

**Effects of a change in water source on the survival and growth of two genetic variants of *Polygonum cuspidatum***

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## ABSTRACT

Acid mine drainage, power plant pollution, sewage, and polluted runoff all contribute to the poor water quality of Decker's Creek and the Monongahela River by increasing the concentration of caustic heavy metals and lowering pH. Native species whose root systems extend into these bodies of water are not only negatively affected by the pollution, but the riparian areas in which they inhabit become highly susceptible to invasion by non-native, invasive species. In our experiment, we used two genetic variants of a species that has already taken advantage of these harsh conditions, *Polygonum cuspidatum*, an invasive plant from Japan. To determine the effects of genotype and a change in water source, in which the sources have different levels of pollutants, sixty plants from a Decker's Creek population and sixty plants from a Monongahela River population were grown in Decker's Creek water, Monongahela River water, and fresh water. There was a significant interaction between water source and genotype with regards to plant height ( $F = 5.6704$ ,  $p = 0.0045$ ). Plants of a given genotype grew the highest when they were grown with water from their native location. Additionally, the interaction between water source and genotype had a significant effect on biomass ( $F = 42.0482$ ,  $p < 0.0001$ ). Plants of Decker's Creek genotype flourished more greatly than the Monongahela River genotype when they were grown in water different from their native source. This indicates that the Decker's Creek genotype can acclimate and adapt more greatly to a sudden change in water quality, and will likely outlast the Monongahela River genotype. The sole existence of the more robust genotype could ultimately lead to a loss in biodiversity by displacing native plants species in the region.

## INTRODUCTION

Water pollution from acid mine drainage, power plant pollution, sewage, and polluted runoff is an expanding crisis in both developed and developing nations (Heath 1997). These forms of pollution particularly play a part in the substandard water quality in the Ohio River Valley (Showman 1997), namely the Monongahela River and Decker's Creek (Stewart and Skousen 2003, Olson and Quirindongo 2004). Many efforts are being made to convalesce these two bodies of water back to health; however, West Virginia's abandoned coalmines and ever-expanding urban development further contaminate the water. This environmental "tug-of-war" has resulted in constant and abrupt fluctuations of water pollutants, pH, and heavy metals in Decker's Creek and the Monongahela River (Stewart and Skousen 2003, Olson and Quirindongo 2004).

These sudden changes in water quality, documented by Stewart and Skousen (2003) and Olson and Quirindongo (2004), can apply significant chemical and physical strain on ecosystems exposed to them (Williams 1992, Lewis *et al.* 2002). Olson and Quirindongo (2004) observed moderate to high heavy metal concentrations and moderate to low pH levels in the Monongahela River. Stewart and Skousen (2003) observed exceedingly high heavy metal concentration in Decker's Creek, several ppm's higher than that of the Monongahela River (Table 1). High heavy metal concentration and low pH have the potential to result in decreased biomass and plant function (Lewis *et al.* 2002) for several reasons. On a molecular basis, power plant pollution and acid mine drainage in particular can severely alter water composition and nutrient availability (Robb and Robinson 1995). When pH levels fall, heavy metals can dissolve more easily, which can then physically prevent the intake of essential nutrients such as magnesium or

calcium (Lewis *et al.* 2002). The depletion of these nutrient cations results in an increase in the mobility of aluminum and an increase in the sulfur content of the soil, both potentially harmful elements to plant growth (Bulger *et al.* 2001). Furthermore, when polluted waters reach riparian habitats, the acid conditions of the water may also cause important nutrients to be leached deep into the soil, preventing uptake by roots (Bulger *et al.* 2001). These disturbances clearly create unfavorable conditions for plant growth, and because the composition of the water has the potential to change so rapidly in riparian habitats (Naiman and Decamps 1997), adaptive organisms capable of obtaining scarce nutrients are favored. Many invasive species have this heightened ability to adapt to sudden changes in water quality and have an advanced capability to uptake essential nutrients (Richardson and Rejmanek 1996, Cleland and Mooney 2001). Therefore, riparian habitats experiencing these disturbances make them vulnerable to invasive organisms (Stephenson *et al.* 1995).

