Growth and allocation in seedlings of three shade intolerant species: the role of biotic and abiotic factors

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Growth and allocation in seedlings of three shade intolerant species: the role of biotic and abiotic factors

by

Nilsen Leonardo Lasso-Rivas

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Major: Ecology and Evolutionary Biology

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Iowa State University
Ames, Iowa
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ABSTRACT

Competitive interactions are considered to be a central force maintaining local diversity and controlling the structure of plant communities. The results of several investigations indicate that the outcome of plant competition can be influenced by biotic and abiotic factors. However, those studies usually evaluate single factors. Since biotic and abiotic factors affect plant competition at the same time, it is important to understand the nature of their inter-relationships. In this dissertation I assessed the effects of ultraviolet (UV) radiation and mycorrhizal colonization on plant competition. The work presented in chapter two evaluates how arbuscular mycorrhizal (AM) symbiosis and UV radiation affect vegetative growth, morphology and allocation in seedlings of *Populus deltoides* (eastern cottonwood), *Salix nigra* (black willow), and *Betula nigra* (river birch), three shade intolerant tree species. The results showed that UV radiation induced changes in leaf morphology and biomass allocation and that AM colonization resulted in the reduction of plant biomass. This work demonstrated that ambient levels of UV radiation can reduce the rate of AM colonization and suggested that, in some species, AM colonization may help to overcome the detrimental effects of UV radiation stress. Chapter three evaluates the effects of competition and UV radiation on early vegetative growth in seedlings of *P. deltoides*. The results showed that UV radiation affected growth and morphology of *P. deltoides* seedlings when seedlings were grown alone, but not when seedlings were grown in competition. Chapter four builds on the findings from chapters two and three to evaluate the individual effects and
interactions of UV radiation and mycorrhizal symbiosis on competition between seedlings. The results found in chapter four showed that UV radiation induced changes in leaf morphology and biomass allocation in all three species, but had no significant effects on growth. The results confirmed that ambient levels of UV radiation diminish the rate of AM fungi colonization. However, neither AM colonization nor UV radiation affected the outcome of plant competition. These findings indicated that the high degree of total competitive stress masked the effects of the UV radiation stress and AM colonization on plant growth. The results indicate that competition and stress factors are not always interactive.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Plant-plant interactions

Interactions among plants influence plant population dynamics, plant community structure, and ecosystem functions (Morris 2003, Kikvidze et al. 2005). Plant-plant interactions can be positive (facilitation and mutualism) or negative (parasitism, competition, and allelopathy). Facilitation is defined as a positive non-trophic interaction that occurs between physiologically independent plants and that is mediated through changes in the abiotic environment or through other organisms (Brooker et al. 2008). Facilitation may act directly through mechanisms such as favorable alteration of light, temperature, soil moisture, soil nutrients, or soil oxygenation, and indirectly through mechanisms such as protection from herbivores, attraction of shared pollinators, root grafts, or beneficial changes in soil mycorrhizal or microbial communities (Callaway 1997). The majority of studies on facilitation between plants have been done in severe environments, such as deserts or arctic or alpine sites (Michalet 2006 and references therein).

Mutualisms are reciprocally positive interactions between pairs of species (Bronstein 2009). Mutualisms are widespread in nature: typical examples include interactions between plants and nitrogen fixing bacteria, plants and mycorrhizal fungi, and plants and pollinators (Herre et al. 1999). In most mutualistic associations, each partner trades a resource to which it has access for another resource that is very difficult
or impossible to acquire on its own (Bronstein 1994). For instance, most plants provide carbon to mycorrhizal fungi in return for phosphorus or other resources; others provide carbon to N₂-fixing rhizobia in exchange for nitrogen (Bronstein and Dieckmann 2004). Mutualism and facilitation share three common features. First, both interactions generate not only benefits but also costs. Costs may include rewards for the partners that confer benefits or the cost of tolerating competition between facilitated and facilitating species. Second, both types of interaction are often context-dependent, i.e., the association may vary from beneficial to detrimental depending on the ecological conditions in which it takes place. Third, both facilitation and mutualism act to ameliorate biotic or abiotic stresses (Bronstein 2009).

Competition is defined as the interaction between organisms brought about by overlapping resource requirements that results in decreased growth, survival or reproductive capacity of one or both of the two organisms. Competition for resources aboveground (light) and belowground (water and nutrients) is believed to be a central force maintaining local diversity and controlling the structure of plant communities (Wilson and Tilman 1993; Latham 1992; Davis et al. 1998; Aerts 1999). Plant competitive interactions are influenced by biotic factors and abiotic factors (Goodwin 1992; Lewis and Tanner 2000; Chen et al. 2008; Scheublin et al. 2007). Those factors can be especially important at the seedling stage, the stage of development that is most sensitive to environmental factors in most plants (Shimono and Kudo 2003), and when most mortality occurs.
Plant stress

The stress concept was initially developed in classical mechanics for describing changes in the shape of physical bodies when subjected to a force; decades later biologists applied the terminology to biological systems (Kranner et al. 2010). There have been many attempts to give a precise definition of plant stress. Levitt (1980) defined stress as “Any environmental factor potentially unfavorable to living organisms.” Lichtenthaler (1996) defined plant stress as “any unfavorable condition or substance that affects or blocks a plant’s metabolism, growth or development.” According to these definitions, environmental factors that deviate from the optimal quantity or intensity for the plant are called stress factors. When stress factors result from living organisms they are called biotic (e.g., herbivory, competition, disease), but if they result from nonliving factors they are considered abiotic (Kranner et al. 2010). Abiotic stress factors may originate from conditions not related to resources (e.g., heat, cold, wind, toxins), or from resource-related conditions such as light or nutrients (Maestre et al. 2009).

Plants are often exposed not only to a single but to multiple co-occurring stress factors. The effects of interacting stresses may be classified as additive, synergistic, or antagonistic (Niinemets 2010). If the deleterious effect of two stresses is equal to the sum of their individual effects. Similarly, an interaction between stresses is considered synergistic if the simultaneous actions of the stresses increase their deleterious effect farther than the simple additive effect of their individual actions. Lastly, if the plant
organism subjected to a single stress factor is capable of increasing its resistance to a different stress factor, the interaction is antagonistic (Alexieva et al. 2003).

The importance of studying plant stresses in combination with environmental factors

The isolation of the effect of individual environmental factors on plant interactions is possible under controlled conditions and must be conducted under tightly controlled conditions, e.g., in pots or in greenhouses (Freckleton et al. 2009). However, studies of plant-plant interactions in greenhouses almost always show competition. This may be because conditions in greenhouses tend to be benign and therefore unlikely to show facilitation (Callaway 2007). In this dissertation, to evaluate the effects of a mutualism and an abiotic stress factor on plant-plant interactions, the effects of mycorrhizal colonization and UV radiation on tree seedlings were evaluated. Seedlings were chosen because that is the stage of plant development that is most sensitive to environmental factors, while shade intolerant species were chosen because they are fast-growing, adapted to high levels of solar UV radiation, and may have a higher response to mycorrhizae than do shade tolerant species (Siqueira et al. 1998, Zangaro et al. 2005). Because positive interactions are less likely to be observed under greenhouse conditions, this study focused on competitive interactions. Understanding the effects of environmental factors in determining the outcome of plant interactions may provide valuable insights into the possible effects of environmental changes on plant communities.
Explanation of dissertation format

In this dissertation, I investigated (i) how ultraviolet radiation and AM colonization affect vegetative growth in seedlings of *Populus deltoides* (eastern cottonwood), *Salix nigra* (black willow), and *Betula nigra* (river birch), three co-occurring shade intolerant tree species; and (ii) how the individual and combined effects of UV radiation and mycorrhizal symbiosis affect competitive interactions between seedlings of these three tree species. The document is presented in four data chapters and a chapter of summary and conclusions and includes one appendix. Chapter two tests the hypotheses that (1) the exclusion of UV radiation positively affects growth of seedlings of *P. deltoides, B. nigra*, and *S. nigra*, (2) the exclusion of UV radiation alters biomass allocation by seedlings (3) UV exclusion results in increased AM colonization of seedlings, and (4) there would be a significant interaction between UV radiation and mycorrhizae, such that the AM colonization will negate, at least in part, the detrimental effects of UV radiation. Chapter three evaluates the effects of competition and UV radiation on early vegetative growth in seedlings of *P. deltoides*. Chapter four tests the hypotheses that (1) UV radiation alters the growth, allocation, and morphology of seedlings in competition, (2) mycorrhizal colonization of roots alters the growth, allocation, and morphology of seedlings in competition; (3) the identity of competitor affects the growth of seedlings; (4) UV radiation negatively affects the rate of mycorrhizal colonization; and (5) interactions among UV radiation, mycorrhizal inoculum and identity of competitor alter the growth of seedlings. Chapter five evaluates differences in arbuscular mycorrhizal and ectomycorrhizal colonization between wild
adults and seedlings of *P. deltoides*. Chapters are presented in a format such as the manuscripts submitted to peer reviewed journals. Results of additional experiments are presented in Appendix A.

**References**


CHAPTER 2. GROWTH AND ALLOCATION RESPONSES OF THREE SHADE INTOLERANT TREE SPECIES TO ULTRAVIOLET RADIATION AND MYCORRHIZAL COLONIZATION

A paper to be submitted to Plos One

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Abstract

The effects of solar ultraviolet (UV) radiation (280-400 nm) and arbuscular mycorrhizal (AM) symbiosis on growth, morphology and allocation of seedlings of three shade intolerant species (Populus deltoides, Betula nigra, and Salix nigra) were evaluated in replicated greenhouse experiments. Plants were grown in a factorial design with and without UV radiation and with normal AM inoculum or with autoclaved inoculum. After three months, the percentage of roots colonized by AM fungi was assessed using the grid line intercept technique. Seedlings exposed to UV radiation had smaller root masses, stem diameters, and root to shoot ratios; and greater specific leaf areas and leaf area ratios. Mycorrhizal colonization resulted in reduced plant biomass. Ultraviolet radiation reduced the percentage of root colonized by AM fungi and induced changes in leaf morphology and allocation; however, the species showed differential sensitivity to UV radiation. It is demonstrated that solar UV radiation can negatively affect the rate of AM colonization.
Key words: arbuscular mycorrhizae, abiotic stress, Populus deltoides, Betula nigra, Salix nigra, seedling growth, greenhouse experiment.

Introduction

Plants live in environments where they are subject to multiple stress factors that can alter their normal physiology (Alexieva et al. 2003). However, plants have developed a suite of mechanisms that allow them to detect and respond to different stress factors, minimizing their destructive effects (Atkinson and Urwin 2012). Besides the stress protective mechanisms, mutualistic relationships with rhizosphere bacteria and symbiotic fungi may help plants to overcome the detrimental effects of some abiotic stress factors (Grover et al. 2010). Since the number and intensity of environmental stresses are likely to increase in the future due to climate change (Lichtenthaler 1996; Alexieva et al. 2001; Dukhovskis et al. 2003), it is relevant to study the effects of abiotic stress factors on plant symbiotic interactions.

Solar ultraviolet (UV) radiation is one important and widespread type of abiotic stress factor for plants (Singh et al. 2006). The UV spectrum is divided into three wavebands: UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm). The UV-C radiation is the most energetic, but is strongly absorbed by the upper atmosphere and does not reach the Earth’s surface. Both UV-B and UV-A penetrate to the troposphere, though most of the UV-B is absorbed by stratospheric ozone (Frederick 1993). The study of the ecological role of solar UV radiation was intensified based on concern over depletion of stratospheric ozone due to the anthropogenic discharge of
chlorofluorocarbons (CFCs) and the resulting increase in ground-level UV-B radiation (Frederick 1993). For that reason, the understanding of the ecophysiological effects of ultraviolet radiation has centered on UV-B irradiance above current ambient levels (Phoenix et al. 2003). However, the intensity of UV radiation reaching the surface of the earth is affected not only by the ozone column but also by other factors such as solar angle, elevation, cloud cover, and pollution of the troposphere by particulate materials (Paul and Gwynn-Jones 2003). Thus, future variations in UV radiation resulting from changes in climate may have more important consequences on terrestrial ecosystems than changes in UV caused by ozone depletion (Ballaré et al. 2011). Research on the effects of enhanced UV-B on seedlings shows changes in morphological traits such as reductions in height, stem diameter, biomass, and leaf area. Those responses can be attributed to (i) UV-B-induced changes in plant hormone metabolism, specifically auxins, which are implicated in different developmental processes (Huang et al. 1997; Jansen 2002), (ii) a shift in carbon allocation toward the production of UV-B-absorbing compounds such as flavonoids and phenolic acids in leaves (Schumaker et al. 1997; Warren et al. 2003; Kotilainen et al. 2008; Morales et al. 2010); and (iii) changes in leaf expansion as a result of UV-B induced increases in cell-wall peroxidase activity (Tegelberg et al. 2001; Wargent et al. 2009; Robson and Aphalo 2012). UV-B radiation may directly damage the light-harvesting chlorophyll a/b protein complex of photosystem II, resulting in a reduction in net photosynthesis rate (Hideg et al. 1993). Less well studied are the effects of UV-A radiation, which also affects living cells and organisms.
Ultraviolet-B supplementation studies provide many useful insights that can be complemented by experiments in which the UV components of the solar radiation are removed by means of plastic filters. Such studies allow for identification of the effects of ambient UV levels on plants (Sullivan 2005; Amudha et al. 2005). Moreover, there is increasing evidence that UV-A also elicits plant responses that sometimes are opposite to those elicited by UV-B, a phenomenon that could be the result of the different photoreceptors that mediate responses to UV-A and UV-B radiation (Ryel et al. 2010). Thus, it is expected that the exclusion of both UV-B and UV-A will produce different results than only UV-B exclusion. For instance, Kotilainen et al. (2008) reported from a field experiment that the concentration of phenolics in leaves can vary in response to exclusion of either solar UV-B or both solar UV-A and UV-B radiation in *Alnus incana* and *Betula pubescens* trees (Kotilainen et al. 2008).

The mycorrhizal symbiosis is probably the most widespread mutualistic association between plants and fungi, with more than 90% of all land plant species presenting some type of mycorrhizal association (Aerts 2002). The arbuscular mycorrhizal (AM) symbiosis is the most common type. In the AM symbiosis the fungi penetrate the cortical cells and form clusters of finely divided hyphae known as arbuscules inside the cells within the cortex, and they also form vesicles both inside and outside the cortical cells (Turk et al. 2006). Mycorrhizal fungi can help plants to cope with biotic and abiotic stresses via plant growth promotion and induced resistance to diseases (Pineda et al. 2010). For instance, mycorrhizae can stimulate plant growth through the enhancement of nutrient uptake, especially phosphorus (Smith et al. 2011).
Similarly, mycorrhizae can improve the water absorption capacity of plants by increasing root hydraulic conductivity, thus improving the ability of plants to withstand drought (Turk et al. 2006; Ruiz-Lozano 2003). Further, mycorrhizal fungi can provide protection against certain root diseases (Jeffries et al. 2003). However, current evidence indicates that increased UV-B radiation can reduce AM colonization (Klironomos 1995; van de Staaij et al. 2001; Zaller et al. 2002). This indirect effect of UV radiation on AM colonization may be attributed to UV-induced changes in the phytohormone balance in the host plant, since evidence indicates that the mycorrhizal colonization may be partly regulated from the plant side by the action of phytohormones (Hause et al. 2007). For instance, abscisic acid (ABA) contributes to the susceptibility of tomato to infection by AM fungi and seems to play an important role in the development of arbuscules (Herrera-Medina et al. 2007). Similarly, auxins such as indoleacetic acid (IAA) may stimulate AM colonization of roots by increasing the number of fine roots during early growth phases (Hause et al. 2007). The studies mentioned above have given some insight into the effect of increased UV-B radiation on mycorrhizal symbiosis. However, there is still limited knowledge about the effect of ambient levels of UV radiation on mycorrhizal fungi in the rhizosphere of tree seedlings, and is not clear whether mycorrhizal symbiosis can help plants to overcome the detrimental effects UV radiation.

