groups. Data were analyzed at weeks 4 and 6 as in our previous experiment. We considered that each individual was an experimental unit and we tested canopy area, leaf area, number of leaves, and number of leaves per canopy area for the effects of full-sibling (df = 2), treatment (df = 2), and their interactions (df = 4). There were very few significant differences (Appendix B) among full-siblings, so we were able to lump full-siblings into populations. To more accurately simulate our first experiment, week 6 data were used in the final analysis.

### 3.30 Results

The mean number of leaves generally increased throughout the growth period for all populations in all treatments, except the 91% shade intensity treatment (Figure 4). There was no significant effect of population on number of leaves. There was a significant effect of shade treatment ($p<.0001$) on number of leaves (Table 5). Multiple comparison tests indicated that when compared to the populations under the 54% shade treatment, the 76% shade treatment group (pdiff, $p<.0001$) and the 91% shade treatment group (pdiff, $p<.0005$) grew fewer leaves as shade intensity increased.

The total number of leaves per $cm^2$ of canopy area was variable, and declined the most under 76% to 91% shade intensity treatments respectively (Figure 5). There was no significant effect of population on number of leaves per canopy area. There was a statistically significant ($p<.0001$) response to shade treatment on number of leaves per canopy area (Table 6). Multiple comparison tests revealed a significant difference between shade treatments. Plants in populations under the 54% shade treatment were larger (pdiff, $p<.0001$) than those in the 91% shade treatment. Populations in the 76% shade treatment were intermediate in size (pdiff, $p<.0001$) when compared to the populations in the 54% shade treatment and in the 91% shade treatment. There was no interaction between shade treatment and population.

There was no effect of population, shade treatment, or population x shade treatment interaction on *C. impatiens*' leaf area.

There was no significant effect of population on final plant weight; however, shade was significant ($p<.0001$) (Table 7). According to multiple comparison tests, all shade treatments were significantly different (pdiff, $p<.0001$) from each other and final plant weight decreased as shade intensity increased. We found a significant interaction ($p<.0132$) between shade treatment and population on final dry plant weight (Table 7). Trends in our multiple comparison analyses of the shade x population interaction indicate that the largest differences among populations (pdiff, $p<.0001$) occurred under the 54% shade treatment group.

Of the 145 plants in our experiment, a total of 7 flowered and all of these occurrences were under the 54% shade treatment. After 9 weeks, only 2 plants from the Bluestone Wildlife population had flowered. Living plants from all populations were allowed to remain in the greenhouse under treatment for an additional 8 weeks by which time 1 more plant

(total of 3) from the Bluestone Wildlife population, 2 plants from the Old Wolf population, and 2 plants from the Spruce Run population flowered.

## 3.40 DISCUSSION

### 3.41 Experiment 2: Cardamine impatiens treated with increased shade and cold treatment.

Our reason for doing a second experiment using a higher percentage of shade treatment were to determine the limit of photosynthetically active radiation (PAR) reduction that *Cardamine impatiens* could withstand without significant mortality. Plants continued to grow and exhibit plasticity throughout the experiment even under the 91% shade treatment (Figures 5, 6). Although none of the plants in the highest shade treatments (76% & 91%) flowered in our experiment, they did continue to grow and experience less mortality overall as compared to our first experiment with no cold treatment (Figures 1, 2, 5, 6). There could be several explanations why *C. impatiens* failed to flower after the cold treatments. Perhaps *C. impatiens* requires a longer amount of time than the time period allowed for our experiment in order to flower. Further, the shade treatments of 76% and 91% may have been greater than what *C. impatiens* from the populations we chose had ever received in their natural environments. If temporal fluctuations in selection pressures are too fast, a population will deviate substantially from and lag behind the optimal phenotype (Lande & Shannon 1996; Tufto 2000). Although only 7 plants flowered in our experiment (all under the 54% shade treatment), *C. impatiens* produces so many seeds that even if 1 plant flowered in a reduced light environment, that may be enough to start a new population, maintain or extend the range of an existing population. Individual plants of *C. impatiens* make large numbers of seed and seeds of *C. impatiens* were found to have a greater seed dispersal distance than other species in its genus, because of its tall infructescences (Kimata 1983).

*Cardamine impatiens* requires a cold treatment for optimal growth and reproduction. Overall, plants lived longer in this experiment than in our previous one (Figures 1, 4), which we attribute to *C. impatiens* being a biennial in the United States (Glenn & Berringer, 2004). Seven plants in this experiment utilized the resources necessary for them to flower under the shade treatments, providing evidence of *C. impatiens* being able to reallocate resources in times of stress. Our results indicate that *C. impatiens* is best suited to flower when under

moderate shade (54% light reduction), as opposed to extensive shading (73% and 91% light reduction). However, these plants may reserve their resources in times of reduced PAR. Other studies have provided evidence of proportional allocation to different plant tissues or assimilation rates under changing environments (Schlichting 1986; Sultan 1987; Bradshaw & Hardwick 1989; Sultan 2000).