*Polygonum cuspidatum*, an invasive species from Japan, is well suited to serve as an indicator for plant success in riparian habitats affected by acid mine drainage and other forms of pollution (Kiviat and Talmage 2004). From observation, *Polygonum cuspidatum* is found in abundance along the banks of both the Monongahela River and Decker's Creek, with much of their root systems extending well into these bodies of water. In fact, it is *P. cuspidatum*'s nature to thrive in areas of high human activity and disturbance (Baker 1974). This is due to the species' impressive ability to adapt to the most abrupt environmental stress in order to survive (Richardson and Rejmanek 1996). *Polygonum cuspidatum* also has the natural ability to uptake essential nutrients efficiently, however scarce or hard they may be to obtain (Kiviat and Talmage 2004). It

grows to sexual maturity quickly, forms thousands of small seeds year-round, and has the ability to germinate in low soil pH and moderate to low water and nutrient availability (Kiviat and Talmage 2004). Its seeds are dispersed by the wind, and can overtake entire areas relatively quickly because of the sheer amplitude of seeds. When these seeds grow into stalks, they form dense stands that dislocate native plants, allowing *P. cuspidatum* to continue growing (Kiviat and Talmage 2004). If stalks are disrupted and break, the individual can re-germinate from portions of its dense rhizome (Hollingsworth *et al.* 1998). For these reasons, the success of *P. cuspidatum* to a change in water source will have several important implications on the overall success of all plants in the area. Since *Polygonum cuspidatum* is presumably the ideal organism to survive and thrive in such a change, it can serve as an indicator as to how well several other species in the area will manage an abrupt change in water quality.

Environmental stress, like a change in water quality, can apply chemical and physical pressure on individuals of a population, which can lead to adaptive changes to compensate for that stress (Abrahamson and Horner 1999). Invasive species are particularly vulnerable to such adaptations because of their flexible behavior and their ability to mass-produce seeds (Baskin 1998, Parker *et al.* 2003). In plants especially, the extent of expression of certain genes is primarily dependent on the habitat in which it interacts (Lotscher and Hay 1997). Within a population of *P. cuspidatum*, intraspecific competition in the form of interference or exploitation can allow more fit individuals to thrive and pass their genes on to future generations (Brockelman 1975). When individuals fight for limited nutrients in polluted waters, the extent of competition is greatly increased; therefore only fit individuals of a certain genotype would prevail and

be able to pass their genes on (Miller and Douglas 1990). Continuing this trend over many generations, the genotype that is less adapted to a certain environmental stress has the potential to die off altogether (Rozen and Lenski 2000). Genetic variation due to various environmental stresses has been shown in previous lab studies. Fang *et al.* (2004) studied growth characters of two genotypes of meadow fescue (*Festuca pratensis*) that were adapted and unadapted to different climates. Growth characters were used as an indicator to extrapolate whether the populations would produce offspring that are genetic variants. The experiment found that organisms that adapted to climate change produced offspring genetically different from those that could not adapt (Fang *et al.* 2004). Similar tests were conducted on *Iris brevicaulis*, producing similar results. Populations of *Iris brevicaulis* increase their overall fitness by adapting to environmental stresses (Johnston *et al.* 2001). Therefore a stress like poor water quality could produce populations with altered genotypes. Since the water quality in Decker's Creek is far different than the water quality of the Monongahela River (Stewart and Skousen 2003, Olson and Quirindongo 2004) it is safe to assume that these two populations of *P. cuspidatum* have different genotypes. Furthermore, the importance of acclimation and adaptation in plants is extremely critical. Since plants are permanently anchored into the ground and cannot escape environmental change, they are forced to adapt. If locally adapted plants only do well in conditions they are used to, a change in these conditions has the potential to be devastating (Epstein 1982). Changes being made daily to the water quality of the Monongahela River and Decker's Creek, both good and bad, have the potential to severely alter the growth response of several plant species around or in these bodies of water.

The main objective of this study was to determine whether two genetic variants of *Polygonum cuspidatum*, one variant from the Monongahela Riverbank and another from Decker's Creek, would exhibit altered growth responses to different water sources: Monongahela River water, Decker's Creek water, and fresh water. We hypothesized that plants of the Decker's Creek genotype would result in significantly greater growth than of the Monongahela River genotype. Secondly, we hypothesized that the Decker's Creek water source would result in plants with significantly less growth than plants grown in Monongahela River water. Lastly, we hypothesized that plants of a certain genotype would exhibit significantly greater growth when grown with water from their native source.

## **METHODS**

To determine whether the effect of a change in water source on plant growth is dependent on plant genotype a two by three, two-way factorial design was generated (Figure 1).