The overall objective of this study was to investigate the individual and interactive effects of ultraviolet radiation and AM colonization on vegetative growth in seedlings of *Populus deltoides* (eastern cottonwood), *Salix nigra* (black willow), and *Betula nigra* (river birch), three co-occurring shade intolerant tree species. Shade
intolerant species were chosen because they are fast-growing, are adapted to high levels of solar UV radiation, and may have a higher response to mycorrhizae than shade tolerant species (Siqueira et al. 1998; Zangaro et al. 2005). In the present study, four hypotheses were tested: (1) the exclusion of UV radiation will alter growth, morphology and allocation of seedlings of *P. deltoides, B. nigra,* and *S. nigra,* (2) AM colonization will alter growth of seedlings of *P. deltoides, B. nigra,* and *S. nigra,* (3) UV exclusion will result in increased AM colonization of seedlings, and (4) there will be a significant interaction between UV radiation and mycorrhizal colonization, such that the AM colonization will negate, at least in part, the detrimental effects of UV radiation.

**Materials and Methods**

**Plant materials**

The three tree species investigated in this study are commonly found in open habitats along stream and river banks in the eastern half of the United States (Jurgensen et al. 1996). *Populus deltoides* Bartram. ex Marshall (eastern cottonwood, Salicaceae) is considered a very shade intolerant species that grows on exposed sites, often in riparian areas. *P. deltoides* has been a subject of intensive research for its end use in timber and paper-pulp industries and as a model bioenergy crop (Bradshaw et al. 2000). *Salix nigra* Marshall (black willow, Salicaceae) is a very shade intolerant species common on river margins; it is also common in swamps, sloughs, and swales, and on the banks of bayous, gullies and drainage ditches. *S. nigra* is the largest and the only commercially important willow of about 90 species native to North America (Burns and Honkala 1990). *Betula*
**nigra** L. (river birch, Betulaceae) is a fast growing species considered as shade intolerant common on bottomlands along stream banks and wet areas in eastern North America (Coyle and Sharik 1982).

**Experimental design**

Experiments were conducted in greenhouses of Iowa State University, Ames, IA. Seeds of *P. deltoides*, *S. nigra* and *B. nigra* were gathered from different localities in Ames (42°02′05″N 93°37′12″W, 287 m elevation). Seeds were sown in cone-shaped pots (Ray Leasch Cone-tainers: 0.15 L volume, 205mm length, 40 mm top diameter; Stuewe and Sons, Corvallis, Oregon, USA) containing a 1:1 sand-soil mixture with pH 6.7 (in water) that was steam autoclaved for 90 min. Two weeks after germination all pots were thinned to contain one seedling with a size that ranged from 1.5 to 1.7 cm. The experimental design was a 2 x 2 x 2 factorial, with two levels of UV radiation (with and without UV), two mycorrhizal conditions (inoculated and un-inoculated), and two greenhouses.

**Growth conditions**

Partial attenuation of solar UV radiation by the greenhouse glass made necessary the use of supplemental UV radiation provided by fluorescent lamps, to provide a treatment representing ambient UV irradiance. Supplemental UV-A/B radiation was provided daily over a 10-h period centered at solar noon using Q-Panel UV-A 340 fluorescent lamps (ranging from 365 nm to 295 nm with peak emission at 340 nm, Q-
Panel, Cleveland, Ohio). Lamps were mounted on wood frames suspended 100 mm above PVC frames that were covered with 0.12 mm cellulose diacetate film, which transmits both UV-B and UV-A radiation. For the UV-exclusion treatment (-UV), pots were placed inside PVC frames that were covered with 0.3 mm polyester transparent film, which excludes both UV-B and UV-A radiation. UV-A/B irradiance was measured with a Solarmeter® Digital Ultraviolet Meter, model 5.7 UVA + B Sensitive Microwatt Version (Solartech, Inc., Harrison Township, Michigan, USA), at the top of canopy at midday every two weeks over the course of each experiment. Midday photosynthetic photon flux density (PPFD, 400-700 nm) was measured under the cellulose diacetate and polyester films using a Field Scout Quantum Meter® (Spectrum Technologies Inc., Plainfield, Illinois, USA). Measurements were taken at the top of the plants every two weeks. To minimize the effects of microenvironment variation, positions of plants within the treatments within each greenhouse were rotated daily.

**Mycorrhizal inoculation**

Mycorrhizal infection was established by adding 6 g of SYMBIVIT® Endomycorrhiza (Symbiom, Lanskroun, Czech Republic), containing a mixture of fragments of colonized roots, mycelia and spores of *Glomus mosseae*, *G. microagregatum*, *G. claroideum*, *G. intraradices*, *G. entunicatum*, and *G. geosporum*. These fungal species are usually compatible with the plant species evaluated. Control seedlings received the same amount of SYMBIVIT® that had been autoclaved.
Growth measurements

Height and number of leaves of all seedlings were measured every 15 days, and plants were harvested after 3 months. At harvest, each seedling was partitioned into leaves, stem and roots. Stem diameter above the root collar, shoot height and the total number of leaves were measured. Leaves were scanned and leaf area (LA) was determined using Compu Eye, Leaf & Symptom Area® software (Bakr 2005). The samples were dried in an oven at 70 °C for at least 48 h and weighed, after which different growth parameters were calculated for each plant (Table 1).

Determination of root colonization

During harvest of the seedlings, a root sample for mycorrhizal quantification weighing 1-2 grams was removed from each seedling and stored in the freezer. Each sample had three subsamples, from shallow, middle, and deep roots. The dry weight of each sample used for mycorrhizal quantification was estimated and added to the total root weight using each mycorrhizal root sample’s fresh weight and the corresponding root mass’s fresh weight/dried weight. The root samples were cleared in 10% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue in acid glycerol (Robertson et al. 1999). Samples were stored in a 1:1:1 solution of water, glycerin, and lactic acid until they were examined. Each root sample was spread across the bottom of an 8.5 cm diameter, gridded petri dish and examined under a dissecting microscope; each intersection between a line and a root was classified as mycorrhizal or non-mycorrhizal. All of the root/gridline intersects were recorded. Then the sample was re-distributed and
the process repeated twice, for a total of three counts. The percent of root length colonized was calculated as the ratio between the number of AM intersections and the total number of intersections multiplied by 100 (Brundrett et al. 1996).

**Statistical analyses**

The statistical software R-2.15.1 was used for the statistical analyses, and the significance level was set at 0.05 (see Supplementary Information). Permutational multivariate analysis of variance (PERMANOVA) was used to identify significant effects of UV radiation, mycorrhizal inoculum, greenhouse and their interactions. Permutation tests for factorial ANOVA (Manly 2007) were performed to help to determine which variables contributed to any significant differences observed in the multivariate analysis. The Tukey HSD test was employed to test for differences among means. Biomass proportions and percentages of root colonization were transformed as necessary to achieve normality and meet the assumptions of parametric statistical analyses. The growth, morphology, and allocation variables tested are described in Table 1. A Meta-analysis, using the software MetaWin 2 (Rosenberg et al. 1999), was performed to summarize the principal effects of UV radiation and mycorrhizal colonization across the three species evaluated.
Results

Environmental conditions

The experiments with *P. deltoides* and *S. nigra* ran from March to May 2013. During that period, mean greenhouse air temperature was 25.6 ± 0.5°C (min. 17.5°C, max. 41°C) and average relative humidity was 51.2 ± 1.7% (min. 21%, max. 91%).

The mid-day PPFD under clear sky conditions, measured under the cellulose diacetate averaged 1325 ± 78 µmol m$^{-2}$ s$^{-1}$ (min. 649 µmol m$^{-2}$ s$^{-1}$, max. 1741 µmol m$^{-2}$ s$^{-1}$); mid-day UV-A/B radiation averaged 15 ± 0.7 W m$^{-2}$ (min. 6 W m$^{-2}$, max. 32 W m$^{-2}$). The mid-day PPFD under polyester film was 7% higher than under the cellulose film; the average was 1438 ± 117 µmol m$^{-2}$ s$^{-1}$ (min. 585 µmol m$^{-2}$ s$^{-1}$, max. 1900 µmol m$^{-2}$ s$^{-1}$), and mid-day UV-A/B radiation was 0 W m$^{-2}$. The experiment with *B. nigra* ran from April to June 2013. During the experiment, mean air temperature was 26.2°C ± 0.4°C (min. 17.4°C, max. 41.8°C) and average relative humidity was 52 ± 0.6% (min. 21%, max. 91%).

The PPFD under the cellulose diacetate 1346 ± 60 µmol m$^{-2}$ s$^{-1}$ (min. 649 µmol m$^{-2}$ s$^{-1}$, max. 1800 µmol m$^{-2}$ s$^{-1}$); mid-day UV-A/B radiation averaged 15 ± 0.6 W m$^{-2}$ (min. 7 W m$^{-2}$, max. 32 W m$^{-2}$). The mid-day PPFD under polyester film was 7% higher than under the cellulose diacetate; the average was 1447 µmol m$^{-2}$ s$^{-1}$ ± 80, (min. 649 µmol m$^{-2}$ s$^{-1}$, max. 1998 µmol m$^{-2}$ s$^{-1}$) and mid-day UV-A/B reading was 0 W m$^{-2}$.

Growth measurements

The results of PERMANOVA for each of the three experiments showed that the suite of dependent variables that were measured (Table 1) was not significantly affected...
either by AM or greenhouse. However, they were significantly affected by UV radiation
(*P. deltoides* F$_{2,60}$ = 23.72, P ≤ 0.01; *S. nigra* F$_{2,50}$ = 14.81, P ≤ 0.01; and *B. nigra* F$_{2,20}$ = 9.51, P ≤ 0.01). Compared with seedlings grown in presence of UV radiation, *P.*
deltoides seedlings grown under UV-exclusion had significantly lower leaf areas,
specific leaf areas (SLA), and leaf area ratios (LAR) (Fig. 1, Table 2). The specific leaf
area is a measure of leaf thickness and/or leaf tissue density (low SLA indicates thick or
dense leaves); leaf area ratio is a measure of photosynthetic surface relative to total plant
respiratory mass. Analyses of variance showed no significant interactive effects of UV
radiation and AM for any of the variables for *P. deltoides* seedlings, and the same results
were found for *S. nigra* and *B. nigra* (Table 2).

Seedlings of *S. nigra*, grown without UV radiation had larger stem diameters,
and greater stems, root and total dry weights than seedlings grown exposed to UV
radiation (Fig. 1, Table 2). In contrast, UV exclusion resulted in significantly greater
SLA and LAR (Fig. 1, Table 2). Compared with seedlings grown with UV radiation,
seedlings of *B. nigra* grown under the UV-exclusion treatment had significantly lower
SLA, and LAR (Fig. 1, Table 2).

**Mycorrhizal colonization**

All seedlings of *P. deltoides*, *B. nigra* and *S. nigra* that were given live AM
inoculant formed mycorrhizas. No AM structures were found in seedlings given
autoclaved inoculant. The results of ANOVA showed that, for seedlings of *P. deltoides*,
the AM inoculum resulted in lower heights (H) and stem diameters (SD) (P < 0.001,
Table 2). There was no difference in heights between AM treatments until midway through the growth period; after that point AM inoculated seedlings presented lower growth levels, and at harvest the average height of the non-inoculated seedlings was higher than that of inoculated seedlings (Fig. 2). There was no significant correlation between the rate of AM colonization and height ($R = -0.27$, $n = 30$, $P = 0.15$). Interestingly, there was a significant difference in total dry weight between AM inoculated and non-inoculated seedlings of *P. deltoides* when grown under UV-exclusion, but there was no difference in total dry weight between the two AM conditions for seedlings grown exposed to UV radiation (Fig. 1).

The results of ANOVAs (Table 2) showed that, for seedlings of *S. nigra*, the AM inoculation had no significant effect on growth or morphological variables. However, relative allocation to roots was lower. The average root to shoot ratios of AM seedlings was 0.70, and the average root to shoot ratios of AM-free seedlings was 0.78. In case of *B. nigra* seedlings, there was no significant difference between the AM inoculated and the un-inoculated control for any of the evaluated variables (Table 1).

**Effect of UV radiation on mycorrhizal colonization**

Seedlings grown under UV exclusion condition had a higher extent of colonization than those seedlings grown in the presence of UV (Table 3). The average extent of colonization for *P. deltoides* seedlings grown without UV radiation was 40%, versus 30% for those grown in presence of UV radiation (Fig. 3). Seedlings of *S. nigra* grown under UV exclusion conditions had an average AM colonization of 11%, versus
4.3% for seedlings grown in presence of UV (Fig. 3). In seedlings of *B. nigra*, AM colonization was not significantly affected by the UV radiation (Fig. 3)

**Overall effects**

In a meta-analysis the outcomes of different experiments are examined to test whether together they demonstrate an effect that is large, moderate, small, or not significantly different from zero (Gurevitch et al. 1992). The meta-analytic method used herein was based on estimation of the magnitude of the effect of interest on each variable from every experiment. The results of the meta-analyses showed that, across the three species tested, UV exclusion had positive effects on stem diameter, root dry weight, and root to shoot ratio, and negative effects on SLA and LAR (Fig. 4). In the case of AM colonization, its effect on the variables evaluated was not significantly different from zero except for final total seedling mass (TDW), which was lower in AM seedlings than in AM-free seedlings (Fig. 5). Similarly, the results of the meta-analysis showed that UV radiation reduced the rate of AM colonization.

**Discussion**

**Effect of UV radiation**

Exclusion of UV radiation resulted in increased biomass for only one of the three species tested, and lower SLA and LAR for all three species (Fig. 2 Table 2). These results provide only partial support for hypothesis (1) that the exclusion of UV radiation would affect growth, morphology and allocation of seedlings of *P. deltoides, B. nigra,*
and *S. nigra*. The results show that UV radiation consistently induced changes in leaf morphology. It is generally accepted that UV radiation is a stress factor that can induce morphogenic responses in leaves such as changes in leaf thickness and elongation (Weih et al. 1998; Bassman et al. 2001; Ren et al. 2006; Yang and Yao 2008; Xu et al. 2010). The type of alterations in SLA and LAR induced by the exclusion of UV radiation reported here are similar to those reported by Schumaker et al. (1997); who found increased leaf thickness in *Populus trichocarpa* cuttings when plants were grown in sub-ambient UV-B. The increase in leaf thickness was explained to result from significantly more palisade parenchyma, which in turn resulted in an increased rate of photosynthesis for cuttings grown in sub-ambient UV-B. Here, only seedlings of *S. nigra* had lower total dry weight when grown with UV radiation. Since reduced biomass accumulation is considered a reliable indicator for plant sensitivity to UV radiation, because it represents the cumulative detrimental effects of UV on plant physiology (Smith 2000), the reduction in total dry weight suggests that seedlings of *S. nigra* may be more sensitive to the effects of UV radiation than seedlings of *P. deltoides* and *B. nigra*. This result suggests that UV radiation could be an important factor influencing the intraspecific interaction between these co-occurring species.

**Effect of AM inoculum**

Although AM structures were observed in all seedlings exposed to live inoculant, it did not result in any increase in growth, and in one case the AM plants were smaller than the non-mycorrhizal control (Fig.1). This result does not support the hypothesis (2)
that AM colonization would alter growth of seedlings of *P. deltoides*, *B. nigra*, and *S. nigra*. Though mycorrhizae are usually considered a mutualistic association in which both species benefit, under particular conditions of high or low P-availability the AM colonization may result in lack of mycorrhizal growth response or even in growth depression (Smith et al. 2009). Therefore some authors point out that the responses of plants to mycorrhizal infection can vary in a continuum from mutualistic to parasitic, with the parasitic relation resulting from carbon costs of mycorrhizal colonization surpassing the benefits of increased phosphorus uptake (Johnson et al. 1997; Schroeder and Janos 2004). Thus, the results here could suggest a parasitic mycorrhizal relationship. However, the mutualism-parasitism continuum of mycorrhizal function is not the only possible explanation for the lack of mycorrhizal growth response. Some authors suggest that, since the direct P uptake by the roots and uptake via the AM fungus are not additive but rather are complementary, growth depression in AM plants may be a function of AM-induced reduction of direct root P uptake that is not compensated for the P delivered via the AM pathway (Smith and Smith 2012). Interestingly, the results here also indicate differential effects of AM colonization on co-occurring plant species. It is well recognized that AM colonization can result in different effects on plants, and those different effects can occur even at the intraspecific level (Munkvold et al. 2004).

