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Bumble Bees of Kanawha County, West Virginia

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Abstract

Bumble bees (*Bombus* spp.) pollinate a wide range of the plant species and are important ecologically and economically. Some Bumble bee species are experiencing severe population declines that have been attributed to several factors. Intensive agriculture has been implicated as a potential contributor to population declines. Changes in habitat quality and landscape context are two consequences of agricultural intensification and have become amplified in the last century. Bees depend on floral resources, therefore floral resource availability influences the success of bumble bee colonies. Parasites may also contribute to the decline of bumble bees, and certain *Bombus* are more susceptible to pathogen spillover than others. To ascertain the current status of *Bombus* in this area, we conducted a census of bumble bee populations in Kanawha County, West Virginia. We hypothesized that if these *Bombus* have declined, the decline is most likely due to the exposure to chemicals released from plants throughout the state. Many of the chemicals synthesized in West Virginia are either wholly, or in part, used as components in a variety of pesticides. Research on *Bombus* needs to be increased with goals of expanding conservation efforts.

Introduction

Bumble bees (*Bombus* spp.) are are the native social pollinators of North America. They play an incredibly vital role in the pollination of plants, flowers and crops all over the world (Bumble Bee Conservation Trust 2014). Along with their relative, the honey bee (*Apis mellifera), Bombus* are very important ecologically and economically (Grixti et al. 2009). Because of their long tongues, bumble bees can pollinate flowers that *Apis mellifera* cannot pollinate as effectively. The pollination services that bumble bees provide have an economic value well into the billions of dollars annually (Goulson 2003).

Several species of bumble bees are experiencing population declines, on a global level (Kleijn and Raemakers 2008). The relative abundance and distributions of several bumble bees species have decreased over the past few decades due to multiple factors. To date, the reason for their decline remains undetermined, but strong correlations have been found in areas of intensive agriculture (Carvell et al. 2011). In particular, many North American bumble bee species have shown rapid population decreases, and collapses in the past decade (Cameron et al. 2011).

Since the 1950's, bumble bees have been observed to be in declining (Brown 2011). In the past twenty years there have been four species in particular that have shown a rapid decrease in abundance: *Bombus occidentalis, Bombus affinis, Bombus pensylvanicus,* and *Bombus terricola* (Brown 2011; Kaufman 2011). These four species populations' have diminished in places by as much as 96%, over a span of twenty years (Kaufman 2011). In a study performed from 2007-2009, covering forty different states and 382 different observation locations, these four species showed a decline ranging from 23-87% over those 3 years (Brown 2011). This huge decrease is cause for serious concern, especially due to the huge impact on the economy that bumble bees have. The specific reason for decline of these bumble bees has been difficult to

deter- mine, especially since some other species, such as *B. impatiens*, are thriving and flourishing (Kaufman 2011). Sydney Cameron, co-author of the study done by the University of Illinois' Department of Entomology, stated that, "We found that yes, indeed, [these four species] are seriously declining, but there are some other species doing very well," (Kaufman 2011). It is very hard to distinguish the reason why some species are suffering so much, while some seem to be unaffected, but one possible reason could be an invasive parasite called *Nosema bombi* (Kaufman 2011). Perhaps contributing to a difference in susceptibility to *N. bombi* is the fact that the four species that are in decline have a much lower genetic diversity that those that are not declining (Kaufman 2011).

Pollinators are important ecosystem components, without them, other species, us, and indeed our economy would suffer greatly (Hatfield et al. 2012). The global economy directly, and indirectly depends on bees of various species to pollinate crop plants (Kaufman 2011). Each year, bees contribute hundreds of billions of dollars to the global economy (Hatfield et al. 2012). Not only do bees bestow an invaluable aid to the success of crops around the world, their pollination services provide food and shelter for various other species of animals and plants (Hatfield et al. 2012). Many animal and plant species depend on bees to pollinate for them, and bees aid plants by serving as pollen vectors, thus creating seeds and fruit on which animals can feed (Hat- field et al. 2012). Therefore a sharp decrease in bumble bee populations can have a large impact on the fitness of many plants and animals worldwide. Bee pollination also helps to maintain genetic diversity in ecosystems around the world (Hatfield et al. 2012).

Natural History of Bumble Bees

The genus *Bombus* comprises a monophyletic group of organisms found in North and South America, Europe and most of Asia (Meeus et al. 2011; Colla et al. 2011, Winfree 2010).

Most species are found in temperate and boreal biomes, with the greatest density of species found in the Mediterranean region and southwestern United States (Winfree 2010). Compared to other species of bees bumble bees are much larger and covered in yellow and black fur (some species can be orange to brown in color also). Bumble bees possess the unique trait of being able to thermoregulate. Thermoregulation is the ability to generate heat and control their body temperature (Hatfield et al, 2012). This characteristic feature of bumble bees contributes to their ability to have longer active seasons, and live in far northern latitudes, and at higher elevations than many other bee species (Hatfield et al, 2012). Bumble bees live as eusocial colonies with distinct task groups divided into castes (Colla et al. 2011). The 3 castes are workers, queen and drones. Queens and males are responsible for reproduction, while all in nest and out of nest duties are performed by workers (Colla et al. 2011). Within workers, specific tasks are performed by bees of different ages, for instance, guarding and foraging duties are usually performed by older individuals (Colla et al. 2011). Bumble bees have annual colonies, which means that there is one generation of bees per year, with only the following year's queen surviving through the winter (Hatfield et al. 2012; Colla et al. 2011). The colony size of bumble bees can range from 50 to over 500 bees, depending on species (Hatfield et al, 2012). *Bombus* require three categories of domain to survive and succeed, those being: floral resources to pollinate and feed from, a place to nest, and a suitable location for the queens to overwinter (Colla et al. 2011). Queen mated bumble bees, emerge in early spring and immediately begin to forage for nectar and search for an appropriate location to start a colony (Colla et al. 2011, Hatfield et al, 2012). Once the queen finds a place to nest and start her colony, she begins laying eggs, and after four days the eggs hatch and the larvae begin to feed on pollen and nectar collected by the queen (Colla et al. 2011). The average total time it takes for an adult bee to develop is about five weeks, depending on the

availability of pollen and nectar (Colla et al. 2011). When the colony is in early stages, the worker bees are born first and they go to work foraging the surrounding floral resources and bringing nectar for the developing larvae (Colla et al. 2011). Later in the colony cycle, males are produced and by this time the colony has reached a level of efficiency that allows their queen to stay in the nest and solely produce larvae (Hatfield et al, 2012). At this point, the production mode switches from yielding worker bees to producing reproductive individuals (Hatfield et al, 2012). The new queens that are produced stay in the colony until the season is over. When they are mated, each then ventures out to find a place to overwinter (Hatfield et al, 2012). Males leave the colony once they reach adulthood, to seek mates (Hatfield et al, 2012). At the end of the season the rest of the colony will die off, along with the founding queen (Hatfield et al, 2012).

Hypotheses for Decline

Agricultural Intensification

Bumble bee declines have been attributed to three potential causes: Agricultural intensification and floral resource availability, pesticides, and parasites (Colla and Packer 2008; Gillespie and Alder 2013; Williams et al. 2012; Kleijn and Raemakers 2008). Consequenses from agricul- tural intensification are generally broken down into two categories: changes in habitat quality and landscape context. Habitat quality and landscape context are independent. For bumble bees, habitat quality is the condition and abundance of the floral resources available to bumble bees and other insects; and landscape context is the conditions, or factors that directly or indirectly affect *Bombus* populations. Both of these factors have been shown to impact bumble bee com- munities (Carvell et al. 2011). Although some research has been conducted on bumble bees, there is not much data taking into consideration another factor: adaptive management (Carvell et al. 2011). Adaptive management is a species' ability to maintain a population,

although adaptations have to be made in adverse conditions. Adaptive management can be applied to all of the factors credited for bumble bee declines, in all areas of the world. Agricultural intensification affects floral resource availability, and floral resources are available at different times, and different areas, and some species of *Bombus* are more selective than others (Williams et al. 2012). Certain species feed on different plants depending on their tongue length, which makes some bees very selective (Colla and Packer 2008, Colla et al. 2011). Some species such as *B. fervidus*, *B. auri- comus* and *B. pensylvanicus* have long tongue lengths, and on the other hand, species like *B. ter- ricola* and *B. affinis* have shorter tongue lengths (Colla et al. 2011). *B. impatiens*, *B. bimaculatus* and *B. vagans* have medium tongue lengths and tend to be very common (Colla et al. 2011). Species with long tongues are much more susceptible to extinction, because they are more specialized in terms of the types of flowers they feed on than short tongued species (Colla and Packer 2008). Floral resource availability affects bumble bees because of the spatiotemporal conditions of the flowers (Williams et al. 2012). Certain flowers will bloom in late spring, and some species of flowers do not bloom until much later in the summer (Williams et al. 2012). A study done on *B. vosnesenskii*, a northern California species, showed that the variation in the availability of floral resources has a direct effect on the size and production of colonies (Williams et al. 2012). This study found that the floral resources available to *B. vosnesenskii* had a positive effect on the amount of worker bees and male bees that a colony produced (Williams et al. 2012). Al- though the overall trend was positive, the time during the season in which the flowers bloomed also was seen to affect the colonies studied. Colonies founded in the early spring were able to have a more abundant and diverse range of resources, thus allowing them to produce more work- er and male bees, and this mass production early on supported the colony when the floral re- sources were not as abundant later in the summer

(Williams et al. 2012). On the other hand, colonies that started late suffered because they struggled to produce as many worker and male bees, so therefore their ability to profit from the floral resources available was greatly lessened (Williams et al. 2012). General trends show that a decrease in floral resources affects the pollination services of bees, and accordingly, directly affects the abundance of the populations (Williams et al. 2012). The experiment performed focused mainly on the effects of colonies based on local floral resource availability, but other factors were recognized as well, such as the foraging patterns and typical tendencies of *B. vesnesenskii* (Williams et al. 2012). Foraging patters of bumble bees differ from species to species, and even bee to bee. The foraging range, for- ager consistency, destinations, flight directions, foraging range and straightness of tracks are all considerations looked at when studying foraging patterns of bumble bees (Osborne et al. 1999). Floral resource availability and landscape context are closely related, therefore the habitat quality for *Bombus* is a very important factor. Habitat quality for bumble bees depends on three things: the available floral foraging resources, colony nesting areas, and hibernacula for queens (Hatfield et al. 2012). These data suggest that the abundance of bumble bees may be affected on some level by an increase in industrialization. This habitat disturbance thus results in the destruction of available habitats for the pollinators to thrive (Hatfield et al. 2012).

Invasive Parasites

Another potential contributor to the decline of bumble bees are invasive parasites. According to multiple studies preformed, invasive parasites are one of the most significant factors contributing to population decreases in *Bombus*, other than habitat loss. (Colla et al. 2006; Winter et al. 2006; Goulson et al. 2008). Parasites that infect bumble bees do not immediately kill the bee that they infect, conversely they live in symbiotic relationships with the bumble bees and slightly

change the behavior of the individuals and/or colonies infected (Gillespie and Alder 2013). This type of symbiotic relationship between the bumble bee and the parasite is parasitism. Parasitism is defined as a relationship between two species where one species benefits and one is burdened with a disadvantage due to the parasite. Although parasitism is the main type of symbiotic relationship that impacts bumble bees, some parasites can develop mutualisms with bumble bees, thus there is a possibility of positive behavioral changes, but they are not as common (Gillespie and Alder 2013). The parasites that attack bumble bees can have multilevel trophic effects, changing the foraging behavior and decreasing the abundance of bee populations (Gillespie and Alder 2013). By altering foraging behavior, bumble bees' pollination efficiencies can take a direct hit (Gillespie and Alder 2013). The foraging behavioral changes causes bumble bees to have a reduced awareness of the bounty of the surrounding floral resources (Gillespie and Alder 2013). For example, the parasite *Crithidia bombi* curtails the bees' ability to decipher between beneficial flowers, and flowers that are not advantageous to them (Gillespie and Alder 2013). This parasite also can put a large delay in the learning process of new flower types in infected bumble bees (Gillespie and Alder 2013). *Crithidia bombi* is a gut parasite that is acquired by bees at floral resources, by means of fecal circulation (Gillespie and Alder 2013). Conopid flies (Diptera: Conopidae) attack free flying foragers on their wings and oviposit eggs inside the abdomen of the bumble bee (Gillespie and Alder 2013). A parasitoid that is blamed for the collapse of *B. occidentalis* is a microsporidian called *Nosema bombi. N. bombi* is transferred from adult bumble bees to larvae in the confines of a colony (Brown 2011; Gillespie and Alder 2013). *N. bombi* was transferred to wild bee populations from commercial populations that were brought to the United States from Europe (Brown 2011). *C. bombi* and *N. bombi* can both directly affect the reproduction of an infected colony, which in turn can have a significant effect on the population

dynamics of bumble bee communities (Gillespie and Alder 2013). The major driving force behind the infectious diseases carried by theses parasites is due to pathogen spillover (Meeus et al. 2011). A pathogen may reside in a reservoir species, and when it reaches a high prevalence it will 'spillover' into a different host species (Daszak et al. 2000). Pathogen spillover can cause species to decline very quickly, and lead to outbreaks of infectious diseases (Meeus et al. 2011). Pathogen spillover depends on the virulence of the pathogen and its potential for control (Meeus et al. 2011). Pathogen spillover also depends on the parasite prevalence in specific species, meaning that some species have higher parasite prevalence and some have lower (Brown 2011). The perplexing issue with parasite prevalence is that some species that have high parasite prevalence are experiencing population declines, but some populations that have the same parasite prevalence are thriving in comparison (Brown 2011). For example, in Alaska, *B. occidentalis* is a species that shows high parasite prevalence, but is still flourishing (Brown 2011). This phenomenon leads to another hypothesis, and that is the fact that certain species, *B. occidentalis* in this example, can be native receptacle of these microsporidian parasites (Brown 2011). Each parasite that affects bumble bees has a different mode of transmission, but all bring change to the pollination services to the flowers and plants that the bees visit (Gillespie and Alder 2013). There is much gray area regarding the affects of invasive parasites on bumble bee species, and another is the element regarding genetic diversity (Brown 2011). "Low diversity is predicted to increase susceptibility to parasites, and a recent study demonstrated a correlation between inbreeding and the prevalence of a trypanosome parasite in bumblebees" (Whitehorn et al. 2011). Parasites, their affect on Bombus bees, along with the ambiguity that accompanies them are a large factor to the population declines all over the world.

Pesticides and Chemicals

Pesticides are the third potential cause for bumble bee declines worldwide. There are two main types of pesticides: insecticides and herbicides (Hatfield et al. 2012). Insecticides are created to kill insects and parasites that pose a threat to crop and plants, but they are also very harmful to bees (Hatfield et al. 2012). Bumble bees are most vulnerable to the use of insecticides in the beginning of the spring when their colonies are just emerging, and the queens are starting to lay eggs and their colonies are very small and not well developed (Hatfield et al. 2012). Most insecticide products are provided with warning labels that explain the harmful effects that the product can have on honey bees, although most do not have warnings that shed light on the detrimental affects that pesticides have on bumble bees (Hatfield et al. 2012).

The goal of this project is to evaluate the diversity of the bumble bees in Kanawha County, West Virginia. Our survey investigated the number of species and their relative abundances.

We are particularly interested in the relative abundance and distribution of two critically endangered species: *Bombus affinis* and *B. terricola.* This project will provide valuable information on the local diversity of bumble bees, and also provide insights that can be used to identify refugia of the declining species, if indeed any are present in Kanawha County.

Methods

Bombus were collected as free flying foragers from various sites throughout Kanawha county during the months of May, June and July 2014. Data was collected on identities of species, locations, frequencies and host plants. Primary collection sites included locations in: Charleston, Dunbar, Institute, Cross Lanes, Nitro, South Charleston and Loudendale. Two particular species were of interest during the survey: *B. terricola* and *B. affinis*, because of

evidence showing severe population decline. Bumble bees were captured by aerial sweep netting and immediately transferred into a vial for identification. Collection was done at various times of the day, mostly in the early afternoon. Most of the individuals were captured and released once collection at that site was finished. The individuals that were not able to be identified in the field were taken back to the lab for identification. Careful attention was paid to the visitation of each site, to ensure that repeat capture of the same individuals was not occurring. Identification of individual bees in the field was determined by using a variety of field guides (Colla et al. 2011). The banding patterns on the abdomen are different for each species. The abdomen has numbered bands, called terga (T). Terga 1, T1, is closest to the thorax, and T6 is the farthest from the thorax (Colla et al. 2011). Figure 2 shows a picture of *B. impatiens* and the specific banding patterns. *B impatiens*, for example, has the thorax and T1 primarily yellow, T2-T6 entirely black (Colla et al. 2011).

The frequency of individuals was calculated using the proportions of each species collected. For example, there were 212 *B. bimaculatus* collected through the survey, and a total of 599 bumble bees, therefore the proportion of *B. bimaculatus* would be 212/599 or 0.354. The frequency was calculated for each species. The frequencies are shown in Table 1.

Diversity index calculations were used to analyze the evenness of each species captured and identified. The Shannon-Wiener index takes into account species richness and proportions (Maryland Sea Grant 2015). Species richness is simply the total number of different organisms present in the study, in this case the species richness (S) = 9. The Shannon-Wiener diversity index (H) is calculated by the formula $H = -SUM [(p_i)*\ln(p_i)]$ (Shown in Figure 1). The frequency, or proportion, of the species is represented by p_i (HIOX Softwares Pvt. Ltd., 2015). To calculate the biodiversity index, first species richness was calculated by adding the total

number of different organisms collected, then finding the proportions of each (HIOX Softwares Pvt. Ltd., 2015). The natural log (ln) of each frequency was calculated, then that value was multiplied by the frequency ($[(p_i)*\ln(p_i)]$) (HIOX Softwares Pvt. Ltd., 2015). The summation of those values ($[(p_i)*\ln(p_i)]$) was calculated, giving the value of the Shannon-Wiener diversity index (H). Using the value of H the 'evenness' of the populations can be calculated. Evenness (E) is calculated using the formula $E = H/H_{max}$ (HIOX Softwares Pvt. Ltd., 2015). H being the Shannon-Wiener index and H_{max} calculated by taking the natural log of species richness ($H_{max} = \ln(S)$) (HIOX Softwares Pvt. Ltd., 2015). The evenness of populations is how similar the abundance's of the different species are, and the value ranges from 0-1 (Maryland Sea Grant 2015). An evenness value of 1 indicates complete evenness of a population, and the value decreases when the community is skewed toward one, or a few species (Synonym 2015). H values typically range from 1.5-3.5, but the minimum value is 0, and values are rarely seen above 4 (Magurran 2004, Maryland Sea Grant 2015). H increases as the richness and evenness increase (Magurran 2004). The Shannon-Wiener index has pros and cons, considering that it is hard to compare communities that differ in species richness (Magurran 2004). Therefore another diversity index, the Simpson index, was used to calculate biodiversity. The Simpson diversity index is less sensitive to species richness than Shannon-Wiener (Magurran 2004). The Simpson index is a measure of dominance, and measures the probability of two individuals from a population infinitely large being selected at random, and those two individuals being from the same species (Magurran 2004). The Simpson index (D) can be calculated by formula D = 1- [(SUM n(n-1)) / (N(N-1))] (Shown in Figure 2) (Barcelona Field Studies Centre S.L 2015). In this formula the total number of a particular species is represented by n, and the total number of all the species in the community is represented by N (Barcelona Field Studies Centre S.L 2015).

The values of the Simpson index range from 0-1, with higher values indicating less diversity in the community (Barcelona Field Studies Centre S.L 2015).

Results

In the duration of the survey, 599 bumble bees were collected. A total of nine different species were found, which are all listed in Table 1 with respective frequencies. Figure 1 shows pictures of each of the different species. Of the nine species collected, 95% of the individuals belonged to three species: *B. impatiens* (0.41)*, B. bimaculatus* (0.35) and *B. griseocollis* (0.19). Two species, *B. auricomus* (0.02) and *B. fervidus* (0.02) represented the next largest proportion of individuals collected. The remaining four species: *B. perplexus* (0.005), *B. pensylvanicus* (0.01), *B. vagans* (0.01) and *B. terricola* (0.005) were found in very low numbers. During the period of collection no *B. affinis* were captured, and only one single *B. terricola* was captured. The lack of these species in our survey suggest that these species are undoubtedly uncommon in this area.

The Shannon and Simpson biodiversity indices were calculated (shown in Table 2 and 3, respectively), and gave values that were not unexpected. The Shannon index value of H= 1.349 shows that the bumble bee fauna of Kanawha County has a very uneven distribution, which is supported by the evenness value of E= 0.614. Both values are on the lower side of their respective scales, meaning that the community is very uneven and is comprised of some very common species and some very rare species. The value for the Simpson index, D= 0.6761, shows the fact that the bumble bee fauna is not very diverse, because the community is dominated by one or a few species. The value of these indices supports our data due to the fact that 95% of our fauna comprised of three species.

Table 1. Individual species numbers and respective frequencies for nine species collected throughout the survey.

Species	Number	Frequency
B. impatients	242	0.41
B. bimaculatus	212	0.35
B. griseocollis	113	0.19
B. auricomus	12	0.02
B. fervidus	14	0.02
B. perplexus	1	0.005
B. pensylvanicus	2	0.01
B. vagans	2	0.01
B. terricola	1	0.005

Table 2. Shannon-Wiener diversity index calculations.

Shannon-Wiener Index-1

Species	Number of Sample	Frequency (f)	ln(f)	(f)*ln(f)	
1) *B. impatiens*	242	0.41	-0.892	-0.366	
2) *B. bimaculatus*	212	0.35	-1.049	-0.367	
3) *B. griseocollis*	113	0.19	-1.661	-0.316	
4) *B. auricomus*	12	0.02	-3.912	-0.078	
5) *B. fervidus*	14	0.02	-3.912	-0.078	
6) *B. perplexus*	1	0.005	-5.298	-0.026	
7) *B. pensylvanicus*	2	0.01	-4.605	-0.046	
8) *B. vagans*	2	0.01	-4.605	-0.046	
9) *B. terricola*	1	0.005	-5.298	-0.026	
N=9					
Total	599			SUM = -1.349	
	Shannon Weiner Index (H) = 1.349		H_{max}	H_{max}	2.197
	Evenness= H/H	0.614			

Table 3. Simpson diversity index calculations.

Simpson Index-1

Species	Number of Sample (n)	n(n-1)	
1) *B. impatiens*	242	58322	
2) *B. bimaculatus*	212	44732	
3) *B. griseocollis*	113	12656	
4) *B. auricomus*	12	132	
5) *B. fervidus*	14	182	
6) *B. perplexus*	1	0	
7) *B. pensylvanicus*	2	2	
8) *B. vagans*	2	2	
9) *B. terricola*	1	0	
Total	N= 599	SUM n(n-1)=	115028
		N(N-1) = 599(598)=	359202
	Shannon Index (D)= .6761		

Figure 1. Pictures of each species found.

B. impatiens

B. griseocollis

B. bimaculatus

B. perplexus

B. fervidus

B. terricola

B. pensylvanicus

B. vagans

B. auricomus

Figure 2. *B. impatiens* (left) *and B. bimaculatus* (right). *B. impatiens:* Head is black, thorax is yellow, and on abdomen first terga band (T1) is yellow, T2-T6 are black. *B. bimaculatus:* Top of the head yellow, thorax with yellow band(s), on the abdomen, T1 is yellow, T2 black laterally with yellow 'W' in middle, T3-T6 black.

Discussion

Several different species of bumble bees have been in decline since the 1950's, and this decline has morphed into complete population collapses for some species (Brown 2011). Over the last two decades these populations' decline has become even more dramatic (Cameron et al. 2011). Research surveys have been performed in the western and midwestern United States, Canada and Europe, but this study is the first of its kind in West Virginia. The condition of the *Bombus* populations in West Virginia is not well known, therefore the goal of this study was to get a better idea of the status of these insects.

This survey covered only a small portion of the state, Kanawha County, which covers 911 square miles. The best efforts were made to evenly cover the entire county, collecting individuals

from many different areas and parks throughout the county. The ultimate goal of this project is to expand over the next few years to areas all over the state, to get a better picture of the abundance and distributions of bumble bees. Although the area of research covered was comparatively a small region, the data we collected is analogous to other projects and studies that have been performed here in the United States.

In the past two decades four species have shown a significant decline in population numbers: *B. occidentalis, B. affinis, B. pensylvanicus,* and *B. terricola* (Brown 2011; Kaufman 2011). Three of these species have been observed to be declining in the eastern region of the United States: *B. affinis, B. terricola* and *B. pensylvanicus* (Colla and Packer 2008). These two species were the focal point, and were found to be largely absent from the survey. Throughout the duration of this project, not a single individual of *B. affinis* was observed and only one *B. terricola* was collected. The single *B. terricola* that was found was collected in South Charleston, and was the only bee collected in that area. (This observation may mean that bumble bees in the city of South Charleston are exploiting robust foraging sites but their nests may be outside of city limits. South Charleston is very well known for its massive Industrial Park and many chemical plants). On the other hand, considering the span of coverage of our study, and the fact that no other *B. terricola* individuals were found, suggests that they are in fact very scarce. Unless there is an unknown refugium in the county, the findings support the sharp decline in *B. terricola*, and possibly the extinction of *B. affinis* from Kanawha County. Although *B. pensylvanicus* was not a focus of our study, only two individuals were collected throughout the entire collection period, which also supports their population decline.

Although the bumble bee species mentioned in the previous paragraph are in severe decline, there are species that are abundant and flourishing. In our survey, 95% of the individuals collected were *B. impatiens* (0.41), *B. bimaculatus* (0.35) and *B. griseocollis* (0.19), and the diversity analyses supported these data. The Shannon-Wiener diversity index (H) typically ranges from about 1.5 to about 3.5, with values closer to 3.5 indicating a more 'even' distribution. The Shannon index value for the community in this project, H= 1.349, was on the very low side, which indicates that the bumble bee community is very uneven and skewed toward a few species. This value supports the findings bases on the dominance of the three species that com- posed 95% of the bumble bee community. The Simpson index, accounts for both the abundance of the species and the evenness of the community; or in other words, the value obtained calcu- lates the probability of two individuals of the same species being collected at random. The Simp- son index (D) values normally range from 0-1, with values closer to 1 indicating a lower diversi- ty in the community. The value of D= 0.6761 indicates a low level of diversity. Taking into ac- count the fact that most of the community was from only three different species, this diversity value is not expected. A community with multiple species that all have similar abundances is go- ing to be much more diverse than a community with some dominant species and some very rare species (Barcelona Field Studies Centre S.L 2015). Both values calculated fully support predic- tions based on the frequencies of each species.

As our data show some bumble bees are declining, but there are some species thriving. This observation raises the question: Why isn't what is lowering the fitness of some species affecting all species equally? (Lozier and Cameron 2009). A great example is *B. occidentalis*, a

species that is experiencing severe declines in the lower 48 of the United States, but in Alaska, this species shows a high parasite prevalence, and is still flourishing and very abundant (Brown 2011). Another factor that could possibly help explain the disappearance of bumble bees is the fact that it may be tied to a phylogenetic basis; for example, the subgenera *Thoracobombus* and *Bombus* are the ones showing the highest level of species declines compared to all other subgen- era of bumble bees. Even though there are so many hypotheses attempting to explain this phe- nomena of population declines, the true causes are still unknown. Thus more research needs to be done to unravel the mystery of our vanishing bumble bees.

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Socioeconomic Barriers to Graduate Education (Higher Education) Access in the UK

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Abstract

There are many different barriers that prevent individuals from obtaining a higher education. This paper will discuss the findings according to the variables which prevents individuals from gaining access to higher education, as well as presenting ways which I believe will combat the issues discussed. Many students feel that due to financial, as well as social, constraints they cannot obtain higher education. Students feel that enrollment and admission practices are set up to prevent them from wanting to enroll in higher education universities. The UK has started to introduce programs to help in funding and in preparation for University.

INTRODUCTION

Our data collection process used quantitative surveys and focus groups to explore access to education among underrepresented college students in London universities. The quantitative portion of the study involved surveys exploring empowerment, specifically, in the university. The collaborators at each institution posted announcements about the study (survey and focus group) by distributing the survey link via anonymous university list. Students will be invited to complete an online survey at a specific link. The survey question consents of approximately 150 questions. Responses are scored on a 6-point Likert scale (1=Strongly Disagree, 6=Strongly Agree). We will use Qualtrics, an online survey program, and we will not collect IP addresses of participants in our database. The survey included items assessing demographic characteristic, as well as empowerment items.

The qualitative portion of this study will shed light on understanding issues of access to higher and graduate education in the United Kingdom. The participants of this study will be adult students at the University of East London and Kingston University who are interested in graduate school. Education, income, and occupation are often the variables used to measure socioeconomic status. Many individuals or groups are placed in social classes based on privilege and power. Those who come from a lower socioeconomic status face many different barriers to accessing higher education. They are faced with lack of funding, preparation, and family support; many individuals decide not to pursue a higher education.

LITERATURE REVIEW

The following literature review will explain the three major explanations to barriers to education and also will review intervention programs. The three major barriers to accessing high education

are financial constraints, social constraints, and enrollment practices. The United Kingdom has established intervention programs to help students prepare for university.

Financial Constraints

Income and access to student support play a significant role in the decision for a student to pursue higher education. Some studies in Scotland have suggested that the financial concerns of the students are two-fold. The first thing to take into account is the daily life as a student. Student's day to day activities include studying, classwork, homework, and this leaves little time for the student to provide themselves with financial support. Affordability can affect whether or not an individual will enter into higher education. Lack of financial support is often given as the reason they have dropped out of University (Mullen, 2010).

The second financial constraint students take into account is the level of debt that they will incur from attending University. This seems to be a potential barrier for those who come from certain socioeconomic backgrounds. Many have been raised in a culture where debt is not viewed as something that is socially acceptable. With this in mind, many decide that the long term financial rewards that one can achieve through obtaining a degree in higher education is not enough reason for them to attend University (Mullen, 2010).

In a study by Alasdair Forsyth and Andy Fulong (2000) of the University of Glasgow *entitled "Socio-economic disadvantage and access to higher education*", over 500 young people participated in the research. In this study the researchers found that many of the disadvantaged young people declined the opportunity to obtain higher education due to the lack of funds and reluctance to take on debt. It was also determined that the cost of leaving home and the daily commuting limited the range of institutions that many could attend.

Many students come from a disadvantaged background and have little support from their families. They feel like they have no access to obtain the monies that will be needed to go to University. Many individuals that do decide to pursue higher education will look at the cost and the quality of the University to make their final determination on attendance.

Social Constraints

Many individuals from disadvantaged backgrounds feel that their social background, which consists of a lack of financial, social and environmental resources, prevents them from obtaining higher education or attending one of the prestigious institutions. They feel like this is a substantial barrier and something that they cannot overcome. Additionally, many individuals classified in certain disadvantaged social groups lack the aspiration to improve their education level, confidence in their ability to do so, and the drive and determination to succeed in higher education. Many have a deficiency in information and awareness of higher education options and do not recognize the benefits of obtaining a higher education (Mullen 2010).

Enrollment and Admission Practices

Some individuals have considered the admission practices at many of the higher education institutions to be an obstacle to those in a lower socioeconomic class. Many higher education institutions rely heavily on prior educational attainment to select their students. They place a great deal of importance on the student's educational background as a determination as to whether or not to admit a student into their institution. This practice fails to recognize the learning potential of those students who come from low attainment rated schools and those students that come from state schools (Mullen, 2010).

Many students from deprived areas are not likely to attend some of the most sought after courses such as medicine or law. The higher education system is open to anyone who achieves a certain level of education, yet many feel intimated by the process. When the student comes from a state school or a school that is not considered from one of the more influential schools, this can cause the student to feel unsure of themselves. The perception alone that admissions processes and recruitment practices at many of the universities are not universal can be disincentive to apply to those from disadvantage backgrounds (Mullen 2010).

Intervention Programs

It is my belief that if more programs are put into place, it will allow individuals access to higher education. We need programs that will assist in funding as well as preparation for University. Many individuals are not aware of funding or have access to funding to help with the cost of University. Even if they do decide to attend and obtain the debt, many are not prepared for what is in store for them. Preparing them is key.

One program in the UK entitled Widening Access Skills in Primary Schools (WASP) initiative is a program that was established in 2009 to develop pedagogical skills in science, technology, engineering, and mathematics (STEM) subjects with children aged 10 to 12 years old. The program engages young people in an interactive way from an early age to encourage them to think positively about the STEM subjects. From 2009 to 2012 a total of 12 schools participated in a series of hand-on workshops to develop a topical advertising campaign. The schools that were selected were located in socioeconomically deprived urban and rural areas with students from disadvantaged backgrounds. Many student's families did not have a history of obtaining higher education (Moffett 2012).

The WASPS program's aim is to provide an opportunity for their pupils to enhance their skills-set in terms of conceptual, creative, marketing, computational, communication and presentation skills. The program's focus is to enhance the quality of the student learning experience. They target, recruit, and support their students who come from diverse backgrounds. They promote learning, professionalism, and employability through the integration of academic theory and relevant professional (Moffett, 2012).

It is programs like the WASPS that are furthermore needed to help widen participation in higher education. Exposing students to more opportunities and giving them the tools that they will need to succeed is a must if we want them to obtain a higher education. Not only do programs like the WASPS help with education, they also help with promoting self-confidence in the students. The more confident the student is in their work and their education, the more they will seek to attend higher education.

Barriers to accessing higher education do exist and removing them is not something that can be solved overnight. It will take the efforts of our government to continue to put into place programs to assist those in need and informing the community about those programs. More funding should be offered to assists those who could not otherwise afford a higher education. As a society, we need to prepare individuals for higher education at an earlier age.

METHODOLOGY

Data Collection

Our data collection approach will be a qualitative research. Qualitative research is intended to reveal a targeted audience's range of behavior. It uses in-depth studies of small groups of people that will help guide and support the construction of your hypotheses. This gives you results that are

descriptive rather than those that are predictive. You are able to have insight as to what the actual person is thinking, you get a better view of their thought process.(www.qrca.org) With this research method we aim to gather an in-depth understanding of human behavior and reasons why the individuals do what they do. What is their reasoning for behaving in such a manner?

The research group chose this type of research for this project because it can produce insightful results. The synergy among respondents can build on each other's comments and ideas. It can cause the respondents to think beyond their own answer. The interview or group discussion process engages respondents to be more active where as in a more structured survey this does not happen. You as the interviewer have the opportunity to probe and ask for more detailed responses. You have the opportunity to observe the respondents and monitor their non-verbal communication as part of their feedback. (www.qrca.org)

We will obtain a focus group to ask a series of questions to. We will get at least five individuals to get their view points on the underlining issues. We want feedback from them. We will ask them a series of questions and record their responses. A focus group is a form of qualitative research in which researchers can ask a group of individuals a series of questions about their ideas, their perceptions on the subject matter and their attitudes towards the subject matter.

A focus group can help researchers test their assumptions and provides them a chance to learn more about the topic or issue at hand. For the researcher this maybe when they test their assumptions. Many look at it as an encouraging discussion on the particular topic and a chance to get feedback from the group. Focus groups have the potential to help solve many unanswered questions that otherwise researchers would not be aware of and can lead to new ideas and theories.

Before you proceed with a focus group there are a few things that you must do. First you need to define the purpose of the focus group. You have to be clear and specific as to why you have

gathered everyone there. You must state the purpose to the individuals that are participating so that they are aware as to what is to be discussed.

You must identify the participants. Think as to how many participants you want in your focus group and how many to invite. Develop a list of characteristics that you want your participants to exhibit. Based on those characteristics, look for members of your focus group.

Prepare your questions for your focus group. You have to determine what it is exactly you are looking for. In order for the focus group to be effective, the focus group questions should clear and concise for everyone to be able to understand them. If is good to start off with open ended questions and then move to the specific questions. (www.cse.lehigh.edu)

We will also conduct personal interviews from a few different individuals. The purpose of these personal interviews are to gain knowledge from the participants. These individuals obtain some form of expertise in the subject matter and their opinions are highly valued. Personal interviews can be done prior to the focus group to aide in designing test questions. They allow for open conversation between the interviewer and interviewee. This can help with changing any questions that maybe confusing to others or have no place in the focus group. (www.survey.cvent.com)

There will be an online survey for people to submit their answers to. With online surveys respondents can input their own data and it can be stored electronically. With this, analysis can be much easier. This is a convenient method for the respondents. They can answer the questions at their own pace and work off of their own schedule. Online surveys can give the respondents a more relaxing feeling. They are more likely to share personal information due to the fact that there is no direct contact with an interviewer.

What the group hopes to accomplish through this research is a better understanding of the UK education system as well as the barriers that prevent individuals from perusing a higher education. With this knowledge we are hoping that strategies can be made to combat the situation and open up the doors for individuals who want to attend a University. Access to higher education needs to be a top priority and should be made ready available for all who seek it. **Demographics**

Thirty-seven students participated in the online survey. Of those surveyed 67.6% were female respondents and 32.4% were male respondents. The ethnic background of the respondents were 56.8% white, 8.1% of respondents were mixed/multi Ethnic Groups, 16.2% of respondents were Black/African/Asian/Black British and 10.8% of the respondents listed their race as other. Ages of the respondents range between 18-48 years of age. The largest age group represented was that of 21 with 13.5% of respondents being that age. Respondents lived in several different boroughs with 18.9% of respondents residing in the Kingston Borough. Respondents reported incomes between 4745-200,000l pounds with 18.9% of respondents reporting that their income from 2013 was less than 4745 pounds, which represented the largest percent of respondents. Thirty-five point one percent of the respondents considered themselves to be a new affluent worker, which includes middle income, sociable, and culturally active. Seventy five point seven percent strongly identified with what they considered to be their social class. Ninety four point six percent of respondents were full time students.

Results

Of the 37 respondents 56.8% stated that they would pursue a Master's degree. Of the respondents surveyed, 35.1% stated that their professors in their education process support them. Twenty nine point 7 percent stated that their friends support them and keep them on track in their education. Forty three point 2 percent strongly agree that their family motivates them to succeed in

the university. Forty three point 2 percent stated that they somewhat agreed that they are supported by an academic staff for their education. Twenty seven percent respondents agreed that they were able to find financial resources to fund their education. Five point 4 percent stated that they did not believe they could find the financial resources to fund their education. Forty point 5 percent stated the University makes an effort to support their students. Forty point 5 percent agreed that the University tried to include students from all backgrounds. Forty five point 9 percent strongly agreed that there is a lot of diversity on the campus as far as racial/ethnic, socioeconomic, and religious status.

Analysis

From the quantitative survey, our respondents consisted of a majority of students who classified themselves as white full time students from middle income homes. These respondents stated that they would pursue post graduate education. Of these same respondents they stated that they agreed that they have a support system within the university as well as within their family and friends. Only a little over 5% of the respondents stated that they did not believe that they would be able to find financial support for their education.

The results from the survey contradict what the previous research stated. From the research, it was believed that most students from lower income families lacked the financial as well as emotional support to attend higher education universities. It was stated that they felt that the universities do not support them and that they would not be able to obtain funding to further their education. Almost half of the respondents from the survey strongly agreed that the campus they attend shows diversity as far as racial/ethnic, socioeconomic and religious status. The results indicate that there is no barriers to students who want to attend a higher education university.