The first factor was genotype and had two levels; one genotype of *Polygonum cuspidatum* was from the Monongahela Riverbank and the other genotype was from the Decker's Creek shore. The second factor was water source and had three levels, Monongahela River water, Decker's Creek water, and fresh water from the greenhouse. Fresh water was supplied by a hose and was used as a control for the experiment being that it lacks the various pollutants the other treatments contain (Stewart and Skousen 2003, Olson and Quirindongo 2004). Several hundred seeds from each genotype were collected from various plant individuals within each population, and only from plants that had much of their stalk extending deep into the water. Seeds from the Monongahela

Riverbank were collected several hundred meters upstream from Decker's Creek to ensure Decker's Creek water contaminants had no effect on these seeds. The water from the Monongahela River and Decker's Creek was collected in buckets right along where the seeds were collected to ensure that treatments correspond with the true conditions. The seeds and two buckets were stored in a greenhouse and the seeds were planted the following day.

One hundred twenty D25 pots were used for planting, with sixty pots designated for Monongahela River seeds and sixty pots designated for Decker's Creek seeds. Of the sixty pots, twenty received Monongahela River water, twenty received Decker's Creek water, and the remaining twenty received fresh water. Thus, there were twenty replicates per genotype for each of three water treatments, resulting in one hundred twenty plants (Figure 1).

However, to ensure germination, *two* seeds were planted per pot, resulting in an initial total two hundred forty seeds. Each seed was planted one-centimeter deep and at opposite ends of the pot, to alleviate much of the competition. The seeds were planted in neutral, pro-mix soil and all one hundred twenty pots were placed in a greenhouse set to ambient CO<sub>2</sub>, light, and temperature. Every three days each set of replicates was randomly rearranged with one another to remove any inequities, such as different lighting, that could arise based on location within the greenhouse.

We were interested in replicating the natural life cycle of these plants, so we decided to subject them to the water treatments right away. Each pot was given fifteen milliliters of the corresponding water source treatment everyday for the first five days after potting. From that point on, the pots were watered with fifteen milliliters per pot

either every day or every other day, depending on necessity. Ten days after planting, our first seed germinated and within the next two to three days most every other seed began to germinate. We continued watering every other day from there on out until every pot contained two plants. Seventeen days after the first sign of germination (twenty-seven days after planting) we thinned out each pot by removing the less robust plant in each. We continued watering every other day and rearranging the pots every third day until the end of the treatment period, forty-two days after planting.

At the end of the treatment period, plant height and biomass were measured. Plants were carefully taken out of each pot and the above ground plant height, from stem base to apical meristem, was measured in centimeters using a standard metric ruler. Each plant was then gently brushed until it was free of soil, placed in a paper bag, and the bags were scattered evenly in a drying oven set to 65 °C for forty-eight hours. At the end of the drying period, the biomass of each individual was measured in milligrams using a Denver Instrument Company A-160 scale.

A two-way analysis of variance statistical analysis was performed using the SAS JMP statistical software version 5.1 (Statistical Analysis Systems 2002). The effect of water source, genotype, and their interaction was determined by comparing differences in average plant heights and biomasses of each treatment. A p-value < 0.05 indicated a statistical significant difference between the various treatments.

## **RESULTS**

The effect of water source on the plant height of *Polygonum cuspidatum* depended on genotype ( $F = 5.6704$ ,  $p = 0.0045$ ). The Decker's Creek genotype exhibited an average of 3.034% higher growth than the Monongahela River genotype.

Interestingly, the lowest average plant height resulted when grown in fresh water, which grew to an average of 3.2995 cm (Figure 2). Additionally, both genotypes outgrew the other genotype when they were grown in their native water source by an average of 10.168% (Figure 2). The independent effect of water source had no significant effect on plant height ( $F = 1.1260$ ,  $p = 0.3279$ ). Additionally, the effect of genotype alone on plant height was not significant either ( $F = 0.8485$ ,  $p = 0.3589$ ).

The effect of water source on biomass differed significantly between the two genotypes ( $F = 42.0482$ ,  $p < 0.0001$ ). Therefore, the effect of water source was dependent on genotype. Plants showed greatest biomass when they were grown in water from their native location (Figure 3). Additionally, biomass was affected by water source regardless of genotype ( $F = 4.5255$ ,  $p = 0.0128$ ). Even though the plants of Monongahela River genotype exhibited their lowest biomass when watered with Decker's Creek water, on average plants grown with Decker's Creek water resulted in greatest biomass (Figure 3). When plants were grown in Decker's Creek water they exhibited an average biomass of 15.0475 mg, 17.42% greater than fresh water, and 5.08% greater than Monongahela River water (Figure 3). Plants grown with fresh water, on average, showed significantly less biomass than when grown with water from Decker's Creek or the Monongahela River. The independent effect of genotype on biomass was also significant ( $F = 13.0780$ ,  $p = 0.0004$ ). The average biomass of Decker's Creek genotype was 15.178 mg while the average biomass of Monongahela River genotype was 12.943 mg (Figure 3). Overall, the Decker's Creek genotype succeeded more greatly than the Monongahela River genotype, regardless of what water source the seeds were grown in.