**Interactive Effects**

The positive effects of the UV exclusion on the rate of AM colonization of *P. deltoides* and *S. nigra* seedlings supports the hypothesis (3) that UV exclusion would
result in increased AM colonization rate of seedlings. This result is consistent with other studies. For instance, Staaij et al. (2001), in a field experiment with *Calamagrostis epigeios* and *Carex arenaria* exposed to enhanced levels of UV-B radiation, reported a reduction in AM colonization rate. The greater extent of AM colonization rate observed in seedlings grown without UV radiation in the current study may be explained as a result of more resources available for mycorrhizal formation. Since the typical response of plants to UV radiation is the production of screening pigments, in the absence of UV radiation a reduction in the concentration of such pigments is expected. For instance, in a field study with cuttings of *Populus trichocarpa*, Schumaker et al. (1997) found a reduction in the concentration of flavonoids in leaves of seedlings exposed to sub-ambient UV-B radiation. A reduction in the production of screening pigments implies the possibility of more resources being available to be allocated to mycorrhizal formation. However, contrary to hypothesis (4), here there were no interactions between AM inoculum and UV radiation for any of the variables evaluated; all observed treatment effects were clearly attributable to either UV or mycorrhizae.

**Overall Effects (Meta-analyses)**

The overall effects of UV radiation on greenhouse-grown tree seedlings included consistent reductions in stem diameter, root biomass, and root to shoot ratio (Figure 4). Similarly, seedlings exposed to UV radiation had reduced SLA and LAR (Figure 4). On the other hand, although the species showed marked differences in their percentages of
root colonized by AM (Fig 3), the overall effect of AM colonization was a reduction on
biomass accumulation (Figure 5).

The results demonstrate that ambient levels of UV radiation can exert an indirect
effect on mycorrhizal fungi in the rhizosphere of tree seedlings.

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also grateful to Drs. Dean Adams and Dianne Cook for assistance with the statistical
analyses and Drs. Thomas Jurik, Brian Wilsey and Richard Hall for manuscript review.
Support was provided by the Department of Ecology Evolution and Organismal Biology
of the Iowa State University.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>Height</td>
<td>cm</td>
</tr>
<tr>
<td>NL</td>
<td>Number of leaves</td>
<td>dimensionless</td>
</tr>
<tr>
<td>LA</td>
<td>Leaf area</td>
<td>cm²</td>
</tr>
<tr>
<td>SD</td>
<td>Stem diameter</td>
<td>mm</td>
</tr>
<tr>
<td>DWL</td>
<td>Dry weight of leaves</td>
<td>g</td>
</tr>
<tr>
<td>SDW</td>
<td>Shoot dry weight</td>
<td>g</td>
</tr>
<tr>
<td>RDW</td>
<td>Root dry weight</td>
<td>g</td>
</tr>
<tr>
<td>TDW</td>
<td>Total dry weight</td>
<td>g</td>
</tr>
<tr>
<td>R:S</td>
<td>Root to shoot ratio (RDW/SDW)</td>
<td>g·g⁻¹</td>
</tr>
<tr>
<td>SLA</td>
<td>Specific leaf area (LA/DWL)</td>
<td>cm²·g⁻¹</td>
</tr>
<tr>
<td>LAR</td>
<td>Leaf area ratio (LA/TDW)</td>
<td>cm²·g⁻¹</td>
</tr>
</tbody>
</table>
Table 2. Summary of statistical analysis. Statistical values tabulated are $F$ ratios from ANOVAs testing for the main effect of UV radiation (UV), arbuscular mycorrhiza (AM), or the interaction between them (UV x AM); degrees of freedom in parenthesis. Significance levels with Bonferroni correction by analysis of variance (**p<0.0001; **P<0.001; *P<0.004).

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>UV (1)</th>
<th>AM (1)</th>
<th>UV x AM (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. deltoides</em></td>
<td>Height (cm)</td>
<td></td>
<td>10.19</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>Stem diameter (cm)</td>
<td></td>
<td>10.43</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Leaf area (cm$^2$)</td>
<td>11.65</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>SLA (cm$^2$.g$^{-1}$)</td>
<td>24.69</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>LAR (cm$^2$.g$^{-1}$)</td>
<td>80.19</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td><em>S. nigra</em></td>
<td>Diameter (cm)</td>
<td>13.74</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Shoot dry weight (g)</td>
<td>14.2</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Root dry weight (g)</td>
<td>20.72</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Total dry weight (g)</td>
<td>20.12</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>SLA (cm$^2$.g$^{-1}$)</td>
<td>26.10</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>LAR (cm$^2$.g$^{-1}$)</td>
<td>40.05</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>R:S</td>
<td></td>
<td>12.48</td>
<td>***</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>SLA (cm$^2$.g$^{-1}$)</td>
<td>11.31</td>
<td></td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>LAR (cm$^2$.g$^{-1}$)</td>
<td>30.57</td>
<td></td>
<td>***</td>
</tr>
</tbody>
</table>

Only terms that are significant at p<0.004 are shown.
Table 3 Effect of UV radiation on extent of AM colonization in *P. deltoides* *B. nigra* and *S. nigra* seedlings. F-values and significance levels for ANOVA (*P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant).

<table>
<thead>
<tr>
<th>Species</th>
<th>F</th>
<th>df</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. deltoides</em></td>
<td>4.56*</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td><em>S. nigra</em></td>
<td>5.95*</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>0.86ns</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>
**Figure 1.** Effects of ultraviolet radiation and AM colonization on height, TDW, SLA, and LAR of *P. deltoides*, *B. nigra* and *S. nigra* seedlings. Each value is a mean ± SE. Means with different letters are significantly different (P < 0.005, post-ANOVA Tukey test).
Figure 2. Effect of AM colonization and UV radiation and on height of *P. deltoides* seedlings. Each point represents the mean, vertical bars denote ± SE. The different letters below the same date indicate statically significant differences between treatments at the P <0.05 level in Tukey’s HSD test. There were not significant differences among treatment until past the first half of the growth period; after that point the cumulative effect of the UV radiations become evident in the non-colonized seedlings.
Figure 3. Effect of UV radiation on extent of arbuscular colonization in *P. deltoides*, *S. nigra* and *B. nigra* seedlings. Each bar is a mean ± SE; means with different letters are significantly different (P < 0.05, post-ANOVA Tukey test).
Figure 4. Effect of UV radiation on growth parameters across the species evaluated. Each line represents a 95% confidence interval (CI) for the effect size of variables from each of the three species; the effect of UV radiation is considered neutral for those variables with CI overlapping zero.
Figure 5. Effect of AM colonization on growth parameters across the species evaluated. Each line represents a 95% confidence interval (CI) for the effect size of variables from each of the three species; the effect of AM colonization is considered neutral for those variables with CI overlapping zero.
References


Smith, S. E., I. Jakobsen, M. Grønlund, and F. A. Smith. 2011. Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for
39


Supplemental information

**R code for statistical analyses of Experiment 1**

```r
rm(list=ls())

populus<-read.csv(file.choose())

head(populus)

options(digits=3)

summary(populus)

#Test for Homogeneity of Variances

bartlett.test(height, data=populus)

bartlett.test(leaves~Myco, data=populus)

bartlett.test(LA~Myco, data=populus)

bartlett.test(Diameter~Myco, data=populus)

bartlett.test(RDW~Myco, data=populus)

bartlett.test(LDW~Myco, data=populus)

bartlett.test(SDW~Myco, data=populus)

bartlett.test(TDW~Myco, data=populus)

bartlett.test(SLA~Myco, data=populus)

bartlett.test(LAR~Myco, data=populus)

bartlett.test(R.S~Myco, data=populus)

bartlett.test(LWR~Myco, data=populus)

bartlett.test(RGR~Myco, data=populus)

#normality test
```
shapiro.test(height, data=populus)
shapiro.test(leaves~Myco, data=populus)
shapiro.test(LA~Myco, data=populus)
shapiro.test(Diameter~Myco, data=populus)
shapiro.test(RDW~Myco, data=populus)
shapiro.test(LDW~Myco, data=populus)
shapiro.test(SDW~Myco, data=populus)
shapiro.test(TDW~Myco, data=populus)
shapiro.test(SLA~Myco, data=populus)
shapiro.test(LAR~Myco, data=populus)
shapiro.test(R.S~Myco, data=populus)
shapiro.test(LWR~Myco, data=populus)
shapiro.test(RGR~Myco, data=populus)

# matrix of linear measurements
populus.data<-as.matrix(populus[,4:16])
Myco<-as.factor(populus[,2])
UV<-as.factor(populus[,3])
competitor<-as.factor(populus[,2])
MycoByUV<-paste(Myco,UV)

# Describe data
cor.populus<-cor(populus.data)
cor.populus
pairs(populus.data)

vcv.populus<-var(populus.data)

vcv.populus

var(scale(populus.data))

dist(populus.data)

#PERMANOVA

library (vegan)

adonis(populus.data~ UV*Myco, data=populus, permutations=99, distance =
"euclidean")

#Permutation tests for factorial ANOVA

mod1 <- lm(height ~ Myco + UV + Myco:UV)

ANOVA <- summary(aov(mod1))

cat( " The standard ANOVA for these data follows ","\n"")

FMyco <- ANOVA[[1]]$"F value"[1]

FUV <- ANOVA[[1]]$"F value"[2]

Finteract <- ANOVA[[1]]$"F value"[3]

print(ANOVA, "\n")

cat( "\n")

cat( "\n")

print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000
FS <- numeric(nreps)  #Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)
FS[1] <- FMyco       # The first F of our 5000
FM[1] <- FUV
FSM[1] <- Finteract
for (i in 2:nreps) {
    newheight <- sample(height, 61)
    mod2 <- lm(newheight ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
    FSM[i] <- b[[1]]$"F value"[3]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM  <- length(FSM[FSM >= Finteract])/nreps
cat(" The probability value for the interaction is ",probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")
mod1 <- lm(leaves ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat( " The standard ANOVA for these data follows ","\n")

FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]

print(ANOVA, "\n")

cat( "\n")

cat( "\n")

print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)    #Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)

FS[1] <- FMyco     # The first F of our 5000
FM[1] <- FUV
FSM[1] <- Finteract

for (i in 2:nreps) {
    newleaves <- sample(leaves, 61)
    mod2 <- lm(newleaves ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]

}

probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract])/nreps
cat(" The probability value for the interaction is ", probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")
mod1 <- lm(LA ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat(" The standard ANOVA for these data follows ", "\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]
print(ANOVA, "\n")
cat("\n")
cat("\n")
cat("\n")
print("Resampling as in Manly with unrestricted sampling of observations. ")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps)  # Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)

FS[1] <- FMyco       # The first F of our 5000

FM[1] <- FUV

FSM[1] <- Finteract

for (i in 2:nreps) {
    newLA <- sample(LA, 6)
    mod2 <- lm(newLA ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]"F value"[1]
    FM[i] <- b[[1]]"F value"[2]
    FSM[i] <- b[[1]]"F value"[3]
}

probS <- length(FS[FS >= FMyco])/nreps

probM <- length(FM[FM >= FUV])/nreps

probSM <- length(FSM[FSM >= Finteract])/nreps

cat("The probability value for the interaction is ",probSM, "\n")

cat("The probability value for Myco is ", probS, "\n")

cat("The probability value for UV is ", probM, "\n")

mod1 <- lm(Diameter ~ Myco + UV + Myco:UV)

ANOVA <- summary(aov(mod1))

cat("The standard ANOVA for these data follows ","\n")

FMyco <- ANOVA[[1]]"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]

print(ANOVA, "\n")
cat("\n")
cat("\n")

print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  # Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000
FM[1] <- FUV
FSM[1] <- Finteract

for (i in 2:nreps) {
    newDiameter <- sample(Diameter, 61)
    mod2 <- lm(newDiameter ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
    FSM[i] <- b[[1]]$"F value"[3]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM  <- length(FSM[FSM >= Finteract])/nreps
cat(" The probability value for the interaction is ",probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")
summary(aov(RGR~Myco*UV, data=populus))
mod1 <- lm(LDW ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat( " The standard ANOVA for these data follows ","\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]
print(ANOVA, "\n")
cat("\n")
cat("\n")
print("Resampling as in Manly with unrestricted sampling of observations. ")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps)  #Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)
FS[1] <- FMyco  # The first F of our 5000
FM[1] <- FUV
FSM[1] <- Finteract
for (i in 2:nreps) {
    newLDW <- sample(LDW, 61)
    mod2 <- lm(newLDW ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$$F value$$[1]
    FM[i] <- b[[1]]$$F value$$[2]
    FSM[i] <- b[[1]]$$F value$$[3]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract])/nreps
    cat("The probability value for the interaction is ", probSM, "\n")
    cat("The probability value for Myco is ", probS, "\n")
    cat("The probability value for UV is ", probM, "\n")
mod1 <- lm(SDW ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
    cat("The standard ANOVA for these data follows ", "\n")
FMyco <- ANOVA[[1]]$$F value$$[1]
FUV <- ANOVA[[1]]$$F value$$[2]
Finteract <- ANOVA[[1]]$"F value"[3]

print(ANOVA, "\n")

cat( "\n")

cat( "\n")

print("Resampling as in Manly with unrestricted sampling of observations.")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  # Set up space to store F values as calculated.

FM <- numeric(nreps)

FSM <- numeric(nreps)

FS[1] <- FMyco       # The first F of our 5000

FM[1] <- FUV

FSM[1] <- Finteract

for (i in 2:nreps) {
    newSDW <- sample(SDW, 61)
    mod2 <- lm(newSDW ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
    FSM[i] <- b[[1]]$"F value"[3]
}

probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract])/nreps
cat(" The probability value for the interaction is ", probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")
mod1 <- lm(RDW ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat(" The standard ANOVA for these data follows ", "\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]
print(ANOVA, "\n")
cat("\n")
cat("\n")
print("Resampling as in Manly with unrestricted sampling of observations.")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps) # Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)
FS[1] <- FMyco # The first F of our 5000
FM[1] <- FUV
FSM[1] <- Finteract

for (i in 2:nreps) {
    newRDW <- sample(RDW, 61)
    mod2 <- lm(newRDW ~ Myco + UV + Myco:UV)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
    FSM[i] <- b[[1]]$"F value"[3]
}

probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract])/nreps

cat(" The probability value for the interaction is ", probSM, 
"
")
cat(" The probability value for Myco is ", probS, 
"
")
cat(" The probability value for UV is ", probM, 
"
")

mod1 <- lm(TDW ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat( " The standard ANOVA for these data follows ", ANOVA, 
"
")

FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]

print(ANOVA, 
"
")
print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  #Set up space to store F values as calculated.

FM <- numeric(nreps)

FSM <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000

FM[1] <- FUV

FSM[1] <- Finteract

for (i in 2:nreps) {
  newTDW <- sample(TDW, 61)
  mod2 <- lm(newTDW ~ Myco + UV + Myco:UV)
  b <- summary(aov(mod2))
  FS[i] <- b[[1]]="$F value"[1]
  FM[i] <- b[[1]]="$F value"[2]
  FSM[i] <- b[[1]]="$F value"[3]
}

probS <- length(FS[FS >= FMyco])/nreps

probM <- length(FM[FM >= FUV])/nreps

probSM <- length(FSM[FSM >= Finteract])/nreps
mod1 <- lm(LAR ~ Myco + UV + Myco:UV)

ANOVA <- summary(aov(mod1))

cat(" The standard ANOVA for these data follows ", "\n")

FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]

print(ANOVA, "\n")

cat("\n")

cat("\n")

print("Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  #Set up space to store F values as calculated.

FM <- numeric(nreps)

FSM <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000

FM[1] <- FUV

FSM[1] <- Finteract

for (i in 2:nreps) {

newLAR <- sample(LAR, 61)
mod2 <- lm(newLAR ~ Myco + UV + Myco:UV)
b <- summary(aov(mod2))
FS[i] <- b[[1]]$"F value"[1]
FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract])/nreps
cat(" The probability value for the interaction is ",probSM,"\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")
summary(aov(LWR~Myco*UV, data=populus))
mod1 <- lm(SLA ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat(" The standard ANOVA for these data follows ","\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]
print(ANOVA, "\n")
cat("\n")
print("Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  #Set up space to store F values as calculated.

