Effects of water from an original and alternate location on the survival and growth of two genetic variants of *Polygonum cuspidatum*

Triad #41

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Abstract

Contaminated Mine Drainage, or CMD, is a particular ecological issue affecting the water quality of riparian zones in Appalachia by increasing the abundance of toxic metals and lowering the pH. Two West Virginia watersheds which contain an overabundance of pollution are the Monongahela River and Deckers Creek, the latter of which displays considerably higher amounts of aluminum, iron, manganese, and zinc. The poor water quality could negatively impact riparian plant species whose root systems extend toward the water in search of nutrients. We studied an invasive plant species abundant along the watersheds, *Polygonum cuspidatum,* which had been exposed to the harsh conditions of the two water sources long enough that the populations had become genetic variants of each other. In this experiment we considered the effects of water source on the growth of two genetic variants of *P. cuspidatum* by planting one hundred and twenty seeds, sixty of each genotype. Twenty seeds of each genotype underwent treatment by fresh water, water from that genotype's original source, or water from the alternate source. A significant interaction was observed between water source and genotype on the biomass of the plants ($F = 42.0482$, $p < 0.0001$). Plants of a certain genotype displayed greater biomass when treated with water from their original source than with water from the alternate source. In addition, the effect of water source on genotype significantly affected the plant height ($F = 5.6704$, $p = 0.0045$). Plants possessing the Deckers Creek genotype exhibited greater plant height when given water from Deckers Creek than water from the Monongahela River. These results indicate that plants possess a genotype unique to their environment which enables them to grow successfully, and in the case of invasive species, spread rapidly even when subjected to harsh conditions such as polluted water. Unless controlled, this could ultimately result in a reduction of biodiversity in riparian areas.

Introduction

Contaminated Mine Drainage (CMD), along with other forms of pollution, flowing into various water sources is an ecological issue of growing importance across the globe (Heath 1995). CMD is particularly apparent in the Appalachian region where coal mining is a vital industry (Olson and Quirindongo 2004, Stewart and Skousen 2003). The water systems in Morgantown, West Virginia are significantly affected by mine pollution characterized by low pH and elevated concentrations of metals such as iron, manganese, aluminum, sulfate, phosphate, arsenic, and lead, among others (Olson and Quirindongo 2004, Robb and Robinson 1995, Stewart and Skousen 2003). Many riparian ecosystems exposed to higher dissolved metal concentration and acidity can experience negative effects from CMD such as a decrease in the diversity and density of flora (Chapin 1991, Mengel and Kirkby 1978, Stephenson, *et al*. 1995).

Two prevalent watersheds in the Morgantown area, the Monongahela River and Deckers Creek, both contain an unacceptable amount of pollution from upstream utility corridors. The predominant toxins researched in these water sources are aluminum, iron, manganese, and zinc (Olson and Quirindongo 2004, Stewart and Skousen 2003). Studies indicate that while the Monongahela River has very high pollution levels, those of Deckers Creek are significantly greater (Olson and Quirindongo 2004, Stewart and Skousen 2003; Table 1).

Though noxious chemicals in the water pose a direct threat to riparian organisms, they additionally may be indirectly harmful by changing the pH of the water. The pH is lowered by the oxidation of iron disulfide in pollution which produces sulfuric acid. The acidic water consequently dissolves other metals from the drainage, making them much more toxic because they are more readily taken up by the roots and passed through cell membranes when in solution (Olson and Quirindongo 2004, Stewart and Skousen 2003). Exposure to toxic metals may potentially alter physiological structures and activities of the plant, including the cell wall and the process of transpiration (Barcelo and Poschenrieder 1990). Ballach and Wittig (1996) studied the effects of lead and platinum on the cellular activities of *Salicaceae;* the metals they used are two of the many dissolved toxins found in the Monongahela River and Deckers Creek. In their experiment, lead and platinum accumulated on the cells walls of the rhizome and decreased the plant's water supply, further affecting the organism by reducing transpiration rates in order to conserve water.

