Comparative Growth Responses of Native *Robinia pseudoacacia* and Invasive *Ailanthus altissima* to the Environmental Pollutant Ethylene Glycol

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Abstract

Invasive species such as *Ailanthus altissima* (Mill.) Swingle have had many detrimental effects on native ecosystems by outcompeting and eliminating native species like *Robinia pseudoacacia.* Increasing industrialization is leading to escalating chemical pollution levels in many ecosystems, for example, ethylene glycol the active ingredient in antifreeze is now commonly found in many areas. This pollution may allow invasive species to proliferate and further harm native ecosystems. We used *A. altissima* an invasive and *R. pseudoacacia* which is a native species to study the effects of ethylene glycol on total growth, root: shoot, and survival. After five weeks of treatment with 200ml of one of the four levels of ethylene glycol concentration solutions we measured and recorded survival percentages, root: shoot, and biomass of the replicates (n=15). After analyzing our results using a 2-way ANOVA we found that the effect of ethylene glycol on total growth and the root to shoot ratio were dependant on species. Our results also suggested after analyzing survival that ethylene glycol did affect survival, but it was not dependant on species. At lower concentrations of ethylene glycol pollution (25mg/l) *R. pseudoacacia* may be able to outcompete *A. altissima* at the seedling stage due to its greater total growth at this level, but at a higher concentration of ethylene glycol (1,300mg/l) *A. altissima* may gain the competitive advantage at the seedling stage because it showed higher total growth than *R. pseudoacacia*. These results suggest that ethylene glycol pollution could change the biodiversity in many areas if land management precautions are not taken to prevent or lessen the pollution levels.

Introduction

Invasive species have been the focus of a significant amount of research both in the past and present, due to their numerous possible detrimental effects on ecosystems. Invasive plants can in many cases outcompete native species, which detracts from the local biodiversity of the area (Faulkner 2002). In some extreme cases, the invasive plant species can totally eliminate a native population and form a pure stand (Tellman 2002). As humans continue to spread to previously uninhabited areas of land and develop it, not only are more invasive species introduced to the area, but the land is also exposed to a range of new pollutants (Grodzinski *et al.* 1990). Some invasive species such as *Ailanthus altissima* (Mill.) Swingle*,* can thrive in polluted terrain in urban and industrialized areas, whereas less hardy native species do not grow and survive as well in these commonly disturbed environments (Lawrence *et al.*1991).

The invasive tree species *A. altissima* was introduced to the United States from China, during the 1900's as an urban ornamental tree (Heisey 1990, Heisey 1996, Lawrence *et al.* 1991). Since its introduction *A. altissima* has spread rapidly throughout most of the United States (Heisey 1990, Heisey 1996, Lawrence *et al.* 1991). *Ailanthus altissima* thrives in disturbed environments, and it has a negative ecological impact because it lowers local biodiversity by displacing many native species (Heisey 1996, Lawrence *et al.*1991). *Ailanthus altissima* is a very competitive tree species because it has definitive traits of invasive species such as the ability to reproduce via root sprouts, a high early growth rate, and it releases allelopathic and herbicidal compound to the surrounding soil that inhibit other plants' growth and germination (Heisey 1990). Overall, these traits help *A. altissima* to outcompete many native tree species (Heisey

1990, Heisey 1996, Lawrence *et al.* 1991).

Robinia pseudoacacia (black locust) is a native tree species that has similar growth requirements as *A. altissima* and indeed, both can often be found growing and competing in the same habitats (Call and Nilsen 2002, Young and Young 1992). Black locust has a positive ecological impact on its surrounding environment because of its ability to fix large amounts of nitrogen (approximately 30 kg N $ha^{-1}y^{-1}$) in symbiotic root nodules (Boring and Swank 1984). This has two positive effects on the local ecosystem; it increases the growth of adjacent trees, and second, it quickly replaces the nitrogen that is normally lost during and after land disturbances (Call and Nilsen 2002). These qualities of *R. pseudoacacia* help to increase species diversity in the surrounding areas (Boring and Swank 1984). Since both *R. pseudoacacia* and *A. altissima* inhabit disturbed areas that are often in urban or industrialized environments then it is likely that the soil they grow in may contain chemical pollutants that could affect their growth (Lawlor 1970, Lawrence *et al.*1991, Young and Young 1992).