Our results demonstrate plasticity and shade tolerance, because *C. impatiens* maintained its canopy area by decreasing the amount of leaves produced under increased shade intensities (Figure 5). Griffith and Sultan (2005) found that *Polygonum persicaria* exhibited very high leaf allocation and leaf area ratio in response to reduced PAR. Although the leaf area of *C. impatiens* was not affected by shade treatment, a declining number of leaves per canopy area provides evidence of plasticity. Plastic responses seem to be expected to have an adaptive value, but trait plasticities are likely to exhibit the same range of relationships with fitness as other traits: some will be neutral, some passive or detrimental, and others adaptive (van Kleunen & Fischer 2005; Nicotra *et al.* 2007).

The overall aboveground biomass of plants under each shade condition may yield even more evidence that *C. impatiens* can adapt to shade through phenotypic changes. The significant difference between final biomass of plants exposed to different shade treatments indicates that a phenotypic change in response to different habitats has occurred.

We accepted our hypothesis that *C. impatiens* would grow and exhibit different phenotypes in different shade treatments. Similar to experiment 1, the number of leaves per area in $cm^2$ decreased as shade levels increased, indicating *C. impatiens* was exhibiting a phenotypic response (Figure 5). We also have evidence of a decrease in growth and survivorship as shade treatment increased, and that different populations responded differently to each shade treatment (Figure 4).

Phenotypic plasticity may be a good indicator of which plants can become invasive. A fundamental hypothesis about the evolution of phenotypic plasticity is that it provides a mechanism for adaptation to spatially or temporally variable environments (Dudley & Schmitt 1996). Plasticity levels are important when studying invasive organisms, because a high level of plasticity may mean that a plant can acclimate easier and faster to changing environments than those species with a low level of plasticity (Baker & Stebbins 1965; Bazzaz 1986; Orians 1986).

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**Chapter 4.00**

**4.00    Conclusions**

Mack (1996) asserts that deliberately sowing an alien beyond its current range is a powerful tool for prediction.  We considered that growing *C. impatiens* in a greenhouse, under variable shade and temperature regimes, was consistent with sowing it beyond its normal habitat or range. There are many aspects to consider when examining phenotypic plasticity. One question is how the expression of plant plasticity varies and feeds back during the life cycle, as plastic response may depend strongly on the timing of its expression (Weinig & Delph 2001; Sultan 2004). We need to fully understand plasticity patterns and their evolution by knowing much more about biochemical and genetic components and how they vary at the individual, population, and species levels (Sultan 2004). We also need to consider the 'costs' of plasticity and whether or not they more likely pertain to the construction of particular phenotypes, or limit  their adaptive effectiveness in certain environments (DeWitt et al. 1998; Sultan & Spencer 2002; Sultan 2004).

Plant plasticity to shaded versus open conditions provides an excellent system in which to examine perception of, and plastic response to, specific environmental cues (Griffith and Sultan 2005).  Phenotypic manipulation that results in the expression of the wrong phenotype in each environment offers a method for testing the hypothesis that phenotypic plasticity is adaptive (Dudley & Schmitt 1996).We feel that our experiment has provided enough evidence to accept our hypothesis that *Cardamine impatiens* would be phenotypically plastic, and hence has potential to be a successful invader in a diversity of different irradiance environments, such as open sites or forests. However, herbarium data suggests that *C. impatiens* is most prevalent in shady environments.

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## 5.00    Annexes

### 5.10    Tables

**Table 1.**    Herbarium Data collected from ~200 responding herbaria, of 350 requests, across the Northeastern US.

| State | # Counties | # Specimens |
|---|---|---|
| Pennsylvania | 21 | 49 |
| West Virginia | 14 | 36 |
| New York | 8 | 14 |
| New Jersey | 6 | 16 |
| Connecticut | 5 | 14 |
| New Hampshire | 2 | 10 |
| Virginia | 2 | 5 |
| Michigan | 2 | 4 |
| Massachusetts | 2 | 4 |
| Vermont | 2 | 2 |
| North Carolina | 1 | 2 |
| Kentucky | 1 | 1 |
| Maine | 1 | 1 |
| Tennessee | 1 | 1 |

**Table 2.**    Experiment 1. Partial ANOVA table for the effect of shade treatment on the number of leaves of *Cardamine impatiens* growing in a greenhouse, p-values are starred at level of significance.