A species commonly found along riparian zones in the Morgantown area and exposed to the contaminated water sources is Japanese Knotweed, *Polygonum cuspidatum* (Beerling *et al.* 1994). *Polygonum cuspidatum*, native to Asia, is now found in abundance in North America due to its weed-like, invasive behavior (Baker 1994, Beerling *et al.* 1995). Invasive species are infamous for their adaptive nature which enables them to out-compete native species for nutrients and to reproduce at a highly efficient rate; they are also threatened by few natural predators in their invasive range, contributing to their survival and distribution (Chornesky and Randall 2003, Mooney and Cleland 2001). Furthermore, invasive species display behavioral characteristics which aid them in competition for space, as well as make them more aggressive predators (Chornesky and Randall 2003, Mooney and Cleland 2001). The spread of *Polygonum cuspidatum* is additionally aided by the nature of its rhizome. Rhizomal fragments, from which an entire plant can regerminate, are readily distributed by birds and the wind (Hollingsworth *et al.* 1998). The stalks of riparian invasive species also form dense, monotypic stands which ultimately displace the native species (Kiviat and Talmage 2004, Meade 1982). These characteristics enable *P. cuspidatum* to significantly affect the biodiversity in any area it inhabits.

Though its invasive nature allows *Polygonum cuspidatum* to thrive in a wide range of environments, both healthy and disturbed, plant populations must have a method of adjusting to stressful conditions posed by the environments in order to remain successful. Plants in a certain area can develop characteristics specific to their unique habitat. Lotscher and Hay (1997) contend that the habitat in which a plant interacts with other species and resources is an essential factor in determining which characteristics the plant will express. An environmental stress such as the modification of a vital resource – water, for example – is sufficient to produce genetic variants (Fang *et al.* 2004). Genetic variation is capable of inducing different responses to a related stress among plants of the same species (Baker 1971, Parker *et al.* 2003). Baker's (1971) and Parker's (2003) research consequently suggests that genetic variants may respond differently to the stress of a polluted water source.

The tolerance of *Polygonum cuspidatum* to heavy metal contamination was studied with results indicating that the plant could grow on soils polluted with copper, zinc, and cadmium (Kubota *et al*. 1988). In 1991, Beerling showed that *P. cuspidatum* actually occurs in much greater abundance than expected on polluted ground and in riparian zones affected by contaminated drainage, suggesting that this species may even have a preference toward stressful environments.

Most plant populations exposed to separate environmental pressures are known to become genetic variants after an extended amount of time in their habitats (Baker 1971, Lotscher and Hay 1997, Parker *et al.* 2003). *Parker* (2003) suggested that work should be done in which an environmental factor was reciprocated between two genotypes of an invasive species to help determine whether that species is adapted specifically to its original habitat or if it can thrive equally well when exposed to the other habitat.

The study of genetic variation in invasive species and pollution presents numerous ecological implications at both a local and global scale. Invasive species create intense competition among neighbor species and therefore have the capability to drastically alter the biodiversity of ecosystems. Genotypic variation is an extremely beneficial ability, particularly for species disturbed by a competing invasive. On the other hand, genotypic variation will also aid invasive species, thereby disadvantaging the native species and contributing to a loss of biodiversity. When so much of the Earth is polluted, it is important to investigate how drastically a plant's genotype can alter the surrounding ecosystem, particularly the plant species with which it directly competes. If growth of an invasive species is unaffected by the toxic water levels, the species may continue to disrupt the biodiversity of the ecosystem. If the growth of an invasive species genetically adapted to survive under harsh conditions was negatively affected by pollution in a water source, it may indicate that the water quality has reached critical conditions.

In this study we examined the effects of water from an original and alternate source on the growth of two genetic variants of the invasive species, *Polygonum cuspidatum*. *Polygonum cuspidatum* seeds were collected from the Monongahela Riverbank and alongside Deckers Creek to acquire plants which would vary genetically in accordance with the stresses posed by their separate habitats. We hypothesized that plants grown with water collected from their respective source would grow more successfully than those plants grown with water from the alternate location. We theorized that the higher toxicity of Deckers Creek water created a genotype which would better prepare a plant such as *P. cuspidatum* for harsh water conditions. As such, we expected that Deckers Creek plants grown with Monongahela River water would thrive more than Monongahela plants grown with Deckers Creek water. In addition, we believed that, on average, plants grown with Monongahela River water would experience healthier growth than plants grown with Deckers Creek water.