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The effect of human population density on the prevalence of the invasive tree *Ailanthus altissima*

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Introduction

An invasive species is an organism that has invaded a non-native habitat. In an invasion, the non-native species out-compete native species, interbreeds with native species, and/or occupy a previously unoccupied niche. Some invasions are natural, such as a flood bringing together species from two previously separated fresh water ponds or species migrating to a new habitat over a land bridge. With the advance of technology and the subsequent increased movement of human populations, many invasions are the result of human activity, such as the introduction of the zebra mussel into the great lakes via a ship's ballast tanks (Elton 1958). It is believed that introduced species are the second greatest cause of species endangerment and extinction worldwide and in the United States. It was estimated that the damage caused by invasive species in the United States was over $120 billion annually (Simberloff 2000).

One such invasive species is *Ailanthus altissima,* or tree of heaven, which belongs to the *Simaroubaceae* family. It is native to central China (Miller 2003) and was first introduced to the U.S. in California in the 1890's and Pennsylvania in 1784. It was found that 58-80% of the continental U.S. is suitable for *Ailanthus* invasion and it has the potential to expand its distribution (Albright et al 2010). It is very common in disturbed sites in West Virginia (Kowarik 1995).

The tree is usually confined to open spaces as it is a light-demanding pioneer species. Likewise, the species is very successful in urban environments and thrives in areas with limited exposed surface soil (Pan et al. 1986). *Ailanthus* utilizes wind and water dispersed samaras as well as vegetative propagation when cut. It is an early successional species that has a high tolerance for air and soil pollutants and is associated with reclaimed mine sites. Due to its success in disturbed, polluted, and high-light areas, and its reproductive methods, *A. altissima* is also often found on the sides of roads (the edge of the forest) and in urban areas. It possesses allelopathic compounds that

contribute to competition with native species (Albright et al 2010). Due to its great success and the importance of controlling invasive species, it is beneficial to study the distribution of the species in order to establish a method of invasion prediction.

"The ability to explain the factors associated with the distribution of invasive exotic plants and predict their distribution spatially is of great importance for prevention, early warning, and management of invasives" (Mack et al. 2000). The purpose of this study is to document and compare the occurrence of *A. altissima* to human population density. *A. altissima*'s adaptations make it very successful at invading highly polluted and highly disturbed areas. Therefore, it should be very successful in areas with a high population density of humans. If a correlation exists between *A. altissima* distribution and human population density, it would provide an opportunity to predict where *A. altissima* could potentially invade, and thus a method to help prevent, control, and eradicate the invasive species.

Methods

Sampling of individual specimens of *A. altissima* over two meters tall was conducted in transects measuring 2 meters by 161 meters (approximately $1/10^{th}$ of a mile) located in and around Kanawha County, WV. Transects were located on the sides of roads at the edge of wooded areas, when present. When the edge of wooded areas were not present, such as in the case of a city block or a residential yard, transects started immediately after the shoulder of the road.

The 161 meter length of the transects ran parallel to the road and were measured either by footsteps or by a car's odometer, while the two meter width ran perpendicular. Prior to sampling, 100 meters was measured using a 100-meter measuring wheel and the distance was walked four times with the number of steps counted and averaged, resulting in a conversion of 120 steps per

100 meters. This conversion was then used to calculate the number of steps in 161 meters. The two meter portion of the transects were measured using a meter stick.

The transects were reached by car and were randomly chosen by setting a trip odometer at the commencement of each day of sampling. The vehicle was stopped at every even-numbered mile and the sampling was conducted when possible. When not possible, such as in the case of road work or dangerous conditions, the location was skipped and the next evened-numbered mile was used. Each transect was located on the right side of the road, relative to the direction of travel, to prevent sampling bias and to ensure safety.

Each transects' locations were recorded by marking a waypoint on a Garmin etrex 20 hand-held GPS. The waypoints were then uploaded to a computer via Garmin's software Basecamp. Once loaded into the Basecamp software, the waypoints were then opened in Google Earth which had a census tract map overlay (Figure 1). The uploaded waypoints were also converted to an excel spread sheet containing the waypoints, their respective locations, and the presence or absence of *A. altissima* at each waypoint. The waypoints displayed on Google Earth's Census Tract map overlay were then individually compared to a map of Census Tracts located at www.census.gov/popmap (Figure 2). The population density of the census tract in which each waypoint was located was documented on the excel spreadsheet.

The spreadsheet was then sorted by population density. The waypoints were then grouped into population density groups of 1000. For each group of 1000, the number of samples in which *A. altissima* was present were divided by the total number of samplings to give the percent of occurrences for each group. Each percentage was then graphed as a function of human population density

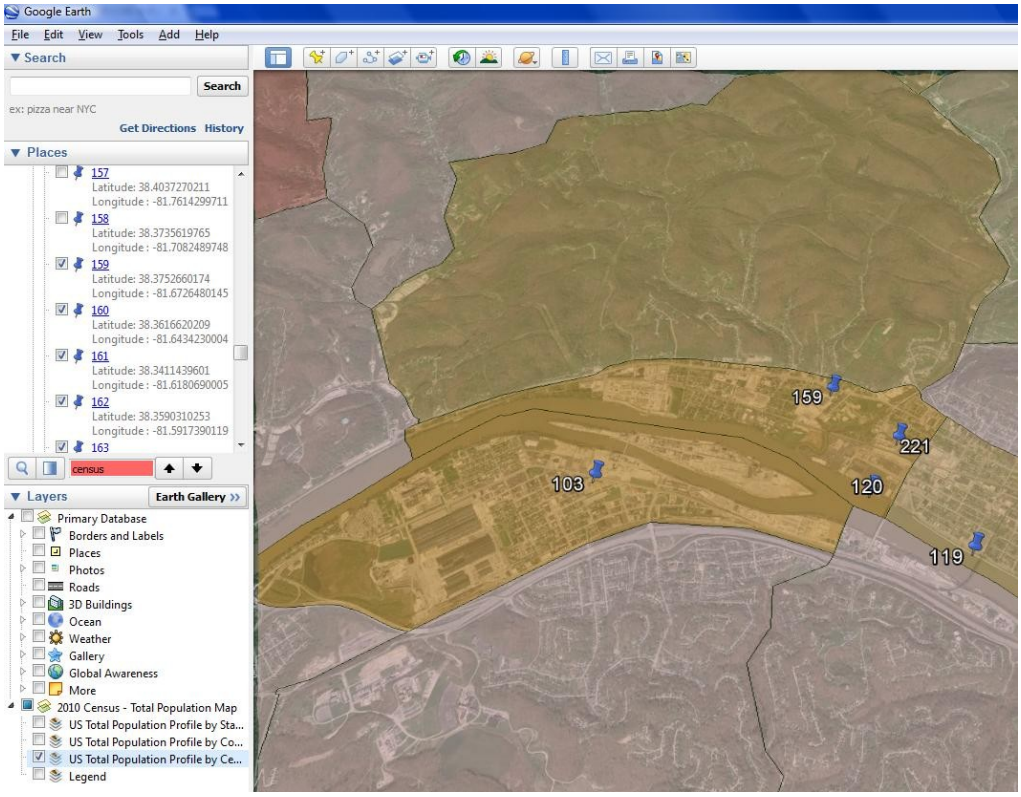

Figure 1. Google Earth with Census Tract map overlay. The map defines the borders of the census tracts. Four waypoints are shown in three different tracts.

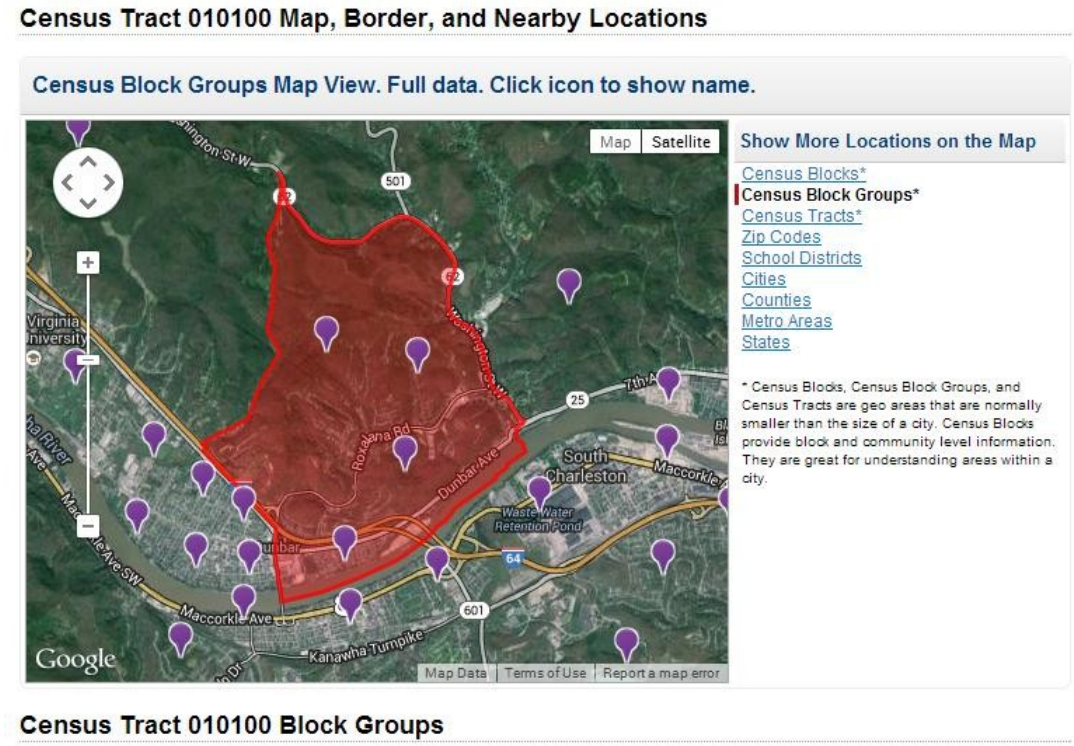

Figure 2. Map from the US Census showing one census tract and the locations of census blocks in the area.

Results:

A total of 218 transects wre sampled in and around Kanawha County, West Virginia (Figure 3). Of these, 52 transects contained at least one measured individual of *Ailanthus altissima* (Appendix A Table 2).

Ailanthus altissima was mostly found in areas that have a human population density of 2000-3999 humans per square mile. According to the data, it is very unlikely to find it in areas that have a human population density above 3000 humans per square mile (Table 1; Figure 6).

The trend line shows that there is negative correlation between human population density but only with an R^2 value of 0.5874.

Table 1. Table displaying the number of transects within a group of census tracts (grouped by 1000), the number of those transects in which A. *altissima* was present or absent, and the percentage of occurrences in the population group. For example, within all the census tracts that had a population density of 0-999 humans per square mile, sampling occurred in 178 transects. Out of those 178 transects, *altissima* was present in 43 and absent in 135, yielding 24% occurrence rate of *A. altissima*.

Population Density (1000's)	Present	Absent	Total Transects	Percent
0-999	43	135	178	24%
1000-1999	7	19	26	27%
2000-2999	1	2	3	33%
3000-3999	1	2	3	33%
4000-4999	0	2	2	0%
5000-5999	0	2	2	0%

6000-6999	0	3	3	0%

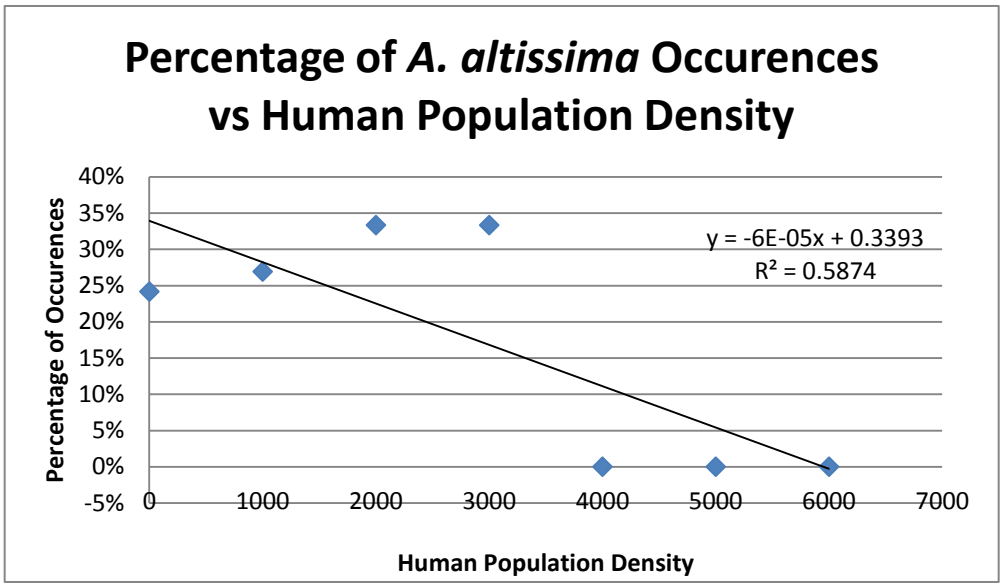

Figure 6. The percentage of occurrences of *altissima* at different levels of human population density.

.

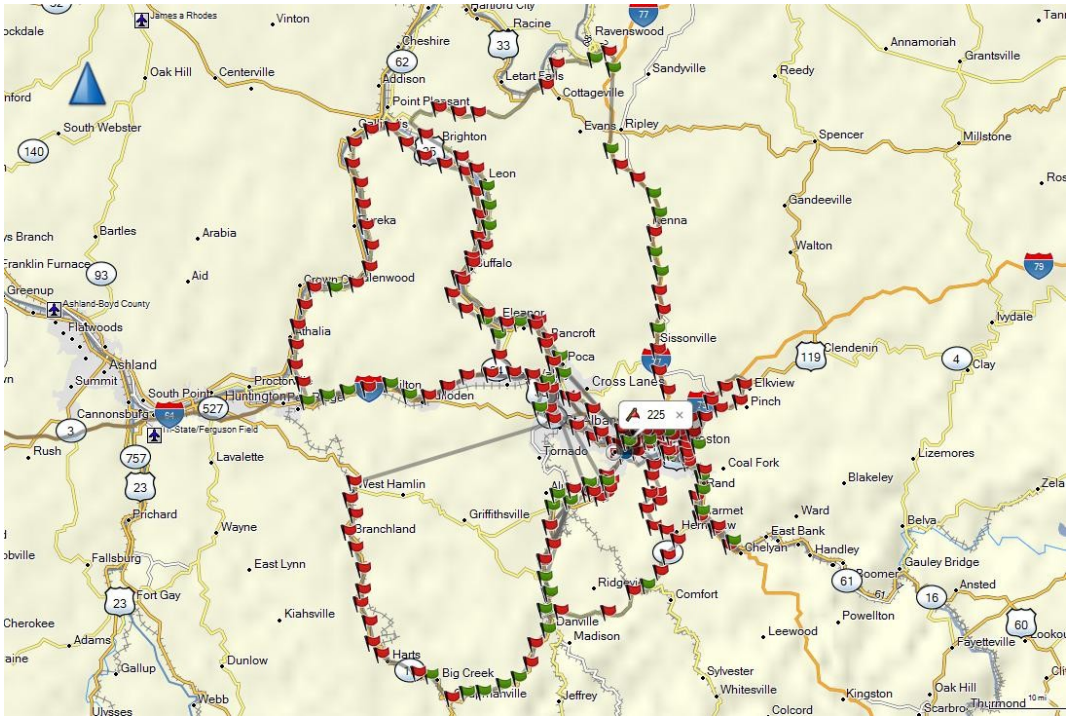

Figure 3. Map of waypoints indicating location of sampling transects. Transects indicated by a red flag had no occurrence of *A. altissima.* Transects indicated by a green flag had an occurrence of *A. altissima.*

Discussion:

The data does not support the hypothesis that there is a positive correlation between human population density and the occurrence of *A. altissima.* However, this finding could be a result of the sampling methods, the sampling size, and the sampling locations. Sampling did occur every two miles in order to ensure it was random; however, many *A. altissima* were observed between transects. Furthermore, there were only 10 transects in areas that had a human population greater than 3000 per square mile. Therefore, a positive correlation could be found with increased sampling.

While the results of this study do not support the hypothesis of a positive correlation between the occurrence of *A. altissima* and human population, an R^2 value of 0.5874 is an indication that the observed data does not fit well with the model, possibly due the sample size. However, if a significant correlation is identified it could provide a "first warning" for the prevention, control, and eradication of *A. altissima.* For example, if human population density reaches a particular threshold in a given area it would provide a warning that the occurrence of *A. altissima* is more likely. Once the "first warning" is given it would be possible to concentrate on more specific indicators such as biotic and abiotic factors within the given area. This method could result in less wasting of time and resources in the prevention, control and eradication of *A. altissima*. It will allow the entities charged with this task to focus on geographic areas that are under greater threat of *A. altissima* invasion.

This method could also be used to predict the invasion of other species, thus allowing more efficient prevention/control/eradication of many other invasive species. For example, if an

invasive species is particularly adapted for invading areas that are highly disturbed and particularly resistant to pollution, it would be beneficial to determine if there is a correlation between its occurrence and human population density, providing a "first warning" when human population density *exceeds* a pre-determined threshold. Likewise, if an invasive species is sensitive to pollution and disturbance, it would be beneficial to determine if there is a correlation between its occurrence and human population density, providing a "first warning" when human population drops *below* a pre-determined threshold.

The prevention, control, and eradication of invasive species are of upmost importance (Mack et al. 2000). The more methods available to aid in such a task, the more effective the efforts will be. Determining whether there is a correlation between an invasive species and human population density is just one of many possible methods that can be utilized to protect native habitats from invasive species.

More data is needed. More transects in each census tract (especially those that have a population density above 3000 humans per square mile) and more *total* census tracts would provide more data. Comparing occurrences of *A. altissima* to human population density using more than just census tracts would also yield more data. Human population density could also be calculated using census blocks, cities, zip codes, counties, and states. The larger the area the less precise the measure of population density but more data is needed. More sampling needs performed and more data needs collected.

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Appendix A

Table 1. Table of each waypoint, its location, presence (Flag, green) or absence (Flag, red) of *altissima,* and human population density of the census tract in which the waypoint is located. The spreadsheet is sorted by human population density within the census tracts.

Latitude	Longitude	Waypoint	Waypoint Flag	Population Density of Census Tract (humans per square mile)
38.08867701	-82.16572504	038	Flag, Red	25.19
38.11638901	-82.18209699	039	Flag, Red	25.19
38.13582103	-82.177497	040	Flag, Red	25.19
38.90992801	-81.724245	137	Flag, Green	30.86
38.59067297	-82.190238	071	Flag, Red	34.75
38.61483296	-82.16581799	072	Flag, Red	34.75
38.64655	-82.16266899	073	Flag, Red	34.75
38.67666501	-82.17232201	074	Flag, Red	34.75
38.76780001	-82.02972497	083	Flag, Red	34.75
38.75673699	-81.99189903	084	Flag, Red	34.75
38.72640003	-81.97182002	085	Flag, Red	34.75
38.692871	-81.965518	086	Flag, Red	34.75
38.66026799	-81.96870396	087	Flag, Red	34.75
38.16350796	-81.63805397	178	Flag, Red	36.77
38.20837	-81.50638098	112	Flag, Green	41.58
38.57919001	-81.64064197	128	Flag, Red	43.52
38.60780398	-81.63780402	129	Flag, Green	43.52

38.63648099	-81.64906997	130	Flag, Green	43.52
38.66643003	-81.64956199	131	Flag, Red	43.52
38.69489296	-81.64046503	132	Flag, Green	43.52
38.72348598	-81.65113301	133	Flag, Green	43.52
38.74700602	-81.68030304	134	Flag, Red	43.52
38.76621399	-81.71106303	135	Flag, Red	43.52
38.70777301	-82.17774804	075	Flag, Red	48.51
38.73927204	-82.18245003	076	Flag, Red	48.51
38.76790001	-82.19737301	077	Flag, Red	48.51
38.79951596	-82.193776	078	Flag, Red	48.51
38.81868898	-82.16322798	079	Flag, Red	48.51
38.82231298	-82.124862	080	Flag, Red	48.51
38.79944103	-82.09963599	081	Flag, Red	48.51
38.77925104	-82.06660802	082	Flag, Red	48.51
38.84399503	-81.96884603	142	Flag, Red	50.36
38.84474102	-82.00466903	143	Flag, Red	50.36
38.85108001	-82.04032104	144	Flag, Red	50.36
38.81155799	-82.06450098	145	Flag, Red	50.36
38.77834202	-81.99992797	146	Flag, Red	50.36
38.76074698	-81.97205898	147	Flag, Red	50.36
38.73429	-81.95332501	148	Flag, Green	50.36
38.70563999	-81.94983998	149	Flag, Green	50.36
38.67563202	-81.94966698	150	Flag, Green	50.36
38.17826699	-81.84981404	021	Flag, Red	51.94
38.14320599	-81.66180803	179	Flag, Green	51.94

38.10683498	-81.84855298	183	Flag, Green	51.94
38.15447596	-81.848269	185	Flag, Green	51.94
38.180225	-81.84908297	186	Flag, Red	51.94
38.20312	-81.83278402	187	Flag, Red	51.94
38.01315799	-82.07757197	035	Flag, Red	52.76
38.04086496	-82.144421	036	Flag, Red	52.76
38.058115	-82.16071098	037	Flag, Red	52.76
38.13029903	-81.840761	023	Flag, Red	54.34
38.10531903	-81.846399	024	Flag, Green	54.34
38.07750704	-81.84241701	025	Flag, Green	54.34
38.05608599	-81.86559096	026	Flag, Red	54.34
38.02750899	-81.87359802	027	Flag, Red	54.34
38.005652	-81.89539097	028	Flag, Green	54.34
37.99512902	-81.92269603	029	Flag, Green	54.34
37.98525296	-81.959363	030	Flag, Green	54.34
38.13206602	-81.83826797	184	Flag, Green	54.34
38.13418698	-81.68778799	180	Flag, Red	54.91
38.10344098	-81.73326602	181	Flag, Red	54.91
38.10490798	-81.81923401	182	Flag, Red	54.91
38.45708102	-81.94635303	054	Flag, Red	64.72
38.45364997	-81.98953198	055	Flag, Red	64.72
38.43760902	-82.02474796	056	Flag, Red	64.72
38.62973498	-81.980216	088	Flag, Red	64.72
38.60089	-82.00436602	089	Flag, Red	64.72
38.57548504	-82.015853	090	Flag, Red	64.72

38.55243498	-81.99169803	091	Flag, Red	64.72
38.53539197	-81.95731698	092	Flag, Green	64.72
38.51442799	-81.93247704	093	Flag, Green	64.72
38.48381996	-81.93071701	094	Flag, Red	64.72
38.492211	-81.64312502	125	Flag, Red	65.4
38.52311902	-81.64375501	126	Flag, Green	65.4
38.55090603	-81.63399202	127	Flag, Red	65.4
38.481899	-81.82100996	007	Flag, Green	67.06
38.50897598	-81.83938703	008	Flag, Red	67.06
38.53543002	-81.85912801	009	Flag, Red	67.06
38.23041298	-81.83944898	019	Flag, Red	68.11
38.23082504	-81.83921403	188	Flag, Red	68.11
38.26008501	-81.83434599	189	Flag, Green	68.11
38.27749799	-81.82545899	190	Flag, Green	68.11
38.16719801	-82.17729399	041	Flag, Red	71.1
38.198302	-82.19097996	042	Flag, Red	71.1
38.22280398	-82.20382397	043	Flag, Red	71.1
38.24821397	-82.191246	044	Flag, Red	71.1
38.27088802	-82.20099198	045	Flag, Red	71.1
38.29577498	-82.19381003	046	Flag, Green	71.1
38.29650303	-82.19426801	047	Flag, Red	71.1
38.29650303	-82.19426801	048	Flag, Red	71.1
38.29650303	-82.19426801	049	Flag, Red	71.1
38.22547303	-81.53897996	110	Flag, Red	75
38.20172299	-81.51341901	111	Flag, Red	75

38.41625101	-81.49464296	213	Flag, Red	75.64
38.440094	-81.48640296	214	Flag, Red	75.64
38.43629298	-81.52108904	215	Flag, Red	75.64
38.41619996	-81.546969	216	Flag, Red	75.64
38.37093299	-81.60482601	163	Flag, Red	79.88
38.39150503	-81.58322099	164	Flag, Red	79.88
38.36901496	-81.57947101	210	Flag, Red	79.88
38.39180997	-81.55773397	211	Flag, Red	79.88
38.279442	-81.76914702	016	Flag, Red	82.29
38.26814898	-81.80234497	017	Flag, Green	82.29
38.26816499	-81.80235998	018	Waypoint	82.29
38.26816499	-81.80235998	020	Flag, Red	82.29
38.26816499	-81.80235998	022	Flag, Green	82.29
38.24427799	-81.56677301	109	Flag, Red	82.29
38.28043702	-81.64162097	170	Flag, Red	82.29
38.26692799	-81.64013796	171	Flag, Red	82.29
38.27829301	-81.65770099	172	Flag, Red	82.29
38.255467	-81.66379798	173	Flag, Red	82.29
38.23021199	-81.65023698	174	Flag, Red	82.29
38.22395397	-81.62790902	175	Flag, Red	82.29
38.20999299	-81.60665704	176	Flag, Red	82.29
38.186582	-81.62503101	177	Flag, Red	82.29
38.28531001	-81.79617397	191	Flag, Red	82.29
38.29395703	-81.76799401	192	Flag, Green	82.29
38.30550796	-81.73607597	193	Flag, Red	82.29

38.29722203	-81.72691899	195	Flag, Red	82.29
38.27914797	-81.73622098	196	Flag, Red	82.29
38.27382596	-81.74910304	197	Flag, Red	82.29
38.27340502	-81.74753202	198	Flag, Red	82.29
38.28625197	-81.73076101	199	Flag, Red	82.29
38.29634101	-81.735649	200	Flag, Green	82.29
38.28076199	-81.760572	201	Flag, Red	82.29
38.33590996	-81.70388804	226	Flag, Red	82.29
38.44158498	-82.12836497	059	Flag, Green	83.33
38.43588897	-82.16854102	060	Flag, Red	83.33
38.43125696	-82.20592598	061	Flag, Green	83.33
38.52860397	-82.29607402	067	Flag, Red	83.33
38.55831396	-82.28320696	068	Flag, Red	83.33
38.58404104	-82.26689	069	Flag, Red	83.33
38.58406501	-82.22898401	070	Flag, Green	83.33
38.92424597	-81.75747296	139	Flag, Green	83.56
38.91666404	-81.82660497	140	Flag, Red	83.56
38.88505404	-81.84538504	141	Flag, Red	83.56
38.00951698	-82.05481896	034	Flag, Green	90.45
38.64652402	-81.95594796	151	Flag, Red	103.18
38.62084698	-81.97914404	152	Flag, Red	103.18
38.59469301	-81.99699498	153	Flag, Red	103.18
38.545853	-81.95210502	154	Flag, Red	103.18
38.53206201	-81.91789898	155	Flag, Red	103.18
38.43173901	-81.62525598	123	Flag, Red	145.98

38.46023404	-81.64134496	124	Flag, Red	145.98
38.78970101	-81.72852396	136	Flag, Green	159.99
38.35084098	-81.57048603	209	Flag, Green	161.41
38.42413704	-82.24315404	062	Flag, Green	194.85
38.41613299	-82.2792	063	Flag, Green	194.85
38.44360602	-82.28721997	064	Flag, Red	194.85
38.46906798	-82.30082396	065	Flag, Red	194.85
38.49892901	-82.29891398	066	Flag, Red	194.85
38.36232	-81.76053797	101	Flag, Red	219.04
37.98371103	-81.98915396	031	Flag, Green	234.81
37.97596397	-82.01656999	032	Flag, Red	234.81
38.53276902	-81.89436402	010	Flag, Green	234.96
38.528538	-81.865382	011	Flag, Red	234.96
38.50096799	-81.84732403	012	Flag, Red	234.96
38.47436802	-81.83307798	013	Flag, Red	234.96
38.45854098	-81.90478801	095	Flag, Red	234.96
38.43382199	-81.83659896	005	Flag, Red	257.58
38.45658297	-81.81450301	006	Flag, Green	257.58
38.30433198	-81.65435401	169	Flag, Red	274.6
38.42407099	-82.05881897	057	Flag, Red	311.69
38.42502099	-82.09389104	058	Flag, Green	311.69
38.33527604	-81.62499304	105	Flag, Green	319.11
38.32499103	-81.58793497	106	Flag, Red	319.11
38.93438598	-81.73180204	138	Flag, Red	319.52
38.40551203	-81.53026496	212	Flag, Red	432.45

38.36413602	-81.61754999	217	Flag, Red	502.59
38.38647002	-81.63089599	219	Flag, Red	502.59
38.40431903	-81.84047802	004	Flag, Red	733.47
38.42280901	-81.807713	051	Flag, Red	733.47
38.42096097	-81.79660597	156	Flag, Red	733.47
38.40372702	-81.76142997	157	Flag, Red	733.47
38.40578201	-81.85783099	098	Flag, Green	809.94
38.32039003	-81.65056304	168	Flag, Red	854.64
38.33334401	-81.59096402	117	Flag, Red	947.47
38.35903103	-81.59173901	162	Flag, Red	947.47
38.34856303	-81.59257997	207	Flag, Red	947.47
38.33815003	-81.58991201	208	Flag, Red	947.47
38.38915902	-81.832521	099	Flag, Red	964.01
38.36854398	-81.69171802	103	Flag, Red	992.64
38.44585396	-81.844126	014	Flag, Green	1224.89
38.44585304	-81.84415399	015	Flag, Green	1224.89
38.454848	-81.87041902	052	Flag, Red	1224.89
38.43490602	-81.853532	097	Flag, Red	1224.89
38.37356198	-81.70824897	158	Flag, Red	1251.8
38.35582	-81.72526298	102	Flag, Red	1269.82
38.35185201	-81.71224697	202	Flag, Red	1269.82
38.34337296	-81.67897803	003	Flag, Red	1607.73
38.341899	-81.68076698	122	Flag, Red	1607.73
38.34071497	-81.68059498	167	Flag, Red	1607.73
38.36435798	-81.67851803	204	Flag, Red	1607.73

38.35588697	-81.684064	223	Flag, Red	1607.73
38.353259	-81.68847296	224	Flag, Red	1607.73
38.35596803	-81.69664096	225	Flag, Green	1607.73
38.367699	-81.66960002	120	Flag, Red	1688.47
38.37526602	-81.67264801	159	Flag, Red	1688.47
38.37151503	-81.66725401	221	Flag, Red	1688.47
38.30416401	-81.56337004	107	Flag, Green	1746.93
38.27428697	-81.57322599	108	Flag, Red	1746.93
38.229883	-81.53426698	113	Flag, Red	1828.34
38.252462	-81.56307299	114	Flag, Green	1828.34
38.283555	-81.56233404	115	Flag, Green	1828.34
38.31366297	-81.55937296	116	Flag, Red	1828.34
38.37005298	-81.62622099	218	Flag, Green	1973.15
38.34919503	-81.63361902	165	Flag, Red	1973.67
38.34776399	-81.63148901	205	Flag, Red	1973.67
38.35636801	-81.65354901	104	Flag, Red	2412.83
38.35167498	-81.65926798	121	Flag, Red	2412.83
38.357287	-81.65999603	222	Flag, Green	2412.83
38.37931397	-81.79061802	100	Flag, Red	3322.42
38.36918	-81.74631799	203	Flag, Red	4439.91
38.36166202	-81.643423	160	Flag, Red	4452.12
38.370633	-81.64430402	220	Flag, Red	5005.02
38.36380804	-81.66178398	119	Flag, Red	5208.85
38.34409699	-81.63220198	118	Flag, Red	6500.93
38.34114396	-81.618069	161	Flag, Red	6500.93

38.34134496	-81.62538397	206	Flag, Red	6500.93

Failure to Obtain Clustering of Unrelated Words Based on Font Color

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Failure to Obtain Clustering of Unrelated Words Based on Font Color

The ability to access one's long-term memory (LTM) may be vital for everyday functioning. Information may be stored in LTM from less than one minute ago and could include everything that a person has ever known or learned. If someone was rendered unable to access their long-term memories, they may not be able to remember how to drive, where they worked, or even their own identity. Since long-term memory is such a significant component of everyday living, experimenters have developed multiple ways to research memory.

One way to examine memory is through free recall tasks. Free recall tasks involve the presentation of items to participants and afterwards instructing the participants to recall as many items as they can in any order. The data from free recall tasks can be analyzed by the number of items recalled, which items were recalled, and the order of the items recalled. Analyses of these results have given insight into some of the trends of item recall. Some examples of these trends are the primacy effect (Deese & Kaufman, 1957), the recency effect (Bjork & Whitten,1974), the picture superiority effect (Paivio & Csaspo, 1973), the generation effect (Slamecka & Graf, 1973), and semantic clustering (Bousfield, 1953). Semantic clustering refers to the phenomenon that when presented a list of words, participants will tend to recall the semantically related words consecutively, that is, in clusters (Bousfield, 1953).

Bousfield (1953) was one of the first researchers to examine semantic clustering. He tested the phenomenon of clustering based on the results of a previous study (Bousfield & Sedgewick, 1944) where the researchers reported that participants instructed to list types of birds tended to group together different categories of birds, such as birds of prey and domestic fowl. After observing this tendency, Bousfield (1953) conducted his experiment to examine categorization by presenting participants a randomized list of words that belonged to one of four categories. He reported that participants tended to have "relatedness increments" in recall, where a recalled item

was far more likely to be followed by a related item rather than an unrelated item. Bousfield (1953) termed this phenomenon semantic clustering. In addition to clustering, he reported that participants that were presented the semantically related list recalled a significantly higher number of words than the participants that were presented the list of unrelated words.

There have been several experiments that have examined how different variables tend to affect semantic clustering. For example, Mathews (1954) examined how the number of categories in a list affected the number of words recalled. He presented a list of words to participants that comprised of either two, three, or six categories. He found that as the number of categories increased, the mean number of words recalled also increased. A later study by Gonzalez and Cofer (1959) examined the difference in clustering scores of lists comprised of different parts of speech. They found that the mean clustering score was significantly higher for nouns than verbs and adverbs.

Slaybaugh (1972) examined the relationship between clustering and word recall rates. He presented three groups of participants the same list of semantically related word. One group was forced to cluster in recall, by giving them a blocked recall task, another group was given the option of clustering, by giving them a free recall task, and the last group was forced to not cluster, by giving them a distributed recall task. Slaybaugh (1972) reported that participants given the blocked recall task recalled significantly more words than participants given the free recall task, who recalled significantly more words than participants given the distributed recall task. Based on these results, Slaybaugh (1972) suggested that clustering may facilitate word recall. The results of Brown, Conover, Flores, and Goodman's (1991) experiment supported this idea because they reported that participants that had higher clustering scores, in both free recall and blocked recall tasks, tended to recall more words than participants with lower clustering scores.

One question that researchers have tried to answer is at what stage of the memory process does clustering, and the possible recall facilitation, occur. Are words sorted together at the time of encoding or are words grouped together at the time of retrieval? While these two stages might not be able to be completely separated, knowing when clustering occurs would expand the knowledge of categorization in memory and potentially aid memorization techniques. In support of the idea that clustering occurs at retrieval, Slaybaugh (1972) has reported that when participants are given retrieval cues, for example through a blocked recall task, participants tend to have higher clustering scores than people given a recall task without any cues. Since participants were given the same encoding procedure, his experiment supports the idea that clustering may occur at the retrieval stage.

In support of the idea that clustering occurs at the encoding stage, Brown, Conover, Flores, and Goodman (1991) reported that participants that have higher clustering scores in a blocked recall task tend to recall more words than participants that had lower clustering scores suggesting that how information is encoded may be what alters clustering and word recall scores. Even though it has been reported that retrieval cues tend to heighten clustering scores (see Slaybaugh, 1972), when participants are given the same retrieval instruction, higher clusterering scores still correspond with a word recall advantage.

Tulving (1962) also emphasized the importance of organization in the encoding stage. He suggested that Miller's (1956) unitization hypothesis may explain the higher recall rates when items are clustered. Miller's unitization hypothesis stated that organizational processes accompanied by repetition may increase memory capacity by increasing the information load of individual units. Tulving (1962) suggested that, at the time of encoding, participants may sort the items that belong to a category into an individual unit, which may cause those participants to have a higher memory capacity.

In addition to examining when clustering occurs and the effects of clustering, researchers have also focused on how to best quantify clustering. In one of his first experiments, Bousfield (1953) quantified his data using an equation he referred to as the Ratio of Repetition (RR; Bousfield, 1953). This equation was later modified by Cohen, Sakoda, and Bousfield (1954). This modified RR equation is shown below:

$$RR = r/n - 1$$

The modified RR equation divides the number of semantic repetition, "r", by the number of recalled items minus one.

A paper by Bousfield and Bousfield (1966) proposed an alternative clustering measurement called Deviation Score (DS). They proposed that an analysis of clustering should control for chance semantic clustering. The equation for DS is shown below:

$$DS = r - E(r) \qquad E(r) = \frac{\sum n_i^2}{n - 1}$$

Bousfield and Bousfield (1966) defined "r" as the number of category repetition and "E(r)" as the chance number of category repetition. E(r) is calculated by adding up the number of recalled words from each category, "n_i", and dividing that number by the total number of words recalled minus one.

In 1971, Frankel and Cole suggested that a design flaw of both RR and DS was that these equations did not account for the number of categories in a list. They explained that if two participants cluster the same number of related words, but recall a different number of categories, a recall list comprised of fewer categories would have a higher clustering score when analyzed using RR and DS.

However, this design flaw did not apply to the Adjusted Ratio of Clustering (ARC; Gerjuoy & Spitz, 1966). The ARC equation divides the DS by "max", which was defined as the difference

between the number of recalled items and the number of recalled categories, minus chance clustering, "E(r)". The equation for ARC is shown below:

$$ARC = \frac{r - E(r)}{max - E(r)} \qquad E(r) = \frac{\sum n_i^2}{n - 1} \qquad max = n - c$$

This equation accounts for the number of categories in a list. ARC scores can range from -1.0 to 1.0, with -1.0 being the minimum amount of clustering and 1.0 being the maximum amount of clustering.

Finally, another equation that accounts for the number of categories in recall is the Modified Ratio of Repetition (MRR; Wallace & Underwood, 1970). This equation divides the amount of category repetition, "r", by the total number of items recalled minus the number of recalled categories. The equation for MRR is shown below:

$$MRR = \frac{r}{\max} \qquad max = n - c$$

The larger the MRR value, the greater the amount of clustering is said to have occurred.

Organization in recall has also been demonstrated in the recall of unrelated word lists over multiple trials and is known as subjective organization (Tulving, 1962). Subjective organization is demonstrated in recall when there are regularities in the order of recall of words over several presentations of the same list in different orders. To date, clustering of unrelated words has been shown through subjective organization (Tulving, 1962). However, the current experiment examined the possibility of clustering of unrelated words based on shared physical characteristics. The basis was that a list of unrelated words comprised of several shared physical characteristics may be clustered based on that shared physical characteristic. This was tested using font color as that shared physical characteristic. There were four groups, Group Color A, Group Color B, Group Unrelated, and Group Related.

The two experimental groups of interest were Group Color A and Group Color B. Participants in these groups were presented a list of 21 unrelated nouns that were randomly assigned, using a random number generator, one of three font colors, either red, blue, or green. The lists used for both color groups were comprised of the same words in the same order with seven words per font color. The only difference between the word list used for Groups Color A and Color B was the arrangement of the font colors.

Group Unrelated and Group Related were used as comparison groups. Participants in Group Unrelated were presented the same list of words as the Color groups, but with all of the words in black font. This group was used to ensure that any recall consistencies of the experimental groups were not due to other variables besides font color.

Group Related was used to compare clustering based on font color to semantic clustering. The list of words presented to participants in Group Related was comprised of semantically related words that belonged to one of three categories, fruits, furniture, or wearable items. There were seven words per category, and a random number generator was used to position them in the list.