The chemical pollutant that we treated the seedlings with was 1,2-Ethanediol better known by its common name as ethylene glycol (Figure 1). It is considered a high volume chemical because ethylene glycol production exceeds one million pounds per year in the United States (O'Neil 2001). The main uses of this chemical are as the active ingredient in both the antifreeze found in vehicles and in de-icing solutions for airliners, runways, and locally on the Personal Rapid Transit system track in Morgantown, West Virginia (Corsi *et al.* 2001a, Corsi *et al.* 2001b, Clarke *personal observation*). Due to these uses it has become a common chemical pollutant from spillage and runoff from these areas (Dobson 2000, Clarke *personal observations*). One of the most important properties of ethylene glycol is that it is strongly hygroscopic, meaning it can absorb twice its weight of water in 100% relative humidity (O'Neil 2001). This considerable hygroscopic property will allow ethylene glycol to water stress the plants by binding a large amount of the water in the soil and making it inaccessible to the plants (Lawlor 1970, Neil 2001, Stock and Briggs 2000). This property of ethylene glycol is very important because although *R. pseudoacacia* inhabits relatively dry areas it relies on a long, deep taproot to get into deep groundwater rather than absorbing water from shallow, surface soil (Cutler 1978). In areas where the groundwater is confined or while the tree is at the seedling stage before it has developed a taproot, then *R. pseudoacacia* may not be able to acquire a sufficient amount of water to survive (Cutler 1978). *Ailanthus altissima* can tolerate drought-stressed environments using alternative strategies that do not exclusively involve using a taproot to get into groundwater (Trifilo *et al.* 2004). It conserves water by shrinking stomatal openings which reduces hydraulic conductance and in turn this lessens water loss due to transpiration (Trifilo *et al.* 2004). In a previous study by Trifilo *et al.* (2004), *A. altissima* showed no significant growth reductions when it was grown in drought-stressed environments.

Overall, there is little known about the effects of ethylene glycol on plants. Also, since *A. altissima* is relatively drought resistant as compared to *R. pseudoacacia* and both plant species inhabit the same ecosystems there may be some important ecological consequences to ethylene glycol contamination in these areas (Call and Nilsen 2002). As a result of these facts the purpose of this study was to test two main hypotheses. First, we hypothesized that ethylene glycol will significantly inhibit the growth of *A. altissima* and *R. pseudoacacia*. Second, that ethylene glycol will inhibit the growth of *R. pseudoacacia* seedlings more than *A. altissima* seedlings. We hypothesized this because ethylene glycol according to its hygroscopic properties will water stress the seedlings and because the growth of *A. altissima* has been found to be unaffected by water stress (Trifilo *et al* 2004).

Methods

To determine if the effects of ethylene glycol on growth are dependant on species we designed a two by four, 2-way factorial experiment (Table 1). The four levels of ethylene glycol concentrations coincide with concentrations found in the field, and are 0mg/l which was the control, 25mg/l which was the concentration that is found along roadways, 1,300mg/l the amount found around production plants, and 19,000mg/l which was the concentration found near airports (Dobson 2000). The two levels of tree species are the invasive *A. altissima,* and the native *R. pseudoacacia*. Fifteen replicates of each species were treated with each level of ethylene glycol (n=15).