Methods

A two-way factorial design was used in order to determine the effects of water source on the invasive species, *Polygonum cuspidatum*. The first factor was water source and had three levels, fresh water, Monongahela River water, and Deckers Creek water. The second factor was genotype and had two levels, Monongahela River genotype and Deckers Creek genotype. The fresh water source from the greenhouse hose, acted as a control in the experiment. There were 20 replicate plants for every treatment combination, totaling 120 plants (Figure 1).

Since we used specific plant genotypes and water sources in the area, seeds and water had to be collected from both the Monongahela River and Deckers Creek. A surplus of seeds were collected from thriving *P. cuspidatum* plants as close as possible to the water source, and water was collected in buckets from the river in areas near the plants from which we obtained the seeds. Monongahela River seeds were collected upstream from Deckers Creek in order to ensure that neither the Monongahela water nor the Monongahela genotype had been affected by the Deckers Creek pollution. The water was stored in a greenhouse at ambient temperature, and the seeds were refrigerated.

One hundred twenty D25 pots were used for planting. The seeds were planted in Pro-Mix soil, approximately one centimeter deep with two seeds per pot, one on each side of the pot. Two seeds per pot were planted, giving 240 seeds a chance to germinate. This helped to ensure that there would be 20 plants to be measured per treatment combination. After planting, the pots and water buckets were placed in a greenhouse at ambient temperature and CO_2 levels with natural lighting through a glass ceiling.

Immediately following planting, the seeds were watered with the appropriate water treatments. We decided to germinate the seeds using the water treatments instead of just using fresh water so the seeds would experience the potential stress offered by an alternate water source for the entirety of the experiment. During watering, each pot received 15 mL of water. The greenhouse was visited every day to check the moisture in the pots, and each pot was given 15 mL of water if needed. The trays were also rearranged every three days to give all treatments equal lighting exposure. Germination was first recorded 10 days after planting. Germination of all treatment combinations occurred continuously in the following weeks. All except two pots had germination 27 days after planting. On Day 27 we thinned out the pots, leaving the healthiest plants, so that each of the pots only had one plant, consequently eliminating space and nutrient competition between the plants. This left 20 plants for each of the six different treatment combinations, yielding the final 120 plants. We allowed the plants to grow and be watered for an additional two weeks, ending a six-week treatment period.

The plants, including both shoots and roots, were then harvested and rinsed. The shoot height was measured, and the plants were then dried in an oven at 65ºC for 48 hours so that biomass could be measured.

The data collected was entered into SAS JMP Statistical Software, Version 5.1 (Statistical Analysis Systems 2002). The effect of water source, genotype, and their interaction with each other was determined using a two-way analysis of variance. A significance threshold of $\alpha = 0.05$ was used to indicate whether the treatments had a statistically significant effect.

Results

The effect of water source on the biomass of *Polygonum cuspidatum* depended on genotype (F = 42.0482, $p < 0.0001$). The Monongahela plants grown with Monongahela River water had an average biomass of 16.605 mg, this is 59.7% greater than the average biomass of Monongahela plants grown with Deckers Creek water and 40.4% greater than the average biomass of Monongahela plants grown with fresh water (Figure 2). Similarly, the average biomass of Deckers Creek plants grown with water from Deckers Creek was 19.7 mg, 63.7% greater than the average biomass of Deckers Creek plants grown with Monongahela River water and 42.8% greater than the average biomass of Deckers Creek plants grown with fresh water (Figure 2). Biomass was affected significantly by genotype regardless of the water source (F = 13.0780, p = 0.0004). The average biomass of the Monongahela genotype plants was only 85.3% of the average biomass of the Deckers Creek genotype plants (Figure 2) showing that the Deckers Creek seeds were more successful than the Monongahela seeds even when water source was not considered. Water source also significantly affected plant biomass regardless of the genotype (F = 4.5255, p = 0.0128). The average biomass of plants watered with Deckers Creek water was 15.048 mg, 5.1% greater than plants watered with Monongahela River water and 17.4% greater than plants watered with fresh water (Figure 2).