## DISCUSSION

The purpose of this experiment was to examine whether genotypic variants of *Polygonum cuspidatum* would respond differently to a change in water source. Our experiment showed that the interaction between water source and genotype resulted in significant differences in plant height and biomass between group means. Nevertheless, two of our previously mentioned hypotheses were rejected. Our first hypothesis stating that the Decker's Creek genotype would result in significantly greater growth than the Monongahela River genotype was rejected in terms of plant height, but was supported in terms of biomass (Figure 2 and Figure 3). Our second hypothesis stating that the Decker's Creek water source would result in plants with significantly less growth than plants grown in Monongahela River water was also rejected (Figure 2 and Figure 3). However, our last hypothesis stating that plants of a certain genotype would exhibit significantly greater growth when grown with water from their native source was supported (Figure 2 and Figure 3).

The independent effect of water source on plant growth is an interesting one to examine because the most polluted water source, Decker's Creek water, resulted in plants with the greatest biomass. This may seem unlikely, but in fact, it is very possible. Gary *et al.* (1979) reported that pollution from industrial waste, like acid mine drainage can show initial increases in biomass. This could be due to the fact that there is a distinct effect of pH and heavy metal concentrations on plant growth. In a previously conducted experiment, *Aspergillus niger* showed initial optimal growth response at a lower pH and when nutritional heavy metals, such as zinc and manganese exist sparingly in the soil (Steinberg 1936). These two heavy metals are found in both the Monongahela River and

Decker's Creek (Table 1). Additionally, since our plants received treatments for only forty-two days, which is relatively young in the lifetime of *P. cuspidatum* (Kiviat and Talmage 2004), a greater biomass in plants grown with the Decker's Creek water is plausible (Gary *et al.* 1979). In order to truly understand the effect of water source on biomass, further research relating water source to biomass of *P. cuspidatum* should be carried out for a longer period of time. If the plants were cultivated for a longer duration and the seedlings were allowed to mature into adult plants, the plants grown with water containing lower heavy metal concentrations would likely have the largest biomass (Lewis *et al.* 2002). Lewis (2002) tested the effect of metal oxides on the biomass of stream biota, namely algae. He found that biomass and metal oxide concentration are negatively correlated in algae, riverside mosses, and liverworts. This can be predicted because it is already known that water consisting of high heavy metal concentrations will lower nutrient availability by causing phosphorus and other nutrients to be leached deep into the soil (Robb and Robinson 1995). Therefore, this aspect of the experiment should be continued for a longer period of time to determine if the expected results are reached later in life.

The collective effect of genotype and water source on the growth of *Polygonum cuspidatum* also produced significant differences in plant growth means. The Decker's Creek genotype exhibited greater growth than the Monongahela River genotype when each was grown with water other than their native source. Therefore, the Decker's Creek genotype is presumably more adaptive to a change in water source than the Monongahela River genotype. This is due to the fact that in the real world, the Decker's Creek genotype is in a location that is far more polluted than the Monongahela River genotype

(Stewart and Skousen 2003, Olson and Quirindongo 2004). When a population has a long life history of tolerating high levels of pollution it gains the ability to acclimate more readily to changes in these conditions (Taylor and Murdy 1975). An experiment testing genotypic variation and its corresponding tolerance to high heavy metal concentrations has been previously studied on grasses (Gregory and Bradshaw 1965). The study compared genotypic variants of a grass, *Agrostis tenuis*, that are tolerant to exceedingly high levels of heavy metals to those that are intolerant, and found that tolerance is clearly a genetically determined character. Grasses that have adapted and succeeded in areas disturbed by high heavy metal concentrations are more tolerant to changes in levels of these metals (Gregory and Bradshaw 1965). Our experiment showed identical results. The Decker's Creek genotype was more adaptive to changes in water source (in effect, changes in heavy metal concentrations and other pollutants) and therefore showed greater biomass and plant height than the Monongahela River genotype.