All *A. altissima* seeds were field collected from a single source in Morgantown, West Virginia during December 2003, and were cold stratified in wet sand for approximately eight weeks. The black locust seeds were acquired from Carolina Biological Supply Company and were stored in a dark cabinet at room temperature. Seeds of both species were allowed to germinate for one week before being planted in sterilized soil in large D-pots. The plants were allowed to take root in the D-pots in a greenhouse exposed to natural sunlight for two weeks before the ethylene glycol treatments began. The plants were watered with 200ml of water twice a week for this period of time. After the initial two weeks the seedlings of both species were treated twice a week with 200ml of the respective concentration of ethylene glycol solutions.

The ethylene glycol solutions were made by diluting the appropriate amount of 99% lab grade ethylene glycol, which was produced by Sigma-Aldrich Milwaukee, WI.

After the 5th week of treatment, all of the plants were completely harvested. The replicates were dried in a drying oven at 65° C for 2 days and then aboveground, belowground, and total biomass measurements were made. This data was used to calculate root to shoot ratios for each replicate. In addition to these calculations, the percent survival from both species and all the levels of ethylene glycol treatment concentrations were calculated.

A 2-way ANOVA was used to determine whether there were statistically significant differences in the biomass and root to shoot ratios among all the treatment groups using the computer program SAS JMP version 5.1 at the threshold value of α =.05. We also conducted a log-likelihood analysis on the survival percentages to determine significance at the threshold value of α = .05 using the computer program SAS JMP version 5.1.

Results

The effect of ethylene glycol on total biomass differed for the two species (F=5.8974, p=.0010). Therefore, the effect of ethylene glycol on total biomass was dependant on species. Both species' total biomass decreased with increasing ethylene glycol concentrations (F=26.2295, p=<.0001). *Ailanthus altissima* showed a 1,300% greater decrease in biomass between ethylene glycol concentrations of 0mg/l and 25mg/l than did *R. pseudoacacia.* Specifically, *A. altissima*'s biomass decreased by 34.1% between the control concentration of 0mg/l and 25mg/l, whereas *R. pseudoacacia*'s biomass decreased by 2.66% at the same levels of ethylene glycol. However, between the ethylene glycol levels of 0mg/l and 1,300mg/l *R. pseudoacacia's* biomass was 23% less than *A. altissima's* biomass (Figure 2).

The effect of ethylene glycol on root: shoot differed between the two species (F=6.4813, p=.0005). The mean root to shoot ratios of both species showed an overall increase with increasing ethylene glycol concentration treatments (F=35.8345, p=<.0001). In particular, *A. altissima*'s root to shoot ratio decreased by 39.2% between the 0mg/l to 25mg/l concentrations of ethylene glycol, but then increased by 35.9% from 0mg/l to 1,300mg/l concentrations. However, *Robinia pseudoacacia's* root to shoot ratio showed an overall increase (Figure 3).

Ethylene glycol significantly affected the survival of both species (χ^2= 78.76, p= <.0001), but the effect of ethylene glycol on survival was not dependant on species (χ^2= 2.138, p= .5444). Survival showed an overall decrease as ethylene glycol treatment concentrations increased. *Robinia pseudoacacia* had higher survival percentages at the lower concentration of ethylene glycol, and its survival percentage was unchanged between treatment groups of 0 mg/l and 25mg/l. *Ailanthus altissima* showed decreasing survival percentages as ethylene glycol treatment concentrations increased. At the ethylene glycol treatment level of 1,300mg/l *A. altissima* had a higher percent survival 73.3% than *R. pseudoacacia* at 66.6%. All of the replicates of both species died at the ethylene glycol treatment level of 19,000mg/l (Table 2).