**** = $p \leq 0.0001$; *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$

| Source | df | F-value | p-value |
|---|---|---|---|
| Treatment | 2 | 41.95 | < 0.0001**** |
| Population | 2 | 1.91 | 0.1524 |
| Trt * Population | 4 | 1.87 | 0.1207 |

**Table 3.** **Experiment 1: Partial ANOVA table for the effect of shade treatment and shade x population interaction on the number of leaves per canopy area of *Cardamine impatiens* growing in a greenhouse, p-values are starred at level of significance. Data were natural log transformed to achieve normality.**

**\*\*\*\* = p ≤ 0.0001; \*\*\* = p ≤ 0.001, \*\* = p ≤ 0.01, \* = p ≤ 0.05**

| Source | df | F-value | p-value |
|---|---|---|---|
| Treatment | 2 | 96.68 | < 0.0001**** |
| Population | 2 | 0.50 | 0.6072 |
| Trt * Population | 4 | 2.85 | 0.0268** |

**Table 4.** **Experiment 1. Partial ANOVA for the effect of shade treatment and population on dry biomass of *Cardmine impatiens* growing in a greenhouse, p-values are starred at level of significance.**

\*\*\*\* = p ≤ 0.0001; \*\*\* = p ≤ 0.001, \*\* = p ≤ 0.01, \* = p ≤ 0.05

| Source | df | F-value | p-value |
|---|---|---|---|
| Treatment | 2 | 38.69 | <0.0001**** |
| Population | 2 | 3.40 | 0.0364** |
| Trt * Population | 4 | 1.00 | 0.4117 |

**Table 5.** **Experiment 2. Partial ANOVA for the effect of shade treatment on number of leaves on *Cardmine impatiens* growing in a greenhouse, p-values are starred at level of significance. Data were natural log transformed to meet normality.**

\*\*\*\* = p ≤ 0.0001; \*\*\* = p ≤ 0.001, \*\* = p ≤ 0.01, \* = p ≤ 0.05

| Source | df | F-value | p-value |
|---|---|---|---|
| Treatment | 2 | 50.04 | <0.0001**** |
| Population | 2 | 1.20 | 0.3064 |
| Trt * Population | 4 | 1.26 | 0.2916 |

**Table 6.**       **Experiment 2. Partial ANOVA for the effect of shade treatment on number of leaves per canopy area of *Cardmine impatiens* growing in a greenhouse, p-values are starred at level of significance.  Data were natural log transformed to meet normality.**

$**** = p \leq 0.0001$; $*** = p \leq 0.001$, $** = p \leq 0.01$, $* = p \leq 0.05$

| Source | df | F-value | p-value |
|---|---|---|---|
| Treatment | 2 | 20.35 | <0.0001**** |
| Population | 2 | 2.60 | 0.0805 |
| Trt * Population | 4 | 0.58 | 0.6768 |

**Table 7.**       **Experiment 2. Partial ANOVA for the effect of shade treatment and shade treatment x population of dry biomass of *Cardmine impatiens* growing in a greenhouse, p-values are starred at level of significance.  Data were log transformed to meet normality.**

$**** = p \leq 0.0001$; $*** = p \leq 0.001$, $** = p \leq 0.01$, $* = p \leq 0.05$

| Source | df | F-value | p-value |
|---|---|---|---|
| Treatment | 2 | 108.64 | <0.0001**** |
| Population | 2 | 0.47 | 0.6287 |
| Trt * Population | 4 | 3.30 | 0.0132** |

## *5.20 FIGURE LEGENDS*

**Figure 1.    Experiment 1. The mean number of leaves and standard error for three populations of *Cardamine impatiens* plants growing in a greenhouse. A: Plants growing under a cage only, B: Plants experiencing 54% shade treatment, C: Plants experiencing 76% shade treatment.**

**Figure 2.** **Experiment 1. The mean number of leaves per canopy area and standard error for three populations of *Cardamine impatiens* plants growing in a greenhouse. A: Plants growing under a cage only, B: Plants experiencing 54% shade treatment, C: Plants experiencing 76% shade treatment.**