The amount of clustering in the current experiment was calculated using multiple equations because there has been little consensus as to which equation is the most valid. The amount of clustering in the current experiment was calculated using RR (Cohen, Sakoda, & Bousfield, 1954), MRR (Wallace & Underwood, 1964), DS (Bousfield & Bousfield, 1966) and ARC (Gerjuoy &Spitz, 1966). Since all of these equations are suggested to measure relative clustering amounts, it was expected that the different equations of clustering would not differ from one-another.

In the current experiment, it was expected that clustering based on font color may be similar to semantic clustering. Thus, it was hypothesized that Group Color A and Group Color B would have similar clustering scores as Group Related. In addition, it was hypothesized that Group Color A and Group Color B would recall a similar mean number of words as Group Related and

that Group Related and the Color groups would recall significantly more words than Group Unrelated.

Method

Participants

Participants for this experiment were 77 students between the ages of 18 and 31 years from a variety of majors that were attending a general studies course, introductory psychology and/or introductory sociology, at a public university. They received extra credit for their participation. Each participant was only tested once. They were offered a list of times to attend the experiment and each timeslot was assigned one of the four groups before testing occurred. Participants were sorted into the groups based on which timeslot they attended. There were four groups in this experiment. Group Color A consisted of 11 males and 7 females, Group Color B had 9 males and 7 females, Group Related had of 7 males and 12 females, and Group Unrelated consisted of 8 males and 12 females.

Materials

An informed consent sheet was given to each participant (see Appendix A). A questionnaire (see Appendix B) was also used that asked participants for demographic information, sex, age, academic major, and whether or not they were color blind. The questionnaire also instructed participants that were color blind to please not participate in the experiment.

This experiment used two sets of words, one set for Group Related and another set for Group Unrelated, Group Color A, and Group Color B. These lists each included 21 common words that were placed onto individual PowerPoint slides with each word presented individually in the same font type (Times New Roman) and size (96) in the center of the slide and set in bold. At the end of each PowerPoint presentation, there was a 30 second countdown with each number on an individual PowerPoint slide,

Group Unrelated's PowerPoint presentation included unrelated words that were no more than two syllables and were presented in black font on a white background (see Appendix C). Group Color A's PowerPoint presentation contained the same words, in the same order as Group Unrelated. However, Group Color's words were randomly assigned one of three colors, either red, green, or blue, using a random number generator. The list consisted of seven words per each font color and there were no more than two of each font color in a row (see Appendix D).

Group Color B's presentation contained the same words, in the same order and font colors as Group Color A. However, the difference between Group Color A's and Group Color B's word list was the order of the colors. The font colors were randomized a second time, rearranging the placement of the colors in the list. Again, there were seven words per font color and no more than two of each font color in a row (see Appendix E).

The final group, Group Related, had a presentation that consisted of words that belonged to 1 of 3 categories, fruits, clothes, and furniture. The word list for the presentation consisted of seven words per category and a random number generator was used to place the words in the list. The word list comprised of no more than two of each category in a row (see Appendix F).

Procedure

At the beginning of the experiment, each group was given the informed consent sheet to sign and informed that they had the ability to leave the testing site at any time. After the informed consent sheets were collected, participants were given the questionnaire to complete. The participants were instructed to turn the questionnaire over to the blank side of the paper after they completed the questionnaire. After all questionnaires were flipped over, the experimenter instructed the participants to watch the PowerPoint presentation. The experimenter manually changed the PowerPoint slide, presenting each word for approximately five seconds.

After the last word in the PowerPoint slideshow was presented, the 30 second countdown appeared and the participants were then instructed to think of each number individually as they were presented. After the countdown reached zero, the participants were instructed to write down as many words from the list as they could recall in any order. The participants were informed that they had unlimited time to complete that portion of the experiment and were asked to give their papers to the experimenter when they were done. The data was then analyzed.

Results

Two types of data were of interest in the current experiment, the mean number of words recalled by each group and the clustering scores for each group according to RR, MRR, DS, and ARC calculations. Clustering scores for Group Related were calculated based on the semantic categories, while clustering scores for Groups Color A and Color B were based on the font colors that were assigned to the words. Group Unrelated was omitted from the clustering analysis, because the list of words given to this group did not consist of any inherent categories.

A one way ANOVA compared the mean number of words recalled by each group and revealed that Group Related (M= 11.8947, SD=3.88580), Group Unrelated (M=9.7000, SD=3.86754), Group Color A (M=7.2778, SD=1.87257), and Group Color B (M=7.3000, SD=2.29645) were significantly different, $F(3,73)= 9.518$, $p< 0.001$ (see Figure 1).

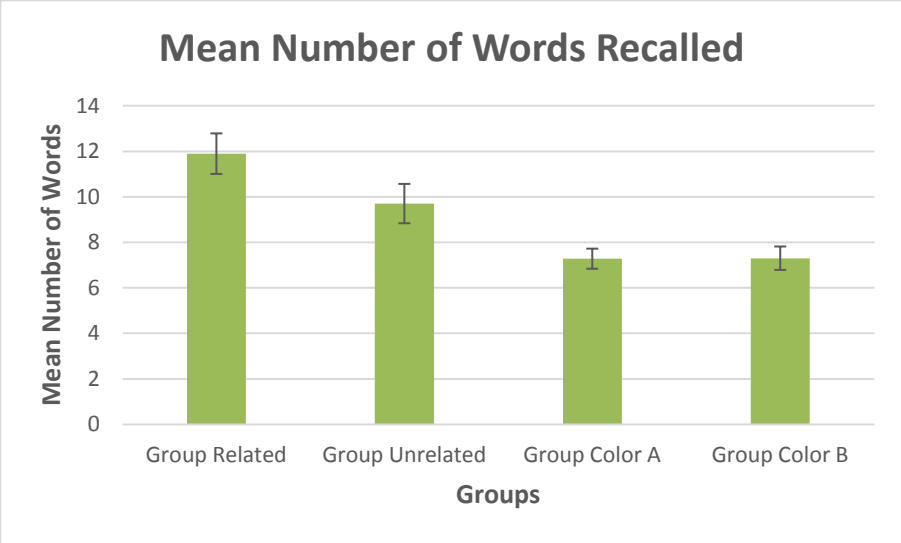

Figure 1-Mean number of words recalled for each of the four groups.

Planned contrasts revealed that Group Related recalled significantly more words than Group Unrelated, $t(73)= 2.188$, $p= 0.032$, Group Color A, $t(73)= 4.483$, $p< 0.001$, and Group Color B $t(73)= 4.580$, $p< 0.001$. Additionally, Group Unrelated recalled significantly more words than both Group Color A, $t(73)= -2.381$, $p=0.020$, and Group Color B, $t(73)= -2.424$, $p= 0.018$. The mean number of words recalled by Group Color A and Group Color B were not significantly different, $t(73)= 0.022$, $p=0.983$.

Similar statistics were found across all four clustering measures. The mean RR differences between Group Related ($M= 0.4837$, $SD= 0.24732$), Group Color A ($M=0.2494$, $SD= 0.10457$), and Group Color B ($M=0.2410$, $SD= 0.13951$) was also statistically significant, $F(2,54)= 11.771$, $p< 0.001$ (see Figure 2).

Figure 2-Mean RR scores for Group Related, Group Color A, and Group Color B

Planned contrasts showed that Group Related had significantly higher RR scores than both Group Color A, $t(54)= 4.066$, $p< 0.001$, and Group Color B, $t(54)= 4.325$, $p< 0.001$. Group Color A and Group Color B did not have significantly different mean RR scores from each other, $t(54)= -0.148$, $p= 0.883$.

The mean MRR scores were also significantly different between Group Related ($M= 0.589$, $SD= 0.28020$), Group Color A ($M= 0.3872$, $SD= 0.16485$), and Group Color B ($M= 0.3780$, $SD= 0.22201$), $F(2,54)= 11.771$, $p< 0.001$ (see Figure 3).

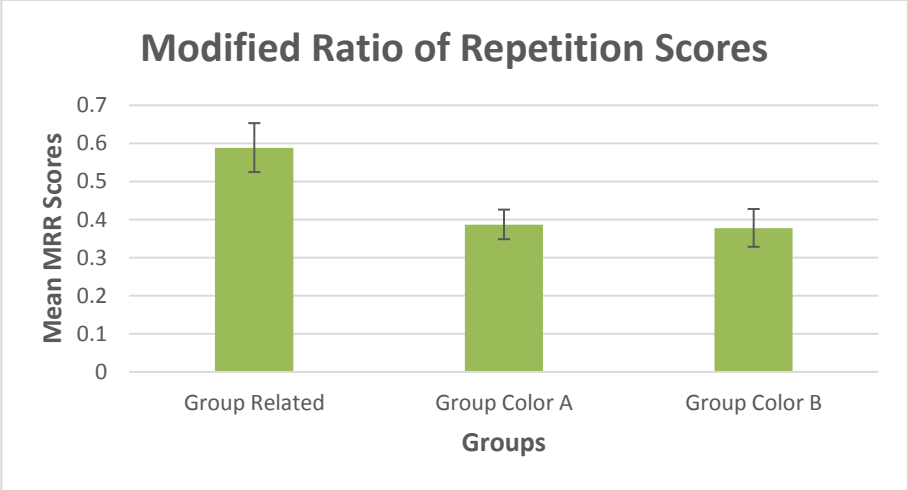

Figure 3-Mean MRR scores for Group Related and the color groups.

The planned contrasts showed that Group Related had significantly higher MRR scores than both Group Color A, t(54)= 2.668, p= 0.010, and Group Color B, t(54)= 2.886, p= 0.006, who did not differ from each other, t(54)= -0.124, p= 0.901.

The mean DS score were also significantly different between Group Related (M= 2.8184, SD= 2.81652), Group Color A (M= -0.1261, SD= 0.75468), and Group Color B (M= -0.1340, SD= 0.91103), F(2,54)= 17.675, p< 0.001 (see Figure 4).

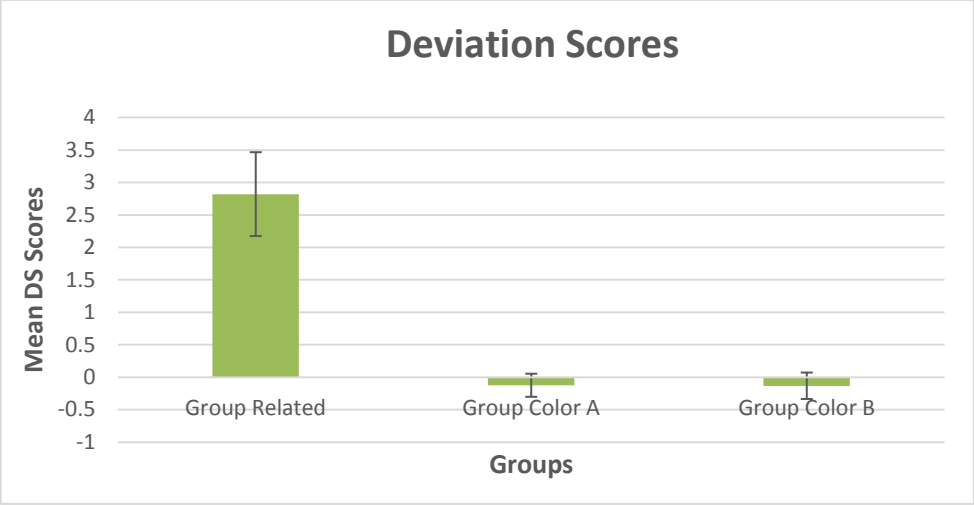

Figure 4-Mean DS scores for Group Related and the Color groups

Planned contrasts revealed that the mean DS score of Group Related was significantly higher than both Group Color A, t(54)= 5.072, p< 0.001, and Group Color B, t(54)= 5.221, p< 0.001, which did not differ from each other, t(54)= -0.14, p= 0.989.

Lastly, an examination of the ARC scores revealed that the scores were significantly different between Group Related (M= 0.3037, SD= 0.50565), Group Color A (M= -0.0533, SD= 0.31612), and Group Color B (M=-0.0195, SD=0.26873), F(2,54)=4.890, p=0.011 (see Figure 5).

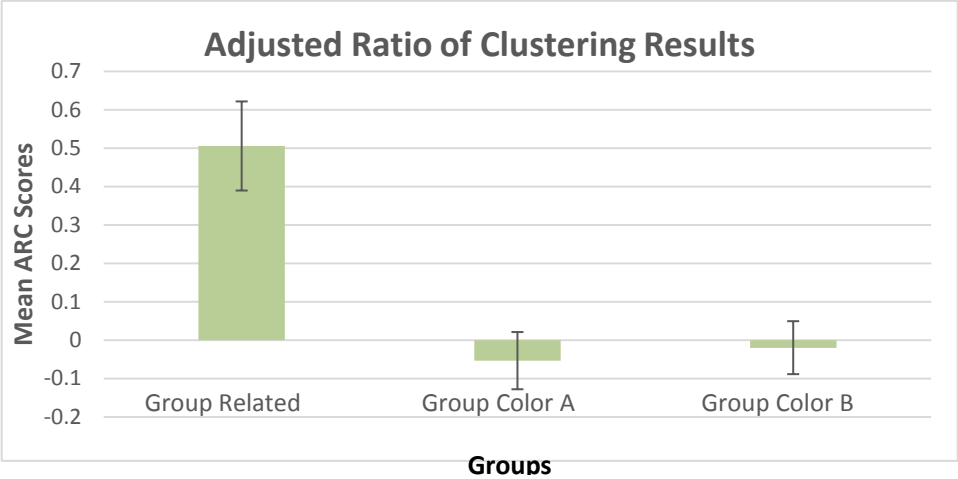

Figure 5-Mean ARC scores for Group Related and the Color groups

Planned contrasts revealed that Group Related had a significantly higher mean ARC score than both Group Color A, $t(54)= 2.801$, $p= 0.007$, and Group Color B, $t(54)= 2.604$, $p= 0.023$. Groups Color A and B did not have significantly different ARC scores, $t(54)= 0.333$, $p= 0.789$.

Discussion

It was hypothesized that Group Color A and Group Color B would have similar clustering scores as Group Related. In accordance with the hypothesis, the results suggested that Group Color A and Group Color B did not differ from each other in regards to their clustering scores, however they both differed from Group Related. Group Related had significantly higher clustering scores than both of the color groups according to all four measures of clustering. In addition, it was hypothesized that Group Color A and Group Color B would recall a similar number of words as Group Related and that all three of these groups would recall more words than Group Unrelated. Consistent with this hypothesis, and past research (see Bousfield, 1953), Group Related recalled significantly more words than Group Unrelated. However, Group Color A and Group Color B recalled significantly fewer words than both Group Related and Group Unrelated.

While there are many possible reasons to explain why Group Related had significantly higher clustering scores than Group Color A and Group Color B, one reason may be that semantic organization may be a more automatic process than organization based on font color. In everyday life people need to associate words to their semantic meaning in order to comprehend sentences, while the font color of those words may be obsolete. This can be examined through the Stroop Effect (Stroop, 1935). Stroop (1935) reported that participants tended to take more time to identify the color of a word when the meaning of the word was not consistent with the font color. This suggests that participants may have associated the words to their semantic meaning before associating the words to their font color. In order for words to be organized by a shared characteristic, perhaps the words first need to be associated to that characteristic. If words are not automatically associated to their font color, then there may need to be a way to associate them together in order to observe color-based clustering in recall.

Based on these observations the question of which testing conditions could allow a word to be associated to its font color is still open. One possible method to associate words to their font color and potentially induce color-based clustering may be through the usage of encoding strategies. Past research (see Miller, 1956; Tulving, 1962; Brown, Conover, Flores, & Goodman, 1991) has emphasized the importance of organization at the time of encoding for clustering scores. Miller (1956) suggested, through his unitization hypothesis, that encoding strategies may alter clustering scores in that words may be associated to each other and formed into individual units at the time of encoding. If semantically unrelated words were not associated to their font color at the time of encoding, then it is possible that these individual units were not formed in Group Color A and Group Color B.

Participants may cluster words together based on font color in a future experiment if they were manipulated to associate the word to that color at the time of encoding. There are several

potential methods to get participants to associate a word to its font color. One potential method to this may be by instructing the participants to assign color fonts to each words color at the time of encoding. If participants decide what font color the words are, it is possible that participants may associate that word to that color more than when the words are simply presented to them. This could be performed in a future experiment by giving participants markers and a stack of notecards turned face down, with one outline of a word in the list per notecard, and be instructing the participants to color the words with the marker that they think is appropriate. Having participants choose the colors of the words at the time of encoding may result in participants associating each word to the color they assigned to it and may result in higher color-based clustering scores than when this encoding strategy is not used.

In addition to the clustering results, there are also several explanations for the pattern of mean word recall rates. It was expected that if Group Color A and Group Color B had similar clustering scores as Group Related, then all three of these groups would also have similar word recall rates. This expectation was based on the research of Brown, Conover, Flores, and Goodman (1991) who reported that participants who have higher clustering scores also tend to recall more words. In the current experiment, Group Color A and Group Color B had lower clustering scores and word recall rates than Group Related. Tulving (1962) suggested that Miller's (1956) unitization hypothesis may explain the positive correlation between clustering and word recall rates. Tulving (1962) suggested that people who cluster words together in recall may have formed individual units of semantically related words at the time of encoding, that may have enabled people that cluster to have a higher memory capacity. Based on this idea, it is possible that Group Related recalled more words and had higher clustering scores because they may have formed individual units of semantically related words at the time of encoding. It is possible that Group Color A and Group

Color B did not have similar word recall rates as Group Related because Groups Color A and Color B might not have formed units that were color-related at the time of encoding.

Additionally to recalling significantly fewer words than Group Related, Group Color A and Group Color B also recalled significantly fewer words than Group Unrelated. This result was not anticipated since Group Unrelated, Group Color A, and Group Color B were given the same list of words, in the same exact order. At worse, it would have been expected that all three of these groups would perform equally poorly. This, however, was not the case and the data suggests that the addition of color had a detrimental effect on word recall. Although there may be several explanations for this result, one possible reason that Group Unrelated recalled significantly more words than Group Color A and Group Color B may have been that the multiple font colors may have caused a distraction. Since the font colors changed as the words were presented to Groups Color A and Color B, participants may have focused on the font colors changing more than the actual word.

If participants were given a distraction in the other groups, then it would be expected that Group Unrelated and Group Related would experience similar word recall deficits. This could be tested in a future experiment by assigning multiple font colors to a list of semantically related words. If multiple font colors does act as a distraction in this task, then it would be expected that participants shown a multi-colored semantically related word list would recall significantly fewer words than participants shown the same list with black font. In a future experiment where participants assign font colors to the words, the multiple font colors may not act as a distraction. Since participants would be instructed at the beginning of the experiment that multiple colors would be involved during testing, the addition of color may not act as a distraction using this methodology.

While significant color-based clustering was not observed in the current experiment, it is possible that the color-based clustering could be observed in future experiments. It is expected that the addition of encoding manipulations may cause participants to have higher color-based clustering scores and subsequently have higher word recall rates as well. If this occurs, then it would demonstrate that clustering in recall can occur for words that share a font color in addition to words that share a semantic category.

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Appendix A:

Informed Consent Form

Concord University

Principal Investigator: Katherine A. Engo

Faculty Supervisor: Dr. Rodney L. Klein

Please read this document carefully and sign at the end to agree to participation in this study.

This is a research experiment. You have the right to withdraw from this research at any time, without prejudice, should you wish. You are entitled to ask questions and to receive an explanation after your participation.

Description of the Study:

This is a quick trial study that should not take more than twenty minutes. You will simply be shown a PowerPoint presentation and further instruction will follow this presentation. There are no possible risks for this study. If this proves to be false, please notify the researcher/ the psychology department. No names will be connected to your performance in this task. If your professor has agreed to extra credit, your name will be collected on a completely separate sheet of paper. You may leave at any time. If you wish to leave, please exit quietly so you do not disturb the other participants.

Your signature below indicates that you voluntarily agree to participate in this study.

Signature:_____ Date:_____

Appendix B:

Questionnaire

Age:_____

Sex:_____

Major:_____

Are you color blind? [ ]Yes [ ] No

- If you are color blind please retire from this research. Thank you for your time.

Please do not put your name on any papers during this experiment, excluding the informed consent sheet, in order to ensure anonymity.

Appendix C:

The list of words in the order they appeared in Group Unrelated's PowerPoint presentation:

Shirt, Table, Statue, Lobster, Bob, Canyon, Iron, Hamster, Soda, Rug, Hula, Paint, Saturn, Rain, Cord, Tulip, Doll, Vent, Book, Flute, Light.

Appendix D:

The list of words in the order they appeared in Group Color A's PowerPoint presentation:

Shirt, Table, Statue, Lobster, Bob, Canyon, Iron, Hamster, Soda, Rug, Hula, Paint, Saturn, Rain, Cord, Tulip, Doll, Vent, Book, Flute, Light.

Appendix E:

The list of words in the order they appeared in Group Color B's PowerPoint Presentation:

Shirt, Table, Statue, Lobster, Bob, Canyon, Iron, Hamster, Soda, Rug, Hula, Paint, Saturn, Rain, Cord, Tulip, Doll, Vent, Book, Flute, Light.

Appendix F:

The list of words in the order they appeared in Group Related's PowerPoint presentation:

Peach, Glove, Table, Shirt, Mango, Bed, Sofa, Banana, Shorts, Hat, Rug, Apple, Counter, Pants, Chair, Orange, Boots, Lamp, Grape, Pear, Jacket.

Immunoglobulin G Biosensor Based on Surface-Enhanced Raman Scattering

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Abstract

Detection of an Immunoglobulin G antigen was carried out using a biosensor based on surface-enhanced Raman scattering (SERS). The biosensor consisted of a gold film with an attached layer of antibody and antibodies with attached SERS probe. The SERS probe, gold nanoparticle@Raman label@SiO_2 core-shell nanoparticles in which the Raman label, malachite green isothiocyanate (MGITC), is embedded between the gold core and SiO_2 shell, produces a peak at 1170 cm^{-1} in the Raman spectrum. When introduced to the system, the antigen was sandwiched between antibody on the film and antibody with attached probe so that the probe remained on the film after washing. The intensity of the resulting Raman peak varied linearly with the logarithmic antigen concentration. Both gold nanospheres and gold nanostars were used in the SERS probe, and it was found that nanostars provided better sensitivity and made the Raman peak more clearly visible. Given the design of the system, this biosensor could easily be adapted to other antibody-antigen pairs making it a valuable tool for quantitative detection.

Introduction

Gold nanoparticle@Raman label@SiO_2 core-shell nanoparticles can be used as Raman probes that possess several desirous characteristics. These probes are stable in water and, depending on the shape of the core, offer high sensitivity.[1] These probes have been used in a variety of sensors, including detection of heavy metals, adenosine triphosphate, cancer biomarkers, proteins, viruses, and microorganisms.[2-5]

Antigen detection with a localized surface plasmon resonance (LSPR) based sensor has been successful with limits of detection as low as 100 pg/mL. This method is label free and uses a layer of silica nanoparticles with a layer of gold on a gold nanochip as the array.[6] Antigens have also been successfully detected with surface-enhanced Raman scattering using a Raman label attached to a gold nanoparticle.[7]

Here, a surface-enhanced Raman scattering based biosensor that uses a probe with a Raman label sandwiched between a gold core and a silica shell is used to detect the Immunoglobulin G antigen. This sensor is different from the one previously described in that the probe is coated with silica before the antibody is attached to protect it and prevent aggregation in solution. Both gold nanosphere@MGITC@SiO_2 and gold nanostar@MGITC@SiO_2 nanoparticles are used as the probe and their performance is compared. Based on the literature, it is predicted that the nanostars will provide better signal enhancement and will therefore be a better choice to use as the probe.[1] Both the probe and a gold film have attached antibody that will sandwich the antigen when exposed leaving the probe on the film. The sensor will then yield Raman spectrum characteristic of MGITC.

Experimental Section

All reagents were used as purchased to make solutions. Gold nanospheres were synthesized using the citrate reduction method.[3] 1 mL of 20 mM $HAuCl_4$ was added to 50 mL deionized water

and brought to a boil. 2 mL of a 1% wt. trisodium citrate solution was added and this was boiled for approximately 30 minutes.

Gold nanosphere@MGITC@SiO_2 core-shell nanoparticles were synthesized by adding 100 µL malachite green isothiocyanate (MGITC) to 25 mL of the gold nanospheres and stirring for approximately 30 minutes. Then 100µL of a solution of 2 µL (3-Mercaptopropyl)trimethoxysilane (MPTMS) in 1 mL ethanol was added in 10 µL aliquots and the pH was adjusted to 8-9. After 30 more minutes of stirring, a solution of 20 µL sodium silicate in 1 mL deionized water was added and the pH was adjusted to 8-9 again. Stirring was continued for another hour and then stopped and the solution was equilibrated overnight. 5 mL ethanol was added and solution was equilibrated about half a day, another 5 mL ethanol was added and the solution was then equilibrated overnight before centrifuging. The gold nanostar@MGITC@SiO_2 core-shell nanoparticles were synthesized by Peng Zheng via the seed growth method.[3]

To attach the antibody to the core-shell nanoparticles, 20 µL 3-Triethoxysilylpropyl succinic anhydride (TEPSA) is added to 1.5 mL nanoparticles and equilibrated overnight. This is centrifuged and the nanoparticles are then re-dispersed in 200 µL *N*-Hydroxysuccinimide (NHS)/1-ethyl3-(3-dimethylaminopropyl)-carbodiimide (EDC) made by dissolving 0.0145 g NHS and 0.0800 g EDC in 2 mL phosphate buffer solution (PBS). This is allowed to equilibrate several hours before centrifuging and re-dispersing in 200 µL of a 10 mg/mL IgG antibody and let set overnight. This is then centrifuged and re-dispersed in PBS solution.

The antibody is attached to gold films by soaking in approximately 2 mL 11-mercapto-1-undecanol ethanolic (MU)/11-mercaptoundecanoic acid (MUA) prepared by dissolving 0.1038 g MU and 0.1084 g MUA in 10 mL ethanol. This is allowed to equilibrate overnight and the films are then washed with ethanol, followed by D.I. water. Then a drop NHS/EDC is added and this is

let equilibrate for several hours before washing with PBS solution and adding a drop of the IgG antibody. This is allowed to equilibrate overnight in a moist environment and then the films are washed with PBS solution when ready to use.

To use the sensor, the film is washed with PBS and dried and 30 µL IgG antigen is added along with the functionalized nanoparticles. 40 µL are used for the nanospheres and 30 µL for the nanostars. This is equilibrated for approximately 20 minutes before washing with PBS and drying. The Raman spectrum is obtained using an excitation wavelength of 785 nm from several points and then the process is repeated with a higher antigen concentration.

Results and Discussion

The design of the sensor can be seen in Figure 1. The antigen is sandwiched between the antibody on the film and antibody on the Raman probe. The probe remains on the film after washing and the Raman spectrum is collected.

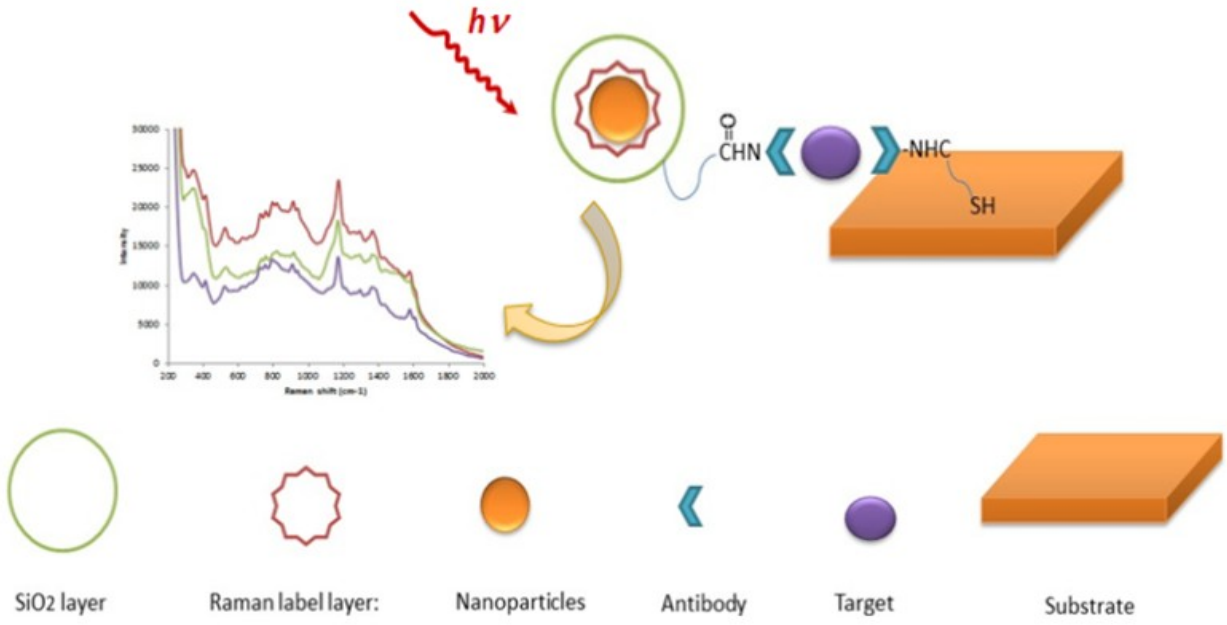

Figure 1: Immunoglobulin G Biosensor

The size of the synthesized nanospheres was confirmed to be approximately 15 nm in diameter using UV-visible spectroscopy. This can be seen in Figure 2, which shows that the peak is at 520 nm.

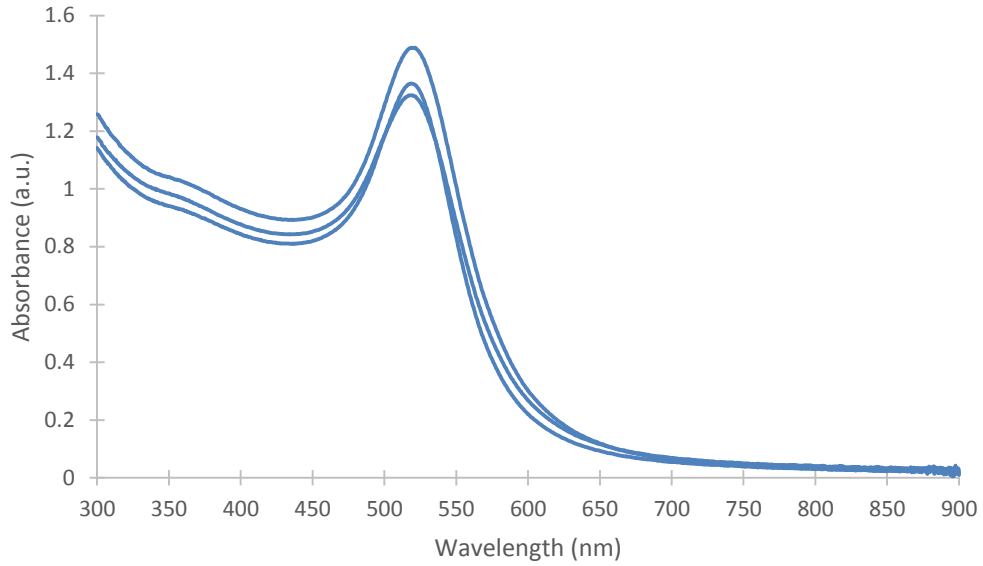

Figure 2: UV-Visible Absorbance Spectra of Gold Nanospheres

In order to ensure that the probe had been successfully synthesized, the Raman spectrum of the probe after functionalization was obtained. The spectrum, found in Figure 3, matched what was expected for MGITC indicating that the label was sandwiched between the gold core and SiO_2 shell. The strong peak at 1170 cm^{-1} from the C=S bond is what is monitored.[8]

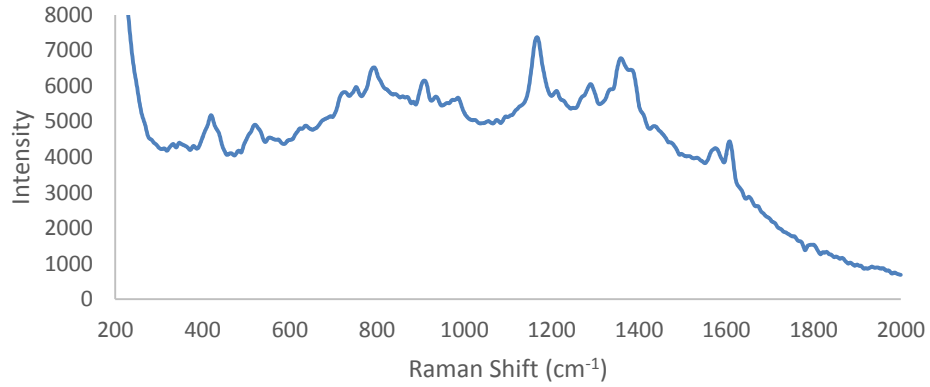

Figure 3: Raman Spectrum of Gold Nanospheres after Functionalization

The Raman spectra of the sensor after exposure to the antigen was taken from several points on the gold film and averaged to give the spectra in Figure 4. As expected, the intensity of the spectra increases as the concentration of the antigen increases.

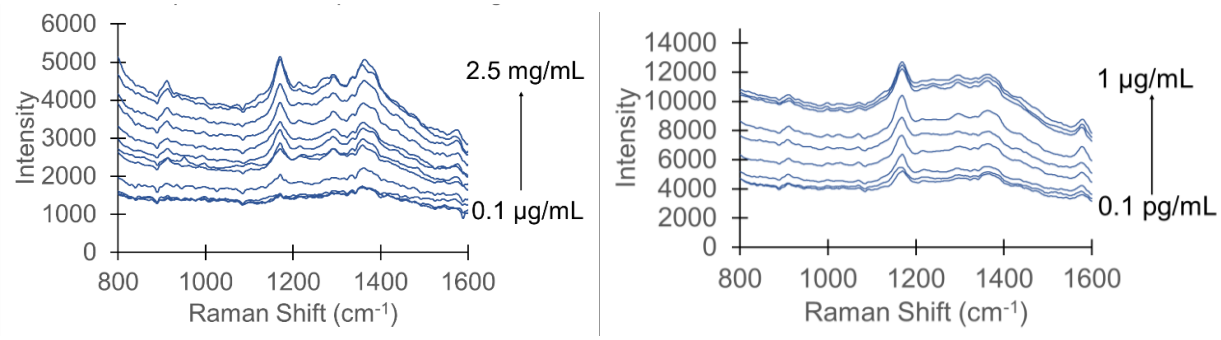

Figure 4: Average Raman Spectra of Biosensor (left: nanosphere; right: nanostars)

As seen in Figure 4, the intensity of the peak is much higher when nanostars are used. This results from the geometry of the nanoparticles, which produce a better signal enhancement. As a result of this enhancement, it is easier to see lower concentrations of the IgG antigen. The results indicate that the limit of detection for the sensor using the gold nanostars as the probe is lower than that of the sensor using the gold nanospheres as the probe.

The intensity at 1170 cm^{-1} was plotted against the logarithmic antigen concentration to produce the plots found in Figure 5. The plot produced from the sensor using the gold nanosphere@MGITC@SiO_2 probe varies somewhat from what would be expected for a linear relationship between concentration and peak intensity. A possible explanation for this could be that the differences in concentration were not as great at the larger concentrations, meaning that the previous concentrations might not be negligible.

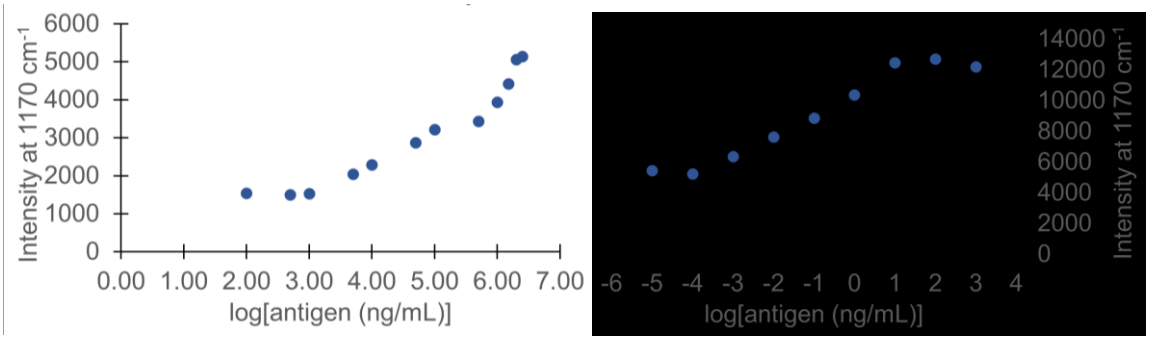

Figure 5: Concentration of Antigen versus 1170 cm^{-1} Peak Intensity (left: nanosphere; right: nanostars)

The linear portion of the plots in Figure 5 were re-plotted with a best fit line. This can be found in Figure 6. The slope for the plot produced with the sensor using nanostars is much higher than that produced with the sensor using nanospheres. This indicates that the sensitivity of the sensor using nanostars is much better.

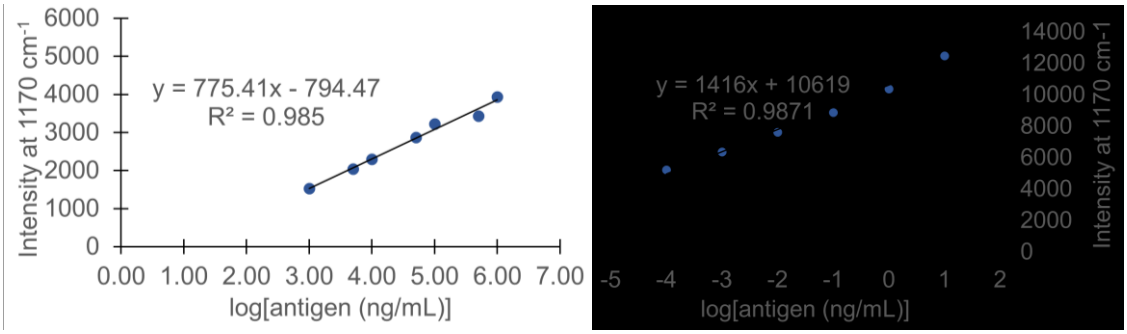

Figure 6: Linear Portion of Concentration of Antigen versus 1170 cm^{-1} Peak Intensity (left: nanosphere; right: nanostars)

Conclusion

The IgG antigen can be detected using a surface-enhanced Raman scattering based biosensor. This surface enhancement is better achieved with the use of nanostars rather than nanospheres in the Raman probe. The nanostars produce a much higher peak intensity than the nanospheres and greater sensitivity. Also, the results indicate that the limit of detection for the sensor using nanostars as the core of the probe is lower than that of the sensor using nanospheres as the probe.

This biosensor can be used to quantitatively as well as qualitatively analyze the IgG antigen. Plotting the logarithmic antigen concentration versus the intensity yields a linear relationship with an equation that can be used to determine the concentration of antigen present.

The next step would be to study the selectivity of the sensor. To do this the procedure would be repeated with a different antigen to see if the sensor still produces a response.