Discussion

According to our results our first hypothesis, that we would see a statistically significant reduction in total growth or biomass when exposed to ethylene glycol, was supported. Our second hypothesis, which was that *R. pseudoacacia* growth would be inhibited by ethylene glycol more than *A. altissima* was also supported according to the results we obtained. The root to shoot ratio measurements are important to explain why ethylene glycol inhibits growth. There is a positive trend between root to shoot ratio and ethylene glycol concentration for both species (Figure 4). This may be a preferential growth response of the plants to increase their root surface area to absorb more water as opposed to increasing their shoot length due to the water stress caused by ethylene glycol binding to water making it inaccessible to the plants (Hsiao and Xu 2000, Stock and Briggs 2000). *Ailanthus altissima* showed less of a root to shoot ratio increase than *R. pseudoacacia* possibly due to its ability to conserve water by shrinking stomatal openings, which was observed in a previous study by Trifilo *et al.* (2004) when *A. altissima* was water stressed. This trait of *A. altissima* suggests why it did not grow as much root tissue when compared to *R. pseudoacacia* because *A. altissima* can effectively reduce its hydraulic conductance and grow using less water (Trifilo *et al.* 2004). We found a significant reduction in growth of both species *A. altissima* and *R. pseudoacacia* due to the drought stress caused by ethylene glycol. Therefore, our results are contrary to the results found by Trifilo *et al.* (2004), which showed no significant growth changes in *A. altissima* when grown under water-stressed conditions. Although, this is assuming that ethylene glycol has no other mechanism to decrease growth other than by drought stressing the seedlings.

Since *A. altissima* had greater biomass and had a higher percentage of survival at a higher ethylene glycol level (1,300mg/l), it may gain a competitive advantage over *R. pseudoacacia* at the seedling stage where they coexist with one another in areas with ethylene glycol pollution levels of approximately 1,300 mg/l. So, in areas where ethylene glycol pollution is high, such as around airports, production plants, and the PRT tracks management precautions should be taken when *A. altissima* is present to prevent it from outcompeting the native flora.

At a lower level of ethylene glycol pollution (25mg/l), *R. pseudoacacia* may have the competitive advantage because it had both a higher survival percentage and showed less of a decrease in biomass from the control group than *A. altissima*. Therefore, in areas with a lower concentration of ethylene glycol pollution *R. pseudoacacia* may be able to outcompete *A. altissima* at the seedling stage. *A*lthough, most competition is for light not water at the seedling stage in the disturbed areas that these species inhabit.

The overall ecological implications of these findings could be important because of the contributions that *R. pseudoacacia* make in forest succession and its ability to fix nitrogen in large amounts (Boring and Swank 1984, Call and Nilsen 2002). These properties are ecologically beneficial to the areas that *R. pseudoacacia* inhabits because it promotes the growth of other species and therefore increases plant biodiversity in the area (Busing 1995, Hodder and Bullock 1997, Manchester and Bullock 2000). Also, invasive species, such as *A. altissima,* can alter the resource supply and nutrient regime of an area by displacing native species (D'Antonio *et al.* 2000, D'Antonio *et al.* 1998, D'Antonio and Vitousek 1992). In addition to this, invasive trees may change the local habitat so that is no longer suitable for native fauna (Trammel and Butler 1995). So, if in areas of ethylene glycol pollution levels of 1,300mg/l *A. altissima* seedlings are indeed able to displace *R. pseudoacacia* seedlings this would minimize the benefits that the local ecosystem gains from *R. pseudoacacia*, while possibly increasing the detrimental effects, listed above, that *A. altissima* could have on the local ecosystem.

Future studies examining ethylene glycol pollution could examine the effects it has on other native species such as *Liriodendron tulipifera* and compare these results to the invasive *A. altissima* or others. Also, it would also be important to know the threshold concentration of ethylene glycol that causes mortality in *A. altissima* and *R. pseudoacacia.* This would be beneficial for land management in taking precautions to prevent the spread of the invasive species, as well as, to prevent deforestation around highly ethylene glycol polluted areas. Finally, it would be interesting to do a study where *A. altissima* and *R. pseudoacacia* are grown in the same pot and exposed to ethylene glycol treatments to determine if direct competition affects the different species growth responses to ethylene glycol.