**Figure 3.**     **Experiment 1. The mean relative canopy area for three populations of** *Cardamine impatiens* **plants growing in a greenhouse and experiencing three different shade treatments**
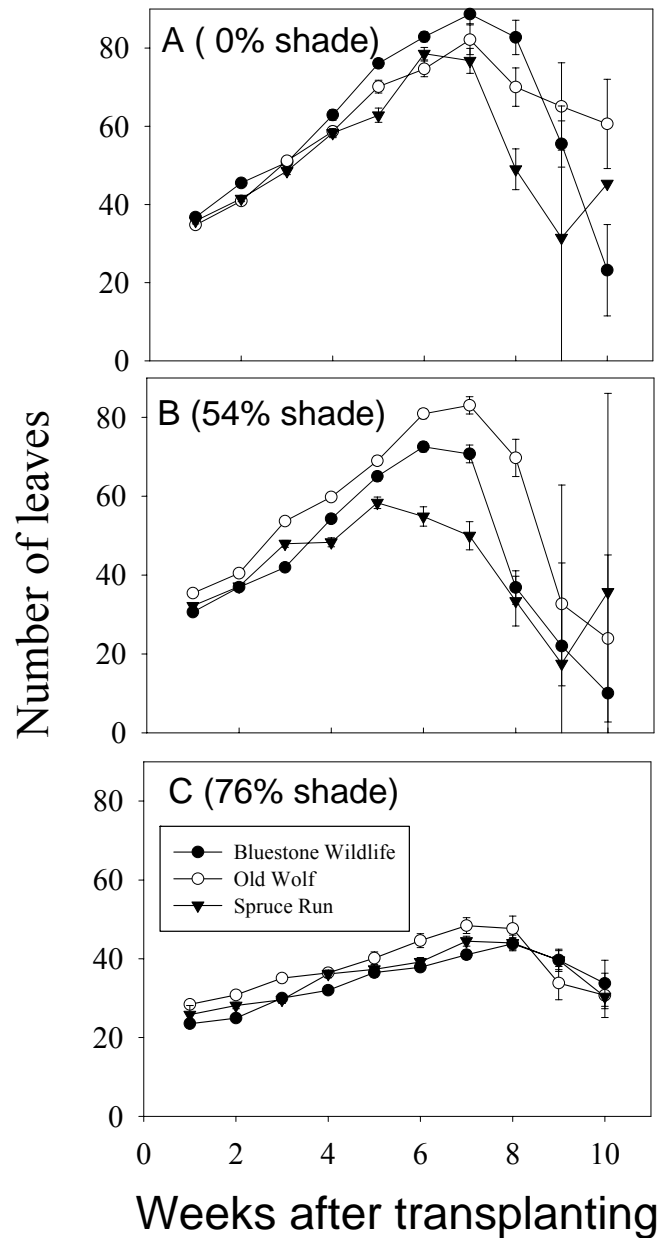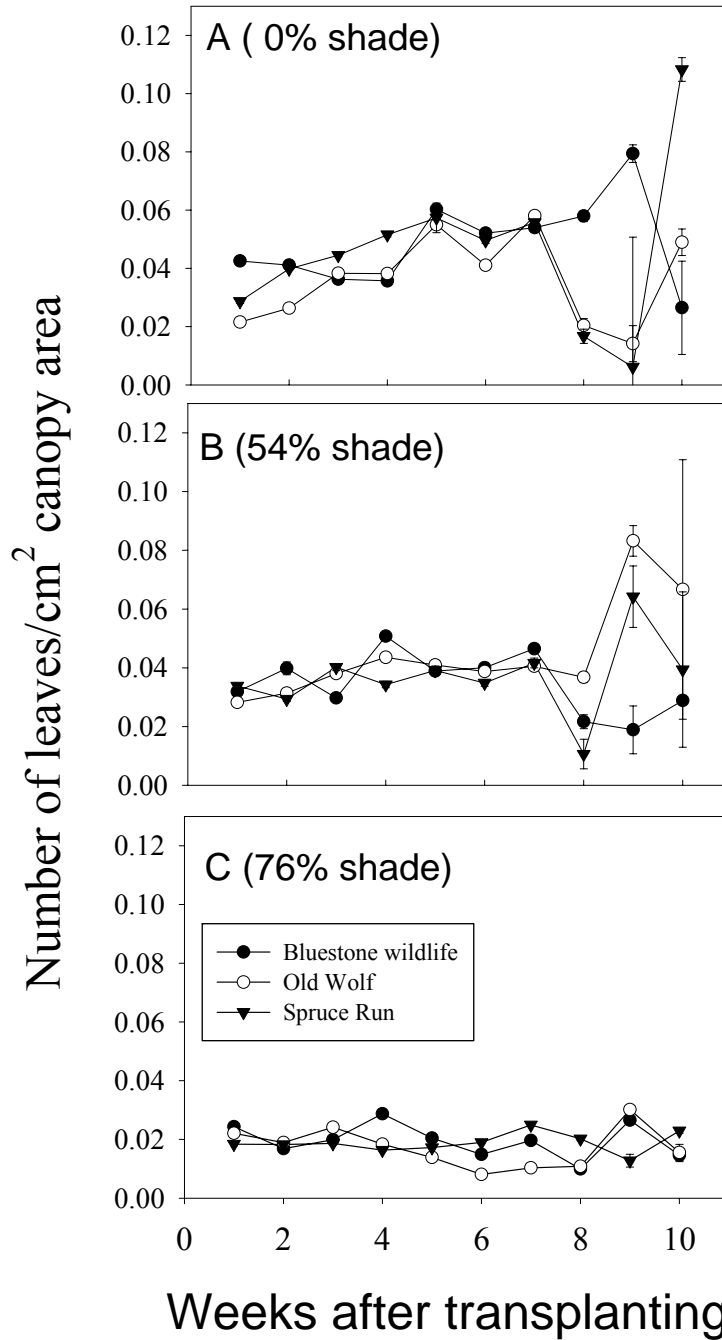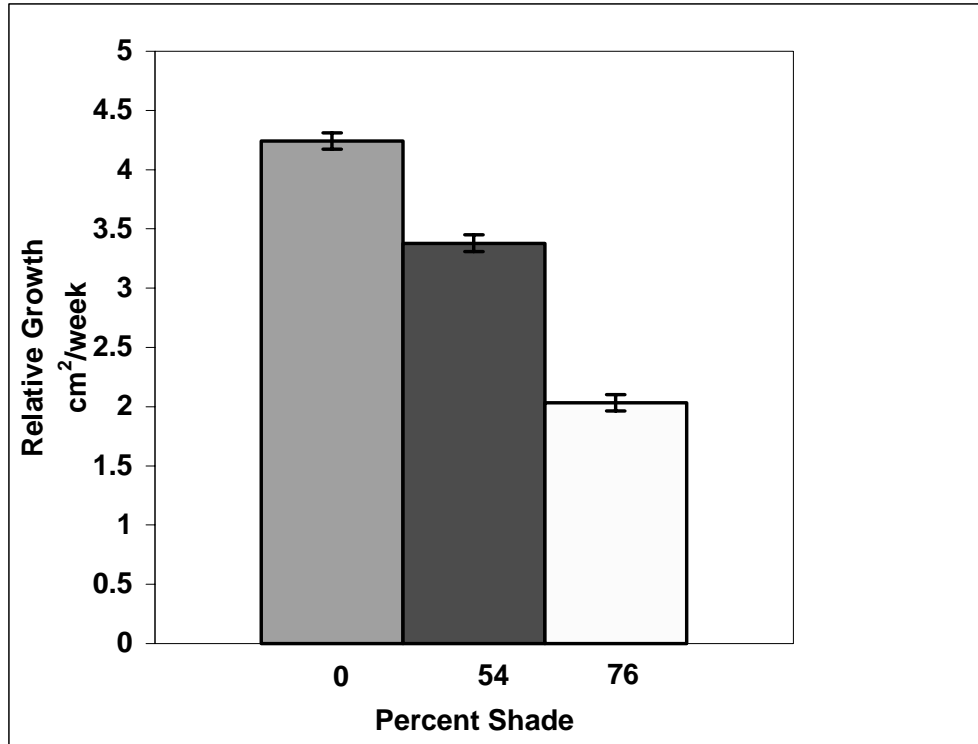
**Figure 4.** Experiment 2. The mean number of leaves and standard error for three populations of *Cardamine impatiens* plants growing in a greenhouse. A: Plants experiencing 54% shade treatment, B: Plants experiencing 76% shade treatment, C: Plants experiencing 91% shade treatment.