FM <- numeric(nreps)

FSM <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000

FM[1] <- FUV

FSM[1] <- Finteract

for (i in 2:nreps) {
  newSLA <- sample(SLA, 61)

  mod2 <- lm(newSLA ~ Myco + UV + Myco:UV)

  b <- summary(aov(mod2))

  FS[i] <- b[[1]]$"F value"[1]

  FM[i] <- b[[1]]$"F value"[2]

  FSM[i] <- b[[1]]$"F value"[3]
}

probS <- length(FS[FS >= FMyco])/nreps

probM <- length(FM[FM >= FUV])/nreps

probSM <- length(FSM[FSM >= Finteract])/nreps

cat(" The probability value for the interaction is ",probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")
mod1 <- lm(RS ~ Myco + UV + Myco:UV)
ANOVA <- summary(aov(mod1))
cat(" The standard ANOVA for these data follows ", "\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
Finteract <- ANOVA[[1]]$"F value"[3]
print(ANOVA, "\n")
cat("\n")
cat("\n")
print("Resampling as in Manly with unrestricted sampling of observations. ")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps) #Set up space to store F values as calculated.
FM <- numeric(nreps)
FSM <- numeric(nreps)
FS[1] <- FMyco # The first F of our 5000
FM[1] <- FUV
FSM[1] <- Finteract
for (i in 2:nreps) {
  newRS <- sample(RS, 61)
mod2 <- lm(newRS ~ Myco + UV + Myco:UV)
b <- summary(aov(mod2))

FS[i] <- b[[1]]$"F value"[1]
FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]
}

probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract])/nreps

cat(" The probability value for the interaction is ", probSM, "n")
cat(" The probability value for Myco is ", probS, "n")
cat(" The probability value for UV is ", probM, "n")
TukeyHSD(aov(height~UV + Myco, data=populus))
TukeyHSD(aov(leaves~UV + Myco, data=populus))
TukeyHSD(aov(LA~UV + Myco, data=populus))
TukeyHSD(aov(Diameter~UV + Myco, data=populus))
TukeyHSD(aov(RGR~UV + Myco, data=populus))
TukeyHSD(aov(LDW~UV + Myco, data=populus))
TukeyHSD(aov(SDW~UV + Myco, data=populus))
TukeyHSD(aov(RDW~UV + Myco, data=populus))
TukeyHSD(aov(TDW~UV + Myco, data=populus))
TukeyHSD(aov(LAR~UV + Myco, data=populus))
TukeyHSD(aov(LWR~UV + Myco, data=populus))
TukeyHSD(aov(SLA~UV + Myco, data=populus))
TukeyHSD(aov(RS~UV + Myco, data=populus))
#group means
library(doBy)

populus<- summaryBy(height ~ UV + Myco, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(leaves ~ UV, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(LA ~ UV, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(Diameter ~ UV + Myco, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(RGR ~ UV, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(LDW ~ UV + Myco, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(RDW ~ UV + Myco, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(SDW ~ UV + Myco, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(TDW ~ UV + Myco, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(LAR ~ UV, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(LWR ~ UV, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(SLA ~ UV, data=populus, FUN=c(length,mean,sd))
populus<- summaryBy(RS ~ UV, data=populus, FUN=c(length,mean,sd))
CHAPTER 3. EFFECT OF UV RADIATION ON COMPETITION BETWEEN SEEDLINGS OF *Populus deltoides* (EASTERN COTTONWOOD)

**Abstract**

The effect of solar ultraviolet (UV) radiation (280-400 nm) on the competitive interaction between seedlings of *Populus deltoides* was investigated in a replicated greenhouse study. Plants were grown at two densities (one or two plants per pot) in a factorial design with two UV conditions (with and without UV). After three months, seedlings grown without UV radiation had more root mass and less leaf area, specific leaf area, and leaf area ratio than seedlings grown with UV radiation. There was an interactive effect between UV exclusion and competition on height growth and on final shoot, leaf, and total dry weight. Those traits were negatively affected by UV radiation when seedlings were grown alone, but were unaffected by UV radiation when seedlings were grown in competition. These results indicate that the stress induced by intraspecific competition masked the effects of the UV stress on seedlings of *P. deltoides* in this experiment.

*Key Words*: UV exclusion, greenhouse study.

**Introduction**

Competition for resources aboveground and belowground is considered a central force maintaining local diversity and controlling the structure of plant communities (Wilson and Tilman 1993). Plant competition is influenced by biotic and abiotic factors, and those factors can be especially important at the seedling stage, which usually is
considered to be the most sensitive stage of plant development (Shimono and Kudo 2003). Since both biotic and abiotic factors influence plant-plant interactions, probably at the same time, it is important to understand the nature of such interactions, i.e., additive, synergetic or antagonistic (Chen et al., 2008; Goodwin, 1992; Lewis & Tanner, 2000). The interrelationships between biotic and abiotic factors depend, in part, upon the particular species involved, and may play a far more important role influencing plant-plant interactions than do individual factors.

Ultraviolet radiation (UV) is an important type of plant stress (Paul & Jones 2003). However, many of the experiments on the effects of UV on plants have focused on the effects of UV-B irradiance above current ambient levels and much less is known about the importance of ambient levels of solar UV radiation (Yuan et al., 1999; Gold and Caldwell 1983). That is important since climate change can affect the levels of ambient UV radiation through changes in cloudiness and albedo without involving ozone (McKenzie et al., 2006). Additionally, most of the studies of the effect of UV-B on plant morphology and growth are focused in the interspecific responses and less is known about intraspecific responses of plants to UV radiation. This aspect is interesting since different phenotypes can differ in their response to UV radiation (Caldwell and Flint 1994; Hofmann et al., 2001).

The objectives of the present study are (1) to determine the effects of competition and UV radiation on vegetative growth in seedlings of Populus deltoides and (2) to evaluate the effect of the interaction of both factors on vegetative growth of P. deltoides seedlings. In the present study, three hypotheses were tested: (1) the exclusion of UV
radiation will alter growth, morphology and allocation of seedlings of *P. deltoides*, (2) competition will result in reduced growth of seedlings, and (3) seedlings grown in competition and exposed to UV radiation will grow less than seedlings grown in competition but without UV radiation.

**Materials and methods**

**Plant description**

*Populus deltoides* Bartram. ex Marsh (Salicaceae, eastern cottonwood) is a very shade intolerant species that requires exposed sites, usually in riparian areas, for seedlings establishment. *Populus deltoides* has been a subject of intensive research for its end use in timber and paper-pulp industries and as a model bioenergy crop (Bradshaw et al. 2000).

**Plant cultivation**

Seeds of *P. deltoides* were collected from different localities in Ames, Iowa (42°02′05″N 93°37′12″W, 287 m elevation). Mixed seeds were sown in cone-shaped pots (Ray Leach Cone-tainers: 0.15 L volume, 205mm length, 40 mm top diameter; Stuewe and Sons, Corvallis, Oregon, USA) containing a 1:1 sand-soil mixture that was steam autoclaved for 90 min. The experimental design was factorial, with two levels of UV radiation (with or without UV), two growth densities (one or two seedlings per pot), and two greenhouses; there were eight replicates. The Cone-tainers were randomly arranged and rotated once a week within each greenhouse.
Supplemental UV-A/B radiation was provided daily over a 10-h period centered at solar noon using Q-Panel UV-A 340 fluorescent lamps (ranging from 365 nm to 295 nm with peak emission at 340 nm, Q-Panel, Cleveland, Ohio) mounted on wood frames and suspended 100 mm above 6 m × 0.9 m PVC frames covered with cellulose diacetate film, which transmits both UV-B and UV-A radiation. For the UV-exclusion treatment (–UV), pots were placed inside 6 m × 0.9 m PVC frames tented with polyester film, which excludes both UV-B and UV-A radiation. UV-A/B irradiance was measured with a Solarmeter® Digital Ultraviolet Meter, model 5.7 UVA + B Sensitive Microwatt Version (Solartech, Inc., Harrison Township, MI), at the top of canopy at midday. Under cellulose diacetate, the mid-day UV-A/B radiation averaged $15 \pm 0.9 \text{ W m}^{-2}$; under polyester film it was $0 \text{ W m}^{-2}$. The mid-day photosynthetic photon flux density (PPFD, 400-700 nm) under clear sky conditions, measured under the cellulose diacetate film averaged $1325 \pm 52 \mu \text{mol m}^{-2} \text{s}^{-1}$. The mid-day PPFD under polyester film at the top of the plants averaged was $1438 \pm 97 \mu \text{mol m}^{-2} \text{s}^{-1}$.

**Growth measurements**

Height and number of leaves of all seedlings were measured every 15 days, and plants were harvested after 3 months. At harvest, each seedling was partitioned into leaves, stem and roots. The statistical software R-2.15.1 was used for all statistical analyses, and the significance level was set at 0.05. Permutational multivariate analysis of variance (PERMANOVA) was used to identify significant effects of UV exclusion, competition, greenhouse and their interactions. Permutation tests for factorial ANOVA
were performed to help to determine which variables contributed to any significant differences observed in the multivariate analysis. The Tukey HSD test was employed to test for differences among means. The variables tested were number of leaves, height, root dry weight (RDW), shoot dry weight (SDW), leaf dry weight (LDW), total dry weight (TDW), leaf area (LA), leaf area ratio (LAR), specific leaf area (SLA), and root to shoot ratio (R: S).

**Results**

The results of PERMANOVA showed that the suite of dependent variables were significantly affected by UV radiation and competition ($F_{2,90} = 17.8$, $P ≤ 0.01$, $F_{2,90} = 9.9$, $P ≤ 0.01$). Compared with seedlings grown in the presence of UV radiation, *P. deltoides* seedlings grown without UV radiation had lower leaf areas, specific leaf areas, and leaf area ratios (Table 4). In contrast, there was significantly greater root dry weight for seedlings grown under UV exclusion compared with those grown with UV. There were no effects of UV on number of leaves, height, shoot dry weight, total dry weight or R: S.

At harvest, seedlings grown in competition had lower leaf area, height, biomass, and number of leaves compared with seedlings grown alone. There was no difference in LAR or SLA between competition treatments (Table 4). There was an interactive effect between UV and competition on height (Fig. 6, Table 4), shoot dry weight, leaf dry weight and total dry weight (Fig. 7, Table 4). Those variables had greater values when seedlings were grown alone without UV radiation, followed by seedlings grown alone...
with UV radiation. Seedlings grown in competition showed no significant differences in growth parameters in response to UV radiation.

**Discussion**

In the current experiment, leaf growth and morphology varied between UV treatments, with plants in the UV-exclusion treatment showing lower SLA and LAR compared with plants grown with UV radiation. These results partial support hypothesis (1) that the exclusion of UV radiation would affect growth, morphology and allocation of seedlings of *P. deltoides*. The results indicate that ambient levels of UV radiation have a significant effect in the leaf morphology of seedlings of *P. deltoides*.

Seedlings grown under competition had lower leaf area, total dry weight, height, leaf dry weight, shoot dry weight, root dry weight, and number of leaves compared to seedlings grown alone. This result supports the hypothesis (2) that competition would result in reduced growth of seedlings. The SLA, LAR, and shoot to root ratio were not affected by competition. The index SLA is associated with important aspects of plant growth and survival and has been proposed as an indicator of plant resource use (Li et al. 2005). The lack of a significant effect of competition on SLA is in accordance with other experiments that show that the SLA is not significantly affected by competitive conditions (Knezevic et al. 1999). In general, the SLA is affected by the light and temperature regime under which plants are grown (Knezevic et al. 1999 and references therein).
The results here showed that competition did not result in a change in the root to shoot ratio compared to seedlings grown alone. Although it is assumed that plants increase allocation to roots in response to belowground competition (e.g., Wilson & Tilman, 1993), some studies indicate that those changes are due to changes in plant size (Müller et al. 2000; Cahill 2003).

There were interactions between UV exclusion and competition on height, leaf dry weight, shoot dry weight, and total dry weight. When seedlings were grown in competition those variables were not affected by UV radiation, but when seedlings were grown alone the presence of UV radiation resulted in lower values for those variables. These results do not support hypothesis (3) that seedlings grown in competition and exposed to UV radiation would grow less than seedlings grown in competition but without UV radiation. Thus, there was not an additive effect between competition and UV radiation for seedlings of *P. deltoides*. These results suggest that the intense physicochemical stresses due to competition may negate the deleterious effects of the UV stress on seedlings *P. deltoides*.

**Acknowledgements**

This research was done in partial fulfillment for a Ph.D. in the Department of Ecology, Evolution, and Organismal Biology at Iowa State University. The author is extremely grateful to Drs. James Raich, Thomas Jurik, and Brian Wilsey for manuscript review. Support was provided by the Department of Ecology Evolution and Organismal Biology of the Iowa State University.
**Table 4.** Summary of statistical analyses of UV effects on *P. deltoides* seedlings grown in competition. Statistical values tabulated are *F* ratios from ANOVAs testing for the main effect of UV radiation (UV), presence or absence of a conspecific competitor (Comp), and the interaction between them (UV x Comp); degrees of freedom are in parenthesis. Significance levels with Bonferroni correction by analysis of variance (**p<0.0001; **P<0.001; *P<0.005).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UV (1)</th>
<th>Comp (1)</th>
<th>UV x Comp(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>-</td>
<td>41.09**</td>
<td>14.5*</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LA(cm$^2$)</td>
<td>-</td>
<td>39.82**</td>
<td>-</td>
</tr>
<tr>
<td>Root dry weight (g)</td>
<td>-</td>
<td>29.8**</td>
<td>-</td>
</tr>
<tr>
<td>Leaf dry weight (g)</td>
<td>-</td>
<td>35.05**</td>
<td>14.58*</td>
</tr>
<tr>
<td>Shoot dry weight (g)</td>
<td>-</td>
<td>56.63**</td>
<td>17.48*</td>
</tr>
<tr>
<td>Total dry weight (g)</td>
<td>-</td>
<td>40.85**</td>
<td>14.4*</td>
</tr>
<tr>
<td>SLA (cm$^2$.g$^{-1}$)</td>
<td>22.06**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LAR (cm$^2$.g$^{-1}$)</td>
<td>23.06**</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Only terms that are significant at p<0.005 are shown.
Figure 6. Effects of ultraviolet radiation and presence of a competitor on seedling growth parameters of *P. deltoides* 3 months after sowing. Each value is a mean ± SE. Means with different letters are significantly different (*P* < 0.005, post-ANOVA Tukey test).
Figure 7. Effect of AM colonization and UV radiation and on height of *B. nigra* seedlings. Each point represents the mean; vertical bars denote ± SE. The different letters below the same date indicate statically significant differences among treatments at the P <0.05 level in Tukey’s HSD test.
References


CHAPTER 4. COMPETITIVE INTERACTIONS BETWEEN THREE SHADE INTOLERANT TREE SPECIES UNDER MUTUAL EXPOSURE TO UV RADIATION AND MYCORRHIZAL COLONIZATION

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Abstract

1. Although some studies indicate that either UV-B radiation or mycorrhizal colonization can affect competitive interactions between plants, little is known about the effects of ambient levels of solar UV radiation on plant-plant competition and even less is known about effects of UV radiation on mycorrhizal colonization, which may affect competitive interactions between plants.

2. In this study, seedlings of three shade intolerant tree species (Populus deltoides, Betula nigra, and Salix nigra) were evaluated in competition under exposure to UV radiation and mycorrhizal colonization. After a twelve-week treatment period, growth, morphology, allocation parameters as well as percentage of mycorrhizal colonization were evaluated.

3. Ultraviolet radiation induced changes in leaf morphology and biomass allocation in all three species, but had no significant effect on seedling growth. Mycorrhizal colonization negatively affected growth of P. deltoides. Ultraviolet radiation reduced the percentage of root colonized by AM fungi in P. deltoides in all three
experiments, but not in the other species. Neither AM colonization nor UV radiation affected the outcome of plant competition in any of the three controlled experiments conducted.

4. Ambient levels of UV radiation can affect leaf morphology and allocation of seedlings biomass and can reduce mycorrhizal colonization levels. However, it is not clear if such changes are enough to have a substantial impact on competitive interactions between those co-occurring species.

Introduction

The study of interactions among plants is fundamental to plant ecology. There are many types of interactions among plants; some of them are positive (facilitation and mutualism), whereas others are negative (parasitism, competition, and allelopathy). Competitive interactions are considered to be a central force maintaining local diversity and controlling the structure of plant communities (Latham 1992; Wilson and Tilman 1993; Davis et al. 1998; Aerts 1999). Due to this viewpoint much research has been devoted to understanding the nature of competitive interactions among plants (Grime 1977; Fitter 1977; Grace and Tilman 1990; Latham 1992; Wilson, S.D. and Tilman 1993; Bazzaz 1996; West 1996; Aerts 1999; Hart et al. 2003). The results of those and other investigations indicate that the outcome of competitive interactions can be influenced by biotic factors such as herbivores, pathogens and mutualists; and by abiotic factors such as nutrient availability and light (Keeler and Holt 1990, Bazzaz and McConnaughay 1992, Wedin and Tilman 1996, Alexander and Holt 1998, Olofsson et
al. 2002, van der Heijden et al. 2003). Since both biotic and abiotic factors affect plant competition, likely at the same time, it is important to understand the nature of their inter-relationships. Such interactions may play a more important role in determining the outcomes of plant-plant interactions than single factors, yet are far less frequently studied.