Acknowledgement

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Mechanical Activation of Covalently Linked Spiropyran in PDMS Networks

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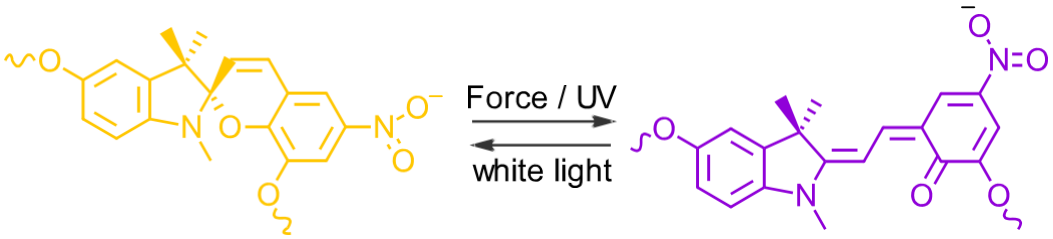
Abstract

Mechanophore covalently linked poly(dimethyl)siloxane (PDMS) was synthesized through vinyl-terminated spiropyran (SP) and mechanical tests were performed under uniaxial extension and compression. Incorporation of the force-sensitive mechanophore, SP allows us to observe how it activates in response to PDMS networks. A silica filled commercialized Sylgard 184 was used as a base material and DCM or xylene was added as plasticizer for specimens containing spiropyran. Uniaxial extension samples shows a vivid color change and gradual increase in in-situ fluorescence intensity, demonstrating the conversion from spiropyran to merocyanineunder extension. In the case of uniaxial compression fluorescence intensity also increases initally, but became saturated at around 60% strain. To understand silica filler effect on force transmission, unfilled PDMS was synthesized with SP through a hydrosilylation reaction. The spiropyran was force-activated as in filled-PDMS, but the sample failed at 40% strain before significant fluorescence was observed.

I. Introduction

As the need for stronger and smarter materials has increased the area of research called mechanochemistry has gained significant attention. Mechanochemistry can be defined as the study of force induced chemical reactions.[1] These chemical reactions may be utilized in areas of catalysis, supramolecular synthesis, regioselective and solvent free synthesis, and as a molecular level probe.[1,2] At the forefront of mechanochemical research is the mechanophore, a molecule that reacts to external stimuli such as mechanical force. Mechanophores can be incorporated into a bulk polymer as a stress detector,[3] thus increasing the reliability of engineering materials. Many force reactive mechanophores have been shown to exhibit useful optical properties when local force is induced.[3] In particular, one mechanophore stands out as being reliable at detecting mechanical deformations in polymeric materials.

Spiropyran has been the subject of many studies when incorporated as a crosslink within polymeric materials.[4,5] As reported in previous studies, when subjected to mechanical force spiropyran undergoes 6Π-electrocylcic ring opening to produce the colorful molecule merocyanine[6] (**Scheme 1**). The product merocyanine is produced through mechanical activation, UV radiation and heat. The reversible reaction occurs with exposure to white light over time. At the core of spiropyran is a spiro bond consisting of a C-N bond and C-O bond. Through the software simulations Steered Molecular Dynamics (SMD), Density functional theory (DFT), and Constrained Optimization (COGEF) the rupture of the C-O induces a ring arrangement to produce merocyanine.[4] Besides color change, merocyanine has reported to be fluorescent with a excitation range of 550-590nm and an emission centered at 610nm.[5]



Scheme 1: Due to an external force or UV radiation, Spiropyran rearranges into the colorful Merocyanine. This process is reversible with exposure to visible light.

Polydimethylsiloxane (PDMS) is an organic polymer which exhibits great mechanical strength, stretchability, and transparency for an elastomer. PDMS is widely used in microfluid devices[7], cosmetics, soft lithography[8], and even silly putty. PDMS can be easily modified through additives that act as a plasticizer, making it more flexible and ductile. The commercialized PDMS Sylgard 184 contains silica filler to enhance its mechanical properties. While making the polymer very practical, the chemical structure becomes complicated and uncertain due to the presence of the filler.

Various modified PDMS networks were tested with and without the mechanophore spiropyran. Sylgard 184 was used as a base material to which the solvents dichloromethane (DCM) and xylene were added as plasticizers while incorporating spiropyran into the material. Like Sylgard 184, a unfilled PDMS was synthesized through a hydrosilylation catalyzed crosslinking reaction[11] with and without spiropyran. The objective of this research was to

observe how spiropyran activates in modified PDMS networks.

II. Methods

Three different types of PDMS networks were tested with spiropyran. The first network was the commercially available Sylgard 184. Sylgard 184 is the basic PDMS polymer but with industrial additives to increase its ultimate strength, making it overall more glassy. It comes with two parts, a base agent containing the PDMS backbone and a curing agent which contains the PDMS crosslink and hydrosilylation catalyst. According to Dow Corning, the recommended ratio between base and curing agent is a 10:1 weight ratio. The spiropyran used in this project was a bis(methyl methacrylate) functionalized spiropyran.

A. Materials

Sylgard 184 base and cure kit was purchased from Dow Corning. Vinyl terminated poly(dimethyl)siloxane with an average molecular weight of 25,000 and viscosity of 850-1150 cSt, poly(methylhydro)siloxane of an average molecular weight of 1700-3200, and xylenes of ACS reagent, ≥98.5% xylenes + etylbenzene basis was purchased from Sigma Aldrich. Methylene chloride (DCM) HPLC grade was purchased from Fisher Scientific.

B. Spiropyran-DCM-Sylgard 184

0.05 wt % (2.4mg) spiropyran was dissolved in 5 wt % (0.316g) dichloromethane (DCM). This solution produced a rich and vibrant magenta color from the open ring form merocyanine. Once the mixture was homogenous, 10 parts (4.37g) of Base was mixed with 1 part (0.437g) Cure. The total mixture was stirred at room temperature for 30 minutes. During stirring the solution turned from light purple to pale yellow. The homogenous solution was then dessicated under vacuum for 1.5 hours. This step is to ensure the evaporation and separation of DCM from the PDMS-Spiropyran mixture. The resulting viscous pale yellow liquid was then poured into a 3-D printed dogbone molds and placed in a 65˚C oven for 4-5 hours. The specimens were then transferred to an 80˚C oven for 8-12 hours until fully cured. Cylindrical samples were obtained using the same synthesis by pouring the uncured product into test tubes.

C. Spiropyran-Xylene-Sylgard 184

Because Sylgard 184 polymer is a substantially strong rubber, a xylene additive was mixed into the Sylgard-Spiropyran mixture. Following the methods from a group at Duke University,[9] 0.4 wt % (14.7mg) of spiropyran was dissolved in 10 wt % (0.365g) xylenes and vortex mixed until the spiropyran was fully dissolved. This yielded a greenish-blue solution, which was then mixed with 10 parts (3.32g) Base and vortexed. 1 part (0332g) of Curing agent was added to the pinkish mixture and vortexed again until complete homogeneity. The mixture was then dessicated under vacuum for approximately 30 minutes to remove any bubbles, placed in molds and transferred to a 65˚C vacuum oven for 16-18 hours until fully cured. Cylindrical samples were obtained then same way as the Spiropyran-Sylgard 184 specimens.

D. Spiropyran-PDMS

The third PDMS network tested was mixture of α,ω-divinyl-polydimethylsiloxane and

polymethylhydrosiloxane (PMHS). This reaction was carried out through hydrosilylation by the catalyst chloroplatinic acid, H_2PtCl_6. The Pt(IV) complex has been documented to be effective at activating Si-H bonds across alkenes.[10] With a calculated PHR of 200:1, we decided to use a 5% PHR, making it a 10:1 ratio. A 0.05 wt % (1.9mg) of Spiropyran was dissolved in 5 wt% (0.19g) of DCM. 10 parts (3.66g) of divinyl functionalized PDMS, 1 part (0.366g) of PMHS, and 30μL of an 8 wt% of H_2PtCl_6 solution was added and mixed for 6-8 hours at room temperature. The pale yellow mixture was then dessicated to remove excess DCM for 1.5 hours; poured into McLube 1711L coated molds, and placed in a 65˚C oven. After 4-5 hours the molds were then transferred to an 80˚C oven for 8-12 hours until cured fully.

E. Mechanical Testing and Optical Measurements

Uniaxial tensile and compressive loading was performed with a Zwick/Roell Z010 at a strain rate of $5\times10^{-3}s^{-1}$. A 532nm laser with an intensity of 8mW was directed towards specimens undergoing mechanical testing. If merocyanine was present the laser caused fluorescence, which was collected through a 570nm filter coupled with a Qimaging EXi Blue CCD camera. Intensity was then measured through the program ImageJ by tracing the center of mass with respect to time to obtain an area of interest's mean intensity. Normalized Intensity was found by measuring the maximm intensity of a UV activated specimen and comparing the mechanically activated intensities through the equation:

III. Results and Discussion

The mechanophore spiropyran was incorporated as a crosslinking agent to induce selective covalent bond scission in three PDMS networks. Spiropyran allows for the detection of the detection was damage through rupturing a C-O bond, undergoing a 6Π-electrocylcic ring, reducing the HOMO-LUMO gap producing a longer emission wavelength into the visible spectrum. Dogbone shaped specimens were made for the three PDMS networks and Sylgard 184 and PDMS without spiropyran as controls. **Figure 1a** demonstrates the Stress vs. Strain curve of the three Sylgard 184 specimens. The control showed to be the most brittle of the three. The PDMS (DCM) and PDMS (Xylene) specimens proved to be more rubbery which is supported by Void Theory and Free Volume Theory. By implementing the solvents DCM and xylene, we increased the free space between polymer stands in PDMS.

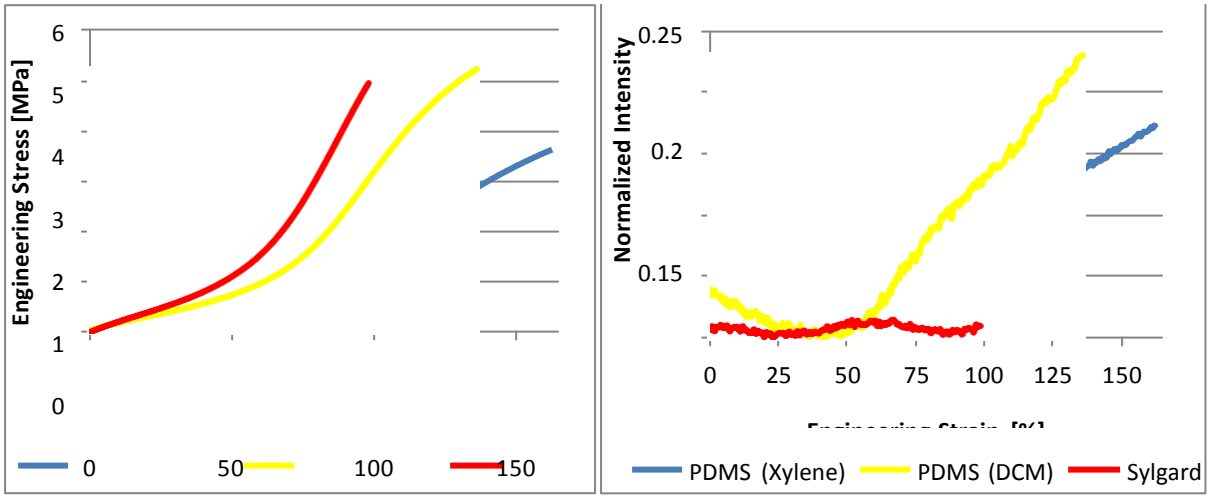


Figure 1 a) Stress vs. Strain curves for the three Sylgard 184 samples. By adding Spiropyran, DCM, and xylene the mechanical properties became more viscoelastic. **b)** Normalized Intensity vs. Strain curve of the three Slygard 184 network

Because of the increased mobility between PDMS strands caused by DCM and xylene it is now possible to analyzed the activation of Spiropyran as a function of mobility. **Figure 1b** denotes that normalized intensity vs. engineering strain plot. As strain increases in both the PDMS (DCM) and PDMS (Xylene) specimens the normalized intensity of fluorescence increases while the Control remains relatively 0. Also observed in **Figure 1b** is the initial decrease in normalized intensity. It is believed that this was due to thinning of the specimen, reducing the concentration of merocyanine while at the same time the local forces within the polymer not being great enough to activate more merocyanine. Comparing PDMS (DCM) to PDMS (Xylene) shows that the PDMS (DCM) yields a higher normalized intensity. When coupled with the adjacent **Figure 1a** it's worth noting that PDMS (Xylene) experiences less stress and more strain compared to PDMS (DCM). Therefore; it's plausible to deduce that activating Spiropyran in a PDMS network is a stress/force driven reaction. This is also demonstrated by graphing the normalized intensity as a function of true stress.

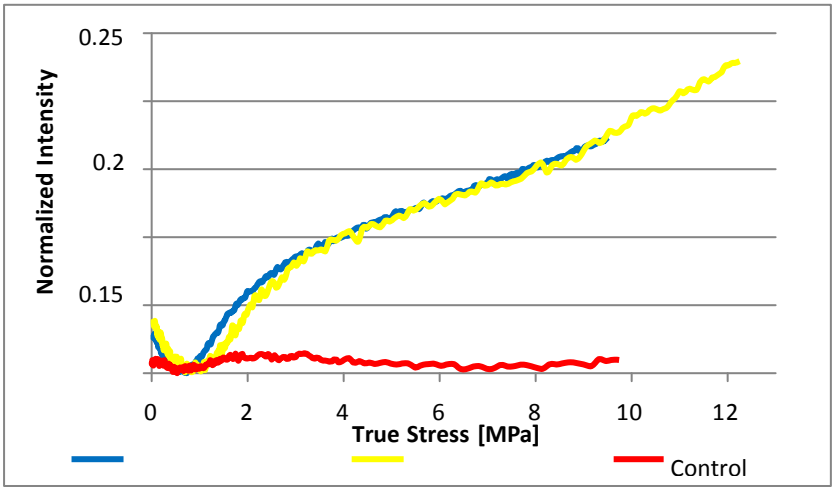


Figure 2 True stress vs. normalized intensity. As true stress increases the normalized intensity increases leading to the assumption that intensity is a function of stress.

During mechanical loading the conversion from spiropyran and merocyanine is a colored reaction. Spiropyran is a yellowish orange color and merocyanine is a deep magenta. **Figure 3** shows all specimens types testing and how they changed color initially, mechachanically activated, and UV activated spiropyran. The first row contains the Sylgard samples without spiropyran, where no color change is seen. The last row contains the PDMS with spiropyran specimens. No color change is seen. There are several ideas as to why the color does not change. The PDMS specimen contains a naturally dark color that could inhibit fluorescence or the specimen may not be able to experience the necessary stress and strain needed to activate spiropyran.

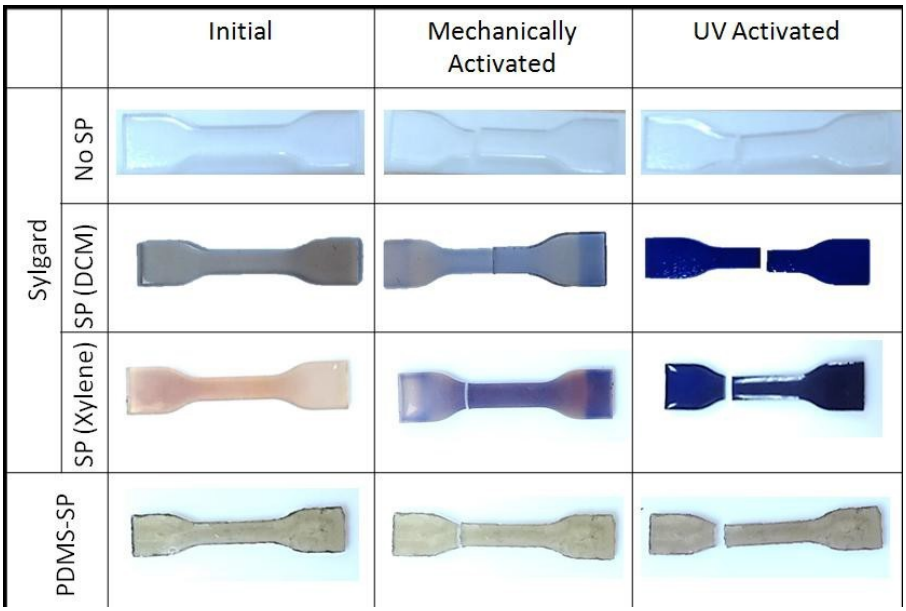


Figure 3 The color change seen from specimens initially, after mechanical loading, and after UV radiation.

The same experiment was conducted with unaxial compressive loading where specimens were cylindrical shaped with a diameter to height ratio of 1:1. **Figure 4** shows the stress vs. strain curve and normalized intensity vs. strain curve. The plasticizing effects that the solvents DCM and xylene are again evident in the stress strain curve, **Figure 4a**. Both the PDMS (DCM) and PDMS (Xylene) specimens experienced greater strains at a lower stress compared to Sylgard. Similarly as stress and strain increases the normalized intensity increases, **Figure 4b**. However, at a strain of approximately 60% the PDMS (Xylene) specimens decrease in intensity. This implies that any merocyanine present would be converted into spiropyran leaving it colorless. Contrarily, the specimens were deep blue indicating an increase in the presence of merocyanine. The decrease in intensity is a reoccurring phenomenon seen in compression samples. This artifact could be due to the experimental setup or natural surface properties. It requires further investigation.

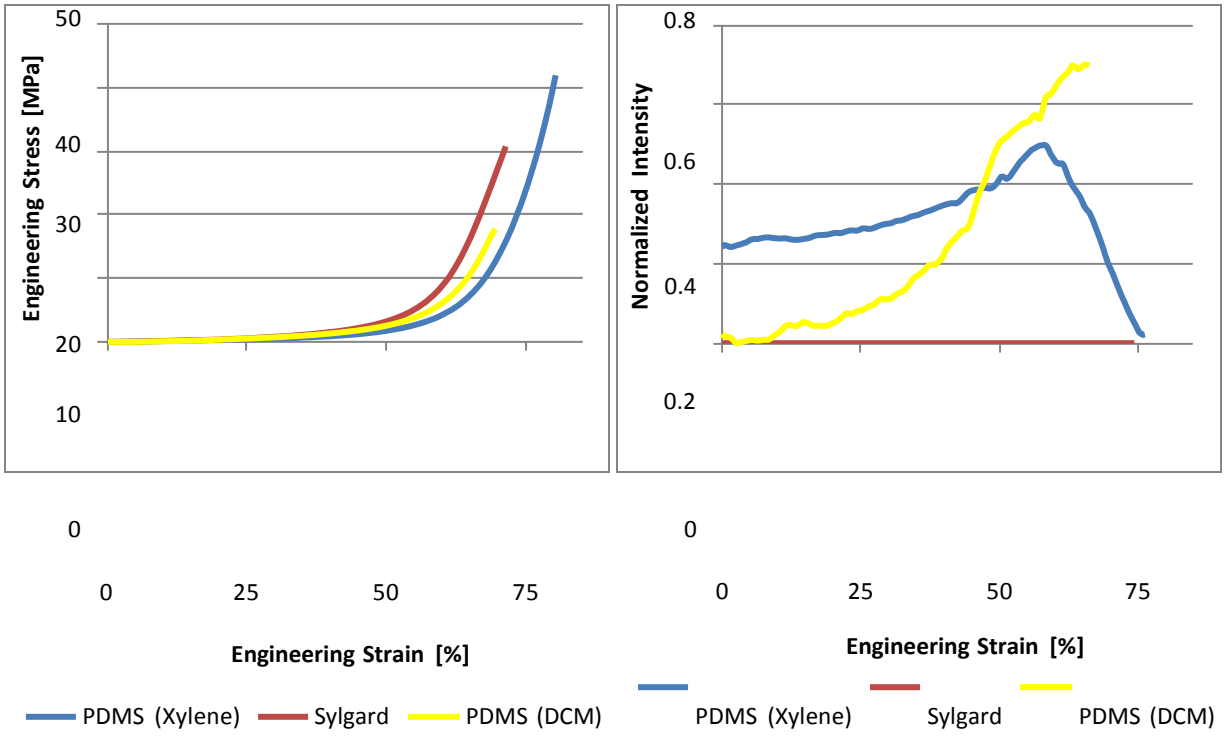


Figure 4 Uniaxial compression data **a)** The stress vs. strain curve of the three Sylgard 184 specimens. **b)** Normalized Intensity vs. strain curve of the three Sylgard 184 specimens.

When normalized intensity is graphed as a function of true stress in **Figure 5** a similar curve is produced. The PDMS (Xylene) specimens experienced a dramatic decrease in intensity as stress increased. The saturation effect is seen in the PDMS (DCM) curve at about 20 MPa. It's unusual that these curves formed when compared to the unaxial extension specimens **Figure 2**. The curves are understandable and predicted in uniaxial extension, but for uniaxial compression unique graphs emerge creating ambiguity. Further testing is needed.

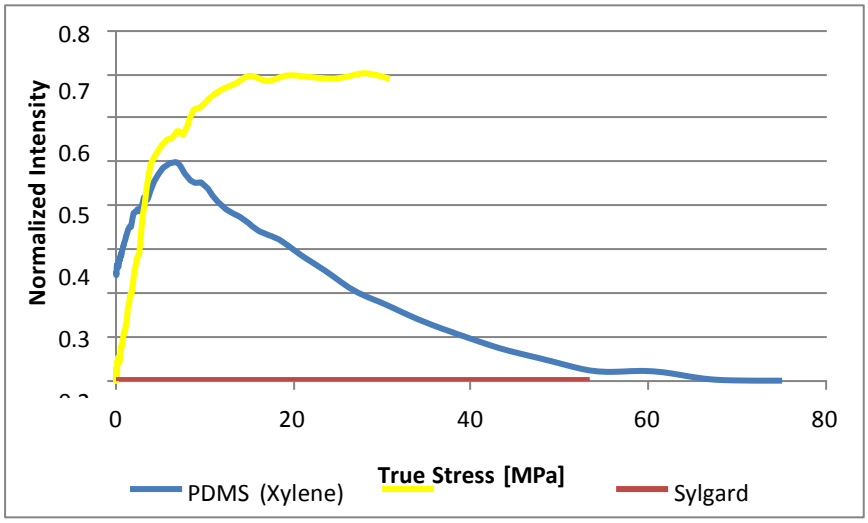


Figure 5 Uniaxial compression. Normalized intensity as a function of true stress for the three Sylgard 184 specimens. Unusual phenomena occur when compared to the curves for uniaxial extension.

As mentioned earlier, Sylgard 184 is a commercially available PDMS which contains many silica fillers to enhance its mechanical properties. The addition of filler adds ambiguity when one wishes to analyze or model how macroscopic force is transmitted to local force where spiropyran is especially useful. To overcome this, an unfilled PDMS was used with spiropyran as a molecular level probe. **Figure 6a** demonstrates the reproducibility of a stress/strain curve between the unfilled PDMS with and without spiropyran. The decrease in mechanical properties is evident when compared to the Sylgard curve in **Figure 1a**. Sylgard 184's measured stress reached 5 MPa and a maximum strain of approximately 100% where the unfilled PDMS had a significantly less stress and strain of 0.25 MPa and 43% respectively. **Figure 6b** shows the change in normalized intensity as strain is increased. The intensity of the PDMS-SP specimen did increase in intensity, however the curve is inconsistent and is not a significant increase when compared to the PDMS curve.

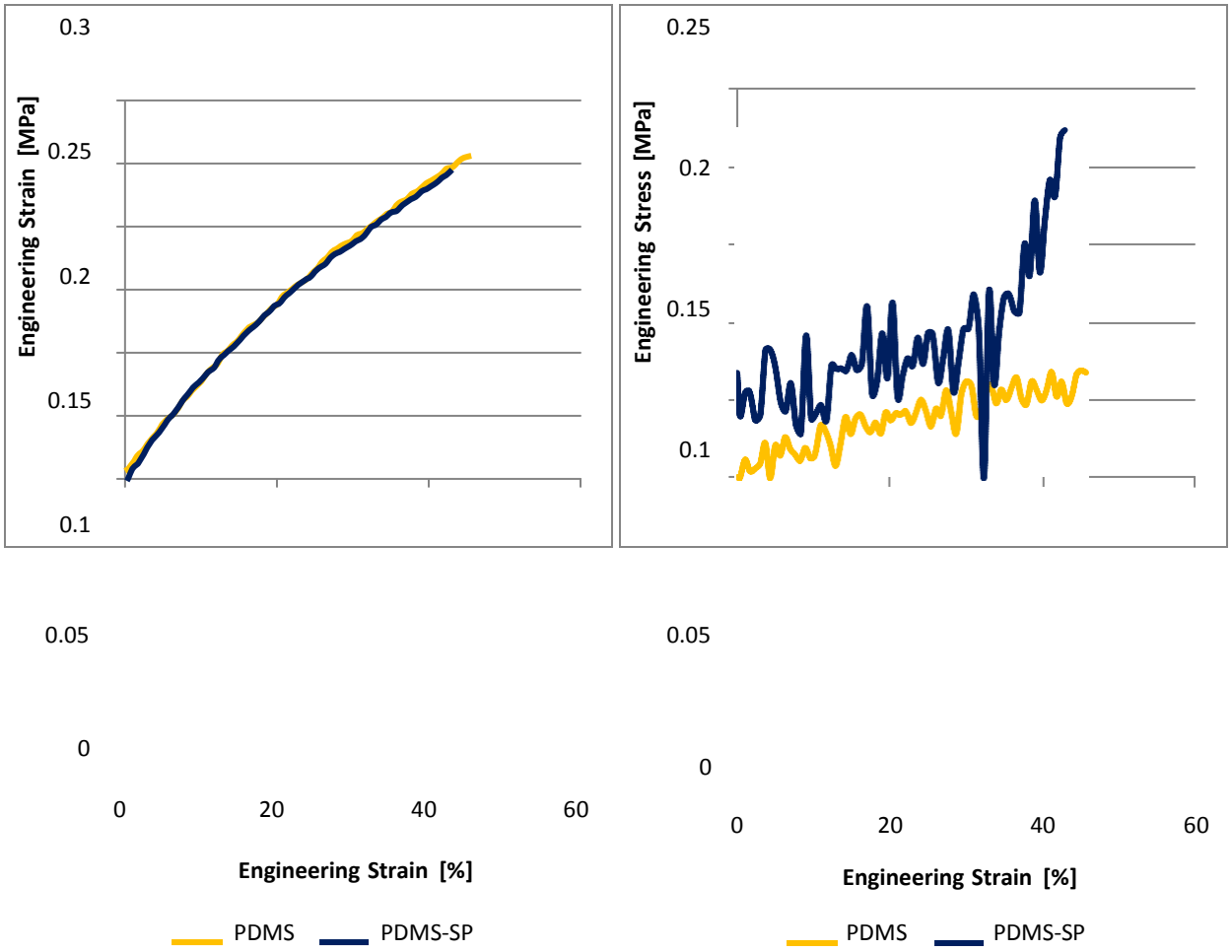


Figure 6 Unfilled PDMS specimens under uniaxial extension **a)** Stress vs.strain curve **b)** Normalized intensity vs. strain curve

The lack of increase in fluorescence of the unfilled PDMS samples with spiropyran could be due to a number of reasons. Because of the dark colored chloroplatinic acid catalyst used for hydrosilylation, the PDMS is characteristic of a naturally dark yellow/brown color. As a result, the natural color of the PDMS could prevent the escape of light from the fluorescent merocyanine. When exposed to UV light, the PDMS-SP samples increased in fluorescence slightly by approximately 50. This implies that the chemical identity of spiropyran has changed possibly due to the catalyst used. The absence of fluorescence increase as stress and stress increase leads to the assumption that the polymer may not be mechanically strong enough to enable activation of spiropyran.

IV. Conclusion

The mechanophore spiropyran was successfully crosslinked into various PDMS networks and molded into uniaxial extension dogbone shaped specimens and cylindrical uniaxial compression specimens. A silica filled commercially available Sylgard 184 was used as a base polymer to which DCM and Xylene was added as plasticizers to specimens containing spiropyran. This enabled observation of how spiropyran is activated with differing PDMS networks. Uniaxial extension specimens PDMS (Xylene) and PDMS (DCM) increased in merocyanine fluorescence as stress and strain increased while the Sylgard 184 specimen without spiropyran flat lined at a relative 0 normalized intensity. Uniaxial compression specimens exhibited unusual behavior in the PDMS (Xylene) samples where normalized intensity peaks at 60% strain then decreased to 0, this artifact may be due experimental setup or surface properties. Unfilled PDMS specimens with and without spiropyran was tested with uniaxial extension. The mechanical strength was significantly less compared to the silica filled Sylgard 184. As strain increased, normalized intensity increased in the PDMS-SP sample, but was too inconsistent and not a significant increase when compared to the PDMS sample. As a whole, color change by mechanically activating spiropyran to merocyanine was observed in PDMS (Xylene) and PDMS (DCM) uniaxial extension and compression specimens.

V. Future Work

To further the investigation of the mechanophore spiropyran in PDMS networks, there are many areas to investigate and improve. The role that transition glass temperature has on the activation of spiropyran has yet to be investigating in depth. By adding the plasticizers such as DCM and xylene to the Sylgard 184, the viscoelasticity increased enabling the material to experience more strain at a lower stress. By monitoring the change in Tg and the activation of spiropyran a study can be conducted in determing how much stress and strain is necessary to activate spiropyran in foreign polymers. The decrease in intensity seen in the PDMS (Xylene) and saturation of intensity of the PDMS (DCM) specimen in **Figure 5** is puzzling and interesting. By investigating the causes of these phenomena one may be able to construct a more realistic and expected increase in fluorescence intensity as stress and strain increase. As mentioned before, this property may be due to the experimental setup or surface property.

Unfilled PDMS containing spiropyran may yield many processing results as to understanding the exact mechanochemical pathway in spiropyran's activation. Modeling this phenomenon would become extremely less complicated compared to the unpredictable structure of Sylgard 184. However, results here are subpar. To improve the probability of obtaining better results a stronger more transparent material is needed. This can be accomplished by using shorter less heavy chains of vinyl terminated PDMS and PMHS. This would reduce the free space between the polymer strands increasing the mechanical properties. The use of an ideal hydrosilylation catalyst is also recommended, ideal meaning a catalyst that is not in solution and can be removed from the bulk polymer. If the catalyst cannot be removed, the catalyst must not affect the optical characteristics of the polymer (e.i. transparency).

VI. Acknowledgements

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Norepinephrine-Induced Modulation of T-lymphocyte Function

By

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Abstract

Stress is known to induce negative immune modulation through the production of stress hormones such as catecholamines and corticosteroids. Norepinephrine (NE) is a stress product that plays a key role in altering immune function but the mechanisms are not well understood. The purpose of this research project was to investigate the role NE may play in the stress response in altering the activities of mouse T-cells in vivo and vitro. It was hypothesized that NE suppresses the proliferation of immune cells by attenuating pro-inflammatory cytokine production or increasing anti-inflammatory cytokine production. Stressed or non-stressed mice were either infected or not infected with *Chlamydia trachomatis* and euthanized for splenic T-cell isolation. T-cells from stressed mice or from non-stressed mice were treated with NE (ex-vivo) in the presence or absence of concanavalin A (conA). After proliferation of the cells for 72hrs, culture supernatants were collected to determine cytokine production by ELISA. The results show that addition of NE resulted in a mitigated production of IL-10, IL-2, and TNF-α compared to the control. However, production of IFN-γ showed no statistically significant difference between treatment groups. The present results indicate that NE significantly alters the production of different cytokines and exerts an overall immunosuppressive effect, likely resulting in diminished cellular proliferation. Further experimentation is underway to delineate the dynamic roles of NE in the immune response and stress-induced modulation.

Introduction

Stress is an ambiguous term, with various definitions depending on the source. For the sake of both concision and physiological relevance, stress can be defined as any stimulus that challenges the homeostasis of an organism that requires an adaptive response to resist the challenge.[30] Stress can be broadly thought of as immunosuppressive, and though there are nuances in the distinction between acute versus chronic stress, overall, stress is well known to increase severity and incidence of disease.[19,23-25] Corroborating this conclusion, recent findings in our laboratory show that cold-induced stress significantly worsens *Chlamydia trachomatis* genital infection outcome and slows convalescence.

Several studies have shown that there is a dynamic interaction between the nervous system, endocrine system, and immune system that subsequently influences the infection outcome. There are two main, distinct effector pathways mediating the stress response. They are the sympathetic nervous system (SNS) response, and thus the catecholamine norepinephrine (NE), and the hypothalamic-pituitary-adrenal (HPA) axis, most notably facilitated by corticosteroids, mainly cortisol.[20] Consistently, the data from our lab using cold-induced stress displayed significantly heighted plasma concentrations of circulating NE and cortisol. In comparison to cortisol, which is quite robust in its immunosuppression, how NE modulates the immune system is far less understood.[23] However, there is growing body of evidence that NE does indeed significantly modulate immune function and contribute to the stress response.[6,11,13] NE has been found to be secreted from SNS nerve terminals in close proximity to circulating leukocytes, including T-cells[7,14,21], where it binds one of its adrenergic receptors (ARs) to transduce its signal. Moreover, on T-cells specifically, essentially the only AR present for NE to bind to is the β2-AR.[11] This receptor is typically G_s (stimulatory) coupled, which upon NE

binding, activates adenylyl cyclase (AC) and causes intracellular accretion of cyclic AMP (cAMP).[6]

The current view is that NE exerts an immunosuppressive and/or anti-inflammatory effect. There are several tentative mechanisms by which this could be brought to fruition. The most robust piece of evidence comes from an interaction with a paramount pro-inflammatory transcription factor of the immune system, nuclear factor-kappa B (NFκB). This factor is required for adequate expression and release of pro-inflammatory cytokines, which function as the messengers of the immune system.[1] There are several reports that indicate NE-β2-AR binding and cAMP pathways inhibit NFκB activation, nuclear translocation, and DNA binding, by a variety of mechanisms.[6,15,17,30] Furthermore, another cardinal transcription factor of the immune system, the nuclear factor of activated T-cells (NFAT), also seems to be directly inhibited via β2-AR stimulation, both by hindering activation and subsequent nuclear translocation, and by impeding its binding to the interleukin-2 (IL-2) promotor region.[4,5,13] IL-2 is the major growth factor of T-cells and several parts of the immune system as a whole, which NFAT is required for its adequate expression[1,18], and NE inhibiting its expression has been corroborated by other studies.[6,11,13] Apart from a direct interaction with these pro-inflammatory transcription factors, other studies have made similar findings in terms of an NE or cAMP-induced immunosuppression, such as augmenting the suppressive modality of T-regulatory cells, and by inhibiting certain proximal T-cell receptor events required for activation.[3,9,25-26,29]

Notwithstanding these mechanisms, NE-induced modulation seems to be far more dynamic and complex. It seems to be able to exert either a stimulatory or suppressive effect that is contingent upon the type of immune cell, or the activation status of the T-cell when the NE is applied.[11,12,16,27] A paragon of this is differential expression of a major and essential cytokine for

many infections, interferon-γ (IFN-γ). They've shown an increase, decrease, or no effect on IFN-γ production depending if the NE interacts with the T-cell after, before, or during T-cell receptor stimulation, respectively.[11,28] However, any synergistic effect NE has on that cytokine production may require the presence of other cytokines as well.[28] Further evidence of a complex interaction comes from findings that NE-depletion increases susceptibility to certain infections[2], and that NE actually increases pro-inflammatory cytokine production in a particular subclass of memory T-cells.[27]

Thus, despite strong evidence from transcription factor inhibition, the exact role NE plays in the stress response and how it modulates T-cell function is largely unknown. As within the body is far more complex picture, with many confounding factors and unknown variables, it is difficult to draw many firm conclusions without data on actual cytokine concentrations, which is what really provides the impetus for the immune system and tells each cell exactly what to do. Ergo, the current research plan was set up to further investigate the role NE may be playing by measuring cytokine concentrations in both stressed and non-stressed mice, either infected or not, and through an isolated interaction of NE in vitro to naïve T-cells. It was hypothesized that NE indeed exerts an immunosuppressive and/or anti-inflammatory effect as measured by cytokine production. Specifically, it was hypothesized that the addition of NE will increase anti-inflammatory cytokine production, IL-10, and decrease production of the pro-inflammatory cytokines, IL-2, IFN-γ, and tumor necrosis factor-α (TNF-α). However, it was also hypothesized that norepinephrine will be more-so anti-inflammatory, as measured via increasing IL-10 concentrations, than immunosuppressive, as measured by decreasing concentrations of the pro-inflammatory cytokines, such that there will be an increased production of IL-10 that parallels,

and likely causes, the decrease in pro-inflammatory cytokines. It was also believed that physiological in-vivo stress will be more robust in its modulation than NE alone.

Materials and Methods

Animals:

Six to seven-week-old female BALB/c mice purchased from Hilltop Animal Lab Inc. (Scottsdale, PA) were used in the study. Animal use was approved by the Institutional Animal Care and Use Committee of BSC. Mice were housed in the vivarium of BSC in the basic science building. Food and water supply, animal care, and ensuring their wellbeing was maintained daily.

Cold water stress treatment and Infection:

The cold water stress protocol used was established for inducing physical or psychological stress by placing them in a shallow container filled with 4 cm of cold water ($1\pm0.5^{o}C$) for 5 minutes each day for 21 days for primary (1^{o}) infection. Control mice were not subjected to cold water stress. Following 21 days of stress treatment, infected group of mice were intravaginally inoculated with $1x\ 10^{7}$ IFU/mL of *Chlamydia trachomatis* inclusion bodies.

T-lymphocyte isolation and proliferation:

Stressed or non-stressed mice were sacrificed using CO_2 inhalation for harvesting splenic cells by standard methods. Subsequently, untouched mouse T-cells were isolated using a dynabead kit of Life Technologies (Carlsbad, C) following the manufacturer's instruction. Isolated cell counts were determined using a TC20 Automatic Cell Counter from BioRad (Hercules, CA) and the counts were at least 1×10^{7}. The experimental groups were treated with concanavalin A (ConA)

and/or norepinephrine (NE) and allowed to proliferate for 72 hours, at which time culture supernatants were collected.

Antigen Presenting Cell (APC) isolation:

Immunologically naïve mice (non-stressed and non-infected) were used to isolate APCs by using mitomycin c. A sub-portion of cells from the non-stressed and non-infected group were set aside before applying the dynabead kit for T-cell isolation for treatment with mitomycin c. The isolated APCs were then applied to certain treatment groups to induce an in-vitro infection, similar to physiological conditions.

Enzyme-Linked Immunosorbent Assay (ELISA):

The concentrations of cytokines produced in culture supernatants of T-cells grown with or without ConA and NE were measured by using an ELISA kit (Invitrogen) following the manufacturer's instructions. Concentrations of cytokines were compared to concentrations of standards and one another. Production of anti-inflammatory cytokine IL-10, and pro-inflammatory cytokines, IL-2, IFN-γ, and TNF-α were measured for each experimental group. Values represent the mean ± standard deviation of samples obtained from 5 mice and each sample was assayed in duplicate.

Statistical Tests:

For each cytokine measured, ANOVA was first used across all groups to detect any statistically significant differences, followed by student's t test to compare individual treatment groups for statistical significance ($p \leq 0.05$).

Proliferation studies and culture supernatant collection

ConA is concanavalin A, which allows proliferation of T-cells during the incubation period. The groups without ConA (ConA-) are simply used as a low-control to ensure ELISA reading sensitivity and accuracy. APC is antigen-presenting cell and Chlamydia is C. *trachomatis*. They are always applied in tandem to induce an in-vitro infection akin to the normal bodily process without many confounding variables. NE is norepinephrine. The time-dependent effect in the non-stress and non-infected group is either NE and ConA applied first, allowed to incubate for 36 hours (half of the total 72 hour incubation time) then later the addition of the APC/Chlamydia to induce the infection, or vice-versa, where the ConA and APC/Chlamydia was applied first and NE was later applied, as well as each being applied at the same time.