**Figure 5.** Experiment 2. The mean number of leaves per canopy area and standard error for three populations of *Cardamine impatiens* plants growing in a greenhouse. A: Plants experiencing 54% shade treatment, B: Plants experiencing 76% shade treatment, C: Plants experiencing 91% shade treatment.

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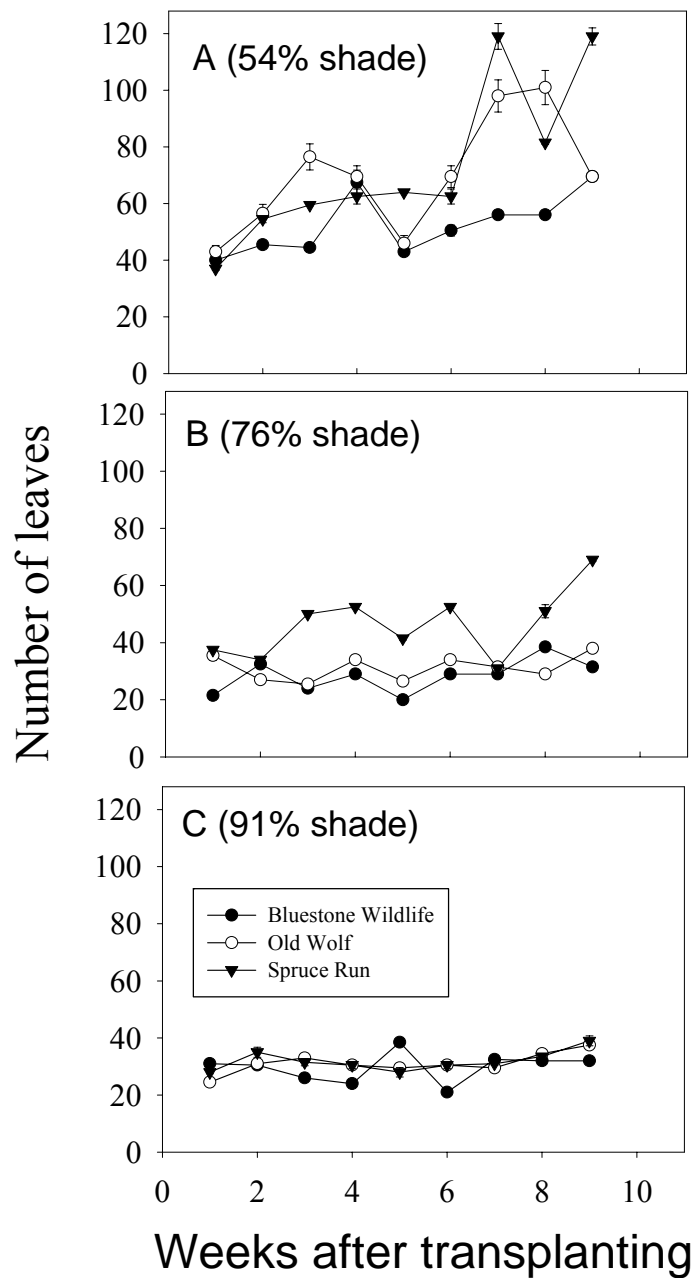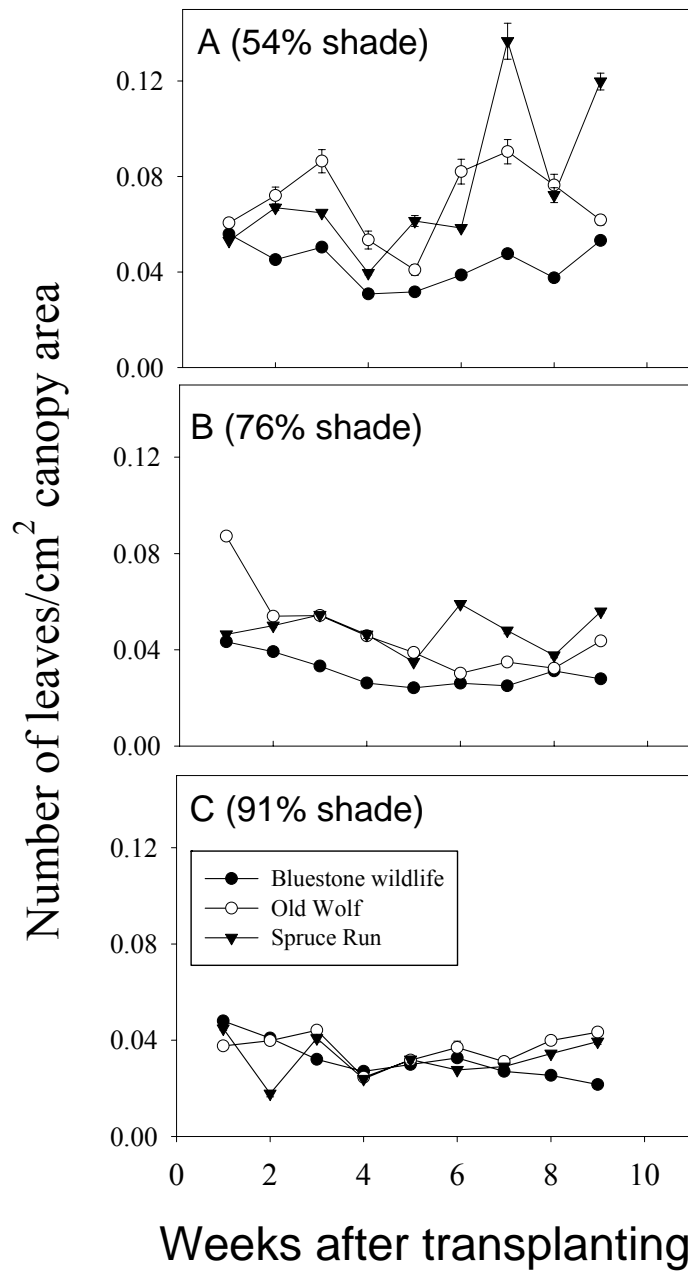**Appendix A.  Preliminary ANOVA results from experiment one**

Experiment 1. Treatments were no shade, 54%, or 76% shade and α were set at p < .05. Populations were defined as the Bluestone Wildlife (BW) population, the Old Wolf (OW) population, and the Spruce Run (SR) population. Preliminary two-way ANOVA results of shade treatment (trt), full sibling (sib), and shade treatment x full sibling (trt x sib) interactions.

| BW sibs | | Week 4 | Week 6 | OW sibs | | Week 4 | Week 6 | SR sibs | | Week 4 | Week 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| **Canopy area** | trt | 0.0015 | <.0001 | **Canopy area** | trt | 0.0979 | 0.0011 | **Canopy area** | trt | 0.0005 | 0.0007 |
| | sib | 0.4571 | 0.0608 | | sib | 0.0495 | 0.0032 | | sib | 0.6880 | 0.9813 |