Mycorrhizae are one of the most widespread plant-fungus mutualistic symbioses on land. The relationship is based on the plant providing carbohydrates and other organic compounds to the fungi, and the fungi enhancing plant uptake of nutrients and water (Habte 2000). Different types of mycorrhizal association occur, distinguished by their specific partnerships, morphology and physiology. These include the ectomycorrhizae (EM) and arbuscular mycorrhizae (AM) (Turk et al. 2006). In EM associations, the fungi invade the cortical region of the host root without penetrating cortical cells; a thick layer of hyphae covers the root surface forming what is known as the sheath or mantle. The AM fungi form no sheath; the fungi penetrate the cortical cells and form clusters of finely divided hyphae known as arbuscules inside the cells within the cortex. They also form vesicles inside or outside the cortical cells (Habte 2000). Since competition for soil resources can lead to a reduction in plant size, and mycorrhizae often improve plant growth by enhancing nutrient supply to the plant, it could be expected that mycorrhizal colonization can change the outcome of competitive interactions between plants (Facelli et al. 1999). Indeed, several studies have shown that AM associations influence plant-plant competition (e.g., Fitter 1977; West 1996; Marler et al. 1999; Guadarrama and Alvarez-s 2004; Scheublin et al. 2007; Daisog et al. 2011; Birhane et al. 2013). These
studies usually report that the plant species that is most responsive to AM colonization is favored in competition with a less responsive species. However, most previous studies focused on crop or grass species, and evaluated pairs of species differing in responsiveness to the mycorrhizal association (Schroeder-Moreno and Janos 2008). Alternatively, hyphae that connect the roots of different plants might reduce the intensity of intraspecific competition by allowing a more even distribution of nutrients (Facelli et al. 1999). There still is limited knowledge about the effect of AM colonization on interactions among co-occurring mycotrophic species.

Although ultraviolet (UV) radiation (200–400 nm) is a minor fraction of the total solar irradiance, it is considered an important type of abiotic stress factor (Paul and Gwynn-Jones 2003). The UV spectrum is conventionally divided into three wavebands: UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm). However, UV-C does not reach the Earth’s surface and much of the UV-B is absorbed by stratospheric ozone (Frederick 1993). Plant responses to UV radiation include reduced plant height, stem length elongation, leaf area, leaf elongation, root length, rhizome internode elongation, and changes in the number of leaves, branches or tillers (Furness et al. 2005; Caldwell et al. 2007). Changes in plant morphology are often accompanied by modification in the partitioning of biomass (Bassman et al. 2001). The above-described changes in plant growth, morphology and allocation in response to UV radiation stress are usually attributed to (i) UV-B- induced changes on auxin metabolism (Jansen 2002), (ii) a shift in carbon allocation toward the production of UV-B-absorbing compounds (Schumaker et al. 1997, Warren et al. 2003, Kotilainen et al. 2008, Morales et al. 2010);
and (iii) changes in leaf expansion as a result of UV-B induced increases in cell-wall peroxidase activity (Tegelberg et al. 2001; Wargent et al. 2009; Robson and Aphalo 2012). As is the case with mycorrhizas, evidence indicates that UV radiation can change the balance of competition between plant species. For instance, Barnes et al. (1988) report that the competitive balance between wheat (*Triticum aestivum* L.) and wild oats (*Avena fatua* L.) shifts to favor wheat under UV-B enhancement, and similar results were reported by Yuan et al. (1999). Similarly, Rinu (2007) found that enhanced UV-B altered the competitive interaction of *Lolium perenne* with *Lotus corniculatus* in favour of *Lolium perenne*. There are two general hypotheses for explaining UV-induced changes in competitive interactions. One is that differential sensitivity of species to UV radiation could result in one species benefiting in the competition for limited resources. An alternate is that UV-B may affect the competitive balance indirectly, by altering the morphology of one or both species without directly influencing photosynthesis (Barnes et al. 1988).

While such studies shed some light on the effects of UV-B on plant competitive interactions, there are intriguing aspects about UV radiation and plant-plant interactions that should be addressed. For instance, plant interactions in response to ambient levels of solar UV radiation (280–400 nm) are not well known. That is important since climate change can affect the levels of ambient UV radiation through changes in cloudiness and albedo without involving ozone (McKenzie et al. 2007). Another intriguing aspect is the possibility of indirect effects of UV radiation on mycorrhizal infection, which could have an effect on interactions between plants (Zaller et al. 2002).
The objectives of this study were: (i) to evaluate the individual effects of UV radiation and mycorrhizal symbiosis on competitive interactions between seedlings of *Populus deltoides* (eastern cottonwood), *Betula nigra* (black willow), and *Salix nigra* (river birch), three naturally coexisting shade intolerant tree species, and (ii) to evaluate the effect of the interaction between these two factors on competitive interactions between co-occurring plants. In this study, the working hypotheses were that (1) UV radiation alters the growth, allocation, and morphology of seedlings in competition, (2) mycorrhizal inoculum alters the growth, allocation, and morphology of seedlings in competition; (3) the identity of competitor affects the growth of seedlings, (4) UV radiation negatively affects the rate of mycorrhizal colonization, and (5) the interaction between UV radiation, mycorrhizal inoculum and identity of competitor alters the growth of seedlings.

**Materials and Methods**

**Plant materials and growth conditions**

The species evaluated occur as pioneer trees in riparian habitats throughout the eastern half of the United States (Jurgensen et al. 1996). *Salix nigra* Marshall (black willow, Salicaceae) is a very shade intolerant, fast-growing tree species common on river margins, in swamps, sloughs, and swales, and on the banks of bayous, gullies, and drainage ditches (Burns and Honkala 1990). *Populus deltoides* Bartram. ex Marshall (eastern cottonwood, Salicaceae) is a very shade intolerant species that grows on exposed sites, often in riparian areas. *Betula nigra* L. (river birch, Betulaceae) is
considered a shade intolerant species that is common on bottomlands along stream banks and in wet areas (Coyle and Sharik 1982).

To test the hypotheses, three experiments were conducted in greenhouses of the Iowa State University, Ames, IA. Seeds of *P. deltoides*, *S. nigra* and *B. nigra* were gathered from different localities in Ames (42°02′05″N 93°37′12″W, 287 m elevation). Additional seeds of *P. deltoides* were collected from different localities in Saverton, Missouri (39°38′47″N 91°16′06″W, 196 m elevation). All seeds were sown in conical containers (0.15 L volume, 205mm length, 40 mm top diameter; Stuewe and Sons, Corvallis, Oregon, USA) containing a 1:1 sand-soil mixture with pH 6.3 (in water) that was steam autoclaved for 90 min. After germination the number of seedlings per pot was reduced to two according to the competition arrangement. Due to differences in time to emergence and in order to start the experiments with seedlings of similar size (1.5 – 1.7 cm), for Experiment Two, seeds of *S. nigra* were sown twenty days earlier than seeds of *P. deltoides*. For Experiment Three, seeds of *B. nigra* were sown ten days earlier than *P. deltoides* seeds.

**Ultraviolet radiation treatments**

Since the greenhouse glass partially attenuated incoming solar UV radiation, supplemental UV-A/B radiation provided by fluorescent lamps was used to provide a treatment approximating ambient UV insolation. Q-Panel UV-A 340 fluorescent lamps (365 nm to 295 nm, with peak emission at 340 nm, Q-Panel, Cleveland, Ohio) provided supplemental UV radiation over a 10-h period daily centered at solar noon. The lamps
were suspended 100 mm above PVC frames covered with 0.12 mm cellulose diacetate film, which transmits both UV-B and UV-A radiation. For the UV-exclusion treatment (-UV), seedlings were placed inside PVC frames covered with 0.3 mm polyester film, which removes both UV-B and UV-A radiation. UV-A/B insolation was measured with a Solarmeter® Digital Ultraviolet Meter, model 5.7 UVA + B Sensitive Microwatt Version (Solartech, Inc., Harrison Township, Michigan, USA), at the top of canopy (below the films) at midday every two weeks over the course of each experiment. Midday photosynthetic photon flux density (PPFD, 400-700 nm) was measured under the cellulose diacetate and polyester films using a Field Scout Quantum Meter® (Spectrum Technologies Inc., Plainfield, Illinois, USA). Measurements were taken at the top of the plants every two weeks. To minimize the effects of micro-environmental variation, position of plants within the treatments were rotated daily.

**Mycorrhizal inoculation**

Arbuscular mycorrhizal inoculation was carried out by adding 6 g of SYMBIVIT® Endomycorrhiza (Symbiom, Lanskroun, Czech Republic), containing a mixture of fragments of colonized roots, mycelia and spores of *Glomus mosseae*, *G. microagregatum*, *G. claroideum*, *G. intraradices*, *G. entunicatum*, and *G. geosporum*. Control seedlings received the same amount of autoclaved SYMBIVIT®.

Ectomycorrhizal colonization was established by adding 6 g of ECTOVIT® (Symbiom, Lanskroun, Czech Republic), which consisted of a mixture of fragments of colonized roots, mycelia and spores of *Sclerodema* spp., *Pisolithus* spp., *Lactarius* spp., *Hebeloma*
spp., and *Laccaria* spp. Control seedlings received the same amount of autoclaved ECTOVIT®.

**Experimental design**

Experiment One was designed to investigate the effects of UV radiation and mycorrhizal colonization (both AM and EM) on intraspecific competition between *P. deltoides* seedlings from two provenances, Ames, Iowa and Saverton, Missouri. The experimental design was a factorial, with two levels of UV radiation (with and without UV), two competition arrangements (inter-provenance and intra-provenance), two AM mycorrhizal treatments, two EM treatments and two greenhouses; there were four replicates. The inoculation treatments were AM fungus, EM fungus, and steam autoclaved inoculum. Experiment One ran from July to September 2012. The environmental conditions are summarized in Table 5.

Experiments Two and Three were designed to investigate the effects of UV radiation and mycorrhizal colonization on interspecific competition. Experiment Two ran from August to October 2012; the competing pair consisted of *P. deltoides* and *S. nigra*. The experimental design was a factorial, with two levels of UV radiation (with and without UV), two competition arrangements (interspecific and intraspecific), two AM conditions (alive inoculum and autoclaved inoculum), and two greenhouses. Experiment Three ran from April to June 2013; the competing pair consisted of *P. deltoides* and *B. nigra*. The experimental design was the same described for experiment Two.
Growth measurements

The number of leaves and heights of each seedling were recorded every 15 days; plants were harvested after 3 months. At the end of the experiment, number of leaves, stem height, stem diameter above the root collar, and the dry biomass of leaves, stems and roots were measured. Leaves were scanned prior to drying and total seedling leaf area (LA) was determined using Compu Eye, Leaf & Symptom Area® software (Bakr 2005). The samples were dried in an oven at 70 °C for at least 48 h before the biomass of leaves, stem, and roots were determined. The same growth parameters were calculated for each plant (Table 6).

Mycorrhizal colonization

At harvest, root subsamples weighing 1-2 grams were removed and stored in the freezer. Each sample consisted of three subsamples, from shallow, middle, and deep roots. The dry weight of each sample used for mycorrhizal quantification was estimated and added to the total root weight using each mycorrhizal root sample’s fresh weight and the corresponding root mass’s fresh weight/dried weight. Samples were cleared in hot 2.5% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue in acid glycerol (Robertson et al. 1999); samples were stored in a 1:1:1 solution of water, glycerin, and lactic acid. To quantify mycorrhizal colonization, each root sample was spread across the bottom of an 8.5 cm diameter gridded petri dish and examined under a dissecting microscope. Each intersection between a line and a root was classified as mycorrhizal or non-mycorrhizal. Three sets of observations were made recording all the root/gridline
intersects; each of the repeated counts was made on the same re-distributed sample (Giovannetti and Mosse 1980). The percent of root colonization was calculated as the ratio between number of AM intersections and number of total intersections multiplied by 100. For assessment of EM colonization, root samples were cut as aforementioned. The samples were then placed in Petri dishes in water for the examination of the tips under a dissecting microscope. EM roots were distinguished from non-mycorrhizal root tips by differences in their color and form (Brundrett et al. 1996).

**Statistical analyses**

Statistical software R-2.15.1 was used for analyses, and the significance level was set at 0.05 (see Supplemental Information). Permutational multivariate analysis of variance (PERMANOVA) was applied to identify significant effects of UV radiation, competitor identity, mycorrhizal inoculum and their interactions. All variables were also analyzed further using Permutation tests for factorial ANOVA (Manly 2007) to help determine which variables contributed to any significant differences observed in the multivariate analysis. The Tukey HSD post-hoc test was employed to detect possible differences among means. No significant effects of greenhouse were observed in any of the experiments, so that factor was removed from consideration and the statistical models were repeated without it. Kruskal-Wallis test and ANOVA were used to determine the effect of UV radiation on EM and AM colonization. The growth, morphology, and allocation variables tested are described in Table 6. To determine the effects of UV radiation on mycorrhizal colonization, the percentage of root colonization
were analyzed using ANOVA. All AM colonization data were arcsin-square root transformed prior to analysis. Non-transformed data are presented in the figures. In case of EM colonization the data did not meet the parametric assumptions after transformation and the Kruskal-Wallis test was used.

**Results**

**Experiment One**

The results of the PERMANOVA for *P. deltoides* seedlings from Ames showed that the response variables evaluated (Table 6) were significantly affected by UV radiation and by AM colonization (PERMANOVA, \( F_{2,96} = 27.9, P \leq 0.05; \ F_{2,96} = 5.5, P \leq 0.01 \)). In the case of seedlings from Saverton, the results of the PERMANOVA showed that the response variables were significantly affected by UV radiation, AM, and identity of competitor (\( F_{2,96} = 41, P \leq 0.01; \ F_{2,96} = 6.5, P \leq 0.05; \ F_{2,96} = 5.7, P \leq 0.01 \)). The results of ANOVA showed that there were no interactions for any of the response variables evaluated (Table 7). Seedlings from Ames and Saverton grown with UV radiation had greater specific leaf areas (SLA) and greater leaf area ratios (LAR) (Fig. 8, Table 7). Seedlings from Saverton grown in the presence of UV radiation also had lower root dry weights and root-shoot ratios (Fig. 8, Table 7).

Ectomycorrhizal colonization was found in all EM-inoculated seedlings, and no EM structures were found in the non-mycorrhizal treatment. However, levels of EM colonization were low in both provenances, with an average of 8% for seedlings from Ames and 6% for seedlings from Saverton. The EM colonization had no effect on any of
the variables evaluated (Table 7). All plants in the AM treatment were colonized by AM fungi, and no AM structures were found in roots from non-mycorrhizal treatment. Seedlings from both provenances had high levels of AM colonization (Fig. 11). Compared with un-inoculated plants, AM-colonized seedlings from Ames had significantly greater leaf areas and leaf dry weights (P<0.005; P<0.005). In contrast, seedlings from Saverton colonized by AM fungi grew less tall (P<0.005).

The identity of competitor affected the growth of seedlings from Saverton, but not of seedlings from Ames (Table 7). Seedlings from Saverton seed that were grown in competition with seedlings from Ames had less biomass, less leaf area, shorter heights, and smaller stem diameters than those grown in competition with seedlings from Saverton (Fig. 8, Table 7).

AM inoculated seedlings from Ames grown without UV radiation had a higher extent of AM colonization than did inoculated seedlings grown in the presence of UV (F=8.66 P<0.01), but this effect was not significant for seedlings from Saverton (Fig. 11). The UV radiation treatment had no significant effects on EM colonization either for seedlings from Ames (\(\chi^2=2.48, \text{df}=1, P=0.11\)) or seedlings from Saverton (\(\chi^2=1.5, \text{df}=1, P=0.22\)).