Overall, since *A. altissima* can outgrow and survive better in areas of ethylene glycol pollution of 1,300mg/l we would expect that in these areas *A. altissima* will outcompete and possibly eliminate *R. pseudoacacia.* This may cause some of the detrimental effects of invasive plant species to occur in the area. But where there is ethylene glycol pollution of 25mg/l we would expect *R. pseudoacacia* to outcompete *A. altissima* and lessen amount of *A. altissima* trees found in these locations. Since there is more land in the vicinity of roads and highways than around production plants we speculate that ethylene glycol pollution will overall not be detrimental to ecological biodiversity, but may actually increase overall biodiversity by giving *R. pseudoacacia* a competitive advantage over *A. altissima* in more areas.

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Table 1- The 2x4, Two Way Factorial Experimental Design With Two Levels of Species and Four Levels of Ethylene Glycol Treatment Concentrations.

	Concentration of Ethylene Glycol			
Species of Tree	**0 mg/l**	**25 mg/l**	**1,300 mg/l**	**19,000 mg/l**
A. altissima	n=15	n=15	n=15	n=15
R. pseudoacacia	n=15	n=15	n=15	n=15

Table 2: Survival Percentages of Species For Ethylene Glycol Treatment Levels				
	Ethylene Glycol Concentration (mg/l)			
Species	**0**	**25**	**1,300**	**19,000**
Ailanthus altissima	86.7	73.3	73.3	0
Robinia pseudoacacia	93.3	93.3	66.6	0

Figure Legend

Figure 1- Chemical structure of ethylene glycol ($C_2H_6O_2$) MW= 62.07

Figure 2- Mean total biomass (g) versus ethylene glycol treatment concentration (mg/l) (± standard error bars). *Ailanthus altissima* is denoted with blue bars and *R. pseudoacacia* with yellow bars.

Figure 3- Mean root to shoot ratios versus ethylene glycol treatment concentrations (mg/l) (± standard error bars). *Ailanthus altissima* is denoted with blue bars and *R. pseudoacacia* with yellow bars.

Figure 4- Root: shoot biomass (g) versus ethylene glycol concentration (mg/l) of *R. pseudoacacia* and *A. altissima* with trend lines for both species showing a positive correlation between the root to shoot ratio and increasing ethylene glycol concentration in both species. The slope of the *R. pseudoacacia* trend line is greater than the slope of the *A. altissima* trend line, meaning that the correlation is greater for *R. pseudoacacia.* The 19,000mg/l ethylene glycol treatment concentration was omitted because none of the seedlings survived.