Experimental Design

Table 1: The table displayed shows the various treatment groups utilized for the experiment. All treatments are done in isolated T-cells.

Stress infected	Stress non-infected	Non-stress infected	Non stress non-infected
ConA+/-	ConA+/-	ConA+/-	APC/Chlamydia+ , NE+/- , ConA+/-
	ConA+/- , APC/Chlamydia+	ConA+/- and NE+	Time-Dependent effect
			NE+ and ConA+
Expected results *Suppression of pro-inflammatory cytokines*	**Expected results** *Suppression of pro-inflammatory cytokines*	**Expected results** *Suppression of pro-inflammatory cytokines w/ NE. Not as robust as physiological stress.*	**Expected results** *Suppression of most pro-inflammatory cytokines w/ NE.*

Results

Analysis of Interleukin-10 tested by ELISA:

IL-10 is the main anti-inflammatory cytokine involved in suppressing inflammation throughout the body. It was hypothesized that NE will increase its expression, though not as high as levels induced by stress.

Table 2: Production of IL-10

The table displays the raw concentration output (pg/mL) of IL-10 from each treatment group.

	SI	Sninf	NSinf	NSNI	SI/Chl/APC	Sninf Chl/APC	Nsinf Chl/APC	NSNI Chl/APC	Nsinf NE	NSNI NE APC/Chl	NSNI -NE APC/Chl
	901.337	321.335	824.548	263.861	341.043	459.211	367.739	402.6	317.724	272.812	235.988
	974.515	352.114	646.316	333.977	298.723	482.373	347.56	394.67	282.548	289.85	251.77
Avg	937.926	336.7245	735.432	298.919	319.883	470.792	357.6495	398.635	300.136	281.331	243.879
Std Dev	51.74466	21.7640396	126.02906	49.57949907	29.92475898	16.37801	14.26870774	5.607356775	24.87318814	12.04768534	11.15955922

Each group displayed includes ConA, as each group without it had a non-detectable concentration, as expected. SI=stress infected, Sninf=stress non-infected, NSinf=non-stress infected, NSNI=non-stress non-infected, NE=norepinephrine, and APC/Chl=antigen presenting cell/*Chlamydia Trachomatis*. The average and standard deviation were also calculated for each group. Statistical significance was found via ANOVA, and individual groups were then compared with student's t test and graphed for individual comparisons.

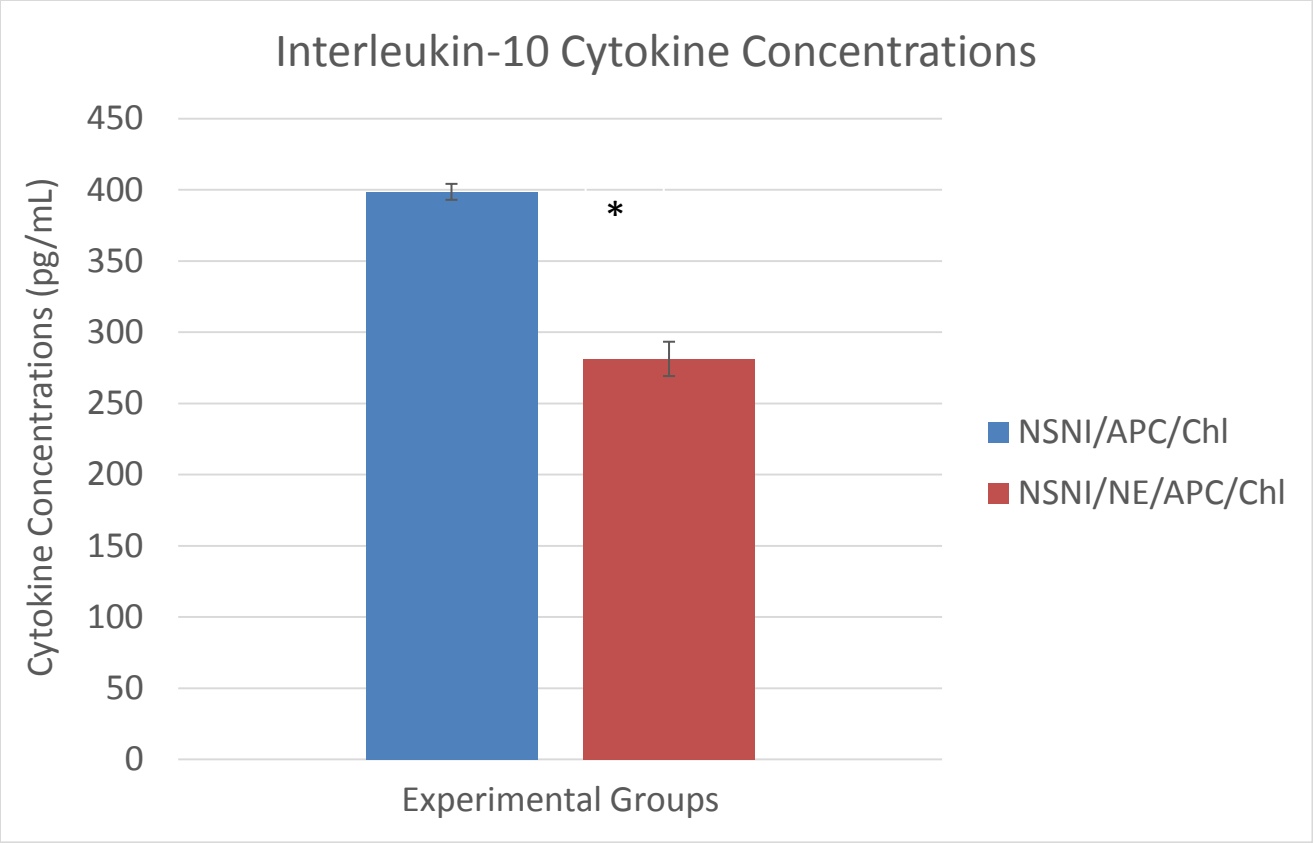

Fig 1:

The graph shown compares a non-stressed and non-infected group with the addition of in-vitro infection using APC/Chlamydia with that same group with the addition of NE. The addition of NE exerted a statistically significant suppression of IL-10 expression ($p=0.025$).

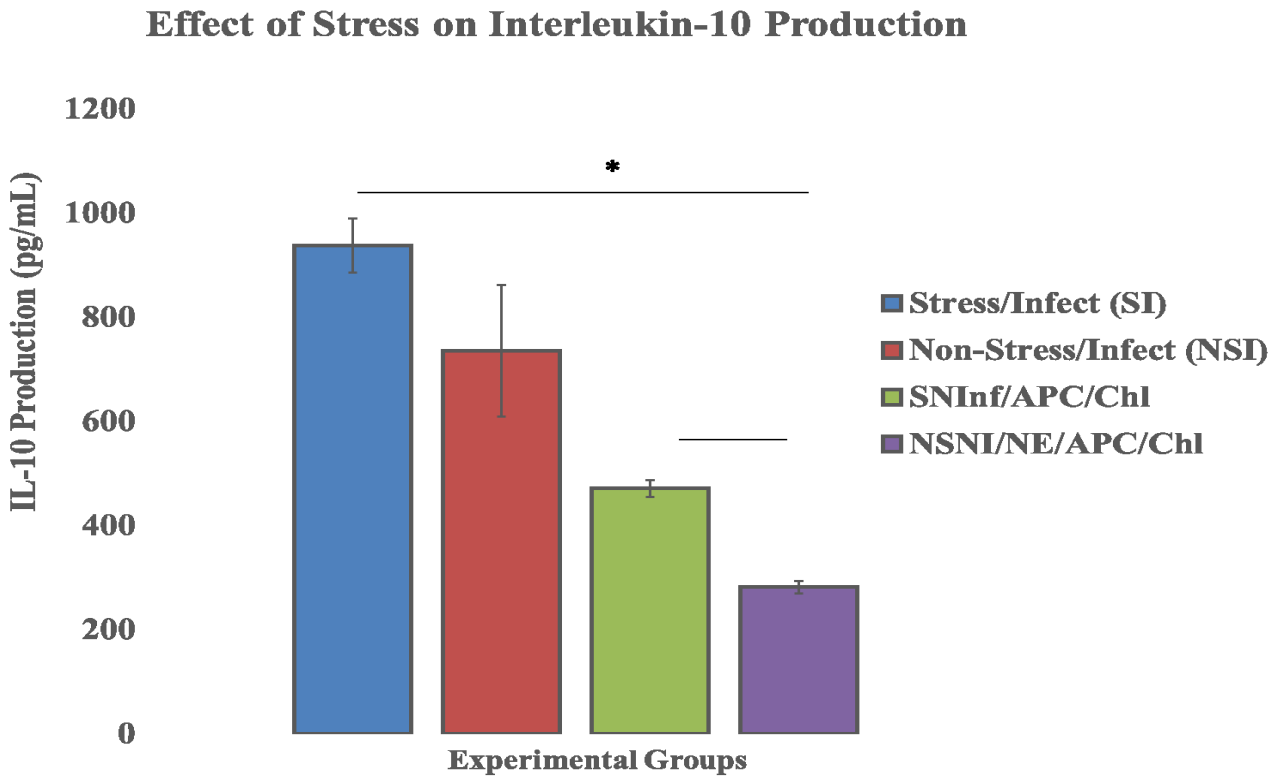

Fig 2:

The graph displayed shows differences in IL-10 expression between the stressed infected, non-stressed infected, stressed non-infected with addition of APC/Chlamydia for in-vitro infection, and non-stressed non-infected with addition of norepinephrine and APC/Chlamydia concurrently, groups. Statistical significance was found between the SI and SNI/APC/Chl groups (p=0.026), the SI and NSNI/NE/APC/Chl groups (p=0.018), and finally between the SNI/APC/Chl and NSNI/NE/APC/Chl groups (p=0.0029).

The data presented indicate that whereas in-vivo stress greatly increases IL-10 expression, as expected, the addition of NE consistently and significantly decreases its expression. This finding is contrary to the hypothesis.

Analysis of Interleukin-2 assayed via ELISA:

IL-2 is the main growth factor of T-cells and various other immune-system components in general, such as natural killer cells and B-cells. It was hypothesized that the addition of NE would suppress its expression.

Table 3: Production of IL-2

The table displays the concentration (pg/mL) of IL-2 cytokine production in non-stressed non-infected cells with addition of APC/Chlamydia and that same group with the addition of NE. The data of other treatment groups are not present because their concentration values were non-detectable and read as zero. Each group was with the addition of ConA. Student's t test was ran between the groups to determine statistical significance.

	NSNI Chl/APC	NSNI NE
	397.218	140.119
	333.371	122.629
Avg	365.2945	131.374
Std Dev	45.14664666	12.3672976

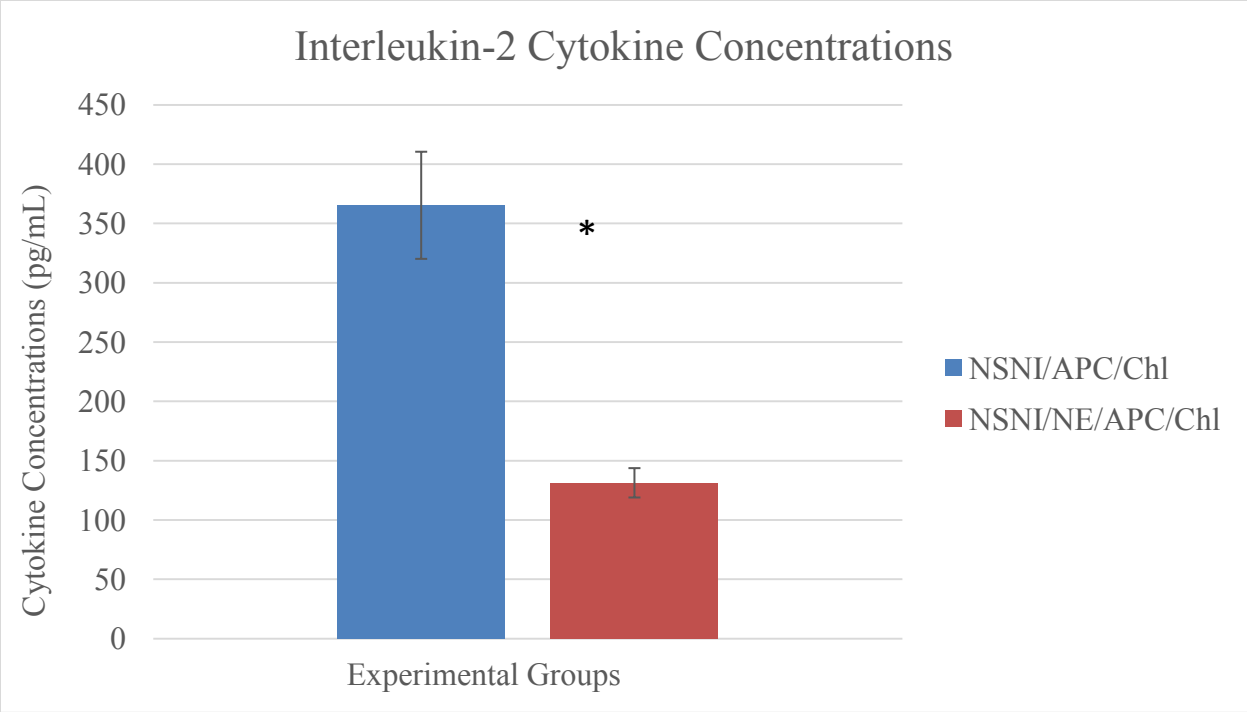

Fig 3:

The graph displays differences in IL-2 expression between non-stressed non-infected cells with addition of APC/Chlamydia versus that same group with the addition of NE. The addition of NE induced a statistically significant suppression of IL-2 expression ($p=0.045$).

The other data between groups were unable to be compared due to non-detectable concentrations, leavening only the two graphed and displayed groups with any detectable IL-2 production. However, the data presented here indicates that, congruent with the hypothesis, NE addition suppresses IL-2 production from T-cells and thus also immune cell proliferation.

Analysis of Tumor Necrosis Factor-α using ELISA:

TNF-α is one of the major pro-inflammatory cytokines of the immune system. It is involved with an immediate and robust inflammatory action to activate, proliferate, and even kill other cells.[1]

Table 4: Production of TNF-α

The table displays the raw concentration output (pg/mL) of TNF-α cytokine production. Each group shown had addition of ConA. ANOVA was first ran to determine overall variance and significance, followed by student's t test amongst certain individual groups.

	SI	Sninf	NSinf	NSNI	SI/Chl/APC	Sninf Chl/APC	Nsinf Chl/APC	NSNI Chl/APC	Nsinf NE	NSNI NE
	937.098	462.195	234.619	219.479	396.328	733.041	564.262	894.749	597.387	318.702
	616.448	576.866	570.333	293.954	360.512	526.678	453.894	977.602	551.273	283.885
Average	776.773	519.5305	402.476	256.7165	378.42	629.8595	509.078	936.1755	574.33	301.2935
Std Dev	226.7337894	81.08464171	237.3856459	52.66178	25.3257365	145.9206767	78.04196123	58.58591814	32.60752211	24.6193368

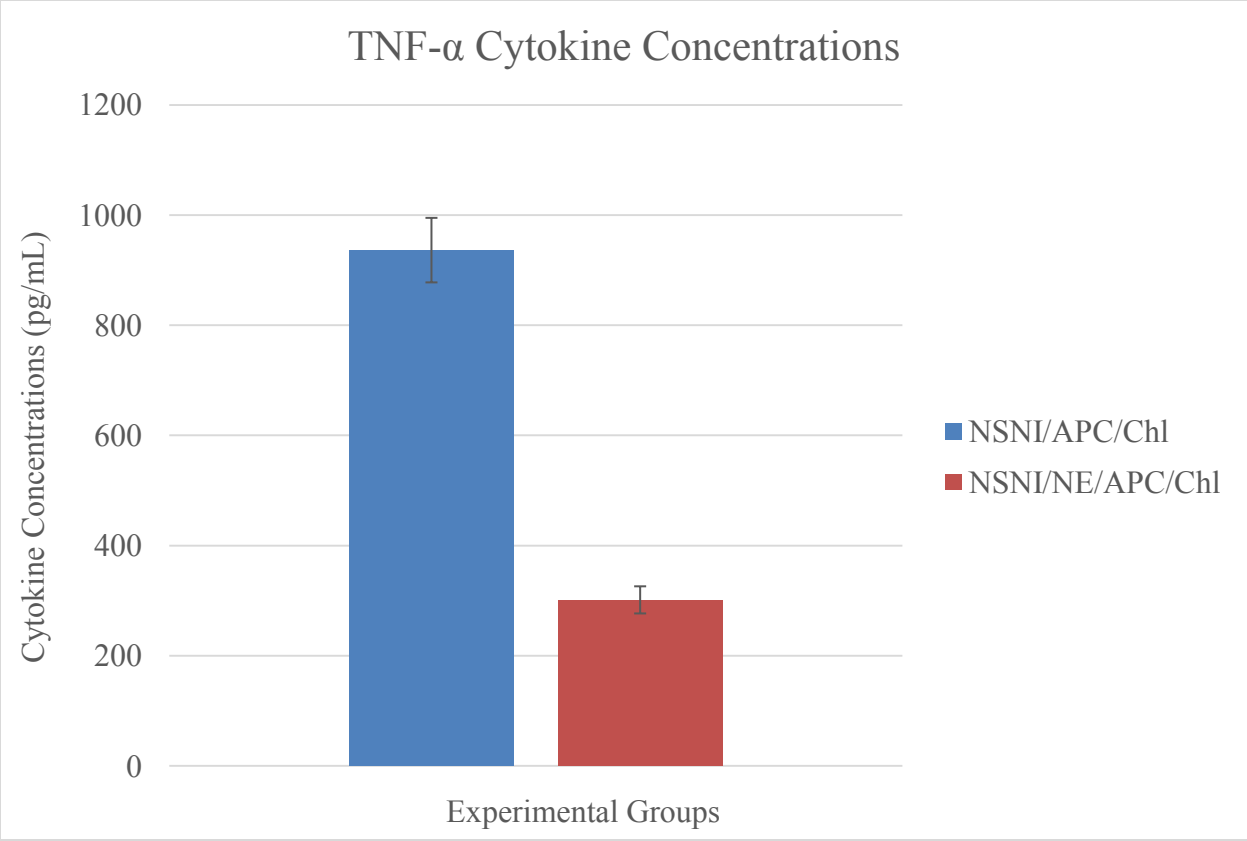

Fig 4:

The graph shows the difference in TNF-α cytokine production between the non-stressed non-infected cells with addition of APC/Chl versus the same group with the addition of NE. The addition of NE caused a statistically significant suppression of TNF-α expression (p=0.022).

Apart from the two groups graphed, sans the completely naïve group (NSNI), there was no other statistically significant differences amongst treatment groups. However, the present data indicate that NE in isolation exerts a robust suppression of pro-inflammatory cytokine TNF-α. This is in-line with the hypothesized results. Regardless, as seen in the concentrations, the in-vivo stress groups did not see a decline in concentrations. Instead, when comparing SI versus NSI groups, though non-significant, the SI group actually had higher TNF-α production. This is intriguing given the exorbitantly heightened level of IL-10 in the SI group.

Analysis of Interferon-γ production using ELISA:

IFN-γ is a pro-inflammatory cytokine that promotes some inflammation and leukocyte proliferation, and is also generally protective and required for clearance of various infections. Most notably, this cytokine orchestrates the anti-viral response, as well as the response to any intracellular pathogen, of which C. *trachomatis* is. Two different ELISA kits were used, one of which is more sensitive than the other in hopes to more scrupulously detect variance.

Table 5: Production of IFN-γ

The table displays the concentrations (pg/mL) of IFN-γ. Each group included the addition of ConA. ANOVA was initially ran to determine statistical significance, however, none were found.

	SI	Sninf	NSinf	NSNI	SI/Chl/APC	Sninf Chl/APC	Nsinf Chl/APC	NSNI Chl/APC	Nsinf NE	NSNI NE
	1728.855	1463.028	1610.496	1073.17	1500.911	1622.695	1874.61	1771.447	1585.775	1574.752
	1699.319	1353.337	1510.436	1112.658	1642.707	1832.981	1777.547	1414.871	1687.761	1562.232
Average	1714.087	1408.183	1560.466	1092.914	1571.809	1727.838	1826.079	1593.159	1636.768	1568.492
Std Dev	20.88511	77.56325	70.7531	27.92223258	100.2649131	148.6946566	68.63391	252.1373076	72.11499	8.852977

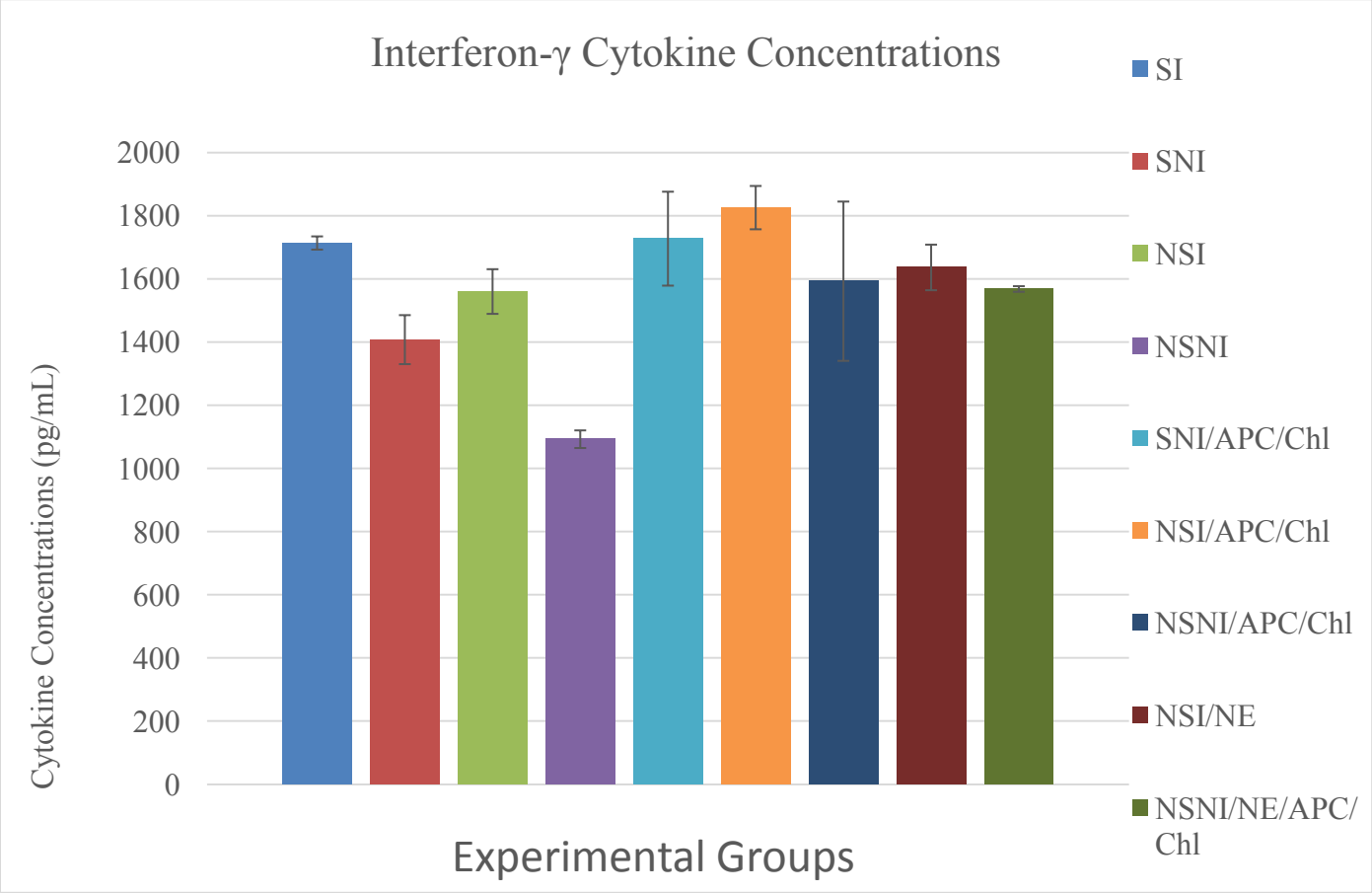

Fig 5:

The graph displayed shows the IFN-γ concentrations of each treatment group. No statistically significant differences were found using ANOVA or student's t test.

Table 6: Production of IFN-γ

The table displays the concentrations (pg/mL) of IFN-γ using the more sensitive ELISA kit. Each group included the addition of ConA. ANOVA was initially ran to determine statistical significance, however, none were found.

	SI	Sninf	NSinf	NSNI	Sninf Chl/APC	NSNI APC/Chl	Nsinf NE	NSNI NE
	217.612	210.085	204.923	197.468	204.744	170.442	201.231	230.695
	171.266	176.786	217.074	193.31	181.051	240.193	179.618	254.28
Average	194.439	193.4355	210.9985	195.389	192.8975	205.3175	190.4245	242.4875
Std Dev	32.77157	23.54595	8.592054	2.94015	16.75348097	49.32140509	15.28269886	16.67711343

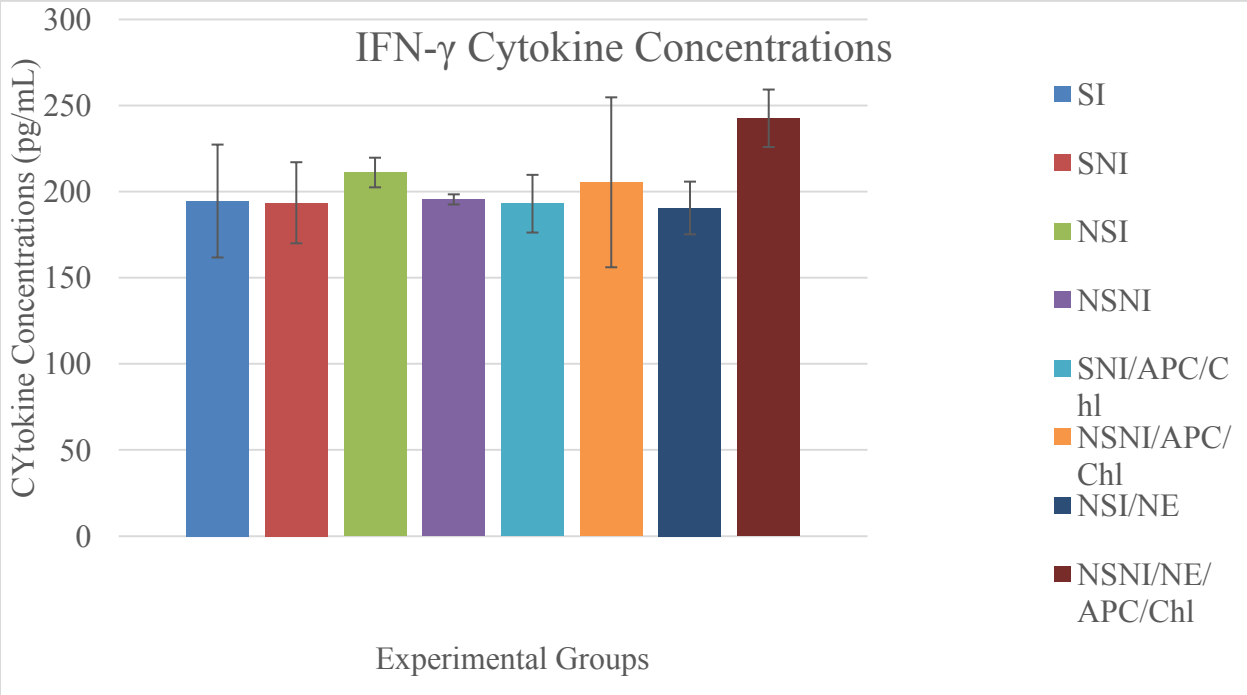

Fig 6:

The graph displayed shows the IFN-γ concentration for each treatment group. No statistically significant differences were found.

Both of the ELISA kits used were consistent in that IFN-γ displayed a resiliency to modulation whether by stress, infection, or NE addition. This is contrary to the hypothesis that both stress and NE would suppress its production.

Discussion

The current research plan was set up to investigate the role NE plays in the stress response and elucidate how it may modulate T-cell function. These results indicate that NE does indeed significantly modulate T-cell function. However, some unexpected results were also gathered. Overall, it was discovered that the addition of norepinephrine to T-cells at the time of infection (APC/Chl addition) significantly suppressed IL-10, IL-2, and TNF-α cytokine concentrations. The cytokine concentration of IFN-γ appeared resilient to changes, as no statistically significant differences were found between any of the tested groups. Furthermore, the use of naïve T-cell groups with the addition of APCs and C. *trachomatis* represents a quasi-in vivo method of infection, as the bona-fide in-vivo process involves the cellular uptake of the antigen into the APC, which subsequently processes its peptides and displays them on its outer membrane for T-cells to bind to and activate/proliferate. Thus, it is a useful method for a more isolated look at NE-induced modulation, because it removes many of the confounding factors present within the body whilst maintaining the typical physiological process of antigen processing and recognition.

The expression of IL-2 exhibited a profound suppression with the addition of NE. No other treatment groups tested had a detectable concentration, making it hard to concretely determine an overall role. However, as noted, the isolated modulation by NE lends credence towards it being veridical in a bodily context. In fact, the present study is congruent with other

investigations, which have found that NE negatively impacts IL-2 expression.[6,9,11] As IL-2 is the major growth-factor for T-cells, this finding implies that NE suppresses T-cell proliferation, distinct from any other mechanism. In fact, one study found that cAMP pathways directly interfere with IL-2 induced signaling.[22] Therefore, NE negatively impacts T-cell, and likely total immune cell, proliferation, leading to a reduction in total leukocyte numbers.

The expression of TNF-α also displayed a stark suppression from the addition of NE in isolation. Few studies have investigated its actual cytokine concentration from NE-modulation. The inhibition of NFκB dictates that NE should have a robust suppressive response on TNF-α secretion, which is observed here. However, a very recent study published showed that in a specific subset of T-cells, memory CD8+ cytolytic T-cells, NE increased TNF-α production.[27] The same study also found that NE decreased IL-2 secretion. The discrepancy may be explained by a potential difference of length of NE stimulation. In the present assay, NE was incubated with the T-cells for the full 72 hours of proliferation, whereas the study noting increased TNF-α production incubated the T-cells for only 16 hours. Not only that, the highly specific subset of T-cells used may have unforeseen differences in signaling, or there may be undetermined cross-talk with other T-cell subsets that influence this response. Adding more complexity to the picture, the fact they were specifically memory T-cells may also have unforeseen implications in this regard. Further studies are highly warranted to delineate those specific quandaries. However, further validation for the finding in the present study comes from studies carried out in natural killer (NK) cells. As NK cells have highly similar signaling pathways involved in activation as T-cells, their data may be extrapolated over, though with caution. One such study found that NE hindered the secretion of TNF-α from NK cells.[8] Interestingly though, the expression of TNF-α remained largely unchanged with only non-significant variance amongst the in-vivo stress groups, which

strays from expectation. Thus, it seems here that NE is more immunosuppressive than a total-body stress response. Conversely, the in vivo stress groups heightened level of IL-10, yet still heightened level of TNF-α may indicate infection clearance. IL-10 typically rises as the infection continues and begins to clear as to begin suppressing the immune response to mitigate tissue damage or autoimmune disorders.

The expression of IFN-γ displayed no statistically significant differences amongst any of the treatment groups. It appeared consistently resilient to stress- or NE-induced modulation as indicated by two separate ELISAs having similar results. The literature indicates IFN-γ is the most dynamic in its response to NE. The current data is however consistent with the preponderance of studies carried out on the topic. In general, it's been shown that the addition of NE at the time of T-cell receptor stimulation has a null or simply subtle effect on IFN-γ concentration.[11] Classically, the differential expression of this cytokine is predicated around distinct NE application with respect to time of T-cell receptor activation. Despite the current research plan being set-up to also investigate these time-dependent changes in cytokine expression, the tests have yet to be run. Upon further data regarding its time-dependent effect being carried out, more robust conclusions may be made. At the current time, however, it may only be said that NE appears to exert no significant influence on IFN-γ expression. In terms of discrepancies with general stress, however, there may have been unintended issues with older T-cell isolation methodology. Specifically, it has now been shown that the nylon-wool method of T-cell isolation causes membrane perturbations that lead to a significant reduction in IFN-γ levels independent from other factors.[31]

The expression of IL-10 due to NE was the most striking. It displayed a consistent and significant decrease in its expression under the influence of NE. As IL-10 is the major anti-

inflammatory cytokine, it was hypothesized that NE would greatly increase its expression. In fact, several studies indicate that β2-AR signaling or cAMP pathways increases the expression of IL-10.[6,17,10] Despite this, the current study may denote cell specific effects and thus be valid. One such study only used non-specific components to globally increase cAMP levels. Another study was carried out in macrophages, not T-cells. Similar to NK cells, macrophages also share many signaling components for activation and proliferation, as well as the β2-AR. However, as the present study shows a robust suppression of IL-10, whereas the study in macrophages noted a robust increase, there may be other confounding factors or disparities between cell types causing the difference, such as the presence of other ARs. The last of these studies noted that inhibiting a particular factor, GSK-3β, increased IL-10 production in T-cells. In the majority of tissues, downstream β2-AR signaling is known to inhibit GSK-3β, however, there may also be differences in this signaling cross-talk in a tissue-specific manner. Thus, it appears that in T-cells, isolated NE inhibits the major anti-inflammatory cytokine, IL-10.

Further evidence for a dynamic and complex role for NE in immune-system modulation comes from a recent review paper.[16] The authors propose a type switching from canonical NE signaling through the β2ARs to increase cytosolic cAMP levels, which is generally suppressive, to an activation of key pro-inflammatory transcription factors that ramp up pro-inflammatory cytokine expression. The basis for their surmise is contingent upon a heightened level of circulating NE, of which would be expected during chronic stress, to flood the β2ARs and cause the signaling switch. As receptor desensitization and type switching from the aforementioned Gs signaling to increase cAMP to Gi signaling to suppress cAMP is a well-known biochemical phenomenon, their hypothesis certainly holds weight. In fact, such a mechanism would help explain many of the incongruent results obtained from several studies involving NE. More

studies are also warranted in this regard to elucidate the validity of the hypothesis, how long NE must be applied before a type-switching occurs, whether it holds true for all members of the immune system or only T-cells, and lastly if it differs based on when the NE binds to its receptor with respect to when the cell is infected. The picture is still clearly nebulous, though the present study answers some questions about a completely isolated viewpoint of NE on general T-cell function.

The present study shows a paradoxical role of NE in modulating T-cells. Though NE, for the most part, indeed exerted an immunosuppressive effect as shown by suppression of TNF-α and IL-2, it also suppressed the anti-inflammatory factor IL-10. Thus, NE seems to be exerting its immunosuppressive effect in a distinct mechanism apart from inducing IL-10. In fact, interpreting only the results of IL-10 would lead one to believe NE is pro-inflammatory, but scrutinizing the data from the pro-inflammatory cytokines demonstrates that is demonstrably false. This indicates that NE is more-so immunosuppressive than it is anti-inflammatory. Though this is partially consistent with the hypothesized results, it was believed that NE wasn't nearly as robust in its ability to impede T-cell proliferation, and more-so exerted any immunosuppression secondarily to an ability to increase IL-10. Therefore, the current study helps delineate a direct effect of NE on the function and proliferation and T-cells by measuring the cytokine output. Furthermore, the present study also sheds more light on a highly dynamic role that NE plays, which underlies further cause for study to completely delineate its mechanism. As the various studies on this topic, this included, seem to indicate great tissue- and cell-specific effects of NE on total immune system modulation, it is clear this factor warrants further scrutiny. Currently, more investigation is underway to investigate the time-dependent effects NE plays in T-cell modulation, as well as potential mechanisms behind receptor switching to promote

inflammation, and general NE-induced modulation of various cell types, including NK cells, macrophages, specific subsets of T-cells (CD4+ and CD8+), and dendritic cells (APCs).

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Affirmative Action in Higher Education

[Race-based Affirmative Action]

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Race-based Affirmative Action in Higher Education

Ever since the *Grutter and Gratz* Supreme Court cases, which challenged the constitutionality of Affirmative Action, a wide body of literature has weighed in on whether or not Affirmative Action is still needed. The literature written tries to answer the question of whether or not Affirmative Affirmation is still needed by using quantitative and qualitative measures.

Goldstein (2006) contends there are two competing perspectives on the need for Affirmative Action. One perspective focuses on the benefits of race-conscious admissions, which assigns priority to eradicating racial disparities in distribution of societal resources. This perspective holds that these programs should continue as long as they are generating returns. This perspective also holds that race preferences should be a temporary strategy and not a fixture of American life. The perspective goes on to say that Affirmative Action should be abolished only when it is no longer needed. Goldstein suggested that there are only two developments that could produce such an outcome: first is the disappearance of the performance gaps between whites and minorities. Second, if race-conscious admissions could have a shelf life or the discovery of alternatives that could serve the interest Grutter recognized, Affirmative Action would no longer be needed. The other perspective holds that there should be specific limits on the durational of Affirmative Action because of the perceived cost of such programs. Proponents of this perspective believe that racial preference creates a pervasive racial spoils system. They worry that race conscious admissions will leave a stain on the admissions process even after the purpose of Affirmative Action has been fulfilled. Proponents of this perspective believe that if race-based Affirmative Action remains intact, then it would defeat the aspiration of achieving a cohesive nation where race does not matter. Kahlenberg (2013) seems to agree with Goldstein,

because Kahlenberg believes that if better solutions are found instead of race-based Affirmative Action that serves the purpose of *Grutter* then Kahlenberg would have no problem with getting rid of Affirmative Action policies.

According to a study done by Epple, Romano and Sieg (2008) in *Diversity and Affirmative Action in Higher Education*, getting rid of Affirmative Action policies may lead to a significant drop in minority students at top tier colleges and universities. This is because Affirmative Action has helped lower tuition for minority students and helped them attend, on average, higher tier schools in comparison to nonminority students. Another study, which was done by the same authors, found that when race is left out of the admissions equation, minority enrollment in high quality colleges drops by 35%. This study negates the idea that race blind or race neutral alternatives can serve the purpose of diversity, because minority presence in these top tier schools dropped tremendously. The problem with this study is that it may be a little bit dated, given that more than a decade has passed.

Hinrichs (2009) echoes the same sentiments in *The Effects of Affirmative Action Bans on College Enrollment, Educational Attainment, and the Demographic Composition of Universities*, when he too finds that Affirmative Action bans cause underrepresented minority enrollment at selective colleges to decline. Hinrichs established that banning Affirmative Action at public universities in the top 50 universities in the U.S. is linked with a 1.74 percentage point decline in Black enrollment. The study also found a decline in Hispanic enrollment of roughly 2.03 percentage points, and a decrease in Native American enrollment estimated to be around .47 percentage points.

Conversely to Hinrichs, Epple, Romano and Sieg, Antonovics and Backes assert in *Effects of Banning Affirmative Action on College Admissions Rules and Student Quality (2013),*

that preventing universities from using race conscious policies does not mean universities do not value diversity. Both authors surmise that an almost inherent response to bans on Affirmative Action is the implementation of race-neutral policies that are supposed to increase diversity by increasing the admission advantage afforded to students who have characteristics that are correlated with being from an underrepresented group. The study by Antonovics and Backes is, however, not representative of all universities and colleges in the U.S. due to the fact that their study was primarily on the University of California and its other campuses where race neutral alternatives have fulfilled the purposes of Grutter and Bakke, which is to achieve a diverse student body.