**Experiment Two**

The PERMANOVA analysis for seedlings of *P. deltoides* showed that seedling growth was significantly affected by UV radiation (\(F_{2,96}=5.46, P<0.01\)) and by AM inoculum (\(F_{2,96}=17.93, P<0.01\)). In the case of *S. nigra* seedlings, the results of the
PERMANOVA showed that the response variables were significantly affected by UV radiation ($F_{2,96}=10.16, P \leq 0.01$), and by identity of competitor ($F_{2,96}=9.30, P \leq 0.01$). Seedlings of *P. deltoides* grown with UV radiation had greater specific leaf area and leaf area ratio, as in Experiment One, and less root dry weight (Fig. 9, Table 8). Similarly, seedlings of *S. nigra* grown exposed to UV radiation had greater SLA and LAR (Fig. 9, Table 8). Seedlings of *P. deltoides* grown in competition with *S. nigra* had lower values for response variables than when grown in intraspecific competition except for leaf area ratio and specific leaf area (Fig. 9, Table 8). In contrast, *S. nigra* had higher values in response variables when grown under interspecific competition than when grown in intraspecific completion, except leaf area ratio and specific leaf area, which were lower (Fig. 9, Table 8).

Mycorrhizal colonization was found in seedlings of both *P. deltoides* and *S. nigra* under the AM treatment, whereas seedlings grown without inoculation had no AM colonization. Seedlings of *S. nigra* had low levels of AM colonization; 8% on average (Fig. 11). AM inoculum resulted in more root biomass of *P. deltoides*, but was not a significant factor for *S. nigra*. There was a significant interaction between AM inoculum and identity of competitor on leaf area ratio in seedlings of *P. deltoides*: AM inoculum enhanced the leaf area ratio of *P. deltoides* seedlings grown in competition with seedlings of *S. nigra* (Fig. 9, Table 8).

Inoculated seedlings of *P. deltoides* grown under UV exclusion had higher levels of AM colonization than seedlings grown with UV ($F_{2,96}=4.33 P<0.01$). Seedlings of *S.
*B. nigra* had low levels of AM colonization in all treatments (Fig. 11), and UV radiation had no effect on AM colonization levels (ANOVA, $F_{2,96}=2.46$ $P=0.12$).

**Experiment Three**

The results of the PERMANOVA showed that response variables of *P. deltoides* were significantly affected by UV radiation ($F_{2,58}=32.5$, $P \leq 0.01$). In the case of *B. nigra*, response variables were significantly affected by UV radiation (PERMANOVA $F_{2,58}=12.76$, $P \leq 0.01$), and by identity of competitor ($F_{2,58}=7.47$, $P \leq 0.01$). Seedlings of *P. deltoides* grown with UV radiation had smaller stem diameters, less root dry weight and less total dry weight than did seedlings under UV exclusion. As in both previous experiments, seedlings exposed to UV had higher specific leaf areas and leaf area ratios than those seedlings grown without UV radiation (Fig. 10, Table 9). In the case of *B. nigra*, UV radiation resulted in higher specific leaf area and leaf area ratio, and smaller shoots (Fig. 10, Table 9). The identity of competitor did not affect response variables of *P. deltoides* (Table 9). On the other hand, *B. nigra* grown in competition with *P. deltoides* had significantly lower values of response variables than when grown with conspecifics, except for leaf area ratio and specific leaf area (Fig. 10, Table 9). Additionally, there was an interaction between UV radiation and identity of competitor for height in seedlings of *B. nigra*. Exclusion of UV radiation resulted in less height growth by seedlings of *B. nigra* when grown with *P. deltoides*.

The average AM colonization rate was 40% for *P. deltoides* and 34% for *B. nigra*; no seedlings grown without AM inoculum had any mycorrhizae. Similarly to
Experiment One, AM inoculum significantly depressed height growth by *P. deltoides* (*P*<0.005) but did not affect *B. nigra* (Table 9). The percentage of AM colonization of *B. nigra* was affected by competitor (*F*<sub>2, 58</sub>= 5.6, *P*=<0.05). Seedlings of *B. nigra* grown in competition with their conspecifics showed higher AM colonization (40%) than when grown in competition with *P. deltoides* (28%).

The exclusion of UV radiation significantly increased the levels of AM colonization in *P. deltoides* (ANOVA *F*<sub>2, 58</sub>= 4.38, *P*<0.05) whereas it had no effect on AM colonization of *B. nigra* (ANOVA *F*<sub>2, 58</sub>= 1.19, *P* = 0.28).

**Discussion**

**Effects of UV radiation on plant growth, allocation and morphology**

Leaf morphology and seedling biomass allocation varied between UV treatments in each of the three experiments. In all cases, seedlings exposed to UV radiation had higher mean specific leaf areas (SLA) and leaf area ratios (LAR) than did seedlings grown under UV exclusion. These results partially support hypothesis (1), i.e., that UV radiation would alter the attributes of seedlings grown in competition. Specific leaf area provides a measure of leaf thickness and density. Different studies indicate that SLA is particularly sensitive to changes in environmental factors and in plant functioning (Milla et al. 2008 and references therein). Leaf area ratio provides a measure of photosynthetic surface relative to respiratory mass. The LAR is a composite parameter that includes a morphological component (SLA) and an allocation component (leaf weight ratio, LWR). Since UV radiation had no significant effects on LWR, observed variations in LAR were
related to variations in SLA. These results are in accordance with Poorter et al. (2012) who, based on a meta-analysis, found that plant variations in LAR resulted most commonly from adjustments in leaf morphology rather than allocation. Effects of UV radiation on seedling growth and allocation were less consistent. In the case of *P. deltoides*, in addition to changes in LAR and SLA, seedlings grown with UV radiation had less root biomass than did seedlings growth with UV, in three of four comparisons, and had less total biomass in experiment three. The reduction in root biomass is in agreement with some studies indicate that root mass is a growth parameter that is sensitive to UV radiation in plants of genus *Populus* (Bassman et al. 2001; Ren et al. 2006). These results indicate that *P. deltoides* was the species most sensitive to UV radiation stress. Neither *S. nigra* nor *B. nigra* exhibited changes in biomass accumulation or allocation when grown exposed to UV radiation.

**Effects of Mycorrhizas on plant growth, allocation and morphology**

The effects of AM colonization on seedling growth were inconsistent among experiments and species. Although seedlings of *P. deltoides* had AM colonization levels higher than 50% in both provenances, AM colonization did not result in the expected increase in growth. Similarly, although in Experiments Two and Three *P. deltoides* and *B. nigra* had AM colonization levels higher than 34%, mycorrhizal colonization did not result in increased growth. These results do not support hypothesis (2), that mycorrhizal colonization alters the growth, allocation, and morphology of seedlings grown in competition. Although positive growth responses are usually associated with
mycorrhizal associations, some authors point out that those associations can vary in a continuum from mutualistic to parasitic depending on a cost-benefit trade-off (Johnson and Graham 1997; Schroeder and Janos 2004). The negative effects of mycorrhizas on some variables here suggest a parasitic relationship.

When subjected to intraspecific competition, the two provenances of *P. deltoides* responded differently from each other. Seedlings from Saverton grown in competition with seedlings from Ames produced significantly less biomass than those grown in competition with seedlings from their same provenance. This result suggests differences in the competitive ability of seedlings from the two provenances. Similarly, the results of Experiment Two showed that identity of competitor affected species growth, morphology and allocation. When grown in interspecific competition with *S. nigra*, seedlings of *P. deltoides* showed lower total biomass than when grown under intraspecific competition. Conversely, *S. nigra* showed higher total biomass when grown in competition with *P. deltoides*. This result suggests that *S. nigra* benefited at the expense of *P. deltoides*. Additionally, AM colonization increased LAR for *S. nigra* when grown under interspecific competition. In Experiment Three the identity of competitor was significant for *B. nigra* seedlings, which had lower biomass when grown in competition with *P. deltoides* than when grown with conspecifics, suggesting that *P. deltoides* was a better competitor. Further, UV radiation depressed height growth of *B. nigra* seedlings when grown in competition with *P. deltoides*. These results support hypothesis (3) that the identity of competitor affects the growth of seedlings and indicate variable patterns of species response to competition.
**Interactive Effects**

All three tree species had higher levels of AM mycorrhizal colonization when grown without UV radiation; in *P. deltoides* that increase was significant (Fig. 11). This result supports hypothesis (4), that UV radiation negatively affects the levels of mycorrhizal colonization. The negative effect of UV radiation on AM colonization found here agrees with results reported for *Acer saccharum* (Klironomos and Allen 1995), *Carex* spp. (Zaller et al. 2002) and *Carex arenaria* and *C. epigeios* (van de Staaij et al. 2001) in response to elevated UV-B radiation. These species showed a reduction in the rate of mycorrhizal colonization that was accompanied by a reduction in the number of arbuscules, which are considered the interface between the plant and fungus and are the sites where the exchange of nutrients occurs (Sanders and Croll 2010). The positive effect of UV exclusion on mycorrhizal colonization can be explained as a result of more resources available for mycorrhizal formation. Since plants typically respond to UV radiation by producing screening pigments, a reduction in the concentration of such pigments as a result of the exclusion of UV radiation would result in more resources available for mycorrhizal formation.

This study was designed to evaluate the effects of UV radiation and mycorrhizal colonization on competitive interactions between co-occurring shade intolerant species. Interestingly, although the results of other studies indicate that UV radiation and mycorrhizal colonization may individually affect the balance of competition between plants, neither AM colonization nor UV radiation affected the outcome of plant competition in any of the three controlled experiments conducted here. The results of the
experiments here showed significant direct effects of UV radiation on leaf morphology, a few significant effects of AM colonization on growth, exclusively in *P. deltoides*, and a few significant interactions between those factors and the identity of the competitor. Although, there was interaction between UV radiation and mycorrhizal colonization, none of the experiments showed significant interactions based on growth variables. These results do not support hypothesis hypothesis (5), i.e., that interactions between UV radiation, mycorrhizal colonization and identity of competitor would alter the growth of seedlings.

Since plant competition is manifested through an increase in physicochemical stresses such as water or nutrient stress, the results indicated that the high degree of total competitive stress in the experimental growth conditions masked the effects of the UV radiation stress and AM colonization on plant growth. These responses could be interpreted as a manifestation of cross-tolerance (Tippmann et al. 2006). The cross-tolerance phenomenon refers to the ability of some plants to limit collateral damage caused by other stresses accompanying a primary stress (Takahashi et al. 1994; Kang et al. 2005; Mangrich et al. 2006). The results presented here show that competition and stress factors need not always be interactive. Some studies evaluating the effect of an abiotic stress on the net outcome of plant-plant interactions demonstrate that the net effect of neighbors was not affected by a stress (Maestre et al. 2005). Thus, ambient levels of UV radiation can affect the leaf morphology and allocation of seedlings of the species evaluated. Also, UV radiation can indirectly induce changes in mycorrhizal colonization. However, due to the lack of any significant effect on competitive
interactions, it is not clear if such changes are enough to have a substantial impact on competitive interactions between these co-occurring species. These findings demonstrate the need for more experimentation using larger pots and longer periods of growth to evaluate if UV radiation induced cumulative effects might affect plant-plant and plant-fungus interactions between these species.

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Table 5. Environmental conditions. Mean air temperature, relative humidity, midday photosynthetic photon flux density (PPFD), and UV-A/B irradiation measured under the cellulose diacetate (+UV) and polyester (-UV) films.

<table>
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<th>Experiment</th>
<th>Air temperature °C</th>
<th>Relative humidity %</th>
<th>PPFD (+UV) µmol m(^{-2}) s(^{-1})</th>
<th>PPFD (-UV) µmol m(^{-2}) s(^{-1})</th>
<th>UV-A/B (+UV) W m(^{-2})</th>
<th>UV-A/B (-UV) W m(^{-2})</th>
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</thead>
<tbody>
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<td>61.1 ± 1.7</td>
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<td>1502 ± 56</td>
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<td>55.5 ± 1.7</td>
<td>1328 ± 43</td>
<td>1460 ± 36</td>
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<tr>
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<td>52 ± 0.6</td>
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<td>1447 ± 80</td>
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Table 6. Growth, morphology, and allocation parameters; abbreviations used and units in which they are expressed.

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<th>Unit</th>
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<tr>
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<td>Stem diameter</td>
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<td>Leaf dry weight</td>
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<td>Total dry weight</td>
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<tr>
<td>R:S</td>
<td>Root to shoot ratio</td>
<td>g.g$^{-1}$</td>
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<tr>
<td>(RDW/SDW)</td>
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<td>SLA</td>
<td>Specific leaf area (LA/LDW)</td>
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<td>LAR</td>
<td>Leaf area ratio (LA/TDW)</td>
<td>cm$^2$.g$^{-1}$</td>
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Table 7. Summary of statistical analysis for experiment one (*P. deltoides* Ames × *P. deltoides* Saverton). Statistical values tabulated are *F* ratios from ANOVAs testing for the main effect of UV radiation (UV), arbuscular mycorrhiza (AM), ectomycorrhiza (EM) competition (Comp) or the interaction between them (UV × AM, UV × EM, UV × Comp, Comp × AM, Comp × EM, UV × Comp × AM × EM). Degrees of freedom in parenthesis. Significance levels with Bonferroni correction by analysis of variance (***p<0.0001; **P<0.001; *P<0.005).

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Only terms that are significant at p<0.005 are shown.
Table 8. Summary of statistical analysis for experiment two (P. deltoides × S. nigra). Statistical values tabulated are F ratios from ANOVAs testing for the main effect of UV radiation (UV), mycorrhizae (AM), competition (Comp) or the interaction between them (UV × AM, UV × Comp, Comp × AM, UV × Comp × AM). Degrees of freedom in parentheses. Significance levels with Bonferroni correction by analysis of variance (***P<0.0001; **P<0.001; *P<0.005).

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Only terms that are significant at p<0.005 are shown.
Table 9. Summary of statistical analysis for Experiment Three (*P. deltoides* Ames × *B. nigra*). Statistical values tabulated are $F$ ratios from ANOVA testing for the main effect of UV radiation (UV), mycorrhizae (AM), competitor (Comp) or the interaction between them (UV × AM, UV × Comp, Comp × AM, UV × Comp × AM). Degrees of freedom in parentheses. Significance levels with Bonferroni correction by analysis of variance (***P<0.0001; **P<0.001; *P<0.005).