| | trt*sib | 0.3846 | 0.0476 | | trt*sib | 0.8846 | 0.2250 | | trt*sib | 0.4162 | 0.5529 |
| | | | | | | | | | | | |
| **Number leaves** | trt | <.0001 | <.0001 | **Number leaves** | trt | 0.0002 | 0.0024 | **Number leaves** | trt | 0.0033 | <.0001 |
| | sib | 0.0041 | 0.5106 | | sib | 0.0671 | 0.5552 | | sib | 0.6072 | 0.2071 |
| | trt*sib | 0.1053 | 0.0542 | | trt*sib | 0.5281 | 0.7146 | | trt*sib | 0.4373 | 0.0911 |
| | | | | | | | | | | | |
| **Leaves per area** | trt | <.0001 | <.0001 | **Leaves per area** | trt | 0.0005 | 0.0002 | **Leaves per area** | trt | <.0001 | <.0001 |
| | sib | 0.5154 | 0.2406 | | sib | 0.8307 | 0.2039 | | sib | 0.6515 | 0.5434 |
| | trt*sib | 0.2472 | 0.2256 | | trt*sib | 0.5960 | 0.9450 | | trt*sib | 0.6432 | 0.5405 |
| | | | | | | | | | | | |
| **Leaf area** | trt | 0.4089 | | **Leaf area** | trt | 0.9887 | | **Leaf area** | trt | 0.8410 | |
| | sib | 0.0345 | | | sib | 0.0240 | | | sib | 0.0040 | |
| | trt*sib | 0.1028 | | | trt*sib | 0.5301 | | | trt*sib | 0.7934 | |
| | | | | | | | | | | | |
| **Final weight** | trt | | <.0001 | **Final weight** | trt | | <.0001 | **Final weight** | trt | | 0.0036 |
| | sib | | 0.0270 | | sib | | 0.3775 | | sib | | 0.4479 |
| | trt*sib | | 0.0335 | | trt*sib | | 0.9121 | | trt*sib | | 0.2629 |