Long (2013) asserts that the *Grutter* and *Fisher* cases have created a clear mandate that the universities must prove that race neutral alternatives are insufficient to produce a diverse class of enrollees before these universities are permitted to use race-based affirmative action. Kahlenberg speaks in a similar vein when he asserts that if a better solution can be found instead of race-based Affirmative Action that serves the purpose of Grutter, then that strategy should be used in lieu of race-based Affirmative Action. Kahlenberg believes that it is feasible to create a critical mass of African American and Latino students in top tier universities without the use of racial preferences.

Kahlenberg (2013) conducted a study of seven schools with race neutral plans; University of Texas, Texas A&M, University of Washington, University of Florida, University of Georgia, University of Nebraska and University of Arizona. Kahlenberg found in 2004, that the University of Texas was able to bring up their Black and Latino representation using the Top 10 Percent plan, along with using class-based Affirmative Action.The study shows that the University of Texas was able to do this without using race based Affirmative Action. Likewise

Texas A&M has been able to gain a greater latino representation since 2005. These numbers are greater than the latino representation in 1996; the last year in which race and ethnicity were used in admissions. Interestingly when the other schools in the study ended their race-based affirmative action programs in favor of race neutral programs, the number of Black and latino student declined but over time the numbers began to get larger and eventually started to do better than race-based Affirmative Action programs that were previously used. An example of this in Kahlenberg's study was the University of Washington; Blacks and latino representation dropped in the first year that race-based policies were banned. However, over time it has matched or exceeded the race-based Affirmative Action policies, in representation of minorities, which were once used. Kahlenberg uses this study to substantiate the idea that it would better if universities pursued more race neutral alternatives such as class-based affirmative action and percent plans.

Lim (2013) addresses the feasibility of percent plans in his studies, and he found that percent plans are not a workable race neutral alternative to Affirmative Action. Lim comes to such a conclusion after he obtained empirical data from the three states that have implemented race-based Affirmative Action. Contrary to Kahlenberg, he finds that percent plans fail to achieve a 'critical mass' of various racial groups. Lim thus sees percent plans as not fulfilling *Grutter* because it fails to achieve a diverse campus. Lim studied percent plans by evaluating the application pools of minority applicants in Texas, Florida and California (the three states with percent plans). Lim found that in 2009, the University of Texas at Austin had accepted 4.6% of Blacks who had applied to the university; 7.8% of all the university's applicants were Black. Hispanics, on the other hand, made up 21.5% of the application pool and 20% out of those that applied got accepted. According to Lim, this is several standard deviations below the proportion of those that applied to those that got accepted. In Florida and California the numbers are similar

to that of Texas'. According to Lim, "In California and Florida, Blacks and Hispanics had lower rates of admission to their states' flagship colleges and universities." Lim highlights this point by giving the example of UC-Berkeley. According to Lim, in 2009, UC-Berkeley's Black enrollment was 2.9%, while Hispanic enrollment was 10.9%. Lim uses these numbers to refute the purpose of percent plan.

Schwarzschild (2006) did a qualitative analysis of class-based affirmative and race-based Affirmative Action, and Schwarzchild is of the conviction that many of the drawbacks to affirmative action are enhanced by the conspicuous visibility of race. Schwarzchild believes that there is a stigma to having received preferential treatment based on race.

Conversely, Schwarzchild asserts that beneficiaries of class preferences would not stand out. Moreover, Affirmative Action based on social class rather than race might be less corrosive to academic standards. Schwarzchild further contends that class preferences would also have the virtue of preferring the less privileged while racial Affirmative Action by contrast does not prefer the less privileged. What racial Affirmative Action does instead, according to Schwarzschild, is show preferential treatment to Black children from affluent backgrounds. This notion by Schwarzchild that race-based affirmative action benefits minorities of privilege somewhat aligns with Bowen and Bok's (1998) findings from their study of Blacks in elite institutions. Specifically, that 86% of Blacks who attended these schools were from affluent families.

Fitzpatrick (2014) also agrees with Schwarzchild because he too thinks race-neutral alternative programs have public support. Fitzpatrick believes that, although race-neutral Affirmative Action may be less divisive and less stigmatizing to its beneficiaries, he suspects that it will not be efficient enough to bring about racial diversity. Fitzpatrick further asserts that the race-neutral programs that are likely to be the least divisive and least stigmatizing are

probably also those that are the least efficient at diversifying. For both of these reasons Fitzpatrick is reluctant to say that race-neutral alternatives are the solution.

Sander and Taylor (2012) assert that Affirmative Action does more harm than good to minorities. The authors believe that whenever minorities are placed at selective colleges, they may not be prepared. Sander and Taylor assert that, due to this lack of readiness, minorities may not perform as well as other students who are prepared for an education at these selective schools. This may eventually lead to these students ending up at the bottom of the class. Sander and Taylor contend that if these minority students were placed at less selective schools, the students would be more prepared for those schools. Sander and Taylor addressed proposition 209[1] in California which was deemed by some commentators as controversial. Sander and Taylor do acknowledge that after race-based affirmative action was enacted in California, the enrollment of minorities has dropped significantly. Sander and Taylor however assert that, despite this fact, what commentators fail to address is drop-out and graduation rates. According to their study after Affirmative Action was banned, the drop-out rate for minorities went down, while the graduation rate for minorities went up.

Maruyama, Burke and Mariani (2005) suggest that one alternative to Affirmative Action programs is pre-collegiate partnership programs. Maruyama, Burke and Mariani did studies on a pre-collegiate partnership program called the Multicultural Excellence Program (MEP). This program has a partnership between an urban school district and 22 higher education institutions. The program started in 1987, and it helped students transition from high school to college. Many of these students have thrived in college, while the drop-out rate is small.

[1] Proposition 209 was a constitutional amendment which prevented public institutions from discriminating on the basis of race or ethnicity (Sander and Taylor).

Research Plan

Research question: Is Affirmative Action still needed in Higher Education?

The research will measure the "still needed" clause by studying the admissions histories of UT-Austin and the UC-schools. Specifically, the research will be sifting through the number of applicants that applied to both school when they had Affirmative Action policies and also after Affirmative Action policies were banned. The research will also evaluate the acceptance rate before and after Affirmative Action was banned.

The researcher will investigate the following schools: University of Texas-Austin and the University of California-Berkley.

This sample size was chosen for two reasons:

1. These universities have been involved in major Supreme Court decisions pertaining to Affirmative Action; namely, *Bakke (1978)* and *Fisher (2013).*

2. UT-Austin and University of California are two of the only schools that provide admissions demographics for various minorities on their online websites. The research will also compare the number of minorities who got accepted with race-based affirmative action policies present, with the number of minority students who got accepted when affirmative action policies were banned.

Methodology

. The data for the research will be obtained through visiting each of the university's respective websites. The information about each school will then be compiled into a excel spreadsheet which will be accompanied by line graphs. The line graphs will give a visual representation of the comparison between the numbers of minority students who enrolled at the

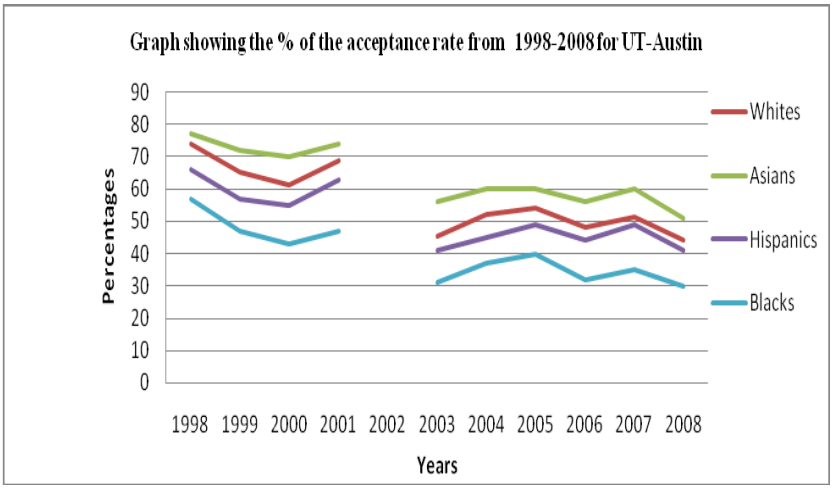
universities when there were race Affirmative Action policies, in comparison to the period after these race based policies were banned.

Limitations

The sample size maybe too small; there are about six or seven other schools that have banned or at one time had but no longer have affirmative action policies, but they do not allow public access to the number of students that applied, only the number of students enrolled. Maybe if the researcher had access to other school's database system, the results may have turned out differently.

Discussion of Results for UT-Austin

Graph 1

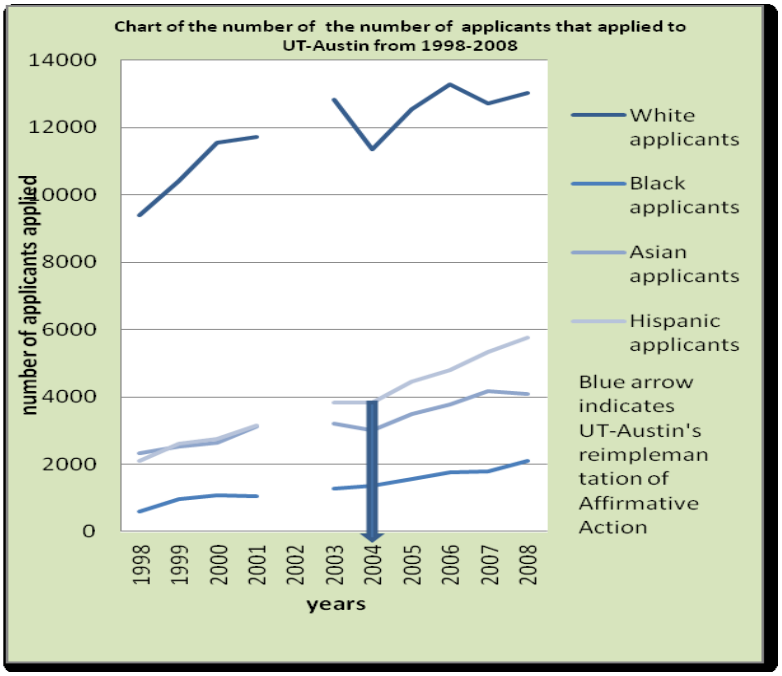


It can be seen from the acceptance rate from 1998 to 2008, that all four demographic groups had a drop in their respective acceptance rates. In 1998 the acceptance rate for white students was 74%, for students of Asian descent it was 77%, for Hispanics it was 66% and lastly for Blacks the acceptance rate was 57%. From 1996-2003 race-based policies were banned, it

probably explains why in 1999 the percentage acceptance rate for Blacks was a paltry 47%, while the Hispanic acceptance rate fell to 57%. The Asian and the white percentage acceptance rate also fell to 72% and 66% respectively. In 2000, all four demographic groups dropped significantly; Asian percent acceptance rate was 70%, whites were 61%, Hispanics were 55% and lastly Blacks were an abysmal 43% acceptance rate. In 2001, the numbers for all demographic groups improved to some degree; for whites, it improved to 69%, for Asians 74%, Hispanics 63% and lastly Blacks improved marginally to 47%. The decline of all demographic groups was odd in the research, until the researcher found that the root cause of the drop maybe the drop is associated with the implementation of the Texas Ten Percent Plan in September .9, 1997. The Ten Percent Plan had two major prongs; the first outlined that: "All institutions of higher education in Texas shall admit any applicant who, in one of the two years preceding the year for which he or she is applying, graduated with a grade point average in the top ten percent of his or her high school class ("Kirwan Institute"). "Applicants still have to take the required standardized tests, however class rank supersedes the standardized tests scores for admission to the schools like UT-Austin." The second prong of the Ten Percent Plan stipulates that the rest of the freshman applicants' class would be selected using a myriad of different criteria such as: parents' income, socioeconomic background and parents' level of education. Based off the statistical data that has been compiled, the researcher has to agree with Lim who espouses, in *Percent plans: A Workable, Race-Neutral Alternative to Affirmative Action,* that Percent plans do not work. According to Lim what it instead does is cause the number of minority enrollment to go down. Surprisingly, the acceptance rate for the white demography also went down, which can be attributed to the fact that the 10 percent plan only accepts the top ten percent of each high school. Due to this fact, many white and Asian students from competitive school districts may

not feel incline to apply to UT-Austin. This development may cause a change in attitude. In 2003, the Grutter and Gratz Supreme Court decisions were rendered; this resulted in the re-implementation of Affirmative Action in 2004. In 2003 however, the numbers for the four demographic groups were jaw-dropping. The white acceptance rate was of 45%, the acceptance rate for Asians was 56%, the Black acceptance rate was 41% and lastly the Hispanic acceptance rate was an abysmal 31%. A possible factor as to why the admissions number were so low in 2003, maybe due to the fact that it was during the Iraq war, and students may have enlisted. In 2004, the numbers improved marginally for whites, Hispanics and Asians but surprisingly though the Blacks admitted went down. In 2005, the numbers for all four demographic groups either improved or stayed the same- whites acceptance rate was 54%, Asians 60%, Hispanics 49, and Blacks 40%. From 2006-2008, there is a proverbial seesaw, because from the graph, there is this up and down fluctuation.

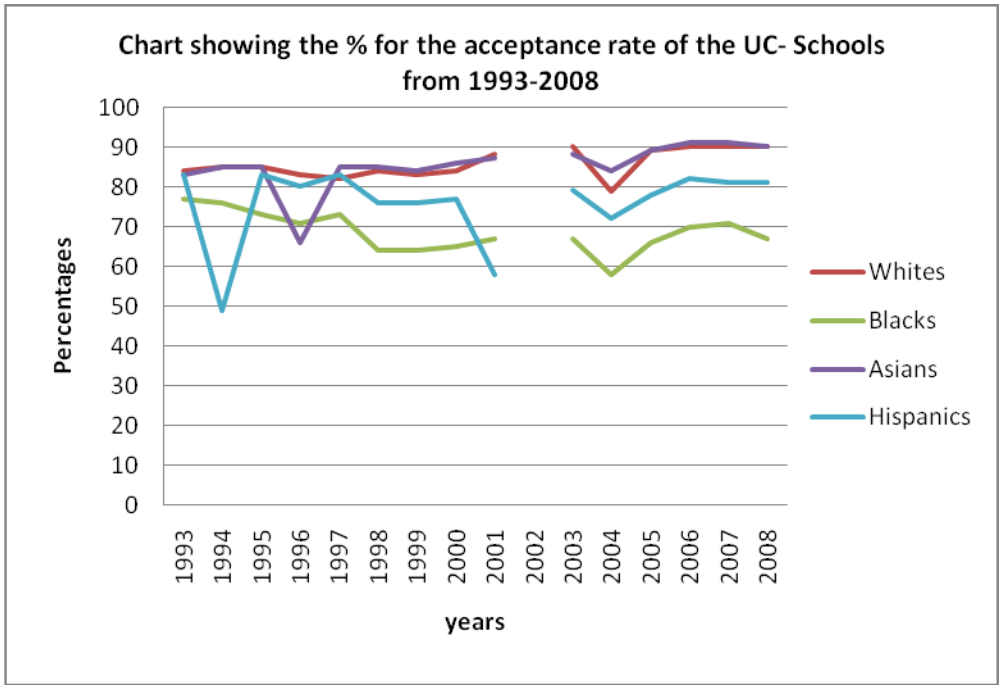
Graph 2



This graph displays the students that applied to UT-Austin from the four major demographic groups. It can be gleaned from the graph that, since 1998, applications to UT-Austin have been on relative rise. In 1998, the number of White applicants were 9,387, by 2008 the number increased to 13,042 applicants. In 1998, the number of Black applicants were 596, by 2008 the number of students increased to 2,110.The number of Hispanic applicants in 1998 were 2,106, by 2008; the number of applicants had skyrocketed to 5,767 applicants. In 1998, the numbers of Asian applicants were 2,326, and by 2008, the number of applicants jumped to 4,088. It appears as if the Ten Percent Plan that was implemented in 1997, did not seem to affect the attitudes of the students who applied to UT-Austin, because the number of students increased over the past 10 years. From these numbers the researcher finds that the percent plan does not have an effect on the attitudes of students applying to UT-Austin. In 2003, when the Iraq war transpired, the number of applicants who applied was 12,823, which is high. According to graph 1, all major demographic groups in 2003 had low admissions. The war may have been a factor, how the research cannot conclusively make that assertion.

The re-implementation of affirmative action in 2004 saw a small increase in Black and Hispanic applicants. The Asian and the white applicants saw their numbers drop slightly in 2004. This may just have been a slight decline in applications in 2004, because in the subsequent years of 2005-2008, the number of white and Asian populations to rose slightly. The researcher cannot specifically attribute a reason why the number of white students improved after 2004. It may just be the case that from 2005-2008 were high enrollment years.

Graph 3



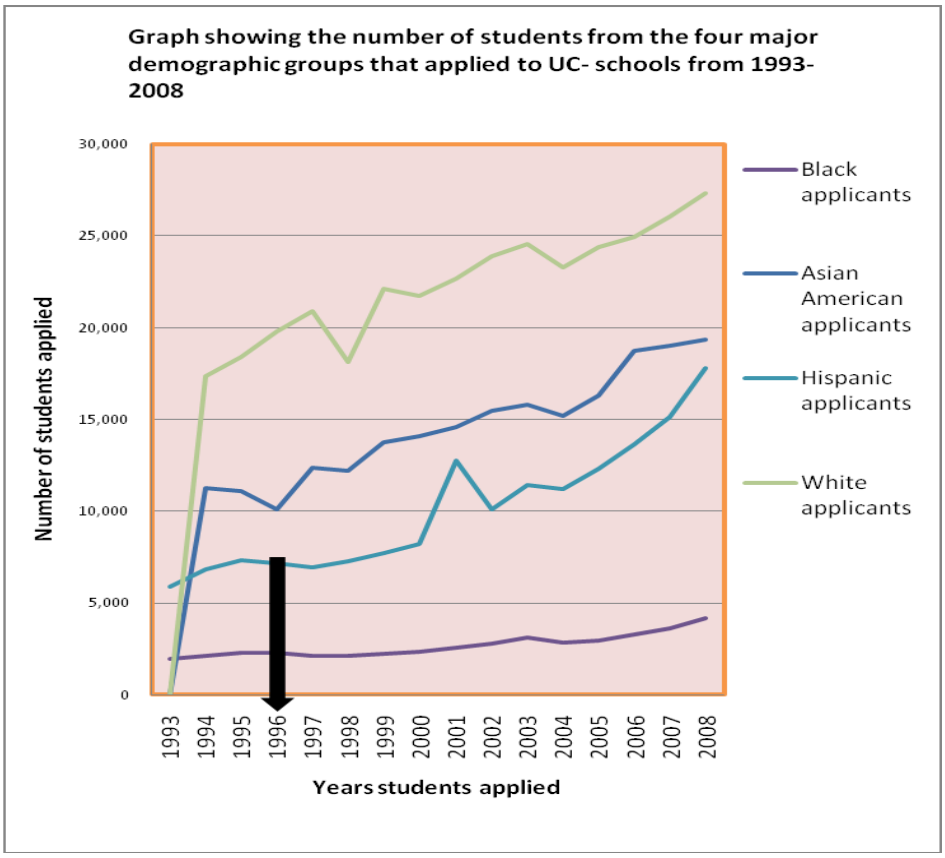
This graph shows the acceptance rate from 1993-2008 for the four major demographic groups. From the graph it can be gleaned that in 1994, the Hispanic percentage acceptance rate dropped from 83% percent in 1993, to a paltry 49 percent. The researcher could not find any reason to explain, why there was a drop. It was probably just a natural drop year for Hispanic admittance. In 1996, there was a big decline for the Asian acceptance rate. The Affirmative Action policies may be a factor that may have led to the drop. The drop however does not continue in subsequent years, so this may just be a normal drop in the acceptance rate for the Asian demography. In 1997, when affirmative action policies were banned, the Asian acceptance rate went up. Thus, the ban on affirmative action policies impacted the acceptance rate for Asians. For Blacks the acceptance rate in 1997 slightly increased to 73%. However in the successive years from 1998-2000, the Black percentage acceptance rate declined significantly. From 1998-2000, the percentage acceptance rate dropped to 63%, then in 1999, 64% and finally

in 2000, 65%. Similarly to the Black acceptance rate, the Hispanic demography also saw decline in their percentage acceptance rate from 1998-2000. In 1998, the percentage acceptance rate fell from 83% in 1997, to 76% in 1998. In 1999 and 2000, the acceptance percentage rates were 76 and 77 percent respectively. These findings indicate that banning affirmative action policies caused the Black and Hispanic demographic group to have a lower acceptance rates. The research can come to this finding because prior to 1998, when Affirmative Action was in place, both demographic groups had higher acceptance rates.

In 2001, similarly to Texas, California too passed legislation for a percent plan. The percent plan was implemented the same year its legislation was passed. This percent plan allows the top four percent of students from each California high school to get accepted to the University of California. In 2001, the only demographic group that significantly went down was the Hispanic demographic group- their percentage acceptance rate was a meager 58%. In 2003, the Hispanic percentage rate went back to its normal percentage rate, and in the subsequent years following, it stayed at its normal acceptance rate, which is in the high 70's. The Black percentage acceptance rate also saw a dip in 2004, from its customary high 60's to 58% percentage acceptance rate. The percentage acceptance for Blacks however went up in successive years following 2004.

These findings, again, suggest that Lim was correct in his assertion that Percent plans do not improve minority admissions. In this case, the Percent Plan caused the admittance of Blacks and Hispanics minorities to go down or make the percentage acceptance rate stay the same.

Graph 4



It can be gleaned from this graph that all demographic groups are on the rise. The graph also highlights that even though a ban of Affirmative Action policies was implemented in 1996, Affirmative Action did not cause the number of minority applicants to go down; instead the number of applicants that applied to the UC- schools stayed relatively the same. The same can be said of the Percent Plan that was implemented in 2001- the number of students that applied to the UC-schools stayed relatively the same before the implementation of the Percent Plan.

Findings

1. Affirmative Action has a significant impact on the acceptance rate of minority groups such as Hispanics and Blacks. In the case of UT-Austin when Affirmative Action policies were banned in 1996, it is seen from 1998-2003, that minorities such as Blacks, Hispanics and even Asian Americans saw their percentage acceptance rates decline. The UC- Schools also saw a slight drop in the percentage acceptance rates for Hispanics and Blacks since legislation was passed in 1996 to ban Affirmative Action.

2. In the case of both UT-Austin and the UC- Schools, whether or not Affirmative Action was in place or not, it did not affect the number of students that applied to both universities- the number of students that applied to each school stayed relatively the same.

3. Percent plans do not translate to increased acceptance rates for minorities, nor do they increase in the number of applicants that applied to both UT-Austin and the UC-schools.

Appendices

University of Texas-Austin

Chart showing the number of students from four demographic that applied to UT-Austin

Years	White applicants	Black apps applicants	Asian applicants	Hispanic applicants
1998	9387	596	2,326	2106
1999	10406	957	2512	2615
2000	11547	1,073	2643	2763
2001	11723	1053	3123	3164
2002				
2003	12823	1275	3198	3822
2004	11348	1375	3021	3827
2005	12552	1552	3,483	4457
2006	13275	1764	3769	4798
2007	12707	1798	4159	5335
2008	13042	2110	4088	5767

Graph showing the number of students from four demographics that applied to UT-Austin

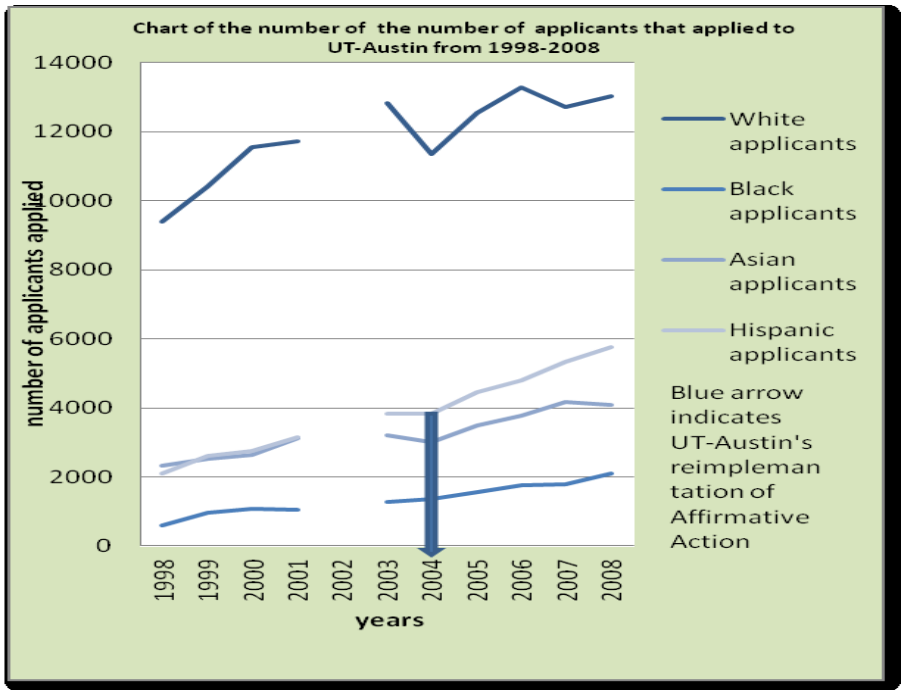


Chart showing the number of students from four demographic groups that were admitted to UT-Austin

Years	Whites admitted	Blacks admitted	Asian Americans admitted	Hispanics admitted
1998	6931	339	1,780	1,393
1999	6,802	452	1,819	1,498
2000	7,027	461	1,862	1,518
2001	8,038	493	2,325	1,978
2002				
2003	5826	397	1775	1572
2004	5,851	511	1,800	1,741
2005	6,745	617	2,076	2,183
2006	6,356	573	2,115	2,117
2007	6441	627	2498	2632
2008	5691	638	2084	2367

Chart showing the number of students from four demographic groups that were admitted to UT-Austin

Graph showing the % of the acceptance rates for demographic groups from 1998-2008 UT-Austin

Years	Whites	Asians	Hispanics	Blacks
1998	74	77	66	57
1999	65	72	57	47
2000	61	70	55	43
2001	69	74	63	47
2002				
2003	45	56	41	31
2004	52	60	45	37
2005	54	60	49	40
2006	48	56	44	32
2007	51	60	49	35
2008	44	51	41	30

University of California Schools

Chart of demographic groups that applied to the UC School

Years	Black applicants	Asian American applicants	Hispanic applicants
1993	1990	10691	5904
1994	2149	11267	6843
1995	2292	11127	7332
1996	2305	10081	7191
1997	2141	12367	6933
1998	2151	12205	7285
1999	2271	13762	7709
2000	2376	14076	8234
2001	2590	14619	12758
2002	2821	15488	10122
2003	3108	15815	11419
2004	2849	15211	11208
2005	2966	16317	12313
2006	3307	18742	13656
2007	3602	19050	15138
2008	4153	19370	17779

Graph showing the number of students admitted from the four major demographic groups from UC-Schools

Years	Blacks admitted	Asians admitted	Hispanics admitted	whites admitted
1993	1534	8840	4904	15,638
1994	1627	9629	3354	14,801
1995	1683	9406	6050	15,606
1996	1628	6712	5744	16,465
1997	1556	10519	5740	17,077
1998	1368	10427	5503	15201
1999	1453	11625	5860	18,305
2000	1536	12085	6397	18,363
2001	1734	12758	7433	20026
2002	1891	13546	8077	21,179
2003	2113	13959	8983	21,979
2004	1647	12719	8048	18,504
2005	1961	14559	9657	21,779
2006	2326	17035	11196	22471
2007	2567	17305	12287	23475
2008	2803	17483	14331	24,568

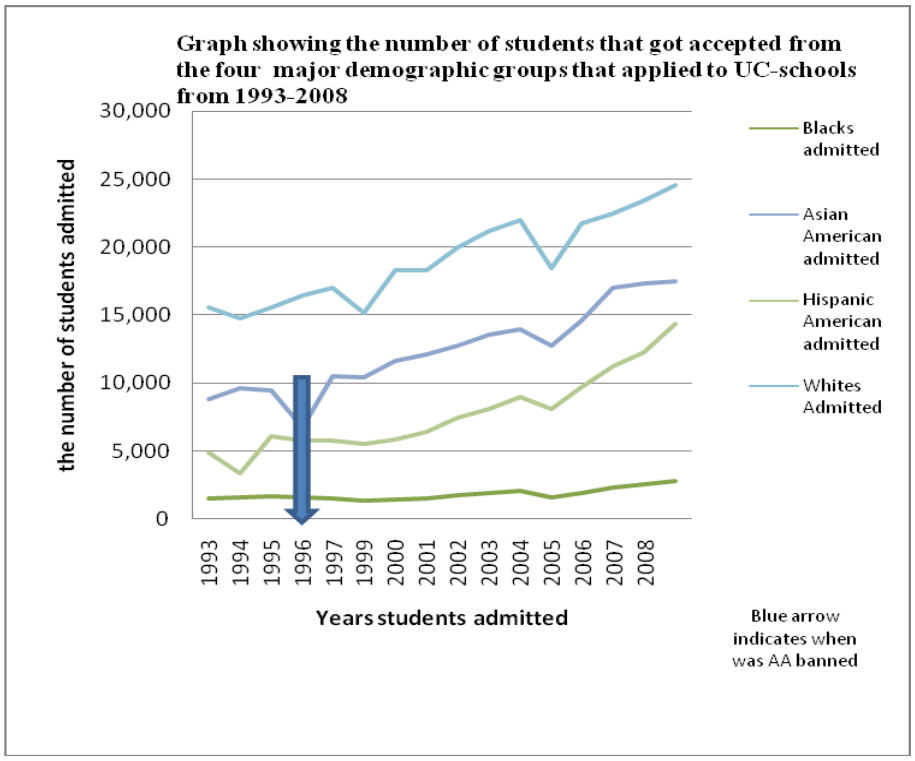
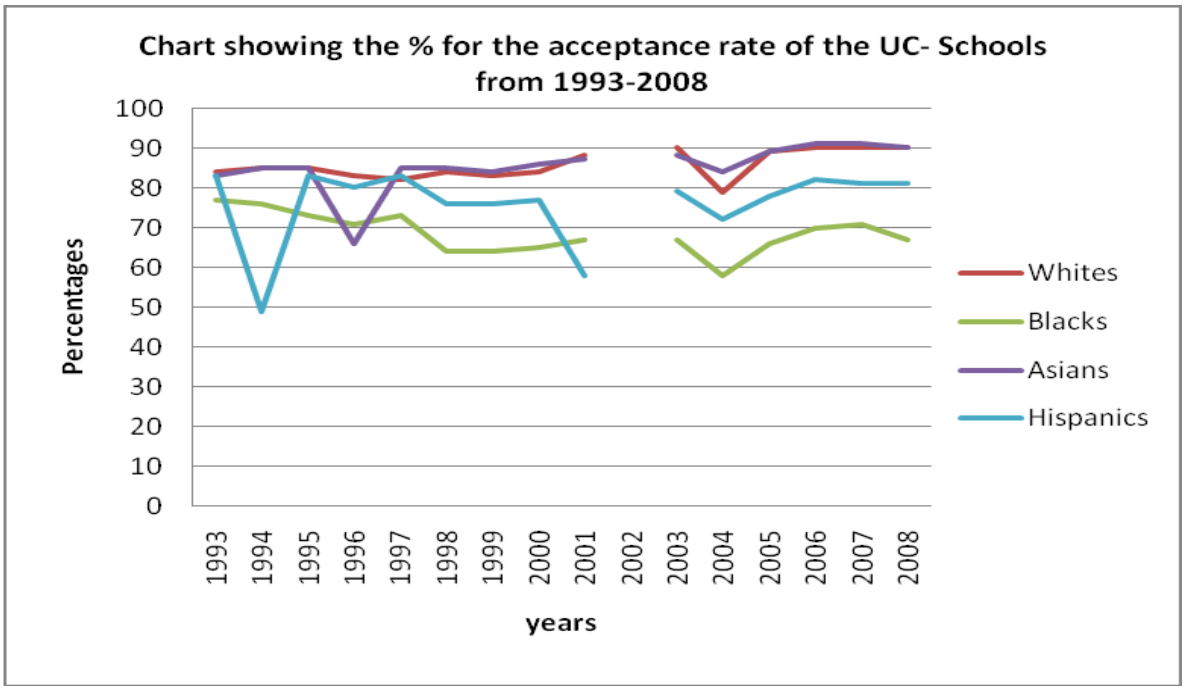


Chart showing the University of California's acceptance rate of the four major demographic groups from 1993-2008

Year	Whites	Blacks	Asians	Hispanics
1993	84	77	83	83
1994	85	76	85	49
1995	85	73	85	83
1996	83	71	66	80
1997	82	73	85	83
1998	84	64	85	76
1999	83	64	84	76
2000	84	65	86	77
2001	88	67	87	58
2002				
2003	90	67	88	79
2004	79	58	84	72
2005	89	66	89	78
2006	90	70	91	82
2007	90	71	91	81
2008	90	67	90	81



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A Change Needed in Secondary Level English Classrooms: Focusing on Student Identity as Theme in Curriculum in Order to Promote Socio-economic Growth among Cultural Groups in the United States

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Honors Thesis

Dr. Barbara Ladner

25 April 2014

"What shall I give my children? Who are poor, who are adjudged the leastwise of the land…. My hand is stuffed with mode, design, device. But I lack access to my proper stone. And plentitude of plan shall not suffice nor grief nor love shall be enough alone to ratify my little halves who bear across an autumn freezing everywhere."

– The Children of the Poor, Gwendolyn Brooks

Introduction:

This paper will argue that the theme of identity, as well as diversity, should be an immense focus of the high school English classroom, and English educators should expand outside the realm of the traditional American literary canon through innovative writing exercises, exploring more modern and regionally specific texts. Along with the suggestion of modifying curriculum at a local level, it is also necessary for schools throughout the country to follow national standards so that students everywhere will have the opportunity to receive equal and good quality educations regardless of socio-economic differences. The examples that have been compiled provide educators' success with confronting low education attainment due to socioeconomic differences, and improving childhood ambitions in disadvantaged communities through the

curriculum of the English classroom. For the sake of this paper, the focus is secondary level English classrooms in Appalachia. Ways in which some of these sources prove useful is through reading and writing examples where educators experimented with unconventional texts and writing exercise in hopes of reaching their students on a personal and academic level. Through these exercises a connection is established between student and text because the students were able to explore themselves, their backgrounds, and their culture while also analyzing the text at hand. The literary examples included in the paper are to remind educators to consider the individual student's backgrounds when choosing literature, but also to not neglect meeting national standards. The English classroom and curriculum should be made up of two aspects; the creative side and also the standard requirements, both of which are needed to fully prepare students for life after their secondary education ends.

1. **Confronting the current debate of national standards in the USA and arguing the importance of national standards to shape local curriculum:**

Up until the last year or so, most people in America were not aware of the fairly new Common Core Standards in English and Math that were adopted into 46 states during 2010. Since the implementation of the standards in schools all across the nation, three states have decided to withdraw from the system as of this year: Oklahoma, Indiana, and South Carolina. All together, teachers of those states were permitted four years to apply their own curriculum to the standards and examine the results before states began withdrawing from the system due to various reasons for disapproval of the Common Core.

"The debate behind Common Core is as old as public education itself: Who controls how—and what—children learn?" (Murphy). Common Core is a set of standards not curriculum that

provides uniformity amongst schools with the intention of preparing students throughout the United States for college and career readiness. Without a set of national standards colleges and employers have no way of gaging what kind of education students have received in the past. Two students living thirty minutes apart should receive the same quality education, but without national standards this is not the case. Standards and testing can differ between counties and even more so between states in some situations. Bill Gates, who spent more than $200 million dollars to promote Common Core was quoted saying, “If you were from Maryland, you didn't have to learn trigonometry, but your neighbors in Virginia did. Maybe they have less triangles" (Murphy).

The standards have transformed into a threat according to some in local politics due to the supposedly high involvement and influence of the national government in education, which has traditionally been a state or local matter. “We definitely need to seize more local control and get the issues back to the local level,” says Ohio Rep. Cliff Rosenberger in *The Columbus Dispatch* (Siegel). Another article in *The Columbus Dispatch* expressing the opposite opinion of Common Core reads: “the goal of Common Core is to improve college and career readiness and critical thinking, while aligning students among states to allow for comparisons of results. Local school boards decide the curriculum needed to meet those standards” (Candisky). The debate over these standards has become so arbitrarily displayed for the public even in solitary newspaper articles that it is impossible for anyone to decipher whether or not the standards are actually improving the education of American students. The standards have become a personal preference for teachers who claim to like or dislike Common Core for various reasons. Most commonly those who oppose the standards argue that they are confusing for students or in other instances, the standards are being criticized as a “one-size-fits-all model that stifles creativity” (Candisky). A

supporter of Common Core, Bob Hill who is superintendent of the Firelands Local School District in Lorain County in Ohio says: "any efforts to halt or disrupt (Common Core) are ill-conceived and a misguided attempt to undermine our core mission of educating children" (Candisky).

As of late, Common Core has become a platform for politicians to stand on and is quickly becoming an ethical debate similar to other controversial topics like abortion, gun rights, and Obamacare. One lawyer in Florida named Glenn Beck was quoted by *Mother Jones Magazine* claiming that Common Core "would attract every one of your children to become as homosexual as they possibly can" (Murphy). Others are labeling Common Core as "Obamacore," (Murphy) "the Obamacare of Education" (Caruba). In politics, Obamacare is also tainted with negative ideas and fears that Americans share when anything is becoming nationalized. Joy Pullmann writes in her essay, "Nationalizing education, like nationalizing anything requires compromise to get enacted. And compromise inevitably sacrifices quality. Quality has to grow from the ground up, through cooperation and competition, or it will never exist." Pullmann also argues "why should centrally controlled, taxpayer-funded, unaccountable-to-the-public committees have the power to define what nearly every U.S. school child will learn?" One way the government seems to have a hand in the longevity of the Common Core Standards is through federal grants like "Race to the Top" which President Obama is only permitting to states who have adopted the official Common Core Standards (Pullmann). "Common Core's critics contend that the administration took advantage of the recession" (Murphy) claiming that grant money was what convinced schools to adopt so quickly.

At a global level, until the introduction of the Common Core Standards, the United States education system was at a standstill, not improving or worsening, while other countries that

support national standards are seeing improvement and success in their attempts to further educate their youth (Jackson). “The Common Core State Standards reflect an understanding among American educators that the highest performing countries create high, consistent expectations for student learning. Moreover, wide discrepancies in state standards are seen as one of the reasons that the United States is not as competitive as it once was on international tests” (Jackson). Among other beneficial improvements like “establishing rigorous, focused and coherent content at all grade levels,” (Jackson) a study performed by PISA found that countries who follow national standards are also striving to “reduce inequality in curricula across socio-economic groups” (Jackson).

Common Core is an attempt for students to not only improve and compete academically throughout the nation, but it is also an attempt for the youth of the United States of America to once again compete with leading countries in education that also follow a set of national standards. Without national standards, not only will the United States get further left behind by high-performing countries, but even at the state level there will continue to be problems with schools that are disadvantaged due to their socioeconomic situations. Therefore, there may never be an improvement in the educational attainment or ambitions of the youth in those areas unless educators are striving to get their students competing at a national level. Students on the east coast of the United States should be getting the same education as students on the west coast. Perhaps the Common Core is not perfected at this moment and could use some modifications and improvements, but what is important is that the country continues on a track of trial and error until national standards are successfully improving the education system in the United States as a whole.