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Only terms that are significant at p<0.005 are shown.
Figure 8. Effect of UV radiation and AM colonization on *P. deltoides* seedlings grown in competition with individuals from the same or a different provenance. Only traits for which at least one treatment factor was significant at $P < 0.005$ are presented. Each symbol is a mean ± SE. Symbols that share letters are not significantly different ($P < 0.05$, post-ANOVA Tukey test).
Figure 9. Growth, morphology, and allocation in seedlings of *P. deltoides* and *S. nigra* grown in competition, under two UV radiation levels and two AM conditions. Only traits for which at least one treatment factor was significant at $P < 0.005$ are presented. Each symbol is a mean ± SE. Symbols that share letters are not significantly different ($P < 0.05$, post-ANOVA Tukey test).
Figure 10. Growth, morphology, and allocation in seedlings of *P. deltoides* and *S. nigra* grown in competition, under two UV radiation levels and two AM conditions. Only traits for which at least one treatment factor was significant at $P < 0.005$ are presented. Each symbol is a mean ± SE. Symbols that share letters are not significantly different ($P < 0.05$, post-ANOVA Tukey test).
Figure 11. Effect of UV radiation on mycorrhizal colonization of *P. deltoides, S. nigra,* and *B. nigra* seedlings. Mycorrhizal colonization measured by percent of root length colonized. Bars that share letters are not significantly different (P < 0.05 post-ANOVA Tukey test).
References


Supplemental information

R code for statistical analyses of Experiment Two

Effects of UV radiation and mycorrhizal colonization on intraspecific competition between *P. deltoides* and *S. nigra*.

```r
# Analysis *P. deltoides*

rm(list=ls())

populusXsalix<-read.csv(file.choose())

head(populusXsalix)

options(digits=3)

summary(populusXsalix)

# Test for Homogeneity of Variances

bartlett.test(heihgt~endo, data=populusXsalix)
bartlett.test(leaves~endo, data=populusXsalix)
bartlett.test(diameter~endo, data=populusXsalix)
bartlett.test(LA~endo, data=populusXsalix)
bartlett.test(LDW~endo, data=populusXsalix)
bartlett.test(SDW~endo, data=populusXsalix)
bartlett.test(RDW~endo, data=populusXsalix)
bartlett.test(TDW~endo, data=populusXsalix)
bartlett.test(LAR~endo, data=populusXsalix)
bartlett.test(SLA~endo, data=populusXsalix)
bartlett.test(R.S~Myco, data=populusXsalix)
```
bartlett.test(LWR~Myco, data=populusXsalix)
# normality test
shapiro.test(heihgt~endo, data=populusXsalix)
shapiro.test(leaves~endo, data=populusXsalix)
shapiro.test(diameter~endo, data=populusXsalix)
shapiro.test(LA~endo, data=populusXsalix)
shapiro.test(LD~endo, data=populusXsalix)
shapiro.test(SD~endo, data=populusXsalix)
shapiro.test(RD~endo, data=populusXsalix)
shapiro.test(TD~endo, data=populusXsalix)
shapiro.test(LAR~endo, data=populusXsalix)
shapiro.test(SLA~endo, data=populusXsalix)
shapiro.test(R.S~Myco, data=populusXsalix)
shapiro.test(LWR~Myco, data=populusXsalix)

# matrix of linear measurements
populusXpopulus.data<-as.matrix(populusXsalix[, (9:22)])
UV<-as.factor(populusXsalix[,8])
endo<-as.factor(populusXsalix[,4])
competitor<-as.factor(populusXsalix [,3])
UVByendoBycompetitor<-paste(UV,endo,competitor)

# Describe data
cor. populusXpopulus <-cor(populusXsalix.data)
cor. populusXsalix
pairs(populusXsalix.data)
vcv. populusXpopulus <- var(populusXsalix.data)
vcv. populusXpopulus
var(scale(populusXsalix.data)
dist(populusXsalix.data)

#load (vegan)
adonis(populusXsalix.data~ UV*endo*competitor, data=populusXsalix,
permutations=99, distance = "euclidean")

# Permutation Tests for Factorial ANOVA
mod1 <- lm(height ~ Myco + UV +Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))
cat( " The standard ANOVA for these data follows ","
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, "\n")
cat( "\n")
cat( "\n")

print("Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling
nreps <- 5000
FS <- numeric(nreps)  # Set up space to store F values as calculated.
FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)
FS[1] <- FMyco  # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4
for (i in 2:nreps) {
  newheight <- sample(height, 186)
  mod2 <- lm(newheight ~ Myco + UV + Comp Myco:UV:Comp)
  b <- summary(aov(mod2))
  FS[i] <- b[1]$"F value"[1]
  FM[i] <- b[1]$"F value"[2]
  FSM[i] <- b[1]$"F value"[3]
FMR[i] <- b[[1]]"F value"[5]
FSMR[i] <- b[[1]]"F value"[6]

probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nreps
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ",probSMR, "\n")
cat(" The probability value for the interaction is ",probRM, "\n")
cat(" The probability value for the interaction is ",probSR, "\n")
cat(" The probability value for the interaction is ",probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")

mod1 <- lm(leaves ~ Myco + UV + Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))
cat( " The standard ANOVA for these data follows ","\n")
FMyco <- ANOVA[[1]]"F value"[1]
FUV <- ANOVA[[1]]"F value"[2]
FComp <- ANOVA[[1]]"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]

print(ANOVA, "\n")
cat("\n")
cat("\n")

print("Resampling as in Manly with unrestricted sampling of observations.")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  # Set up space to store F values as calculated.
FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)

FS[1] <- FMyco     # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4

for (i in 2:nreps) {

newleaves <- sample(leaves, 186)
mod2 <- lm(newleaves ~ Myco + UV + Comp Myco:UV:Comp)
b <- summary(aov(mod2))
FS[i] <- b[[1]]$"F value"[1]
FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]
FSR[i] <- b[[1]]$"F value"[4]
FMR[i] <- b[[1]]$"F value"[5]
FSMR[i] <- b[[1]]$"F value"[6]
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nrep
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ",probSMR, ":")
cat(" The probability value for the interaction is ",probRM, ":")
cat(" The probability value for the interaction is ",probSR, ":")
cat(" The probability value for the interaction is ",probSM, ":")
cat(" The probability value for Myco is ", probS, ":")
cat(" The probability value for UV is ", probM, ":")
mod1 <- lm(LA ~ Myco + UV + Comp Myco:UV:Comp)

ANOVA <- summary(aov(mod1))

cat( " The standard ANOVA for these data follows ", "\n"")

FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]

print(ANOVA, "\n")

cat( "\n")

cat( "\n")

print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  #Set up space to store F values as calculated.

FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000

FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4
for (i in 2:nreps) {
  newLA <- sample(LA, 186)
  mod2 <- lm(newLA ~ Myco + UV + Comp Myco:UV:Comp)
  b <- summary(aov(mod2))
  FS[i] <- b[1]"F value"[1]
  FM[i] <- b[1]"F value"[2]
  FSM[i] <- b[1]"F value"[3]
  FSR[i] <- b[1]"F value"[4]
  FMR[i] <- b[1]"F value"[5]
  FSMR[i] <- b[1]"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nrep
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps
cat("The probability value for the interaction is ", probSMR, 
"
")
cat("The probability value for the interaction is ", probRM, 
"
")
cat("The probability value for the interaction is ", probSR, 
"
")
cat("The probability value for the interaction is ", probSM, 
"
")
cat("The probability value for Myco is ", probS, 
"
")
cat("The probability value for UV is ", probM, 
"
")

mod1 <- lm(Diameter ~ Myco + UV + Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))
cat("The standard ANOVA for these data follows ", 
"
")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, 
"
")
cat(""
")
cat(""
")
print("Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  # Set up space to store F values as calculated.

FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)
FS[1] <- FMyco # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4
for (i in 2:nreps) {
  newDiameter <- sample(Diameter, 186)
  mod2 <- lm(newDiameter ~ Myco + UV + Comp Myco:UV:Comp)
  b <- summary(aov(mod2))
  FS[i] <- b[[1]]$"F value"[1]
  FM[i] <- b[[1]]$"F value"[2]
  FSM[i] <- b[[1]]$"F value"[3]
  FSR[i] <- b[[1]]$"F value"[4]
  FMR[i] <- b[[1]]$"F value"[5]
  FSMR[i] <- b[[1]]$"F value"[6]
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nrep
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ", probSMR, "\n")
cat(" The probability value for the interaction is ", probRM, "\n")
cat(" The probability value for the interaction is ", probSR, "\n")
cat(" The probability value for the interaction is ", probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")

mod1 <- lm(LDW ~ Myco + UV + Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))

cat(" The standard ANOVA for these data follows ", "\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, "\n")
print("Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)    #Set up space to store F values as calculated.

FM <- numeric(nreps)

FR <- numeric(nreps)

FSM <- numeric(nreps)

FSR <- numeric(nreps)

FMR <- numeric(nreps)

FSMR <- numeric(nreps)

FS[1] <- FMyco          # The first F of our 5000

FM[1] <- FUV

FR[1] <- FComp

FSM[1] <- Finteract1

FSR[1] <- Finteract2

FMR[1] <- Finteract3

FSMR[1] <- Finteract4

for (i in 2:nreps) {

newLDW <- sample(LDW, 186)

mod2 <- lm(newLDW ~ Myco + UV + Comp Myco:UV:Comp)
b <- summary(aov(mod2))
FS[i] <- b[[1]]$"F value"[1]
FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]
FSR[i] <- b[[1]]$"F value"[4]
FMR[i] <- b[[1]]$"F value"[5]
FSMR[i] <- b[[1]]$"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nreps
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps
cat(" The probability value for the interaction is ", probSMR, ", \n")
cat(" The probability value for the interaction is ", probRM, ", \n")
cat(" The probability value for the interaction is ", probSR, ", \n")
cat(" The probability value for the interaction is ", probSM, ", \n")
cat(" The probability value for Myco is ", probS, ", \n")
cat(" The probability value for UV is ", probM, ", \n")
mod1 <- lm(SDW ~ Myco + UV +Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))

cat( " The standard ANOVA for these data follows ","\n")

FMyco <- ANOVA[[1]]$"F value"[1]

FUV <- ANOVA[[1]]$"F value"[2]

FComp <- ANOVA[[1]]$"F value"[3]

Finteract <- ANOVA[[1]]$"F value"[4]

print(ANOVA, "\n")

cat( "\n")

cat( "\n")

print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)    #Set up space to store F values as calculated.

FM <- numeric(nreps)

FR <- numeric(nreps)

FSM <- numeric(nreps)

FSR <- numeric(nreps)

FMR <- numeric(nreps)

FSMR <- numeric(nreps)

FS[1] <- FMyco       # The first F of our 5000

FM[1] <- FUV

FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4

for (i in 2:nreps) {
    newSDW <- sample(SDW, 186)
    mod2 <- lm(newSDW ~ Myco + UV + Comp Myco:UV:Comp)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
    FSM[i] <- b[[1]]$"F value"[3]
    FSR[i] <- b[[1]]$"F value"[4]
    FMR[i] <- b[[1]]$"F value"[5]
    FSMR[i] <- b[[1]]$"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nreps
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ", probSMR, "\n")
mod1 <- lm(RDW ~ Myco + UV + Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))
cat(" The standard ANOVA for these data follows ","n"")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, "n")
cat("n")
cat("n")
print("Resampling as in Manly with unrestricted sampling of observations.")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps)  #Set up space to store F values as calculated.
FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp

FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4

for (i in 2:nreps) {
  newRDW <- sample(RDW, 186)
  mod2 <- lm(newRDW ~ Myco + UV + Comp Myco:UV:Comp)
  b <- summary(aov(mod2))
  FS[i] <- b[[1]]$"F value"[1]
  FM[i] <- b[[1]]$"F value"[2]
  FSM[i] <- b[[1]]$"F value"[3]
  FSR[i] <- b[[1]]$"F value"[4]
  FMR[i] <- b[[1]]$"F value"[5]
  FSMR[i] <- b[[1]]$"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nreps
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps
cat(" The probability value for the interaction is ", probSMR, "n")
cat(" The probability value for the interaction is ", probRM, "n")
cat(" The probability value for the interaction is ", probSR, "n")
cat(" The probability value for the interaction is ", probSM, "n")
cat(" The probability value for Myco is ", probS, "n")
cat(" The probability value for UV is ", probM, "n")

td1 <- lm(TDW ~ Myco + UV + Comp Myco:UV:Comp)
ANOVA <- summary(aov(td1))
cat(" The standard ANOVA for these data follows ", "n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, "n")
cat("n")
cat( "\n"")

print( "Resampling as in Manly with unrestricted sampling of observations. ")

# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  #Set up space to store F values as calculated.

FM <- numeric(nreps)

FR <- numeric(nreps)

FSM <- numeric(nreps)

FSR <- numeric(nreps)

FMR <- numeric(nreps)

FSMR <- numeric(nreps)

FS[1] <- FMyco       # The first F of our 5000

FM[1] <- FUV

FR[1] <- FComp

FSM[1] <- Finteract1

FSR[1] <- Finteract2

FMR[1] <- Finteract3

FSMR[1] <- Finteract4

for (i in 2:nreps) {
  
  newTDW <- sample(TDW, 186)

  mod2 <- lm(newTDW ~ Myco + UV + Comp Myco:UV:Comp)

  b <- summary(aov(mod2))
FS[i] <- b[[1]]$"F value"[1]
FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]
FSR[i] <- b[[1]]$"F value"[4]
FMR[i] <- b[[1]]$"F value"[5]
FSMR[i] <- b[[1]]$"F value"[6]
}

probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nreps
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ", probSMR, "\n")
cat(" The probability value for the interaction is ", probRM, "\n")
cat(" The probability value for the interaction is ", probSR, "\n")
cat(" The probability value for the interaction is ", probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")

mod1 <- lm(LAR ~ Myco + UV + Comp Myco:UV:Comp)

ANOVA <- summary(aov(mod1))

cat(" The standard ANOVA for these data follows ", "\n")
FMycoc <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, "\n")
cat( "\n")
cat( "\n")
print( "Resampling as in Manly with unrestricted sampling of observations. ")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps)    #Set up space to store F values as calculated.
FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)
FS[1] <- FMycoc          # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4
for (i in 2:nreps) {
    newLAR <- sample(LAR, 186)
    mod2 <- lm(newLAR ~ Myco + UV + Comp Myco:UV:Comp)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
    FSM[i] <- b[[1]]$"F value"[3]
    FSR[i] <- b[[1]]$"F value"[4]
    FMR[i] <- b[[1]]$"F value"[5]
    FSMR[i] <- b[[1]]$"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nrep
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ",probSMR, 
"n")
cat(" The probability value for the interaction is ",probRM, 
"n")
cat(" The probability value for the interaction is ",probSR, 
"n")
cat(" The probability value for the interaction is ", probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")

mod1 <- lm(SLA ~ Myco + UV +Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))
cat(" The standard ANOVA for these data follows ", "\n")
FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]
print(ANOVA, "\n")
cat("\n")
cat("\n")
print("Resampling as in Manly with unrestricted sampling of observations. ")
# Now start resampling
nreps <- 5000
FS <- numeric(nreps)  # Set up space to store F values as calculated.
FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)
FS[1] <- FMyco       # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4
for (i in 2:nreps) {
  newSLA <- sample(SLA, 186)
  mod2 <- lm(newSLA ~ Myco + UV + Comp Myco:UV:Comp)
  b <- summary(aov(mod2))
  FS[i] <- b[[1]]$"F value"[1]
  FM[i] <- b[[1]]$"F value"[2]
  FSM[i] <- b[[1]]$"F value"[3]
  FSR[i] <- b[[1]]$"F value"[4]
  FMR[i] <- b[[1]]$"F value"[5]
  FSMR[i] <- b[[1]]$"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nreps
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps

cat(" The probability value for the interaction is ",probSMR, "\n")
cat(" The probability value for the interaction is ",probRM, "\n")
cat(" The probability value for the interaction is ",probSR, "\n")
cat(" The probability value for the interaction is ",probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")

mod1 <- lm(RS ~ Myco + UV + Comp Myco:UV:Comp)
ANOVA <- summary(aov(mod1))

cat(" The standard ANOVA for these data follows ", "\n")

FMyco <- ANOVA[[1]]$"F value"[1]
FUV <- ANOVA[[1]]$"F value"[2]
FComp <- ANOVA[[1]]$"F value"[3]
Finteract <- ANOVA[[1]]$"F value"[4]

print(ANOVA, "\n")
cat( "\n")
cat( "\n")

print(" Resampling as in Manly with unrestricted sampling of observations. ")
# Now start resampling

nreps <- 5000

FS <- numeric(nreps)  # Set up space to store F values as calculated.
FM <- numeric(nreps)
FR <- numeric(nreps)
FSM <- numeric(nreps)
FSR <- numeric(nreps)
FMR <- numeric(nreps)
FSMR <- numeric(nreps)

FS[1] <- FMyco  # The first F of our 5000
FM[1] <- FUV
FR[1] <- FComp
FSM[1] <- Finteract1
FSR[1] <- Finteract2
FMR[1] <- Finteract3
FSMR[1] <- Finteract4

for (i in 2:nreps) {
    newRS <- sample(RS, 186)
    mod2 <- lm(newRS ~ Myco + UV + Comp Myco:UV:Comp)
    b <- summary(aov(mod2))
    FS[i] <- b[[1]]$"F value"[1]
    FM[i] <- b[[1]]$"F value"[2]
FSM[i] <- b[[1]]$"F value"[3]
FSR[i] <- b[[1]]$"F value"[4]
FMR[i] <- b[[1]]$"F value"[5]
FSMR[i] <- b[[1]]$"F value"[6]
}
probS <- length(FS[FS >= FMyco])/nreps
probM <- length(FM[FM >= FUV])/nreps
probSM <- length(FSM[FSM >= Finteract1])/nreps
probSR <- length(FSR[FSR >= Finteract2])/nreps
probMR <- length(FMR[FMR >= Finteract3])/nrep
probSMR <- length(FSMR[FSMR >= Finteract4])/nreps
cat(" The probability value for the interaction is ", probSMR, "\n")
cat(" The probability value for the interaction is ", probRM, "\n")
cat(" The probability value for the interaction is ", probSR, "\n")
cat(" The probability value for the interaction is ", probSM, "\n")
cat(" The probability value for Myco is ", probS, "\n")
cat(" The probability value for UV is ", probM, "\n")

tukeyHSD(aov(height~UV + Com, data= populusXsalix))
tukeyHSD(aov(leaves~UV + Comp, data= populusXsalix))
tukeyHSD(aov(LA~UV + Myco, data= populusXsalix))
tukeyHSD(aov(Diameter~UV + Comp, data= populusXsalix))
TukeyHSD(aov(LDW~UV + Comp, data= populusXsalix))
TukeyHSD(aov(SDW~UV + Comp, data= populusXsalix))
TukeyHSD(aov(RDW~UV + Comp, data= populusXsalix))
TukeyHSD(aov(TDW~UV + Myco, data= populusXsalix))
TukeyHSD(aov(LAR~UV + Comp, data= populusXsalix))
TukeyHSD(aov(LWR~UV + Myco, data= populusXsalix))
TukeyHSD(aov(SLA~UV + Myco, data= populusXsalix))
TukeyHSD(aov(RS~UV + Myco, data= populusXsalix))
CHAPTER 5. MYCORRHIZAL COLONIZATION DIFFERENCES BETWEEN SEEDLINGS AND ADULTS TREES IN COTTONWOOD (*Populus deltoides Bartram*)

Abstract

A field survey was conducted to evaluate the differences in arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) colonization levels between mature plants and seedlings of *Populus deltoides*. Roots were sampled from native *P. deltoides* adult trees and seedlings along stream and river banks at ten different sites in Ames, Iowa, USA. Seedlings of *P. deltoides* had higher levels of AM colonization than did adults. In contrast, EM colonization of *P. deltoides* seedlings was extremely low while adult trees had high levels of EM colonization.