Figure 1

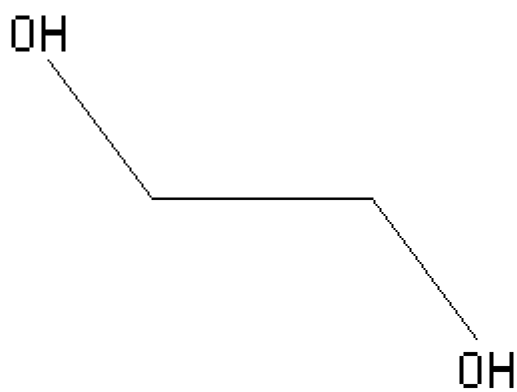
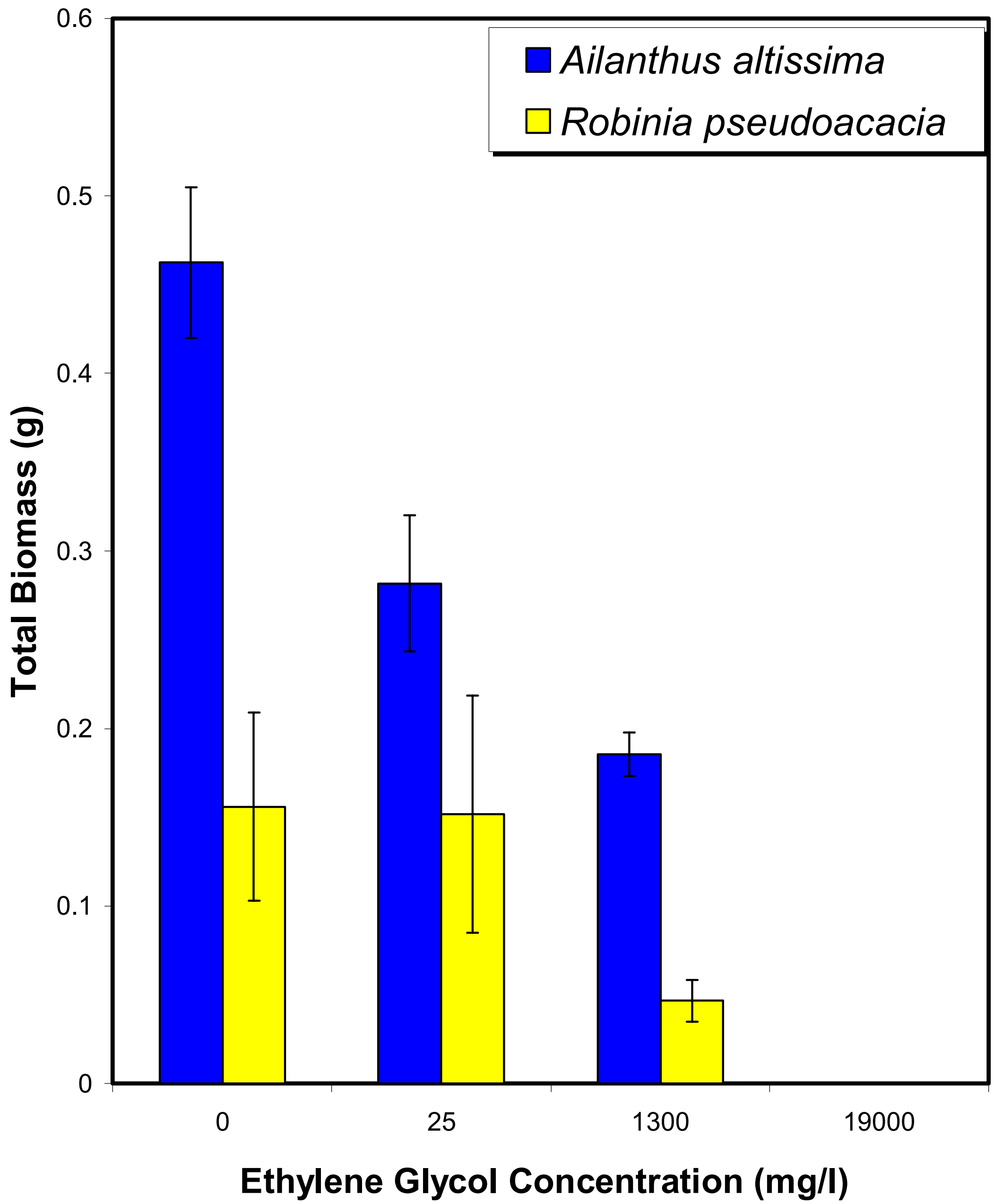


Figure 2



0.6
0.5
0.4
0.3
0.2
0.1
0
Total Biomass (g)
Ailanthus altissima
Robinia pseudoacacia
0
25
1300
19000
Ethylene Glycol Concentration (mg/l)