The effect of water source on plant shoot height significantly depended on genotype (F = 5.6704, p = 0.0045). Monongahela plants watered with Monongahela River water had an average shoot height 5.7% greater than Monongahela plants watered with Deckers Creek water and 16.0% greater than Monongahela plants watered with fresh water (Figure 3). Likewise, Deckers Creek plants watered with Deckers Creek water displayed an average shoot height that was 14.8% greater than Deckers Creek plants watered with Monongahela River water and 2.6%

greater than Deckers Creek plants watered with fresh water (Figure 3). Shoot height was not significantly affected by genotype regardless of water source (F = 0.8485, p = 0.3589), nor did water source have a significant effect on shoot height regardless of genotype (F = 1.1260, p = 0.3279).

Discussion

The experiment was designed to determine the effects of water source from an original and alternate location on two genotypes of the invasive species, *Polygonum cuspidatum*. The two-factor hypothesis stated that each of the genotypes of *P. cuspidatum* plants would experience a different growth response when treated with water from their original location than with water from the alternate location. The overall results of this experiment indicated, with a 5% confidence level, that a genetic variant of *Polygonum cuspidatum* was better adapted to its natural water source than to a separate water source with different degrees of toxicity. There was a significant interactive effect between the two factors, however, genotype and water source by themselves did not affect both biomass and plant height significantly.

Genotypic variants distinguish themselves according to environment-specific traits which enable them to be successful amid unfavorable conditions. After a period of time, a population may develop a genotype advanced enough to prefer its own habitat over another, regardless of the amount of stress that habitat provides. Therefore we hypothesized that plants of a particular genotype would develop more healthily when nurtured with water from their own habitat than if they were given water from an alternative location. The hypothesis was supported upon observing that the Monongahela River genotype had greater biomass and plant height when grown with its native Monongahela River water than with Deckers Creek water; likewise the

Deckers Creek genotype experienced greater biomass and plant height when grown with Deckers Creek water (Figure 2, Figure 3). The results illustrate that genetic variation can cause a population to respond uniquely to different water sources and, when looked at in a broader sense, to many different ecological conditions. Our results show that *Polygonum cuspidatum* has specific environmental preferences. This suggests that invasive species are not necessarily always pre-adapted to luxuriate in every type of situation, a theory which contradicts the speculation of a general-purpose genotype. The general-purpose genotype describes the genotype of an invasive plant as the result of the long-term evolution of genes which grant the individual the ability to thrive among a variety of environmental conditions equally (Baker 1965, Baker 1994, Parker *et al.* 2003). Though many invasive species may adopt the general-purpose genotype, the results of this study show that some invasive species, such as *P. cuspidatum*, can either select or adhere to the strategy of local, rapid adaptation. This finding may have several implications including the potential for increased invasions due to additional mechanisms by which a species can invade.

The experiment also tested another hypothesis which stated that a population that had been exposed to harsher conditions would result in a hardier genotype and experience greater growth on average than the relatively less perturbed population. Our results showed that the Deckers Creek genotype's average biomass was significantly greater than that of the Monongahela River genotype (Figure 2). The results were in agreement with our hypothesis. Since water flowing through Deckers Creek has substantially higher metal concentrations than the Monongahela River (Table 1), it can be expected to produce a genotype more capable of handling a highly stressed existence. It has been theorized that the production and growth of a genotype is dependent upon its exploitation of resources (Lotscher and Hay 1997). In expansion of this idea, a unique characteristic of the Deckers Creek genotype is that it may have developed a use for some of the “toxins” in the polluted water, making them not so toxic to that population (Kubota *et al.* 1988).