2. Efforts made in West Virginia to help students who need remedial classes during their first year of college

The Common Core Standards have been adopted and modified to fit into West Virginia schools, and yet newspapers and other forms of media still want to present a sense of opposition. Previous standards have not been successful in creating progress within the educational system of West Virginia, but what problem does the state have with admitting their compliance to a set of national standards if they are indeed using them? It is no secret in this state, and more specifically in the southern counties of West Virginia, that many students graduate from high school and find themselves in remedial classes for math and English in their first year of college. Otha Thornton, president of the National Parent Teacher Association, was quoted in the *Charleston Gazette* saying: "We owe our children an education that promises each child, when they get a diploma, that it actually means something," Thornton said. "It means that they are ready for college and that they do not need to spend money on remedial classes that don't count toward a degree" (Quinn).

In some places, diplomas do in fact contain different meaning in the qualifications and capabilities of the owner, hence the need for nationalized standards. Facing the problem of students falling into the sinkhole of remedial classes also requires one to face the initial problem: students are not being prepared with traditional standards. Up until the introduction of the Common Core, what other initiatives can West Virginia argue have been made to prevent students from entering college and immediately spending their time and money on remedial classes? This of course, is if they make it to college at all after their secondary education ends.

Once accepted into a university or technical school, there are certain courses that are necessary for every degree. For example, at West Virginia State University, English and Science majors alike are required to have taken or tested out of one college-level math class specific to their degree. For English majors pursuing a MA or PhD, they will also have to take the GRE, an entrance exam that is required for almost all graduate programs, which has a "quantitative reasoning section that measures problem-solving ability and focuses on basic concepts of arithmetic, algebra, geometry and data analysis" (About the GRE Revised General Test). The point is, although this paper is mainly focusing on the English side of the Common Core Standards at the high school level, it is also important to recognize other weaknesses in the education system that further show a need for better overall standards for West Virginia's students. Students who graduate from high school and begin at community college or a university may not be college ready still. In some situations, high school scores on the ACT or SAT in English or math may not be high enough for students to take their general education credits or the prerequisites to most of the classes in their degree. According to an online article written by Bruce Vandal, "traditional prerequisite remediation has failed West Virginia students-- with 64% of all community and technical college system students requiring remedial education and only a 13% graduation rate, many students are never finding their way out of remedial education."

Due to efforts made by James Skidmore, Chancellor of the West Virginia Community and Technical College System, West Virginia has become part of an initiative to aid students who are being forced to take remedial classes in order to begin their college degrees. Vandal wrote: "Beginning this fall, community and technical college presidents will implement plans to ensure that 70% of students currently placed into remedial education receive academic support while

enrolled in college-level courses, as a corequisite. West Virginia joined eight other states at a *Gateway Course Success: Scaling Corequisite Support Academy* sponsored by CCA last summer." The program is not to be an alternative type of education, but instead a fundamental redesign that will ultimately support students who are not appropriately prepared for college courses (Vandal).

Unlike the Common Core, this effort made by West Virginia has managed to stay out of the reach of legislative dictation because the state's community and technical colleges are independent institutions (Vandal). "Without these policy tools at his disposal, Chancellor Skidmore and his team have had to use data, the expertise of national corequisite reformers and their powers of persuasion to mobilize support. West Virginia's example demonstrates that large scale reform can occur in even the most decentralized state higher education systems" (Vandal).

3. Parents' concerns of Common Core Standards lacking creativity and introducing the theme of identity in the English classroom:

One of the many concerns of parents and educators regarding the English standards of the Common Core is whether or not these standards allow for creativity in classroom curriculum. Creators and advocators of the standards have said many times that Common Core is a set of guidelines that should be used when deciding curriculum, not the actual curriculum itself. Although students should be getting the same education all throughout the country, the standards still do not account for socioeconomic or cultural background differences. Therefore it is up to teachers to decide how to reach their students on a personal and individual level through exercises and texts that will allow students to identify themselves, their families and their cultures while also exploring diversity within classroom. The rest of this paper will focus on

creativity in English classrooms located in the Appalachian region of the United States and will deal with literature and exercises intended to appeal to Appalachian students on a universal and regional level; focusing on the identity of the student as well as diversity when deciding curriculum. This type of focus when designing English curriculum can be applied to other cultural groups outside of Appalachia, and has proved to be successful in the following examples that have been provided throughout the remainder of this paper.

4. Introducing the Appalachian culture:

According to *An American Vein*, a book of critical readings of Appalachia, at one time, the people of the Southern Appalachian mountain region were "the most written-about but least understood people in America." Stories with characters who were fictitious in their existence, but never-the-less born out of this region, started out "larger-than life" and portraying this "heroic image" that would eventually evolve into the "national archetype of the noble pioneer" (Miller). Somewhere along the way, the Appalachian identity became tainted with stereotypes portraying the typical hillbilly who was consumed with ignorance, illiteracy, idleness, and thanks to the help of the media, normally jobless, shoeless, and toothless.

To this day, these dehumanizing stereotypes live on in the minds of some people who are not native to the region or for those who have never visited the area. The problem with this is that the youth of this region are exposed to these ideas early on and can be unconsciously crippled by them. This section of the paper is going to briefly explore the importance of educating students of Appalachia about their history as a region and cultural group, and will focus on the unsuccessful routes that school systems can take if they do not consider the special needs of their students when exposing them to literature. The main argument is that students

need to be exposed to different kinds of literature that they can identify with in addition to reading classics. The proposal is not to eliminate all of the ideas of the canon in schools but to also incorporate certain contemporary and regional authors in schools' reading lists, and to experiment with writing exercises and activities that encourage students to positively explore their identities. The ultimate goal of this change is to find a way to encourage personal growth and provide motivation for children coming from all socioeconomic backgrounds of Appalachia.

5. **The power of coal in West Virginia and other parts of the Appalachian region, and its influence in regional legends and folklore:**

There are still places in the United States of America where, although technology may be prevalent, awareness of what is happening in the world can still evade the people living there. Isolation used to be an important factor in the existence of this peculiar group of people known as Appalachians, but in today's world with almost everyone having access to an iPhone and cell reception, even a hillbilly should be able to communicate with the outside world, right? And with this access to unlimited information, he is also given the ability to step outside of this stereotypical cycle of poor living conditions and negative ideas that have seemed to plague some parts of rural Appalachia even to this day, isn't he? He now has the opportunity to move in whichever direction he chooses on society's ladder of class systems, but will he? Words like otherness, idleness, and isolation are prominent parts of the idea that the outside world has established for this region if the world even chooses to acknowledge it at all. And why should the world take notice of a place where the very people there choose to overlook and underappreciate their own culture, history, and literature?

Historically, Appalachia has been a region that has endured a brutal struggle, some generations having to deal with dictating coal companies who forced miners to risk their lives in dangerous coal mines every day for almost no pay at all, unless you count the company's currency designed to prevent the mining families from using their money anywhere other than the company store. The Appalachian people were trapped in a system where their race did not matter even in a time of segregation: white Appalachians worked underground with the blacks and the Asians. There was a distinction between the two groups that made up the coal mining operations: the Appalachian and other racial minorities being controlled by the powerful, ruthless coal companies. This was the typical setup where those in power have to dehumanize their victims in society's view in order to justify their actions, hence the promotion of the negative stereotypes of the mountain people.

Historically in America this type of treatment and dehumanization has occurred with other minority groups: blacks, Japanese, Chinese, Native Americans, and etcetera. Ironically, Appalachians have never been accepted into the rest of white American society, and some places have acknowledged people of Appalachian heritage as a minority group. In the early 90's "Cincinnati adopted an equal opportunity ordinance that for the first time gives Appalachians the same protection against housing and job discrimination as other minority groups. The law represented an official admission of widespread prejudice in a city where 20 percent to 35 percent of the 364,278 population has Appalachian heritage" (Fleishman). Many Appalachians were forced to leave their homes in the mountains to migrate to cities like Cincinnati to look for jobs, but were discriminated against because of their accents and the negative stereotypes that afflicted their heritage.

In the state of West Virginia, if any political figure wants to see success on Election Day he or she better be a supporter of coal. West Virginia has been mined since the 18th century and is second in the country in coal production under Wyoming. West Virginia is also one of the poorest states in the nation, but ironically for much of America's existence it has largely been depended upon for its natural resources. "No state has given more to the American Dream and gotten less back from it than West Virginia," *Mother Jones* quoted a member of the West Virginia Action Group and a former coal operator in 1999. "The corrupt political system, the dead streams, the severed mountains, the fraud, the dust, the noise, the air pollution -- what for? All to supply the voracious American appetite for cheap electricity." This article portrayed the controversial topic of strip-mining (mountaintop removal) and its negative impacts on the environment, while also briefly covering the industry's transition from mostly man labor to machines that produced more efficient above-ground excavating, but resulted in detrimental job loss for miners. "A key cause of the job loss is the increasing automation of the coal industry — fewer miners are needed to do the same work. Add that to the fact that some of Appalachia's coal basins are running out, and that competition from cheaper coal out west is fierce, and annual production could continue to fall another 46 percent by 2020" (Valentine).

Circumstances like this, the theme of man against machine, have long been a part of Appalachia's culture and rich oral history. Most commonly known would be the folktale of the legendary, steel driving man, John Henry. Retold by S.E. Schlosser, the beginning of the tale goes, "He was born a slave in the 1840's but was freed after the war. He went to work as a steel-driver for the Chesapeake & Ohio Raildroad, don't ya know. And John Henry was the strongest, the most powerful man working the rails." According to legend, one day a salesman visits the camp with a steam-powered drill that he claims can out-drill any man (Schlosser). John Henry

though could not let that be true and so he competed with the steam-powered drill, using two hammers to muscle through the mountain to create the Big Bend Tunnel which in reality is located in Summers County of West Virginia. Henry succeeds in beating the machine, but in the end dies from a blood vessel bursting in his brain.

Introducing folklore that is culturally relevant can be an innovative way to expose students to timeless and regional ideas and themes. Tales like John Henry would be ideal for English classrooms due to the easy reading level, and because within a small amount of text, there is a large amount of information and ideas that can be examined. Discussions and topics can be limitless, beginning with what is the difference between fact and fiction. What makes a tall tale? And is John Henry a realistic character?

By providing regionally specific curriculum to the English classroom, students will be able to better understand and examine their own identities through their own cultural lens in comparison to others. Understanding in return produces appreciation in terms of how a person views a culture. It is important for students, Appalachian or any background, to first become aware of their diversity if they are ever going to understand how their own or any other culture plays into the multiplicity of people in the world.

6. **Efforts to eliminate negative effects of socioeconomic differences within disadvantaged communities and how their ideas can be applied to education:**

In 1968, a study titled "*Design for Action*" was published by the Office of Research and Development in the Appalachian Center of West Virginia University. It was written and performed by Thomas E. Woodall who was born and raised in a "hollar" in Lincoln County, West Virginia. Woodall, along with a small group of staff to assist him, participated in a "unique

community program employing an approach that attacks the demoralizing effects of alienation and apathy by involving the individual directly in communal efforts designed to accomplish cooperatively designed objectives" (Woodall 3). The project timeline consisted of five years and was to "focus on ways of working effectively with individuals, families, and groups that have been bypassed and left out by society."

According to the study, the characteristics of the two communities which were labeled A and B were both rural non-farm communities located in two counties adjacent to Kanawha County and both were below the U.S. average for family income and individual educational attainment. "The communities have a high percentage of young people… but housing quality is low" (Woodall 3). The problem that Woodall found with the living conditions of the people in the community resulted because they were "not aware of services and programs available to them" or if a service was discovered the organization did "not really know the problems, needs, and feelings of the people" of the communities (Woodall 4). The staff performed home visits where they were always promoting problem-solving within the families and stimulating "the process of self-discovery, growing insight, and increased self-awareness on the part of the local citizens" (Woodall 6).

At this point, it may be difficult to see the relevance of this study to this paper's proposal of improving educational attainment and childhood ambitions in rural Appalachian schools, but Woodall's idea and this paper do go hand in hand. "These people need help in organizing, in learning to function as acting members in a group endeavor, and in realizing that professionals do care about the resident's vital and immediate needs rather than the middle class oriented values and objectives that are sometimes advocated for the disadvantaged" (Woodall 12). This is the ongoing theme of this paper, the ability for society to ask every individual regardless of their

cultural or socioeconomic background to conform to ideas that do not necessarily apply to them or their resources. This has always been strongly evident in the literary canon which is the only exposure of literature that some children of rural Appalachia receive before graduating and becoming adult United State citizens who may or may not end up struggling to adapt like the people in Thomas Woodall's community study.

With the implementation of national standards, students in disadvantaged communities would still be given the opportunity to receive a quality education. However, the question remains will socioeconomic and culture differences stand in the way of these students actually acquiring the same education as a student who was brought up in a well-off family who expects such achievements? There is a well-known correlation between the lack of education and poverty. Children living in rural or disadvantaged areas have a better chance of coming from these types of communities where education is not something that is strongly advocated, and yet, these children are required to live up to the same testing standards that are being imposed on schools throughout the country even before the implementation of the Common Core Standards. If the cycle of poor living and low ambitions cannot be broken with the youth of disadvantaged areas, then looking at the bigger picture, there may not be a great change in the community or the region as a whole, much less an increase in the student's ability to compete nationally or globally in any field after high school. This study by Woodall was performed and written 46 years ago and signs of this type of problem can still be seen in rural communities in West Virginia, and the rest of Appalachia, today.

Many children who come from disadvantaged homes can be in some ways crippled because of their social status. More than likely the youth has heard this idea that if you are poor you are lazy, which is not a fact but is an idea that is constantly insisted by American society as a

characteristic of the people of Appalachia. This is sometimes due to the high percentage of people who benefit from government programs like welfare which has come to be a noticeably large part of the lifestyles of some Appalachians. Some children in places like rural Appalachia, or similarly in other disadvantaged communities throughout the country, have come to the conclusion that their material conditions are the only way to be established and recognized. Sadly, they accept the conditions in which they are born into continuing to be labeled as disadvantaged or in worse circumstances they are oblivious to their situation at all. The goal of any teacher should be to become aware of their students situations and help them realize their potential regardless of their socioeconomic differences. Unless someone encourages these students to step outside of their comfort zones in their educations they may continue feeding into the cycle of low education attainment with little to no change in their ambitions. In order to conceive a well-rounded student in the English classroom, the literature needs to be diversified to fit student's needs.

7. Initiative launched in West Virginia to aid in the education of Black male students:

A very good example of Thomas E. Woodall's community efforts and ideals tailored to the education system is through an initiative launched by West Virginia native and Marshall University alumnus Charles C. Meyers Jr. called the American Dream Movement (Gordon). *Black Starz* quoted Meyers in the "Blerd" (simply put, a blerd is a black nerd) section of their online magazine, explaining that he hopes the program gives black male students "a sense of hope and direction when they need it most" (Gordon). Meyers believes there are times that a student just needs a positive push or better guidance to be able to succeed in life (Gordon). In an article titled, "5 Things Black Nerds May Have to Deal with Growing Up," the writer describes a social environment where students, more or less, are at war with themselves as

they are deciding who they want to be. “You were never fully accepted by any social group because the black kids mislabeled you as “acting white” and the other kids couldn’t relate to your cultural background (Leo).

The American Dream Movement will welcome 12 students from the Huntington area ranging from 8th through 12th grade. The program is meant to motivate and inspire these select students’ throughout their secondary education and provide “positive reinforcement, education and guidance” (Gordon). “In today’s society, the dedication and encouragement to achieve the ‘American Dream’ has dissipated in the hearts of many African American males in Huntington, West Virginia,” said Meyers (Gordon). “Now, more than ever, it is critical for African American males growing up in society to understand the importance of faith, family, education and having a career” (Gordon). Meyers also organized the “Words of Reflection Writing Contest” which was sponsored by Marshall University. The purpose was for students to reflect upon their lives and write about obstacles they want to overcome, obstacles they have overcame, inspirational people in their lives, or the impact that they want to make in life (Wellman).

8. The need for English educators to recognize the differences in cultural backgrounds and socioeconomic situations in the classroom:

Historically, Appalachians have been viewed as a very peculiar group of people, but never fully considered as minority group although their diverse cultural characteristics favor one immensely. One thing that needs to be recognized by educators and seems to be apparent in the English education of minority groups like those of blacks and Hispanics for example, is that comprehension and understanding are more likely to be achieved when students are able to relate

to the literature at hand on a personal level. In America, the white male has always been the source of universal ideas, but what young, black girl living in poverty is going to relate to any idea coming out of a middle class, well-educated, male mouth? Likewise, what young, white girl living in poverty in Appalachia is going to relate to this hegemonic idea of self that most canonical texts portray? The problem is that although these universal ideas may be important parts of the American canon and culture, this does not mean that they are attractive topics for every reader, especially ones from disadvantaged families where perhaps educational attainment is an anomaly.

Historically, the canon, which "was essentially an aesthetic organism tended by literary and academic gardeners" (Krystal) of white males, has been forced to change as American society has evolved as well. How relevant is a list of texts solely written by white male voices to the idea of universality in a country that is booming with countless social backgrounds and cultures? At one time, this literature was considered to be the only and most highly thought of texts in American society worthy of the literary canon according to white male voices. With the movement of women's rights and with the abolishment of slavery, suddenly new writers of another sex and different ethnicities rush to the scene of the writing world, and become equally worthy of such an important list. As if these women and people of other ethnicities were not writing before.

Regardless of the relevance of traditionally canonical texts, children have to first enjoy reading and understand what they are reading before they can be made into critical readers and appreciators of such texts like those of the canon. In an article by Arthur Krystal titled "*What is Literature?*," he writes in defense of the canon saying: "We want important writing to explore the human condition, and we want our writers to function as curious explorers of the soul," but

what is not realized by promoting only classical texts in schools is that teachers may be failing in producing such results with young readers and writers, if they even continue reading and writing throughout their lives at all once their educations have ceased. "That's what literature is about, isn't it?—a record of one human being's sojourn on earth, proffered in verse or prose that artfully weaves together knowledge of the past with a heightened awareness of the present in ever new verbal configurations" (Krystal). The problem with most canonical texts is that they only express one type of human testimony and typically choose to ignore all others.

9. Success in promoting reading and writing with young black students due to experimentation of untraditional literature and writing exercises:

Students in rural or disadvantaged areas are more likely to have lower literacy levels and this can lead to a student who dislikes reading all together. The idea of exploring one's self through reading and writing has been a huge success with African American culture in the past and present. There seems to be a growth in literacy levels and an increase in interest in literature and writing within the youth of black culture thanks to the experimentation of writing exercises and texts that are specifically targeting ideas that they as a cultural group can identify with.

In an article written by Summer Wood and Robin Jocius titled "Combating 'I Hate This Stupid Book!': Black Males and Critical Literacy" the authors write "too often, instruction designed to improve literacy achievement for black male readers and writers focuses on skill-based learning, ignoring cultural, social, and personal development." The article argues fighting "students' assumptions about reading and texts, society's preconceived notions of black males, and traditional views of literacy success" by stepping outside of the traditional texts of school systems which seem to only always favor one specific group of American society. Wood and

Jocius emphasize the importance of students becoming "socially conscious text users" and also being able to identify their cultural histories within texts that they are experiencing. The article argues that sometimes teachers are unable to meet the specific needs of black male students and can cause the student to feel alienated in a literary classroom. "The problem of how to increase literacy achievement of African Americans is embedded in social, cultural, economic, and historical dynamics" (Wood) and this idea could also easily apply to any racial or minority group who is growing up in a rural area or coming from a disadvantaged background.

Educators can constantly push traditional texts at students arguing the value that is within them, but for some students who dislike reading or who cannot see how this information is relevant to them, the correct method would be to present a text that within its pages a student can see themselves, their families or cultures. Educators should make a point to introduce "books that connect directly to students' cultural and linguistic backgrounds" in order for them to connect in a more meaningful way (Wood).

Similarly concerning black culture, an article titled "Creating Spaces for Black Adolescent Girls to 'Write It Out!'" tells about the experiences of a group of young black girls, who varied in age (11-17) and also varied in their literary abilities, who were asked to come together in a group effort in an attempt for each girl to explore their own identities and build resiliency within their individual lives and also within their cultural backgrounds through writing. "The four themes that grounded the institute's curriculum were identity, resiliency, solidarity, and advocacy" (Muhammad). The program was designed so that the girls could write openly and honestly about anything they wanted and with this freedom they were able to explore themselves and the world around them. Muhammad wrote: "Identity was never a separate pursuit by black women as they advanced literacy development" most of the curriculum designed for this writing

course was centered on historical literary attempts made by black men and women in the past. The purpose of writing societies that were established by black women of the past was the idea of literary presence in literary societies. This was important because "black women did not wish to merely exist in the country but sought to exert their presence and make their mark on history to tell their narratives, rather than have their stories told by others, which could fail to give accurate accounts of their experiences" (Muhammad).

The idea of letting others or outsiders define a cultures identity has been an enormous contributing factor of the negative stereotypes and ideology that is always tainting Appalachian culture. Promoting creative writing exercises and groups in Appalachian schools would be a positive way for students to explore their culture's past and also find their own individual voices within their writing.

10. Exploring "The Freedom Writers Diary Teacher's Guide":

Starting in Long Beach, California in 1994, first-time teacher Erin Gruwell made a remarkable impact on a diverse group of students who later referred to themselves as Freedom Writers; authors of the diary entries that were published in Gruwell's *New York Times* best seller titled "The Freedom Writer Diaries". Gruwell first met this group of students in her freshman English class which was meant for students who were basically the "rejects," "unteachable," and who were labeled by some other educators within the school as "too stupid to read a book from cover to cover" (Gruwell 1). Gruwell began teaching at Woodrow Wilson High School not long after the Los Angelos riots in 1992 and the enforcement of integration laws. Majority of her class was made up of students who came from disadvantaged situations, most of them either affected or affiliated with gang violence due to racial and socioeconomic issues. However hopeless,

Gruwell had faith in her students and she knew that they were capable of learning what she wanted to teach them even though "they felt as if they had no reason to care about school" because for them "the potential rewards of college and a career seemed remote, even alien" (Gruwell 1).

The students in Gruwell's English class made it clear more than once that what they were being taught was irrelevant to their lives and experiences, and this is what sparked Erin Gruwell to step outside the box of normal classroom curriculum and modify her lessons in order to apply the subject of English to what her students dealt with once they left the classroom. "They needed an educational philosophy that promoted tolerance and encouraged them to believe in themselves" (Gruwell 1).

Gruwell first wanted to get to know her students and what they were dealing with in their home and neighborhood environments. She asked them to play games, the Line Game in particular, that allows students to "speak volumes without even saying a word" (Gruwell 34). The basic setup of this game is make a line in the center of the room, she used a line of tape, and ask the students questions starting with popular culture questions which can make it easier for later when the students are asked questions that are more personal; questions "about everything from absent parents to learning disabilities to juvenile hall" (Gruwell 34). Students who wish to respond step on the line for "yes". By playing this game students were able to relate to one another, establish a sense of trust within the classroom, and Erin Gruwell became more familiar with her students and their educational needs. Gruwell wrote in her teacher's guide, "I was able to make connections between their previous experiences and the new skills they were developing in the classroom" (Gruwell 7).

One of the adjustments that Erin Gruwell made in her classroom was modifying her reading lists. She writes that one of her students said, "Why do we have to read books by dead white guys in tights?" (Gruwell 7). Gruwell realized that she would have to be creative in finding material that would cause the students to want to invest in what they were learning so that they could apply it to the world around them. One Freedom Writer student wrote in Diary #14, "This story ['The Last Spin'] is a trip. I've never read something in school that related to something in my life" (Gruwell 7). Gruwell decided to use literature that was associated with young people who lived through war because her students lived in a "war zone" on the streets and in their homes themselves. "By reading about war, terror, and civil strife, my students discovered parallel worlds and their own ability to empathize with strangers" (Gruwell 138). Gruwell included literature with topics about the Holocaust, the Cambodian genocide, the Bosnian Civil War, Japanese Internment Camps, and U.S. racism. Gruwell also provided her students with individual journals that were to be read only by her. Through personal responses and writing exercise in the journals, Gruwell was able to see that her students were responding to the literature and that they were becoming more active in their educations; discovering things about themselves and about the people whose stories they were reading from past, present, and fictional worlds. Diary responses were unique according to how students wanted to express themselves. Sometimes students compared themselves to characters in the text. One student wrote in Diary #38:

"Zlata and I seem to have a lot in common because while Zlata was living through a war in Sarajevo, I was living through a different kind of war—the L.A. riots…. I can understand how afraid and scared she was to see her city go up in flames, because my city was on fire, too."

The poem below was written by a student in Diary # 78's entry. It began as a poem exercise where Gruwell used a shell format to help the students explore their own writing skills and creativity. "Use this shell to share the experiences and emotions of your life… For example:

An Innocent

_____

A young soldier
Looking for someone to see who I am.
On the first day of school I get the cold shoulder.
To comfort me a friend who does all he can." (Gruwell 86)

Below is part of a Freedom Writer's poem titled "An Innocent Freedom Writer":

"He goes to Wilson High School with a messed up trail,

And meets a guardian angel named Erin Gruwell.

He learns about the Holocaust, Anne Frank, and the Jews.

Now comes the time that he should choose.

He meets Anna, Terry, Tommy, and others.

These are the innocent boy's new sisters and brothers.

A 0.5 now a 2.8—

Change is good for those who wait.

He's back to innocence, but still has fear,

That death is upon him, drawing near."

Although Erin Gruwell and the Freedom Writers of Wilson High school had an exceptionally rare situation, lessons can still be learned from their experiences and applied to English classrooms throughout the nation with one thing in mind: the identity and background of the student. Students are willing to learn if educators are willing to teach, but it takes more than a standard curriculum to reach the minds of some students. "I believed that every one of them could succeed, regardless of their socioeconomic status, race, ethnicity, academic record, or

personal history. Because I believed in my students they believed in themselves and success was inevitable" (Gruwell 6).

Due to Erin Gurwell's hardwork and dedication, she was able to help her students shed what negative stereotypes they had been given and helped them become "critical thinkers, aspiring college students, and citizens for change" (Gruwell 242). The once deemed "unteachable" students who contributed to *The Freedom Writers Diary: How a Teacher and 150 Teens Used Writing to Change Themselves and The World Around Them* all received high school diplomas and some have gone on to earn master's degrees and pursue PhDs. Some original Freedom Writers still contribute to the Freedom Writers Foundation where they help to organize teacher training workshops and visit schools to spread the Freedom Writer message (Gruwell 244). The Freedom Writers Foundation was established and intended to replicate the success Erin Gruwell had with her students, the original Freedom Writers, in hopes of reaching and inspiring teachers and students throughout the country, and ultimately any place that needs a change in the English classroom in order to successfully reach out to their students.

11. Success in Appalachian English classrooms with relating regional themes and ideas to curriculum:

In 1966, in the Appalachian Mountains of north Georgia, an English teacher named Eliot Wigginton founded a quarterly magazine whose writers were made up of his ninth and tenth grade students as a part of their literary curriculum in hopes that the magazine would get them involved and interested in literature, their culture, and their identities. Fresh out of college himself and pre-magazine, Wigginton noticed the lack of desire and enthusiasm his students had for the texts that he was presenting to them in class. In a desperate attempt to reach his students,

he proposed working on the very first issue of what came to be the *Foxfire* magazine which was named after "a tiny organism that glows in the dark and is frequently seen in the shaded coves of these mountains" (Wigginton 11). The focus of the magazine was local folklore and the students ventured out into the community to interview and really talk to their relatives and neighbors, discovering in their conversations things like "superstitions, old home remedies, weather signs, a story about a hog hunt, a taped interview with the retired sheriff about the time the local bank was robbed—and directions for planting by the signs" of the zodiac (Wigginton 11). The school could not fund the magazine so students took it upon themselves to go out and find donations for their first issue, accumulating four hundred and fifty dollars to print their first six hundred copies of the magazine, which sold out and was reprinted immediately.

Throughout the life of the magazine, Wigginton writes that there were plenty of times where they went broke and some organization would come along and help them out. One of the organizations in particular, the National Endowment for the Humanities, gave them two grants, one being $10,000. *Foxfire* was recognized by other magazines like *National Geographic School Bulletin, Scholastic Scope,* and *Whole Earth Catalogue*, but what Wigginton wanted people to be aware of was that this newspaper was ran by high school students, "students who are going to college knowing they can be forces for constructive change; knowing that they can *act* responsibly and effectively rather than being always *acted upon*" (Wigginton 12). Since the creation of the magazine in 1966, *Foxfire* has now turned into a book series known as the *Foxfire Book* which as of now has twelve published volumes, all of which are still written by students and focusing on the oral histories and folklore of Appalachian culture. Wigginton writes that he feels similar projects could be duplicated successfully in many other areas of the country. The

students in this project gain "an invaluable, unique knowledge about their own roots, heritage, and culture" (Wigginton 13).

> Wigginton wrote: "Is the subject, English, ignored in the process? Hardly. In fact, the opposite is true. English, in its simplest definition, is communication—reaching out and touching people with words, sounds, and images" (Wigginton 13).

There are other ways to incorporate identity themed writing exercises into literary curriculum that are not as involved as the *Foxfire* project, but still not tedious like traditional ways of looking at classical texts. This has been proven by a professor named Elizabeth Oakes who is native to Kentucky and teaches at Western Kentucky University. In her essay, she explains that her class read Shakespeare's *Othello* during their semester and in attempts to have her students identify with the literature, she asked her students if they could relate to the otherness that is portrayed in Othello's character, and not on a racial basis but instead in a way that an individual can be separate from or not fit in with the dominant group.

In the same way that Othello contrasts in the play with white society as the Moor, a person from rural Kentucky can be alienated from the hegemonic ideas of American society. Oakes writes about the students' ability to recognize and sympathize with Othello due to the fact that they also can be considered the "other" in their own society. "Who am I when I read Shakespeare," Oakes wrote. "I grew up on a farm in rural northern Kentucky, a real farm with cows and chickens. My parents raised tobacco and had a garden, from which my mother canned much of our food. However, when I read, say, Othello, am I reading from that perspective or from that of the Ph.D. from Vanderbilt who has taught and read and, by now, traveled, and who goes back to the farm only for the yearly picnic? But even though I think I am reading from the

latter, I can be stereotyped as just a grown up version of the little barefoot girl who never left the farm."

What Oakes emphasizes is the importance of reading canonical texts as they relate to current times and also on a cultural level with whoever is reading. She asked her students to write a two-page paper, exploring Othello's otherness and also their own "from an aspect of ourselves that we have tried to deny or outgrow instead of from the 'academic self" that through their education they have attained. "Although we see ourselves from the subject position and thus consider ourselves individuals and therefore different from each other, the process of objectifying means that those who make "us" into a "them" see us as objects, as all alike, i.e., different from–and inevitably inferior to–them" (Oakes). One of Oakes' students named Mike Sobiech wrote in his paper:

"Othello is like me. I know that we are very different. He's black, and I'm white. He's a general, and I'm a civilian. He's dead, and I'm alive. He's fiction, and I'm real. He's a Moor, and I'm a Kentuckian. We are different people, but we also share things in common. We are both physically stereotyped: Othello has "thick-lips" (1.1.68), and I have yellow/yellow-brown/ brown/black teeth. We are both considered capable of bestiality: a farm animal might be my sexual partner, and Othello is "an old black ram [. . .] tupping" Desdemona, a "white ewe" (1.1.90-91). The paternity of our children is suspect; mine might be the result of incest, while Othello's marriage to Desdemona will result in the devil making Brabantio a grandfather (1.1.93). We are both dangerous: I am thought to have guns all over my house, while Othello is capable of murder… The real tragedy of the play is not that other people thought less of Othello: stupid people are going to think stupid things. The real tragedy is that he cared what they

thought. The real tragedy is that he let them–not just Iago, but his culture— manipulate him" (Oakes).

Through writing exercises like these where the reader's own identity is important in understanding the themes of such plays like *Othello*, success is obvious in educators attempts to bring universally and traditionally accepted ideas of such living texts, like those of Shakespeare, and relating them to current and local experiences of students of rural Appalachian backgrounds.

12. Updating reading lists of canonical literature by including modern and regional texts:

Traditional high school reading lists have long since been filled with classical texts such as *Beowulf*, *Animal Farm*, and *The Scarlet Letter* and although all of these texts have proven to withstand the test of time and have secured their spot in canonical literature, educators still need to consider modifying their classroom curriculums in order to meet the specific needs of their students. Curriculum should be designed to not only reach students on a universal level, but also on a personal, local, and cultural one as well. "The lack of relevance seems to rest in the environment and the method by which they are being taught; therefore, the integration of modern texts in conjunction with the classics can revive America's English classrooms" (Yuhas). This statement was taken from a student's capstone project which highlights the importance of modern text integration in America's classroom. Taking that one step further, when considering what literature to include in curriculums, we have to also consider the location in which these students live and their cultural and circumstantial differences. Every American student is not the same and yet they are all being presented the same kind of literature, and expected to make a connection. "Teachers have the obligation to make the texts and themes relevant through the

inclusion of modern texts alongside canonical literature, which will allow students to make a personal connection with a variety of contemporary and classic texts" (Yuhas).

One of the best examples of contemporary literature and authors that uses canonical texts as a theme in his novels themselves are written by newly popular, young adult literature author, John Green. Green quotes classical authors like Shakespeare and Walt Whitman, using classical texts to create the paths in which his own characters will take throughout their adventures in his novels. "Deeply philosophical and richly textured, *Looking for Alaska* is an intense coming-of-age story about a group of teens, a boarding school, and one boy's search for the "Great Perhaps." First-time novelist Green depicts the intensity of adolescence seldom seen in contemporary literature" (McGraw-Hill). In his novels, Green presents issues and ideas that are common parts of adolescent life like understanding one's purpose, teenage suicide, terminal illness, adventure, and independence. Green also does not fall short in enticing the reader from the very first page most often using wit and humor as a hook to reel readers in. The inclusion of authors like Green in American classrooms would be beneficial to any area of America and to any student regardless of socioeconomic backgrounds because of the universal ideas of youth that are at play within the novel.

For more convincing on incorporating modern texts into English curriculum, the *Huffington Post* compiled a list of young adult novels that express the traditional and universal themes of canonical texts. An innovative exercise that could be included in curriculum is the comparison and contrast of the modern literature and the classic text. The article argues the modern literature addresses some of the same themes the classics do, including race, female sexuality, mental illness, and love. First on the list is John Green's *The Fault in Our Stars* being paired with Shakespeare's *Romeo and Juliet*. "The novel, which takes its title from another Shakespeare

play, Julius Caesar, continues in the tradition inaugurated by Romeo and Juliet, illustrating love's ability to overcome seemingly insurmountable obstacle" (Books). Another brilliantly, notable equivalent to Mark Twain's *The Adventures of Huckleberry Finn* is *The Absolutely True Diary of a Part-Time Indian* by Sherman Alexie. "Alexie gives readers another renegade to look up to -- an aspiring cartoonist who flouts the expectations of his Native American community -- while offering a glimpse of another, no less problematic, aspect of America's racial landscape" (Books).

Although the inclusion of contemporary novels within the classroom would be a very good start for students who have trouble connecting with the text at hand, the still too universal ideas of the novels do not allow for a more in depth look at the student's specific culture and identity. Modern authors like John Green still portray a mostly universal idea of what it is like to be a teenager growing up and living in today's America. In order to be even more thorough in exploring a student's identity, it is necessary to also include unique texts that reflect cultural ideas that are specific to a certain area and a certain group of people. In this case, within Appalachia, students should be exposed to regional writers and their stories which were coined within the very same mountainous region that the students call home.

A text that would be an excellent edition to any Appalachian schools' reading list would be *The Short Stories of Breece DJ' Pancake*. Pancake was native of Appalachia and born in Milton, WV. He attended West Virginia Wesleyan College and Marshall University before teaching English at two military academies and then receiving a fellowship to the University of Virginia, according to an article written by Jon Michaud in *The New Yorker*. The biographical aspects of Pancake alone would be relevant to any Appalachian English classroom, where

students could learn about writers that originated in their region and their success within the literary world.

On a literary level, Pancake allows students to experience regional dialect, perhaps similar to their own. Within his short stories is "an uncanny hybrid of dirty realism and Southern gothic" (Michaud) that highlights human tragedy and nature. In *The New Yorker*, Michaud wrote: "Not quite Southern and, likewise, neither Eastern nor Midwestern, Appalachia is a largely overlooked region in our literary culture." The circumstance of the characters plays a huge role in the plot of Pancake's stories whether it is dealing with their educational, financial, or romantic situations. Students of rural Appalachia would undoubtedly see themselves, their families, or perhaps where they grew up within Pancake's stories. By being exposed to literature that expresses ideas that are rooted within their own culture, students will be more aware of their own identities and then can explore a world of literature that perhaps they never even knew existed; a world that very well could be much like their own.

Conclusion:

"Students desire literature they can relate to personally" (Yuhas) but it is up to educators to acknowledge this mostly unspoken request and make modifications to classroom reading lists and writing exercises according to the special and unique needs of the students. Students need to be exposed to different kinds of literature that they can identify with in addition to reading classics in schools in order to have a positive effect on overall educational attainment and ambitions. Elimination of all traditional ideas is not necessary, but it is important to incorporate certain contemporary and regional authors in schools' reading lists to provide well-rounded curricula. It is also equally important to include writing exercises and activities that

encourage students to positively explore their own identities. As an educator in the English classroom, the goal is to create innovative and fresh ways of looking at tradition ideas that are relevant to the individual student's identity rather than repetitively expecting students to practice ancient, tedious methods of exploring only hegemonic ideas. Through the use of national standards that allow room for creativity in English classroom curriculum, students all over the country will be receiving quality educations filled with rich literature that is specific to whom they are as individuals. With the implementation of these modifications throughout the nation, there will be a positive change in how students with socioeconomic differences view reading and writing, how they compare their worlds to others, and ultimately, how they see themselves.

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Deciphering the Early Eruptive History of Mount St. Helens using Microanalytical Geochemistry

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Abstract

Tephra deposits provide key information for documenting volcano eruptive histories, and to understand volcanic processes and hazards. Widespread tephra layers from major eruptions are used around the world for correlation and dating in geology and archaeology. Applications include earth surface processes, past environmental/ climate changes, and human history.

Mt. St. Helens has a long history with many major eruptions and is a key source of volcanic ash used for tephrochronology across North America. Because eruptions that were closely-spaced in time have very similar volcanic glass compositions, distinguishing tephra from different eruptions can be challenging. Very careful analytical work resulting in relatively high precision data and excellent session-to-session reproducibility are needed.

We are working with samples of set C tephras (approximately 35 to 50 ka) collected in 2008 from key proximal reference sites described by Mullineaux (1996) including Road 25, Road 81, Road 90, and Muddy River Quarry as well as several reference samples collected by Mullineaux himself. We also have medial to distal samples of set C and older tephras from the 220,000 year sediment record at Carp Lake, from Cape Horn Creek and Green Mountain, Washington and from other locations in eastern Washington (Busacca et al., 1992), eastern Oregon (e.g. Kuehn & Negrini, 2010), and southern Idaho.

Some of the major layers are clearly distinguishable by major-element glass compositions alone (e.g. Cy and Cs) whereas others show more overlap (e.g. Cw and Cy).

Introduction

Tephra

Tephra refers to the ash and pumice erupted from a volcano that later became deposited onto the ground. Eruptions producing tephra typically last a few hours to a few days, but can last up to several months (Lowe 2011). Larger fragments are deposited close to the volcano decreasing in particle size farther from the eruption site.