Figure 3

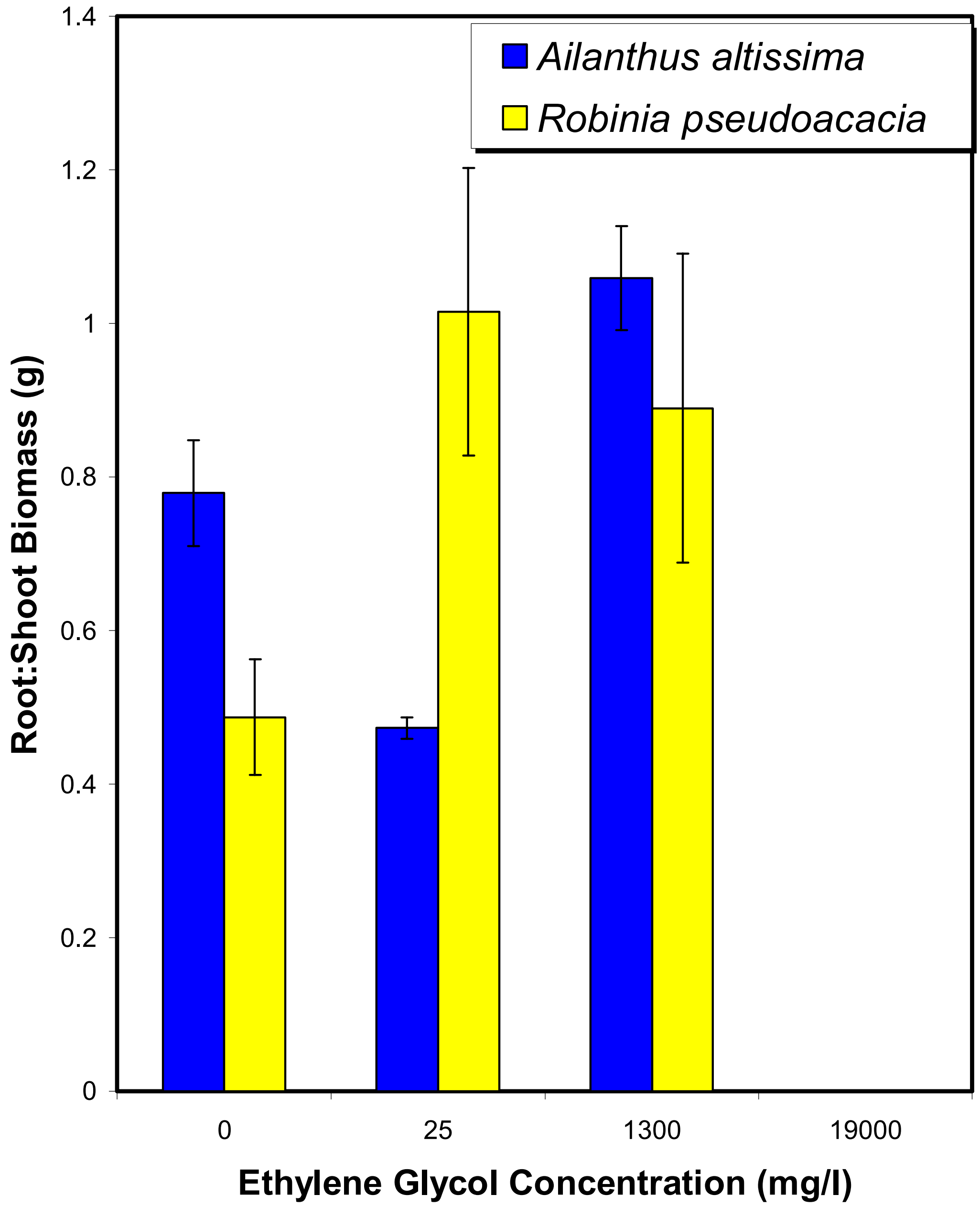
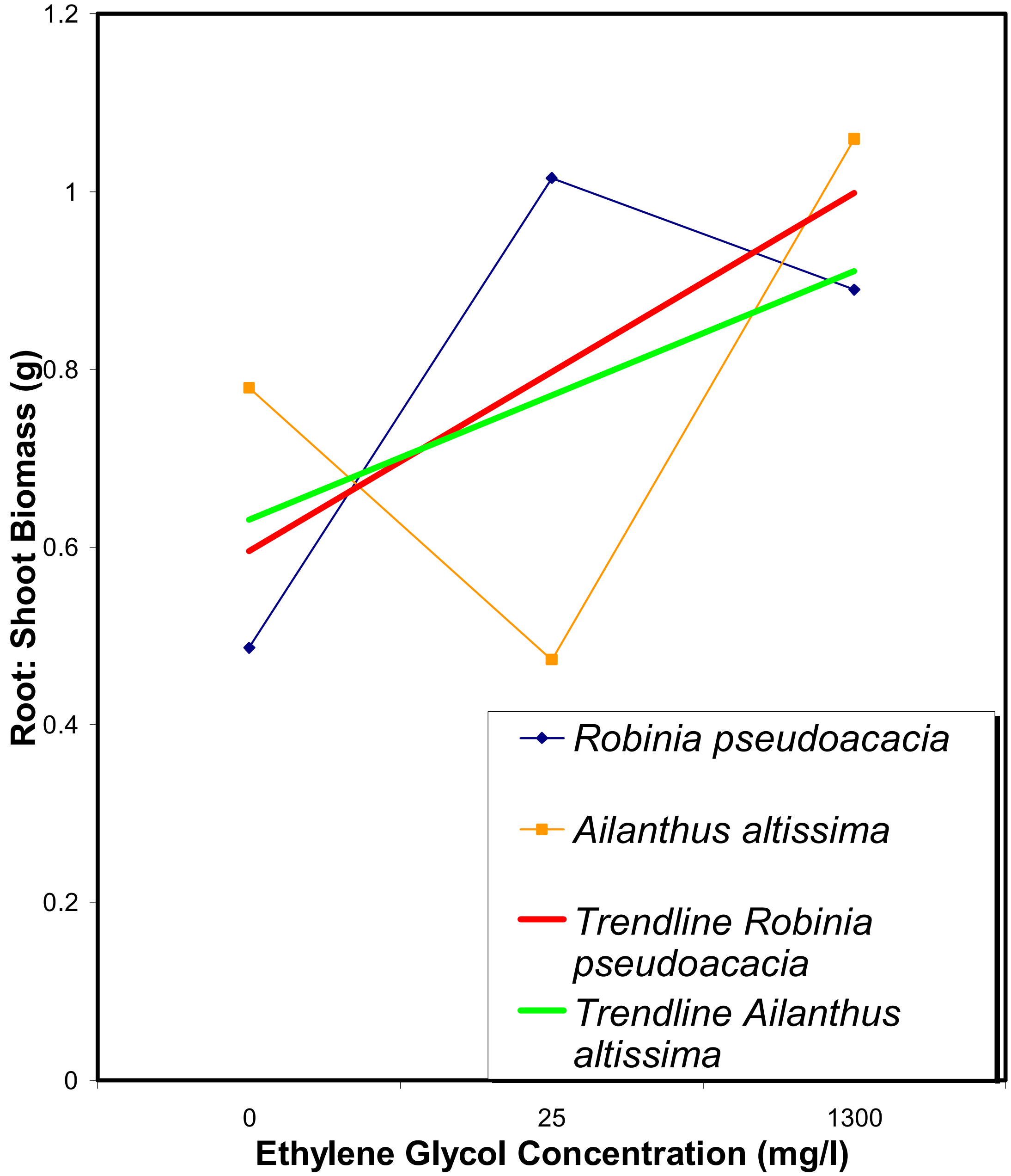

Ailanthus altissima
Robinia pseudoacacia
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Root:Shoot Biomass (g)
0
25
1300
19000
Ethylene Glycol Concentration (mg/l)

Figure 4



1.2
1
0.8
0.6
0.4
0.2
0
Root: Shoot Biomass (g)
0
25
1300
Ethylene Glycol Concentration (mg/l)
Robinia pseudoacacia
Ailanthus altissima
Trendline Robinia pseudoacacia
Trendline Ailanthus altissima