**Appendix B.   Preliminary ANOVA results from experiment two**

Experiment 2. Treatments were 54%, 76%, or 91% shade and α were set at p < .05. Populations were defined as the Bluestone Wildlife (BW) population, the Old Wolf (OW) population, and the Spruce Run (SR) population. Preliminary two-way ANOVA results of shade treatment (trt), full sibling (sib), and shade treatment x full sibling (trt x sib) interactions.

| BW sibs | | Week 4 | Week 6 | OW sibs | | Week 4 | Week 6 | SR sibs | | Week 4 | Week 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Canopy area** | trt | 0.0397 | 0.0572 | **Canopy area** | trt | 0.0117 | 0.5181 | **Canopy area** | trt | 0.7974 | 0.6175 |
| | sib | 0.9643 | 0.3493 | | sib | 0.5586 | 0.4966 | | sib | 0.6863 | 0.5788 |
| | trt*sib | 0.5661 | 0.3066 | | trt*sib | 0.4347 | 0.8397 | | trt*sib | 0.4448 | 0.2201 |
| | | | | | | | | | | | |
| **Number leaves** | trt | <.0001 | <.0001 | **Number leaves** | trt | 0.0002 | 0.0023 | **Number leaves** | trt | <.0001 | <.0001 |
| | sib | 0.6632 | 0.7823 | | sib | 0.5782 | 0.4984 | | sib | 0.6856 | 0.1051 |
| | trt*sib | 0.9670 | 0.8804 | | trt*sib | 0.9273 | 0.9275 | | trt*sib | 0.3061 | 0.5284 |
| | | | | | | | | | | | |
| **Leaves per area** | trt | 0.0040 | 0.0142 | **Leaves per area** | trt | 0.0598 | 0.0161 | **Leaves per area** | trt | <.0001 | 0.0033 |
| | sib | 0.3476 | 0.9517 | | sib | 0.9787 | 0.2543 | | sib | 0.8097 | 0.2937 |
| | trt*sib | 0.9454 | 0.2685 | | trt*sib | 0.8190 | 0.9546 | | trt*sib | 0.3342 | 0.9385 |
| | | | | | | | | | | | |
| **Leaf area** | trt | 0.0079 | 0.0360 | **Leaf area** | trt | 0.0350 | 0.3905 | **Leaf area** | trt | 0.0082 | 0.3884 |
| | sib | 0.3672 | 0.0160 | | sib | 0.8368 | 0.4786 | | sib | 0.0144 | 0.5792 |
| | trt*sib | 0.9910 | 0.4104 | | trt*sib | 0.7139 | 0.7948 | | trt*sib | 0.1043 | 0.6134 |
| | | | | | | | | | | | |
| **Final weight** | trt | | <.0001 | **Final weight** | trt | | <.0001 | **Final weight** | trt | | <.0001 |
| | sib | | 0.9069 | | sib | | 0.2089 | | sib | | 0.7560 |
| | trt*sib | | 0.5764 | | trt*sib | | 0.2638 | | trt*sib | | 0.1340 |

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**Curriculum Vitae**

KERRI MILLS HUFFMAN

2/14/08

Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061,
email:  kehuffma@vt.edu

Education

Virginia Tech (VPI), Blacksburg VA, 2003-present. Master of Science, Biology.
Advisor: Erik Tallak Nilsen

Virginia Tech (VPI), Blacksburg, VA, 2000-2003. Bachelor of Science, Biology, Advisor
George Simmons

Virginia Western Community College, Roanoke VA, 1998-2000. Associate of Science,
Science, *summa cum laude. A*dvisors:  Lou Bass and Rich Crites

**Employment**

Research Specialist Senior, Virginia Tech (VPI), Blacksburg, VA, September 2007 – present.
Strawberry genomics project, molecular biology, P.I. Richard Veilleux.

Laboratory Assistant ClearWater Testing, LLC., Christiansburg, VA, February – September
2007. Tested drinking water for *e. coli* and total coliform.