Interestingly, previous lab studies have shown that as a certain genotype forms from adaptation and becomes tolerant to a certain environmental stress, it may be accompanied by an increased sensitivity to another stress (Snyder and Hendricks 1997). Further research could be done on Decker's Creek and Monongahela River genotypes by not only changing water source, but also adding another environmental stress, like UV-B radiation to the experimental design. Creating this proposed three-way factorial design will more closely parallel the genotype's true conditions and will reveal more realistic growth responses.

The results of our experiment have given rise to many implications about the growth of genotypic variants of *P. cuspidatum* to altered water sources. Our results

demonstrate that Decker's Creek genotype plants are more tolerant to a change in water source than plants of Monongahela River genotype. The greater adaptive quality of Decker's Creek genotype could presumably allow it to outlast the Monongahela River genotype in areas of changing water quality. Acid mine drainage, power plant pollution, sewage, and polluted runoff are causing declines in the water quality of Decker's Creek and the Monongahela River (Stewart and Skousen 2003, Olson and Quirindongo 2004). However, accompanying the pollution, organizations like "Friends of Decker's Creek" and other groups are working to improve water quality. Both ends of the environmental spectrum are causing abrupt changes, both good and bad, to the water quality in our area. By doing so, several important ecological consequences could result. In response to changes in water source, Decker's Creek genotype could outlast the Monongahela River genotype due to its greater competitive ability. If the Decker's Creek genotype population were to eliminate the Monongahela River genotype as their populations grow and overlap, eventually only the more adaptive Decker's Creek genotype would exist. Reduced genetic variation within the species could make it more susceptible to disease or other disturbances (Williams 2001). The Decker's Creek genotype may be able to more successfully tolerate the stresses they experience now, but they may be less adaptive to other stresses they may likely face in the future. The potential loss in genotypic diversity within the species could result in its demise if a sudden disturbance, intolerable to the genotype, were to occur (Williams 2001).

Additionally, our experimental results show that Decker's Creek genotype grows to greater heights faster, and produces greater biomass than the Monongahela River genotype. Therefore, the genotype with greater biomass, a more extensive root system,

and greater height could be the only one existing. This could have detrimental effects on native plants unaccustomed to competing against the stronger, more adaptive genotype of *P. cuspidatum*. Since the biomass of Decker's Creek genotype is much larger and its above ground growth is greater, increased exploitation competition for nutrients and light could exist between native species that had previously never competed with this genotype. This could severely alter the distribution and development of riparian plant communities by displacing or even eliminating native species (Kiviat and Talmage 2004). The elimination of native species and the sole existence of *P. cuspidatum* in riparian habitats could increase flooding by congesting river flow due its dense stands and thick stalks (Kiviat and Talmage 2004). Furthermore, the heightened probability that *P. cuspidatum* could decrease species diversity in the region could alter trophic cycles and herbivory patterns (Sakai *et al.* 2001). Therefore, if this invasive species were to eliminate a genotype that was less adaptive and less robust there could be severe negative effects on the life and fitness of native species and on general ecosystem dynamics. In fact, this could occur even without taking the damaging effects of water pollution into account because the Decker's Creek genotype outgrew the Monongahela River genotype even in unpolluted water (Figure 2 and Figure 3).

The level water pollution due to acid mine drainage, power plant pollution, polluted runoff, and sewage is fluxing continuously in our natural environment (Heath 1997). This, along with the introduction of an invasive species to previously uninhibited natives could have detrimental effects on biodiversity. In addition, as the Decker's Creek population and Monongahela River population spread and overlap, it is likely that the more adaptive genotype will outlast the less adaptive genotype, creating further loss of

genetic variation in the region. These extreme losses in biodiversity, both of native species and within the species of *P. cuspidatum*, could make the surviving Decker's genotype more vulnerable to future disturbances that they may not be genetically capable of adapting to (Williams 2001). Therefore, it is of utmost importance to conduct further research comparing growth responses of the Decker's Creek genotype and native species to polluted waters. This could suggest a lot about competition dynamics, and could help predict possible directions biodiversity is headed in the region. Furthermore, it could serve as a basis to explain the likelihood that an invasive species will completely remove a native species in regions of polluted waters.