Key words: arbuscular mycorrhiza, ectomycorrhiza, river banks

Introduction

The mycorrhizal symbiosis is a mutualistic association between fungi and higher plants (Turk et al. 2006). There are different types of mycorrhizal associations, the most common being ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) associations (Habte 2000). Most plant species form only one type of mycorrhizal association, but there are a few plant genera capable of forming both types of association including *Acacia, Casuarina, Gompholobium, Owenia, Tilia, Ulmus, Eucalyptus, Alnus, Helianthemum, Salix*, and *Populus* (Brundrett et al. 1996; Lodge and Wentworth 1990). In many of these dually colonized host plants, AM fungi initially colonize seedlings and then are replaced or supplemented by EM (Lodge and Wentworth 1990, Santos et al.)
One hypothesis suggested for explaining this phenomenon is that AM may be more common in young plants because AM fungal spores are ubiquitous and can rapidly colonize roots from spores, while ectomycorrhizal fungi may require more time for colonization. Once EM fungi are established, EM fungi may prevent colonization of newly-formed roots by arbuscular mycorrhizal fungi. Thus, the AM-EM succession may be linked to spatial competition for infection sites and differential colonization levels by the two types of fungi (G. A. Chilvers 1987). Another hypothesis explains the arbuscular mycorrhizal-ectomycorrhizal succession as resulting from changes in host physiology and carbohydrate productivity with age (Lodge and Wentworth 1990). Additionally, mechanical barriers as well as chemical compounds produced by the host or by a resident fungi may preempt the colonization of roots by other fungi (Santos et al. 2001 and references therein). In summary, the fact that some plant genera are able to form functional mycorrhizal association with two mycorrhizal types raises important questions about the benefits that the mycorrhizal symbiosis provide to the host and also about the competitive interactions that occur between the two types of fungi.

*Populus deltoides* Bartram. ex Marshall (eastern cottonwood, Salicaceae) is a very shade intolerant species that requires exposed sites created by seasonal floods for seedlings establishment (Karrenberg et al. 2002). Since exposed sediments in river corridors are a difficult substrate for plant colonization i.e., because of the hydrology regime of flooding and drought, AM associations may facilitate seedling establishment. Arbuscular mycorrhizal fungi can tolerate saturated soil conditions better than EM fungi. The purpose of this study was to evaluate the differences in arbuscular mycorrhizal
(AM) and ectomycorrhizal (EM) colonization between wild adult trees and seedlings of *Populus deltoides*.

**Materials and methods**

Roots were sampled from native *P. deltoides* trees and seedlings along streams and river banks in Ames Iowa (42°02'05"N 93°37'12"W, 287 m elevation). Five locations were selected and eight random cottonwood trees were sampled at each site. Only roots that could be traced from the trunk were collected (total N=40). The same procedures were used for seedlings i.e., five location were selected (three sites in the Squaw Creek and one site in Worrell Creek and South Skunk River respectively) and eight random seedlings were sampled at each site (total N=40). Root samples between 0.2-0.3 grams were cleared in hot 2.5% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue in acid glycerol (Robertson et al. 1999). For examination, each root sample was spread across the bottom of an 8.5 cm diameter gridded petri dish and viewed under a dissecting microscope. Each intersection between a line and a root was classified as mycorrhizal or non-mycorrhizal. Sets of three observations were made recording all the root/gridline intersects; each of the replicated records was made on the same re-distributed sample (Giovannetti and Mosse 1980). *P. deltoides* roots also hosted non-mycorrhizal fungi in their roots: septa and clamp connections were observed in some hyphae, indicating the presence of fungi other than AMF. For assessment of EM colonization, root samples were placed in Petri dishes for the examination of the tips under a dissecting microscope. Ectomycorrhizal root tips were distinguished from non-
mycorrhizal root tips by differences in their color and form (Brundrett et al. 1996). To test for differences in colonization levels between adults and seedlings, the Mann-Whitney U test was conducted using the statistical software R-2.15.1.

**Results and discussion**

Seedlings of *P. deltoides* had higher levels of AM colonization than did adults (W=111, P<0.001, Fig. 12). In contrast, very few seedlings were colonized by EM fungi. The average extent of EM colonization for seedlings was 0.1%, while the average EM colonization for adults was 59% (Fig. 12). These results are in accordance with Piotrowski et al. (2008), who reported a shift in abundance between arbuscular mycorrhizae and ectomycorrhizae in *P. deltoides*, during floodplain succession. The authors proposed two possible mechanisms contributing to the shift from AM to EM: (i) that a higher percent of soil organic matter and surface litter in old sites stimulates organisms that compete with AM, and (ii) the chemistry of cottonwood litter may suppress AM, since soluble phenolic compounds present in *Populus* foliage can inhibit fungal spore germination and hyphal growth. An alternate explanation to the phenomenon is that the dominance of AM or EM is influenced by soil moisture, being the AM associations most frequent at the extremes of the moisture gradient (Lodge 1989). The results of the current study demonstrate that the relative abundances of AM and EM differ between seedling and adult *P. deltoides* and indicate that AM fungi may have a significant role in facilitating the establishment of *P. deltoides* seedlings.
Figure 12. Arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) colonization of adults and seedlings of *P. deltoides* from five locations in Ames, Iowa. Bars that share letters are not significantly different (P < 0.001 Mann-Whitney test).
References


CHAPTER 6. SUMMARY AND CONCLUSIONS

Study One

The overall objective of study one was to investigate the individual and interactive effects of ultraviolet radiation and AM colonization on vegetative growth in seedlings of *Populus deltoides* (eastern cottonwood), *Salix nigra* (black willow), and *Betula nigra* (river birch). Four hypotheses were tested: (1) the exclusion of UV radiation would alter growth, morphology and allocation of seedlings of *P. deltoides*, *B. nigra*, and *S. nigra*, (2) AM colonization would alter growth of seedlings of *P. deltoides*, *B. nigra*, and *S. nigra*, (3) UV exclusion would result in increased AM colonization of seedlings, and (4) there would be a significant interaction between UV radiation and mycorrhizal colonization, such that the AM colonization would negate the detrimental effects of UV radiation. The results showed that UV radiation affected growth, morphology, and allocation of the three species evaluated, and that mycorrhizal colonization reduced seedling growth but there were no interactions between factors. Thus, hypotheses (1) and (3) were supported by the data. By testing these hypotheses it was possible to determine not only that ambient UV radiation can affect seedling growth and morphology of the three species evaluated, but also that the species tested presented differential sensitivity to solar UV radiation. *S. nigra* was the species most affected by UV radiation, while *B. nigra* was the species least affected. However, when the experiment was replicated (Supplemental Appendix A) many growth parameters of *B. nigra* were affected by UV radiation. Thus it is not clear if *B. nigra* was more or less tolerant of UV radiation than were the other species. The results of the original
experiment showed that, in general, AM inoculum had a negative effect on plant growth under the growing conditions of these experiments. The results also indicated differential effects of AM colonization on co-occurring plant species, with *P. deltoides* being the species most affected by AM colonization. Interestingly, the results of replication of the experiment with *B. nigra* (Supplemental Appendix A) showed that AM colonization had a positive effect on growth, as was originally expected. The results of Study One were interesting because of their possible implications with respect to plant-plant interactions. That is, interspecific differences in sensitivity to UV-B radiation are proposed as a mechanism to explain the reported effects of UV-B radiation on plant competition. Similarly, interspecific differences in responsiveness to mycorrhizal association are proposed as a mechanism to explain how mycorrhizae can influence the outcome of plant competition.

**Study Two**

The objective of the second study was to determine the effects of competitive interactions and UV radiation on early vegetative growth in seedlings of *Populus deltoides*. Three hypotheses were tested: (1) the exclusion of UV radiation would alter growth, morphology and allocation of seedlings of *P. deltoides*, (2) competition would result in reduced growth of seedlings, and (3) seedlings grown in competition and exposed to UV radiation would grow less than seedlings grown in competition but without UV radiation. The results showed that seedlings grown without UV radiation had more root mass and lower leaf area, specific leaf area, and leaf area ratio than seedlings grown with UV radiation. The results also showed the interaction between UV
radiation and competition on height, shoot dry weight, leaf dry weight, and total dry weight. Those parameters were affected by UV radiation when seedlings were grown alone, but were unaffected by UV radiation when seedlings were grown in competition. The results support hypothesis (1) and hypothesis (2) but do not support hypothesis (3). Thus, the results indicated that the stress induced by competition may mask the effects of the UV stress on seedlings of *P. deltoides*.

**Study Three**

The third study was designed to evaluate the individual effects of UV radiation and mycorrhizal symbiosis on competitive interactions between seedlings and to evaluate the effect of the interaction of both factors on competitive interactions between co-occurring plants. Five hypotheses were tested in study number two: (1) UV radiation alters the growth, allocation, and morphology of seedlings in competition, (2) mycorrhizae alter the growth, allocation, and morphology of seedlings in competition, (3) the identity of competitor affects the growth of seedlings, (4) UV radiation negatively affects the rate of mycorrhizal colonization, and (5) interactions among UV radiation, mycorrhizal inoculum and identity of competitor alter the growth of seedlings. The results supported hypotheses (3) and (4) and partially supported hypothesis (1). However, neither UV radiation nor AM colonization affected the outcome of plant competition. Although the results of the first study (plants grown alone) showed that UV radiation affected seedling growth, morphology, and allocation, the results of study two (effect of UV radiation on competition between seedlings) and study three showed that
the stress induced by competition masked the effects of the UV stress and mycorrhizal colonization.

It is evident that UV radiation is a ubiquitous stress factor that induces morphological changes in the aerial part of plants, especially leaves. Yet the effects of UV radiation were not exclusive to leaves; the response to mycorrhizae also was affected by UV radiation. This study demonstrated that ambient levels of UV radiation can negatively affect the rates of mycorrhizal colonization in tree seedlings. However, neither UV nor mycorrhizae had a substantial impact on plant-plant interactions, probably due to the high degree of total competitive stress in the experimental growth conditions.

**Study Four**

The objective of study four was to evaluate the differences in arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) colonization rates between mature plants and seedlings of *Populus deltoides*. The results of this study demonstrated that the relative abundances of AM and EM differ between adult plants and seedlings and indicate that AM fungi may have a significant role in facilitating the establishment of *P. deltoides* seedlings.

**Future Work**

The results suggest that competition stress can mask the effects of UV radiation stress and AM colonization on growth, morphology, and biomass allocation of seedlings
of the three shade-intolerant tree species evaluated. However, it is possible that the physical constraint imposed on roots by the small containers could have had a net negative effect on seedling growth. Another possible deficiency in the experimental design is that in this type of design the density of the plants was fixed, whereas plants may behave differently at varying densities. Furthermore, the experiments in this investigation were short-term and it is possible that UV radiation could alter competitive interaction over a longer period of time. Therefore, future work could avoid these issues by growing the two species in a 1:1 ratio at five densities as suggested by Firbank and Watkinson (1985), using a greater pot volume during a longer time period.

It was demonstrated that ambient levels of UV radiation can affect the rate of AM colonization of seedlings of the three species evaluated. Future work will intend to determine whether ectomycorrhizal colonization is also affected by ambient levels of UV radiation. Additionally, since the level of ambient UV radiation in sunlight is relatively higher in tropical regions than in temperate regions, it might be expected that tree seedlings of tropical regions show greater response to UV radiation exclusion than temperate region seedlings. Thus, future work in Colombia would aim to evaluate the effects of UV radiation on mycorrhizal colonization in tree species differing in their shade tolerance through an elevation gradient.

Lastly, future experiments could be designed to determine which mechanism is responsible for the indirect effect of UV radiation on AM colonization; specifically, the aim would be to test the hypothesis that changes in AM colonization rates are result of UV-induced changes in the phytohormone balance in the host plant.
APPENDIX

Results of replication experiments for *P. deltoides* and *B. nigra*

**Growth measurements**

The results of the PERMANOVA for the second trial of *B. nigra* showed that a suite of measured dependent variables was significantly affected by UV radiation ($F_{2,70} = 20.44$, $P \leq 0.01$), and a slightly different set of variables was influenced by AM colonization ($F_{2,70} = 16.22$, $P \leq 0.01$, Table 1). Seedlings of *B. nigra* colonized by AM fungi had higher values of stem diameter, leaf area, shoot dry weight, leaf dry weight, and total dry weight than un-colonized seedlings (Table 1). Similarly, *B. nigra* seedlings grown under UV-exclusion had higher values of stem diameter, height, leaf dry weight, shoot dry weight, root dry weight, and total dry weight than seedlings grown exposed to UV, but had less specific leaf area and leaf area ratio (Fig. 1). There was no significant interactive effect of UV radiation and AM colonization for any of the variables for *B. nigra* (Table 1, Fig. 2). Compared with seedlings grown in the presence of UV radiation, seedlings of both species were taller when grown under UV-exclusion (Fig. 1).

In the case of *P. deltoides*, only seedling height was affected by the presence of UV radiation, and no variables were affect by the presence of AM. There was a significant interaction between UV radiation and AM colonization for leaf area ratio (Table 1). There was no difference in LAR values between AM colonized and un-colonized seedling when grown without UV radiation; however, when seedlings were grown with UV radiation non-mycorrhizal seedlings had higher values of LAR (Fig. 1).
**Table 1.** Summary of statistical analysis for replication experiments with *P. deltoides* and *B. nigra*. Statistical values tabulated are \(F\) ratios from ANOVAs testing for the main effect of UV radiation (UV), arbuscular mycorrhiza (AM), or the interaction between them (UV x AM); degrees of freedom in parentheses. Significance levels with Bonferroni correction by analysis of variance (***p<0.0001; **P<0.001; *P<0.004).

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>UV (1)</th>
<th>AM (1)</th>
<th>UV x AM (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. deltoides</em></td>
<td>Height (cm)</td>
<td>38.66***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LAR (cm(^2).g(^{-1}))</td>
<td>-</td>
<td>-</td>
<td>13.05***</td>
</tr>
<tr>
<td><em>B. nigra</em></td>
<td>Diameter (cm)</td>
<td>33.4***</td>
<td>14.3***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>24.4***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LA (cm(^2))</td>
<td>-</td>
<td>34.12***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Root dry weight (g)</td>
<td>47.44***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Leaf dry weight (g)</td>
<td>18.70***</td>
<td>25.33***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Shoot dry weight (g)</td>
<td>40.79***</td>
<td>23.69***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total dry weight (g)</td>
<td>56.57***</td>
<td>17.62***</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SLA (cm(^2).g(^{-1}))</td>
<td>24.87***</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>LAR (cm(^2).g(^{-1}))</td>
<td>36.83***</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1. Effects of ultraviolet radiation and mycorrhizal infection (AM) on height, RDW, SLA, and LAR of *P. deltoides* and *B. nigra*. Each value is a mean ± SE. Means with different letters are significantly different (P < 0.004, post-ANOVA Tukey test).
Figure 2. Effect of AM colonization and UV radiation and on height of *B. nigra* seedlings. Each point represents the mean, vertical bars denote ± SE. The different letters below the same date indicate statically significant differences between treatments at the P < 0.05 level in Tukey’s HSD test. Past the first half of the growth period the cumulative effect of the UV radiations become evident in the non-colonized seedlings.