We additionally hypothesized that plants watered with Monongahela River water would be healthier than plants watered with Deckers Creek water. The data did not support this theory and instead showed that plants grown with Deckers Creek water had a significantly greater biomass than plants grown with Monongahela River water (Figure 2). The relatively exorbitant amount of pollution in Deckers Creek makes this a very surprising observation, but previous research offers suggestions as to why this occurred. The invasive species, *Polygonum cuspidatum,* has been found in locations strongly influenced by waste, mine drainage, and other forms of pollutions at such a high frequency that many scientists believe this species could possibly be attracted to environments which non-native species find threatening (Beerling 1991, Beerling, *et al.* 1994, Chornesky and Randall 2003, Mooney and Cleland 2001, Rubino, *et al.* 2002). A reason for this is that some invasive species may find many of the chemicals to be nutritious. Past experiments have been successful in isolating a Cu-binding protein in *P. cuspidatum* (Kubota, *et al.* 1988). Kubota’s (1988) team was able to locate the protein and measure its content in the cytoplasm. They found that the amount of protein increased with increased copper levels, suggesting that this invasive plant can not only tolerate copper, but may also have an affinity toward it.

Genotype and water source, when considered individually, were both found to significantly affect biomass, but neither of those factors had an independent effect on plant height (Figure 3). Though Fang’s (2004) research team ascertains that plant height is a trait significantly affected by genotypic variances, our results say the opposite. A plausible explanation has been termed the "lag effect"; this supposition expects some invasive species to remain at a reasonably low population for years before exploding (Cousens and Mortimer 1995, Mooney and Cleland 2001, Reichard and White 2001, Scott and Panetta 1993). Perhaps plant height of *Polygonum cuspidatum* is not greatly affected by single factors such as genotype or water source until later in their life cycle. Since our research only consisted of data from six weeks of plant growth, future work should include a long-term approach to this experiment in order to acquire more information about the lag effect in relation to *P. cuspidatum* and other invasive species.

Invasive species have the potential to alter the environment tremendously, a characteristic which makes them a vital ecological topic. *Polygonum cuspidatum* was introduced from Asia to foreign areas where it experiences little to no natural predation to control it (Beerling, *et al.* 1994, Beerling, *et al.* 1995). It has made its way into the United States, Britain, and many other parts of the globe (Beerling, *et al.* 1994). It is estimated that, due to their aggressive behavior, invasives have come to dominate at least 3% of the ice-free surface of Earth (Mooney and Cleland 2001). Making its home in riparian zones, this species can exaggerate floods by clogging river channels; flooding can then cause erosion in watersheds, changing the sedimentation of the river and ultimately affecting the species occupying the river (Kiviat and Talmage 2004, Meade 1982).

The two genotypes produced by the separate water sources had a large effect on the growth of *Polygonum cuspidatum* (Figure 2, Figure 3). Every living organism greatly relies on water to survive, making it the most fundamental resource on the planet. The fact that such disparate genotypes had arisen due to water source shows that devastating water quality in both Appalachia and across the biosphere is not an issue to be overlooked. The poor state of the water did not inhibit survival of the species, which may seem to lessen the threat of the toxins, but growth under such undesirable conditions has brought about very durable genotypes that can withstand pressures that other species can not. This aids the spread of invasive species to an even higher degree which lead us to realize that the need to improve water conditions around the world is of even greater urgency. A supplemental experiment would be to test the effect of genotype on water source with a *non-invasive* species. Our study shows that invasives are able to rapidly adapt to stressful conditions, but additional research is needed to determine if a non-invasive species would have the tools necessary to alter their genome in order to cope with the chemicals in the water. If that species were to exhibit depressed fitness, it would add ecological relevance to the quality of water and possibly compel society to respond.

Despite the toxicity of the water, our invasive species was able to germinate and grow successfully. Using *P. cuspidatum* as a model species, this implies that other noxious species around the world have the ability to effectively invade areas experiencing both high and low stress levels. It is well-known that the successful invasion of a species to a new area will conclude in a loss of biodiversity by affecting the growth and distribution of native species (Chornesky and Randall 2003, Kiviat and Talmage 2004, Mooney and Cleland 2001). Hybridization, niche displacement, competitive exclusion, predation, and extinction of native species are all results of invasions, and such a loss of biodiversity could have disastrous repercussions including an alteration of the evolutionary pathway (Mooney and Cleland 2001).