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<b>Table 1: Concentrations of select metals found in Decker's Creek and Monongahela River (Stewart and Skousen 2003, Olson and Quirindongo 2004)</b>				
	<b>Aluminum</b>	<b>Iron</b>	<b>Manganese</b>	<b>Zinc</b>
Decker's Creek	3.100 ppm	4.600 ppm	0.600 ppm	0.200 ppm
Monongahela River	0.101 ppm	0.200 ppm	0.061 ppm	0.051 ppm

**Figure 1.** Experimental design diagram relating two genotypes of *Polygonum cuspidatum*, Decker's Creek genotype and Monongahela River genotype, to three water sources; Decker's Creek water, Monongahela River water, and fresh water.

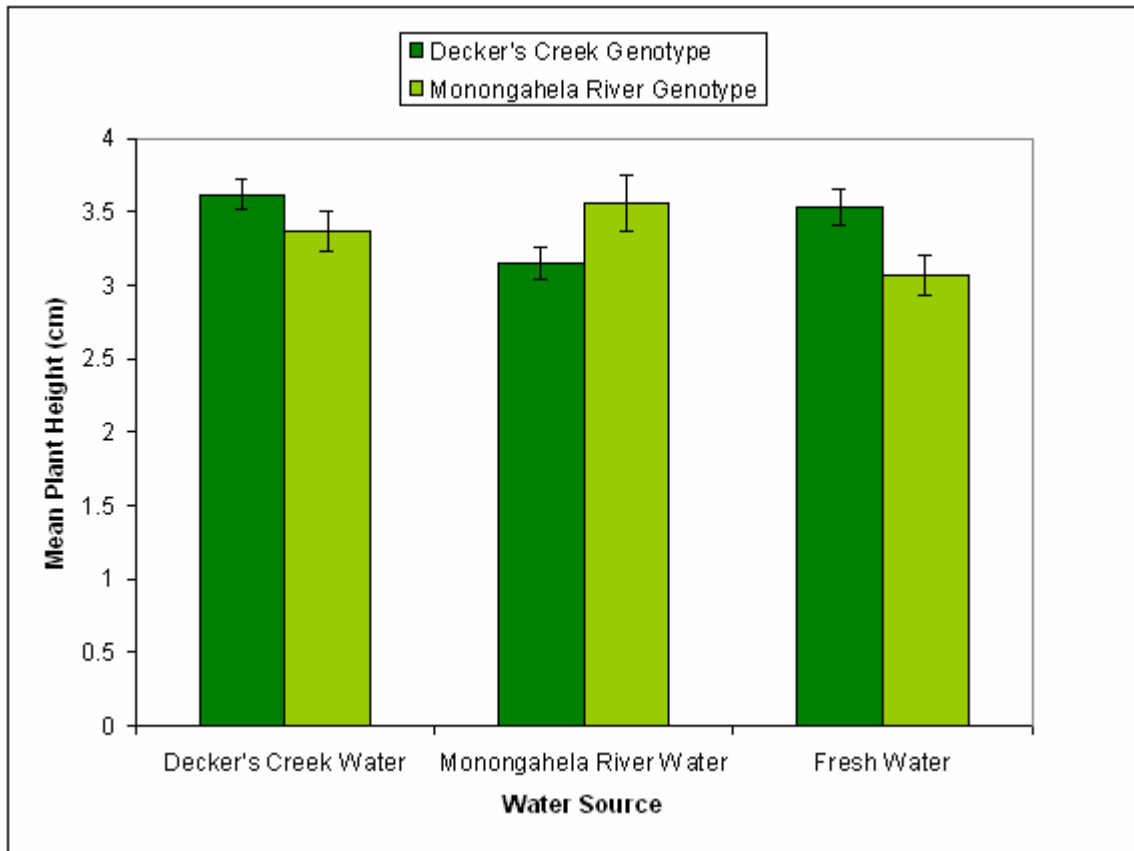
**Figure 2.** Mean plant heights (cm) (+/- standard error) for Decker's Creek and Monongahela River genotypes of *Polygonum cuspidatum* in Decker's Creek water, Monongahela River water, and fresh water.

**Figure 3.** Mean biomass (mg) (+/- standard error) for Decker's Creek and Monongahela River genotypes of *Polygonum cuspidatum* in Decker's Creek water, Monongahela River water, and fresh water.

**Figure 1**

<b>Experimental design diagram displaying water source vs. genotype and the number of replicates per combination.</b>				
		<b>Water Source</b>		
		Decker's Creek Water	Monongahela River Water	Fresh Water (Control)
<b>Genotype</b>	Decker's Creek	N = 20	N = 20	N = 20
	Monongahela River	N = 20	N = 20	N = 20

**Figure 2**



**Figure 3**