Research Assistant, Savannah River Site, SC, January 2007. Plant Ecology, P.I. Dr. Bob Jones.

Graduate Teacher, Virginia Tech (VPI), Blacksburg, VA, Fall 2003 - Fall 2006.Biological
Principles Lab, Fall semesters 2003, 2004, 2005; Plant Taxonomy Lab, Spring
semesters 2004, 2006; Plant Ecology Lab Spring semesters 2005.

Field Assistant, Virginia Tech (VPI), Blacksburg, VA, July-August 2003. Insect plant
        Interactions, P.I. Dr. Lynn Adler.

Laboratory Assistant, Virginia Tech (VPI), Blacksburg, VA, May-July 2003. Aquatic Insect
        identification, Mary Schaeffer.

Laboratory Assistant, Virginia Tech (VPI), Blacksburg, VA, September 2001-May 2003.
        Molecular biology, P.I. Dr. Jim Westwood.

Undergraduate Teacher, Virginia Tech (VPI), Blacksburg, VA, Fall semester 2002.Biology
        Freshman Seminar.

Research Assistant: UVA Biological Station, Mountain Lake, VA, Summer 2002. Plant
        Taxonomy, P.I. Dr. Betsey Von Holle.


**Grants and Scholarships**

2006   Virginia Native Plant Society, Research Grant 2004.  $500.00

2004   Virginia Native Plant Society, Research Grant 2004.  $500.00

2003   Multicultural Academic Opportunities Program. Tuition Scholarship

2003   Full GTA position in Biology Department.  2003

2002   Deborah A. Koller Scholarship. $1500.00

1999   Virginia Native Plant Society Scholarship, 1999.  $250.00

**Professional Service**

Spring 2005.  Presented a poster at the Biological Sciences Annual Research day, Virginia Polytechnic Institute, Blacksburg, VA; Title: Can The Exotic *Cardamine impatiens* Adapt To Become An Invasive Species?

Spring 2005. Guest lectured for Dr. Art Buikema's General Biology course and Erik Nilsen's Plant Ecology course.

Spring 2005. Invited and hosted Virginia Tech EEB seminar speaker, Dr. Betsey Von Holle.

Fall 2005.Seminar given to the Virginia Native Plant Society, Regional meeting, Roanoke VA;  Title: *Cardamine impatiens* – potential invasive species.

Summer 2005. Guest lectured to two classes (approximately 20 students/class) of high school students, enrolled in an Upward Bound course for one week. Developed coursework and lectured to 6 classes.

Fall 2004.   Seminar given to the Virginia Native Plant Society, Regional meeting, Roanoke, VA; Title: A Naturalist's View of Research Conducted at Mountain Lake Biological Station.

**University Service**

Summer 2005. Organized volunteer work schedules of approximately 15 people in order to provide assistance for the Association for Biology Laboratory Education (ABLE) Conference held at VT. Provided refreshments, transportation, and laboratory assistance for three days, utilizing undergraduate and graduate students.

Fall 2003-2005 Actively participated in multicultural meetings as a scholar of the VT Multicultural Academic Opportunities Program, and VT Minorities in Agriculture, Natural Resources, and Related Sciences.

**Departmental Service**

Spring 2005.Served on the Biological Sciences Research Day Committee, assisted in organizing the day's events, speakers, poster presenters, and evening banquet. Organized volunteer work schedules of approximately 15 people in order to provide refreshments, transportation, and logistical support for the event.

Spring 2005, 2006. Biology Graduate Student Association (BGSA) President for the Department of Biological Sciences, Virginia Tech.

Summer 2005. Planned logistics for and organized volunteers for our Annual BGSA Biological Sciences departmental picnic.

Spring 2004. BGSA Vice President for the Department of Biological Sciences, Virginia Tech. Spring 2004. Assisted with Biology Department Undergraduate Transfer Student Orientation, Undergraduate, and Graduate Student Orientation.

**Community Service**

Spring 2004. Community Outreach Program: Guest Speaker to 7th graders at Auburn Middle School about the importance of education, March 2004.

Fall 2004. Riparian Zone Restoration: Planted tree seedlings along Tom's Creek as part of restoration project.

**Vitae**

Kerri Mills Huffman grew up in Franklin County, VA. She attended Virginia Western Community College, in Roanoke, VA, where she completed an Associate of Science degree in Science, graduating *Summa cum laude*. She then transferred to Virginia Tech where she obtained a Bachelor's degree in Biology. During her time at Virginia Tech she worked in a plant pathology, physiology, and weed science lab, Virginia Tech, where she learned the valuable skills necessary to perform molecular biology. However, she was also interested in the field of Ecology. So, she went back to the Virginia Tech Biological Sciences department and sought a Master's degree in the field of plant ecology

Kerri's currently works in a Virginia Tech laboratory in the Department of Horticulture, under title of "Research Specialist Senior" on a strawberry genomic project. Her specialty is molecular biology. She lives with a friend just outside the town of Blacksburg, in Montgomery County, VA, and she is happy with her life and her work.