Polygonum cuspidatum is one of the foremost invasive species in Great Britain (Beerling, *et al.* 1994). This is not surprising considering our results regarding genotypic variation in *P. cuspidatum* and its ever-enlarging distribution. The unanticipated point is that, even though invasions have numerous negative consequences, the spread of this invasive species may not be wholly unfortunate. The Institute of Terrestrial Ecology in Britain researched *Polygonum cuspidatum's* potential use as a source of renewable energy and considered it to be one of the most productive terrestrial plant species in Britain (Beerling, *et al.* 1994). Investigation of invasive species as energy sources should continue because it may lessen the need for coal and other polluting industries.

Contaminated Mine Drainage and invasive species are serious factors impacting the ecological world. Environmental stresses, such as poor water quality brought about by CMD, distinguish populations of invasive species by pressuring the individuals until they adapt and form a genotypic variant. *Polygonum cuspidatum* genotypes from more demanding ecosystems exhibited greater stamina to harsh conditions. In addition, plants of a certain genotype grew more successfully when grown with water from their original habitat due to gene adaptability. Though some treatments were more successful than others, all of the invasive plants prospered. The ability of an invasive species to survive under such harsh conditions poses a serious threat to the biodiversity and fitness of ecosystems in every part of the world. A benefit of invasive species is being studied in which certain invasive species may be used to produce an alternate energy source, cutting down on the amount of coal and other fossil fuels that are mined, and finally decreasing the amount of CMD into water sources. Extended research could prospectively lead to a solution for improving water quality around the world while simultaneously utilizing an overabundant, noxious plant. For these reasons, the continued study of the interaction between invasive species, pollutants, and genotypic variants should remain a focus of the biological community.

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Table 1 – Average Concentrations of Select Metals Found in the Water Sources				
	Aluminum	**Iron**	**Manganese**	**Zinc**
Monongahela River	0.101 ppm	0.200 ppm	0.061 ppm	0.051 ppm
Deckers Creek	3.100 ppm	4.600 ppm	0.600 ppm	0.200 ppm

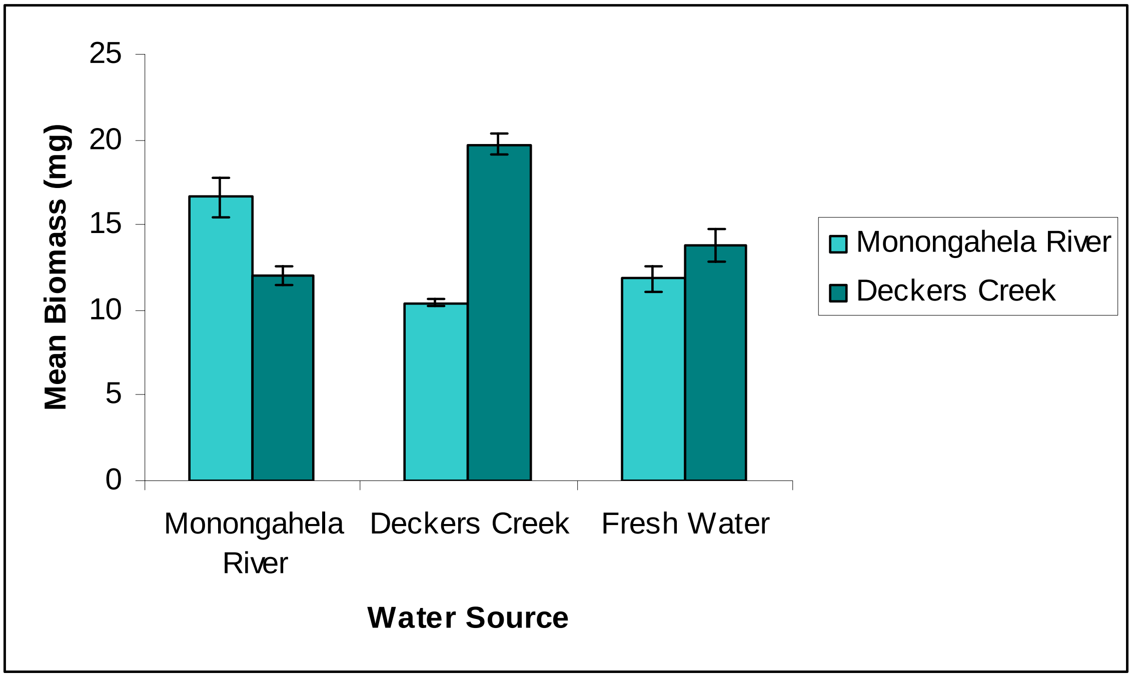
(Olson and Quirindongo 2004, Stewart and Skousen 2003).

Figure 1. Experimental design displaying *Polygonum cuspidatum* genotype vs. water source and number of replicates per treatment combination.

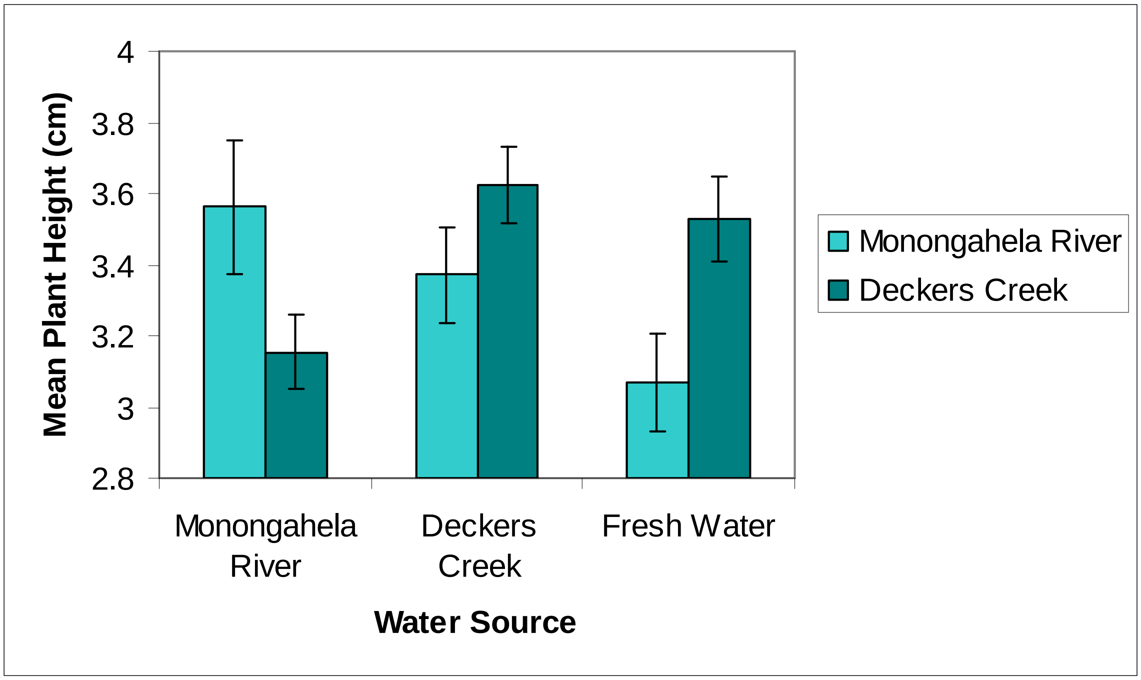
Figure 2. Mean biomass (+/- standard errors) for two genotypes of *Polygonum cuspidatum* grown with water from each of the three sources.

Figure 3. Mean shoot height (+/- standard errors) for two genotypes of *Polygonum cuspidatum* grown with water from each of the three sources.

		Water Source		
		Fresh Water	Monongahela River	Deckers Creek
Genotype	Monongahela River	n = 20	n = 20	n = 20
	Deckers Creek	n = 20	n = 20	n = 20



25
20
15
10
5
0
Mean Biomass (mg)
Monongahela River
Deckers Creek
Fresh Water
Water Source
Monongahela River
Deckers Creek


Mean Plant Height (cm)
4
3.8
3.6
3.4
3.2
3
2.8
Monongahela River
Deckers Creek
Fresh Water
Water Source
Monongahela River
Deckers Creek