Particle size determines the classification of tephra. Any particle under 2mm is determined to be ash, fine ash is less than 1/16 mm, and coarse ash is between 1/16 mm to 2 mm. Lapilli ranges from 2 mm to 64 mm whereas particles that are larger than 64 mm are called blocks and bombs (Mullineaux 1996).

A sequence of tephra is divided into sets, layers and beds. Strata similar in age is a set and records an episode of more than one eruption. Layers are beds that can be traced as stratigraphic units, but units describe as beds cannot be traced laterally. (Mullineaux 1996)

The thickness of the layers vary with distance. Factors other than distance can be responsible for the depositional thickness variation such as downslope sloughing during fall, being carried down by rain, shielding of ground next to tree trunks, and excess accumulation between trees by detaching of detritus from tree limbs. Once it has been deposited weathering processes can be responsible for removal of the tephra causing variations in thickness. Erosion by ice, wind, water, and landslides are prevalent causes (Lowe 2011).

Tephrochronology

Tephrochronology is a stratigraphic method using deposited tephra to date and correlate. It relies on the principle of superposition and the ability to characterize the tephra using properties identified in a lab. Methods for this include determining the geochemistry of glass shards within the tephra using an electron microprobe and other analytical instruments. The process is most useful when a numerical age is obtained on a layer of tephra using dating methods. Even with unknown numerical dating the layers can still provide a time-plane (Lowe 2011).

Tephrochronology has many important applications across a range of fields. The study can link and date archeological, geological, paleoecological, and paleoclimatic sequences. Distinguishable layers of tephra will help decipher a time frame for these studies. Tephra beds hold a good preservation record of artifacts if young enough. Beds may contain a conserved pollen record that scientist use to determine the ecological habitat and climate during a certain time interval (Clynne 2008).

Mount St. Helens

Mount St. Helens has been one of the most active and frequently erupting volcano in the Cascade Range. There is a distinct strata of over 100 tephra deposits produced by the pyroclastic eruptions of Mount St. Helens over the past 45,000 years. Most of the tephra has been deposited east of a north south line due to the westerly winds. The distribution of the tephra is indicative of fall deposits (Mullineaux 1996).

Donal R. Mullineaux, the tephrochronologist responsible for much of the tephra characterization of Mount St. Helens, has published papers on the classification and understanding of Mount St. Helens by focusing on the tephra deposits throughout the eruptive history of the Volcano. Mullineaux has derived a classification system for the tephra of Mount St. Helens that have become a uniform standard for tephrochronology research of the volcano.

Until around 2,500 years ago the composition of the eruption was only dacite and silcic andesite. From then forward there is an irregular compositional history where andesite began to occur and alternate with dacite and basalt. Mount St.Helens tephra deposits include dense lithic fragments and slightly vesicular rocks with some mineral crystals and glass. Pale clasts of pumice and dark clasts of scoria are also commonly present (Mullineaux 1996).

Mount St. Helens is easy to identify based on the prominence of ferromagnesian phenocryts which may consist of augite, biotite, cummingtonite, hornblende, hypersthene, and olivine (Mullineaux). Biotite and cummingtonite are not seen commonly in other Cascade range volcanos thus are key identifiers for Mount St. Helens tephra (Mullineaux 1996).

A lettering system is used to describe the tephra strata which depicts field descriptions and not chronological sequence. Radiocarbon ages will provide a chronological dating method, but this method still provides some uncertainty. Studies show in samples older than 30, 000 years the ages can be uncertain up to thousands of years whereas samples 4,000 to 30,000 years have uncertainties of hundreds of years. For more accurate dating in recent samples ages derived from growth rings counts of trees in the tephra falls can be used (Mullineaux 1996).

Mount St. Helens Deposits Sets 1980- 35,000 Years Ago

The most well-known eruption of Mount St. Helens is the 1980 event, but before this many eruptions happened throughout time. A distinguishable layer called layer T accounts for a dacitic eruption with voluminous tephra took place in the 1800's and was the last recorded layer before the 1980 eruption (Mullineaux 1996).

The early 16th century records a mafic composition that differs from previous eruptions. Refereed to as set X, it contains fine grained andesitic beds that have only been recognized near the volcano. After this there is set W, a dacitic set produced by eruptions in the 15th century. Initially it deposited large voluminous pumice layers which became overlain by smaller pumice tephra layers. The W layers have been mapped hundred of kilometers downwind of the eruption site. The sugar bowl dome was emplaced about 1,200 years ago by a small volume dacictic tephra layer labeled D (Mullineaux 1996).

Tephra set B was characterized by andesite, dacite, and basaltic tephra that occurred with abundant lava flows, but few pyroclastic flows. The set includes small to moderate volume layers. The layers are thicker and coarser than the underlying set P. No individual layers were able to be distinguished past few tens of kilometers past the volcano. (Mullineaux 1996)

Set P eruptions, creating few tephra layers and numerous pyroclastic flows, occurred between 3,000 to 2,500 years ago during the Pine Creek Stage. What tephra beds were produced were made of fine grained ash. Set P has been recognized several hundred kilometers downwind but no distinguishable layers were traced farther than this (Mullineaux 1996).

Around 3,000 to 4,000 years ago through the Spirit lake eruptive stage the eruption of set Y occurred producing abundant tephra and little pyroclastic flows in contrast to the Pine Creek stage eruptions. The set has two prominent layers of coarse voluminous pumice with small interbedded layers (Mullineaux 1996).

The sets of S and J were deposited during the early and late parts of the Swift creek stage 13,000 to 10,500 years ago following a 6,000 year dormant period that created large volume dacite pumice layers near the volcano that consist of lapilli. Each of these sets has been identified up to hundreds of kilometers to the east of Mount St. Helens (Mullineaux 1996).

Tephra Set K was produced 19,000 to 13,000 years ago during the later part of the Cougar stage. The set is small in volume with no layers that can be separately described and cannot be located outside of the immediate area of the volcano (Mullineaux 1996).

Set M eruptions during the early Cougar stage (20,500 to 19,000 years ago) produced moderate volume dacite layers of pumice, lithic lapilli, and ash that do not exceed a few tens of centimeters thick near the volcano, but ash beds have been found as far away as Nevada (Mullineaux 1996).

Mount St. Helens Set C 35,000 yrs to 200,000 yrs

The Ape Canyon stage deposits point towards at least two periods of volcanism. One ranging from 300-250 ka the other from 160- 35 ka where there may have been a gap of inactivity spaced between the two periods. Otherwise, the record of 250 ka to 160 ka could have been buried by younger volcanic deposits or not yet been identified (Clynne 2008).

Set C deposits are found east of the volcano and clockwise to the southwest at altitudes of typically less to 600 m. The apparent distribution reflects preservation and not the amount deposited at the time of the eruption. Pumice fragments and other dense materials are highly weathered. Pumice layers of lapilli and bombs that several tens of centimeters thick lie 10 to 15 m from the volcano. The basal ash layers here are 100 cm thick. It is distinguishable from the overlying tephra sets of M and K due to the abundance of biotite. No tephra is beneath set C so it lies on soil developed in colluvium or glacial drift. Layers within the set can be seen in Table 1 (Mullineaux 1996).

Table 1: Layers within Set C Tephra as Identified by Mullineaux

The Study Purpose

The purpose of this study is to gain a better understanding of the early eruptive history of Mount St. Helens. Set C and earlier tephras are the most poorly understood in the history of the volcano. Knowing the eruptive history can help define the timing in which the layers were deposited and things associated with the tephra. The widespread ash layers will be able to indicate the size of eruption and the area affected at a particular time. Because eruptions often

produce different chemistry, many eruptions can be distinguished based on their chemical composition.

Geochemical analysis were performed on Mount St. Helens set C tephras using an electron microprobe at Concord University. Glass shards in the tephra were analyzed and the chemistry was determined to help distinguish the layers within the set. The data was used to determine which eruptions may be distinguishable from one another.

Methodology

Sample Collection

Samples analyzed were obtained from collections by several people from various location throughout multiple years. Samples collected by Mullineaux in the 1970's and 1980's were analyzed to be used as references. Nick Foit from Washington State University provided samples from eastern Washington. Eleven samples used had been previously described in the paper *Correlation of Distal Tephra Layers in Loess in the Channeled Scabland and Palouse of Washington State* (Busacca et al. 1992). Other samples were collected by Cathy Whitlock from Montana State and Caitlin Orem of Arizona State. Elmira Wan from the USGS provided some of the sample analyzed. The majority of samples were collected and provided by Stephen Kuehn from Concord University (Table 2).

Proximal samples were collected by Kuehn in 2008 based off of Mullineaux's sample locations. (Figure 1) Locations were Mullineaux's road 25 site, Mullineaux's road 90 site, Mullineux's road 81 site, Mullineaux's Muddy River Quarry Site, Near Mullineaux's Swift Site, Cape Horn Creek, Summer Lake Oregon, and Carp Lake Washington.

Sample Number	Collector or Reference	Collector's Organization	Received From
CU1003-CU1024	Steve Kuehn	Concord University	
CU1029	Steve Kuehn	Concord University	
CU1031	Stephen Kuehn	Concord University	
CU1034	Stephen Kuehn	Concord University	
CU1042-CU1047	Don Mullineaux	USGS	Nick Foit (Washington State)
CU1048-CU1058	Busacca et al 92	Washington State	Nick Foit (Washington State)
CU1059-CU1070	Various	Various	Nick Foit (Washington State)
CU1108-CU1111	Cathy Whitlock	Montana State University	Elmira Wan (USGS)
CU1182	Stephen Kuehn	Concord University	
CU1185-CU1186	Stephen Kuehn	Concord University	
CU1199-CU1210	Stephen Kuehn	Concord University	
CU1212-CU1221	Stephen Kuehn	Concord University	
CU1292-CU1294	Caitlin Orem	University of Arizona	

Table 2: List of Sample Collectors and Sample Providers

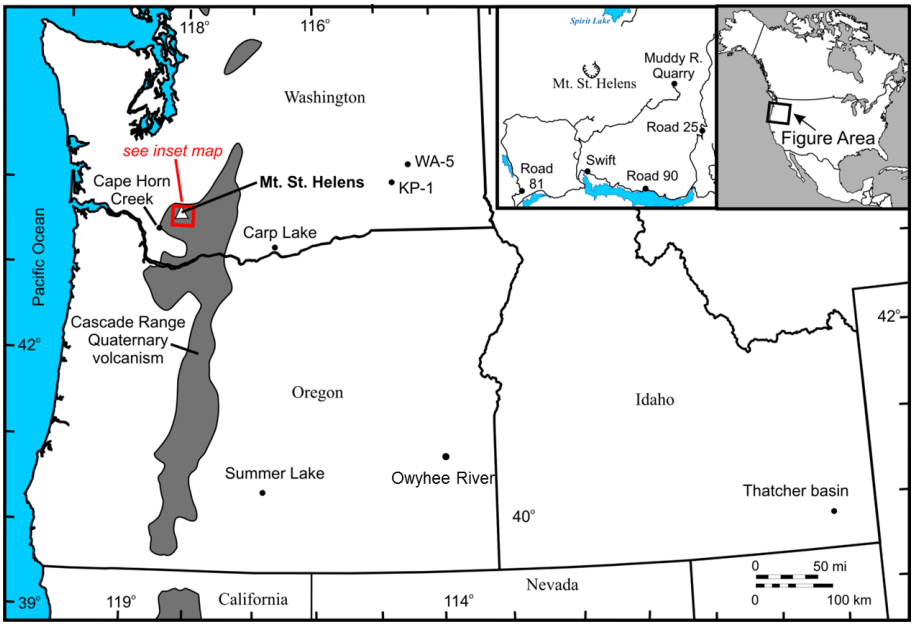


Figure 1: Sample Collection Locations

Sample Preparation

The majority of the samples were sieved to remove the particles smaller than 20 microns. Coarser samples from close to the volcano were crushed before the sieving began. The samples were then mounted in acrylic mounts using epoxy and polished in stages ending at a

final grit of 0.3 micron alumina. Mounts were labeled, carbon coated, and then put into the microprobe for analysis.

Analytical Method

Samples were analyzed for SiO2, TiO2, Al2O3, FeO, MnO, MgO, CaO, Na2O, K2O, P2O5, and Cl using the ARL-SEMQ electron microprobe at Concord University. The current procedure in use since June 2014 is tuned to maximize precision and day-to-day consistency. Analytical conditions are 14 kV accelerating voltage and 10 nA beam current. The procedure uses four WDS spectrometers and a 30 mm2 Bruker SDD EDS detector with spectrometer #1 PET used to measure K, Ca, and Ti, #2 RAP to measure Na and Mg, #3 LIF to measure Fe and Mn, and #4 PET to measure P and Cl, and EDS to measure Al and Si. To provide better precision in the same analysis time, all elements, including minor elements, use modeled MAN (mean atomic number) backgrounds instead of measured backgrounds with a blank correction used to maintain trace-level accuracy. Because the glasses are significantly hydrated, water-by-difference is incorporated into the X-ray matrix corrections.

Because the Mt. St. Helens tephras typically have inflated pumice with very thin glass, surfaces available for analysis are typically smaller than 10 microns and sometimes as small as 4-5 microns. Because these samples require small beam diameters and rhyolitic glasses are unstable during exposure to the beam, the analytical procedure uses a time-dependent-intensity (TDI) correction for Na. Si, Al, and K are also measured at the beginning of each analysis with Si and Al measured for only 8 seconds.

Four reference glasses are analyzed at the beginning and end of each analytical session (day): Lipari obsidian ID3506 (Harvard), BHVO-2g (USGS), NKT-1g (USGS), and Orthoclase glass (USGS/Corning). To maximize session-to session consistency, each session as a whole is adjusted to match the reference glasses to their reference compositions using a multi-standard consensus (weighted average).

The result is outstanding reproducibility over multiple years (Figure 2), even with changes to the analytical procedures. Si and Al were switched from WDS to EDS in mid 2012, and MAN backgrounds were implemented late 2012. The improvement in precision aided by MAN backgrounds can be seen by comparing the mid-2012 and the 2013 results.

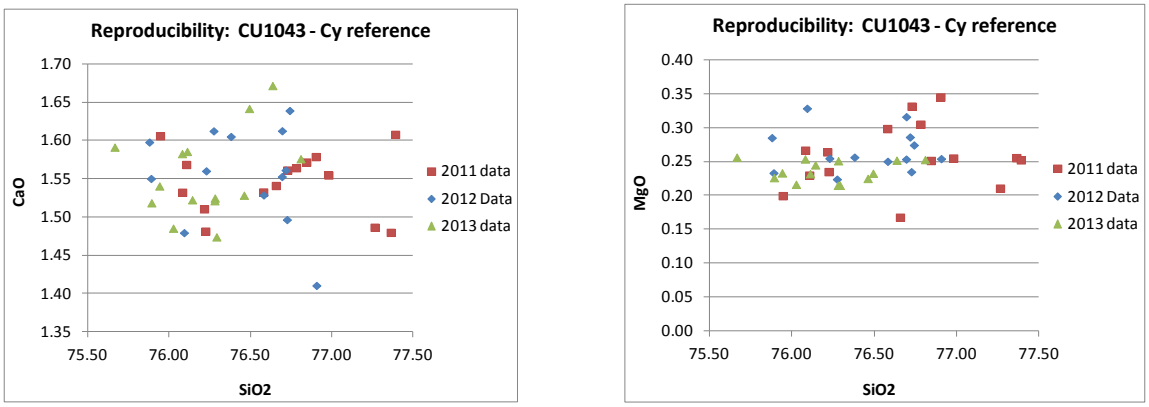


Figure 2: Reproducibility of samples over multiple years with changes to the analytical procedure

Data

Analysis of the glass shards (Table 3 and Figures 2 and 3) show mostly subtle but reproducible differences between layers which distinguish some of the samples. With this information we are able to differentiate the layers of tephra from one another. Among the

Table 3 - Normalized tephra glass data as weight%, normalized to 100% totals

Pub. ID	Sample Number		SiO2	TiO2	Al2O3	FeO	MnO	MgO	CaO	Na2O	K2O	P2O5	Cl	Total	H2O diff	FeO+ MgO	Na2O+ K2O	n
Mullineaux Reference samples																		
	CU1042 (10-20-11)	**Average**	**76.29**	**0.16**	**13.86**	**1.15**	**0.03**	**0.40**	**1.70**	**3.91**	**2.36**	**0.08**	**0.08**	**100**	**4.98**	**1.55**	**6.27**	**15**
		StDev	0.58	0.03	0.48	0.06	0.01	0.05	0.05	0.19	0.10	0.03	0.02		2.10	0.09	0.24	
	CU1042 (6-11-14)	**Average**	**76.37**	**0.15**	**13.97**	**1.12**	**0.04**	**0.36**	**1.61**	**3.91**	**2.37**	**0.06**	**0.07**	**100**	**5.04**	**1.47**	**6.28**	**15**
		StDev	0.32	0.02	0.21	0.06	0.02	0.02	0.04	0.29	0.08	0.02	0.01		0.71	0.06	0.31	
Cs	**CU1042** (combined)	**Average**	**76.33**	**0.15**	**13.92**	**1.14**	**0.04**	**0.38**	**1.66**	**3.91**	**2.37**	**0.07**	**0.08**	**100**	**5.01**	**1.51**	**6.27**	**30**
		StDev	0.46	0.03	0.37	0.06	0.02	0.05	0.07	0.24	0.09	0.02	0.02		1.54	0.08	0.27	
	CU1043 (10-21-11)	**Average**	**76.67**	**0.10**	**13.59**	**0.89**	**0.03**	**0.26**	**1.54**	**4.36**	**2.46**	**0.02**	**0.09**	**100**	**3.67**	**1.15**	**6.82**	**15**
		StDev	0.47	0.03	0.36	0.05	0.02	0.05	0.04	0.15	0.13	0.04	0.04		1.40	0.08	0.23	
	CU1043 (6-23-12)	**Average**	**76.45**	**0.10**	**13.52**	**0.86**	**0.03**	**0.26**	**1.55**	**4.56**	**2.57**	**0.05**	**0.06**	**100**	**6.10**	**1.13**	**7.13**	**13**
		StDev	0.35	0.04	0.21	0.04	0.03	0.03	0.06	0.24	0.12	0.02	0.01		0.82	0.06	0.26	
	CU1043 (6-12-14)	**Average**	**76.22**	**0.09**	**13.84**	**0.92**	**0.04**	**0.24**	**1.55**	**4.49**	**2.51**	**0.03**	**0.08**	**100**	**4.61**	**1.16**	**7.00**	**14**
		StDev	0.31	0.01	0.18	0.05	0.01	0.02	0.06	0.39	0.10	0.02	0.01		0.62	0.06	0.41	
Cy	**CU1043** (combined)	**Average**	**76.45**	**0.10**	**13.65**	**0.89**	**0.03**	**0.25**	**1.55**	**4.46**	**2.51**	**0.03**	**0.08**	**100**	**4.74**	**1.14**	**6.97**	**42**
		StDev	0.42	0.03	0.29	0.05	0.02	0.04	0.05	0.28	0.12	0.03	0.03		1.41	0.07	0.33	
Cb	**CU1044**	**Average**	**78.51**	**0.19**	**12.33**	**1.15**	**0.05**	**0.24**	**1.02**	**3.66**	**2.68**	**0.07**	**0.11**	**100**	**3.72**	**1.40**	**6.34**	**11**
		StDev	1.66	0.02	1.14	0.18	0.01	0.08	0.44	0.58	0.34	0.01	0.02		1.44	0.23	0.37	
Cw	**CU1045**	**Average**	**76.59**	**0.11**	**14.07**	**1.02**	**0.04**	**0.29**	**1.75**	**3.66**	**2.36**	**0.04**	**0.10**	**100**	**6.64**	**1.31**	**6.02**	**14**
		StDev	0.69	0.03	0.54	0.13	0.02	0.08	0.16	0.43	0.11	0.02	0.04		3.13	0.20	0.40	
	CU1046	**Average**	**76.15**	**-0.03**	**14.40**	**1.07**	**-0.02**	**0.20**	**1.75**	**3.68**	**2.40**	**0.06**	**0.45**	**100**	**6.63**	**1.27**	**6.08**	**10**
		StDev	0.41	0.08	0.46	0.03	0.04	0.05	0.25	0.26	0.08	0.01	0.21		0.86	0.05	0.30	
	CU1047	**Average**	**75.67**	**0.11**	**14.04**	**1.21**	**0.04**	**0.39**	**1.90**	**4.22**	**2.31**	**0.06**	**0.07**	**100**	**8.73**	**1.59**	**6.52**	**13**
		StDev	0.65	0.04	0.27	0.27	0.02	0.10	0.17	0.22	0.06	0.02	0.01		0.84	0.37	0.24	
Ct	**CU1046/CU1047** (combined)	**Average**	**75.88**	**0.05**	**14.20**	**1.15**	**0.01**	**0.30**	**1.83**	**3.98**	**2.35**	**0.06**	**0.23**	**100**	**7.82**	**1.45**	**6.33**	**23**
		StDev	0.60	0.09	0.40	0.21	0.04	0.13	0.22	0.36	0.08	0.02	0.23		1.35	0.32	0.34	
Mullineaux Road Site 25																		
	CU1003 (1-31-12)	**Average**	**76.09**	**0.09**	**14.63**	**0.92**	**0.04**	**0.30**	**1.73**	**3.83**	**2.27**	**0.04**	**0.09**	**100**	**6.84**	**1.22**	**6.10**	**21**
		StDev	1.08	0.04	0.77	0.05	0.03	0.04	0.08	0.39	0.11	0.04	0.02		1.65	0.06	0.39	
	CU1003 (9-18-14)	**Average**	**76.22**	**0.10**	**13.83**	**0.90**	**0.04**	**0.27**	**1.72**	**4.43**	**2.40**	**0.05**	**0.08**	**100**	**5.17**	**1.16**	**6.83**	**15**
		StDev	0.31	0.01	0.15	0.03	0.01	0.02	0.06	0.29	0.05	0.03	0.02		0.80	0.04	0.27	
	CU1004	**Average**	**76.66**	**0.10**	**13.54**	**0.86**	**0.03**	**0.26**	**1.59**	**4.43**	**2.40**	**0.07**	**0.08**	**100**	**5.47**	**1.11**	**6.84**	**16**
		StDev	0.33	0.01	0.25	0.04	0.01	0.02	0.06	0.24	0.06	0.05	0.01		1.23	0.05	0.27	
	CU1005	**Average**	**75.85**	**0.13**	**13.89**	**1.01**	**0.04**	**0.33**	**1.61**	**4.58**	**2.43**	**0.06**	**0.09**	**100**	**5.28**	**1.34**	**7.01**	**16**
		StDev	0.43	0.02	0.21	0.05	0.02	0.04	0.07	0.33	0.11	0.03	0.03		1.46	0.09	0.34	
	CU1006	**Average**	**76.53**	**0.12**	**13.67**	**0.96**	**0.03**	**0.27**	**1.56**	**4.30**	**2.45**	**0.05**	**0.08**	**100**	**4.93**	**1.23**	**6.75**	**13**
		StDev	0.49	0.02	0.23	0.05	0.01	0.03	0.08	0.35	0.11	0.03	0.01		0.97	0.07	0.32	

Mullineaux reference samples (Figure 3 A-C), Cy has greater Na2O+K2O and less FeO than Cw and Cs. Cw and Cs overlap. Cb and Ct have broader compositional ranges.

Pub. ID	Sample Number		SiO2	TiO2	Al2O3	FeO	MnO	MgO	CaO	Na2O	K2O	P2O5	Cl	Total	H2O diff	FeO+ MgO	Na2O+ K2O	n
Mullineaux's Road 90 Site																		
	CU1007	**Average**	**76.15**	**0.10**	**13.96**	**0.91**	**0.04**	**0.28**	**1.68**	**4.26**	**2.46**	**0.10**	**0.09**	**100**	**4.95**	**1.19**	**6.72**	**13**
		StDev	0.26	0.01	0.17	0.03	0.02	0.01	0.03	0.25	0.06	0.04	0.01		0.66	0.03	0.22	
	CU1008	**Average**	**76.55**	**0.11**	**13.69**	**0.89**	**0.03**	**0.27**	**1.65**	**4.27**	**2.40**	**0.06**	**0.09**	**100**	**5.25**	**1.09**	**6.25**	**15**
		StDev	0.34	0.02	0.15	0.03	0.01	0.01	0.04	0.29	0.05	0.02	0.01		0.98	0.29	1.69	
	CU1009	**Average**	**76.35**	**0.10**	**13.79**	**0.89**	**0.03**	**0.28**	**1.52**	**4.42**	**2.50**	**0.05**	**0.08**	**100**	**4.64**	**1.17**	**6.92**	**15**
		StDev	0.31	0.02	0.13	0.04	0.02	0.04	0.04	0.38	0.07	0.02	0.01		0.56	0.07	0.40	
	CU1010	**Average**	**75.79**	**0.14**	**13.94**	**1.03**	**0.04**	**0.33**	**1.65**	**4.50**	**2.44**	**0.06**	**0.09**	**100**	**4.71**	**1.36**	**6.95**	**17**
		StDev	0.43	0.03	0.24	0.05	0.03	0.03	0.06	0.35	0.10	0.02	0.02		1.02	0.07	0.38	
Mullineaux's Muddy River Quarry site																		
	CU1011	**Average**	**76.14**	**0.12**	**13.73**	**0.91**	**0.05**	**0.29**	**1.70**	**4.45**	**2.50**	**0.05**	**0.08**	**100**	**4.54**	**1.20**	**6.95**	**14**
		StDev	0.46	0.03	0.19	0.06	0.01	0.04	0.11	0.29	0.08	0.02	0.01		0.49	0.09	0.27	
	CU1012	**Average**	**76.35**	**0.08**	**13.86**	**0.88**	**0.03**	**0.27**	**1.72**	**4.24**	**2.47**	**0.04**	**0.07**	**100**	**5.12**	**1.15**	**6.71**	**16**
		StDev	0.21	0.01	0.18	0.04	0.02	0.01	0.05	0.24	0.07	0.01	0.01		0.81	0.04	0.24	
	CU1013	**Average**	**76.50**	**0.09**	**13.75**	**0.88**	**0.02**	**0.28**	**1.74**	**4.20**	**2.44**	**0.02**	**0.08**	**100**	**7.29**	**1.17**	**6.64**	**10**
		StDev	0.32	0.01	0.24	0.04	0.02	0.01	0.02	0.28	0.03	0.02	0.02		1.00	0.04	0.26	
	CU1014b	**Average**	**76.50**	**0.09**	**13.67**	**0.88**	**0.03**	**0.27**	**1.71**	**4.37**	**2.39**	**0.03**	**0.07**	**100**	**7.28**	**1.16**	**6.76**	**10**
		StDev	0.31	0.02	0.15	0.02	0.01	0.02	0.04	0.31	0.04	0.02	0.01		0.87	0.03	0.29	
	CU1015	**Average**	**76.34**	**0.09**	**13.69**	**0.84**	**0.04**	**0.27**	**1.87**	**4.43**	**2.33**	**0.03**	**0.09**	**100**	**5.83**	**1.11**	**6.77**	**13**
		StDev	0.25	0.02	0.13	0.03	0.01	0.01	0.14	0.17	0.06	0.01	0.03		1.22	0.04	0.16	
	CU1016	**Average**	**76.55**	**0.10**	**13.67**	**0.85**	**0.04**	**0.28**	**1.67**	**4.43**	**2.31**	**0.03**	**0.09**	**100**	**5.50**	**1.13**	**6.75**	**15**
		StDev	0.31	0.02	0.16	0.04	0.02	0.01	0.05	0.35	0.04	0.02	0.02		0.77	0.04	0.35	
	CU1017	**Average**	**76.27**	**0.10**	**13.77**	**0.95**	**0.05**	**0.32**	**1.73**	**4.20**	**2.47**	**0.09**	**0.08**	**100**	**5.58**	**1.26**	**6.66**	**11**
		StDev	0.49	0.02	0.18	0.06	0.02	0.04	0.08	0.36	0.33	0.04	0.01		0.57	0.09	0.31	
	CU1018	**Average**	**75.81**	**0.12**	**14.26**	**1.10**	**0.04**	**0.35**	**1.80**	**4.09**	**2.29**	**0.08**	**0.09**	**100**	**6.44**	**1.45**	**6.38**	**8**
		StDev	0.58	0.03	0.49	0.09	0.02	0.03	0.10	0.49	0.08	0.03	0.02		1.64	0.12	0.43	
	CU1019b	**Average**	**79.32**	**0.19**	**12.03**	**1.02**	**0.06**	**0.21**	**0.77**	**3.61**	**2.63**	**0.08**	**0.12**	**100**	**4.40**	**1.23**	**6.24**	**8**
		StDev	1.07	0.01	0.53	0.10	0.02	0.03	0.18	0.50	0.12	0.02	0.02		1.07	0.12	0.42	
	CU1020	**Average**	**74.40**	**0.15**	**15.45**	**0.83**	**0.03**	**0.18**	**2.27**	**4.81**	**1.77**	**0.05**	**0.08**	**100**	**3.56**	**1.01**	**6.58**	**9**
		StDev	1.71	0.02	1.20	0.13	0.02	0.07	0.52	0.54	0.41	0.01	0.02		1.08	0.19	0.46	
	CU1021	**Average**	**75.64**	**0.13**	**13.85**	**1.40**	**0.06**	**0.39**	**1.26**	**3.59**	**3.46**	**0.11**	**0.15**	**100**	**5.19**	**1.53**	**6.04**	**6**
		StDev	0.61	0.04	0.23	0.47	0.03	0.16	0.34	0.57	1.20	0.05	0.08		0.54	0.88	2.74	
Mullineaux's Road 81 Site																		
	CU1022	**Average**	**75.88**	**0.15**	**14.08**	**1.13**	**0.04**	**0.36**	**1.62**	**4.25**	**2.37**	**0.06**	**0.09**	**100**	**6.46**	**1.49**	**6.62**	**17**
		StDev	0.40	0.02	0.34	0.06	0.02	0.03	0.08	0.38	0.07	0.02	0.04		1.92	0.08	0.39	
	CU1023	**Average**	**75.72**	**0.17**	**13.96**	**1.07**	**0.04**	**0.34**	**1.65**	**4.39**	**2.53**	**0.06**	**0.08**	**100**	**5.98**	**1.41**	**6.93**	**15**
		StDev	0.57	0.04	0.27	0.13	0.02	0.06	0.09	0.32	0.10	0.03	0.04		1.46	0.18	0.31	

Pub. ID	Sample Number		SiO2	TiO2	Al2O3	FeO	MnO	MgO	CaO	Na2O	K2O	P2O5	Cl	Total	H2O diff	FeO+ MgO	Na2O+ K2O	n
Cape Horn Creek																		
	CU1024	**Average**	**76.58**	**0.10**	**13.63**	**0.98**	**0.04**	**0.23**	**1.51**	**4.19**	**2.65**	**0.03**	**0.09**	**100**	**0.00**	**1.21**	**6.84**	**13**
		StDev	0.47	0.02	0.25	0.06	0.02	0.02	0.08	0.32	0.14	0.02	0.03		0.00	0.07	0.32	
Green Mountain Site																		
	CU1029	**Average**	**76.56**	**0.10**	**13.77**	**0.95**	**0.03**	**0.27**	**1.59**	**4.16**	**2.46**	**0.05**	**0.07**	**100**	**4.85**	**1.14**	**6.15**	**13.00**
		StDev	0.31	0.02	0.22	0.10	0.01	0.03	0.09	0.12	0.10	0.04	0.01		1.14	0.34	1.78	
Near Mullineaux's Swift Site																		
	CU1031	**Average**	**76.87**	**0.17**	**13.38**	**1.06**	**0.04**	**0.31**	**1.49**	**3.99**	**2.53**	**0.06**	**0.14**	**100**	**4.88**	**1.38**	**6.52**	**15**
		StDev	0.35	0.01	0.13	0.04	0.02	0.01	0.03	0.39	0.08	0.02	0.02		0.79	0.04	0.37	
Summer Lake, OR																		
Cy	**CU1032/CU1033**	**Average**	**76.53**	**0.10**	**13.64**	**0.87**	**0.04**	**0.25**	**1.54**	**4.26**	**2.64**	**0.06**	**0.08**	**100**	**5.30**	**1.13**	**6.90**	**16**
		StDev	0.16	0.02	0.26	0.05	0.02	0.02	0.03	0.31	0.15	0.03	0.01		0.80	0.06	0.31	
	CU1034	**Average**	**76.78**	**0.11**	**13.66**	**0.97**	**0.05**	**0.25**	**1.56**	**3.87**	**2.67**	**0.03**	**0.08**	**100**	**5.59**	**1.21**	**6.53**	**9**
		StDev	0.43	0.01	0.27	0.09	0.01	0.01	0.04	0.53	0.09	0.01	0.01		0.42	0.09	0.49	
Carp Lake, Washington																		
	CU1108	**Average**	**77.01**	**0.11**	**13.92**	**0.88**	**0.04**	**0.26**	**1.66**	**3.56**	**2.48**	**0.02**	**0.08**	**100**	**5.16**	**1.14**	**6.05**	**10**
		StDev	0.14	0.01	0.12	0.03	0.02	0.01	0.05	0.15	0.04	0.02	0.01		0.56	0.04	0.14	
	CU1109	**Average**	**77.47**	**0.10**	**13.92**	**0.99**	**0.03**	**0.24**	**1.58**	**3.03**	**2.56**	**0.02**	**0.08**	**100**	**6.78**	**1.23**	**5.59**	**12**
		StDev	0.57	0.02	0.19	0.04	0.03	0.01	0.08	0.58	0.13	0.02	0.01		1.06	0.04	0.67	
	CU1110	**Average**	**78.09**	**0.13**	**13.05**	**0.91**	**0.01**	**0.19**	**1.30**	**3.55**	**2.61**	**0.08**	**0.08**	**100**	**3.97**	**1.10**	**6.16**	**16**
		StDev	1.56	0.02	0.70	0.10	0.03	0.05	0.62	0.61	0.34	0.22	0.02		0.78	0.14	0.44	
	CU1111	**Average**	**75.42**	**0.13**	**14.45**	**1.18**	**0.02**	**0.38**	**1.86**	**4.27**	**2.15**	**0.07**	**0.09**	**100**	**5.29**	**1.56**	**6.41**	**10.00**
		StDev	0.46	0.02	0.19	0.07	0.02	0.03	0.08	0.33	0.07	0.03	0.02		0.68	0.09	0.35	
	CU1176-CU1179	**Average**	**77.05**	**0.11**	**13.65**	**0.92**	**0.03**	**0.27**	**1.64**	**3.88**	**2.36**	**0.04**	**0.08**	**100**	**6.96**	**1.19**	**6.24**	**45**
		StDev	0.78	0.04	0.41	0.07	0.03	0.03	0.09	0.62	0.35	0.03	0.02		1.69	0.07	0.74	
	CU1181	**Average**	**76.56**	**0.12**	**13.75**	**0.97**	**0.03**	**0.28**	**1.73**	**4.14**	**2.32**	**0.04**	**0.09**	**100**	**6.74**	**1.25**	**6.46**	**18**
		StDev	0.81	0.04	0.27	0.07	0.02	0.01	0.07	0.60	0.29	0.04	0.02		1.05	0.07	0.78	
	CU1185	**Average**	**76.81**	**0.11**	**13.52**	**0.96**	**0.03**	**0.24**	**1.67**	**4.12**	**2.44**	**0.05**	**0.07**	**100**	**6.08**	**1.20**	**6.56**	**11**
		StDev	0.35	0.02	0.30	0.08	0.02	0.05	0.12	0.23	0.09	0.03	0.02		1.88	0.12	0.24	
	CU1186	**Average**	**76.89**	**0.10**	**13.50**	**0.78**	**0.04**	**0.16**	**1.49**	**4.15**	**2.81**	**0.04**	**0.07**	**100**	**4.18**	**0.87**	**6.38**	**11**
		StDev	0.73	0.02	0.50	0.11	0.03	0.07	0.22	0.33	0.38	0.02	0.01		1.05	0.32	2.03	
	CU1199	**Average**	**76.61**	**0.09**	**13.49**	**1.06**	**0.04**	**0.23**	**1.56**	**4.21**	**2.61**	**0.04**	**0.09**	**100**	**5.85**	**1.13**	**5.96**	**14**
		StDev	0.31	0.02	0.23	0.05	0.02	0.01	0.07	0.35	0.07	0.03	0.02		1.19	0.44	2.35	
	CU1200	**Average**	**76.33**	**0.10**	**13.61**	**1.21**	**0.02**	**0.24**	**1.61**	**4.21**	**2.56**	**0.06**	**0.07**	**100**	**5.55**	**1.45**	**6.77**	**13**
		StDev	0.46	0.03	0.31	0.10	0.01	0.01	0.10	0.30	0.10	0.05	0.01		1.27	0.11	0.28	
	CU1201	**Average**	**76.72**	**0.12**	**13.67**	**0.81**	**0.03**	**0.16**	**1.74**	**4.11**	**2.53**	**0.05**	**0.08**	**100**	**4.54**	**0.96**	**6.64**	**14**
		StDev	1.28	0.02	1.01	0.13	0.03	0.06	0.43	0.35	0.27	0.03	0.02		1.30	0.18	0.29	
	CU1202	**Average**	**76.07**	**0.15**	**13.96**	**1.24**	**0.04**	**0.38**	**1.77**	**3.93**	**2.33**	**0.06**	**0.09**	**100**	**5.32**	**1.61**	**6.26**	**8**
		StDev	0.74	0.02	0.39	0.09	0.03	0.06	0.12	0.54	0.19	0.03	0.02		2.06	0.14	0.42	

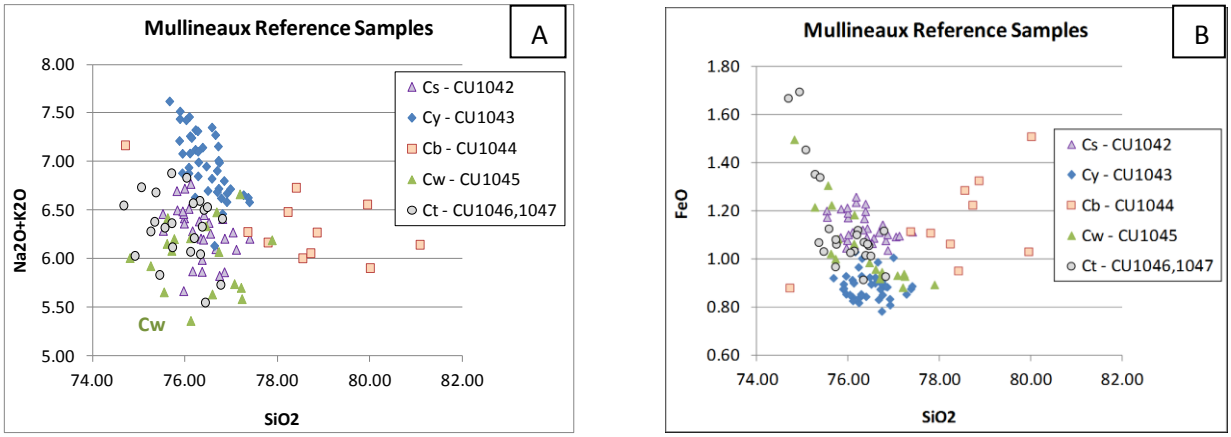
Pub. ID	Sample Number		SiO2	TiO2	Al2O3	FeO	MnO	MgO	CaO	Na2O	K2O	P2O5	Cl	Total	H2O diff	FeO+ MgO	Na2O+ K2O	n
	CU1203	**Average**	**76.43**	**0.12**	**13.71**	**1.06**	**0.03**	**0.31**	**1.72**	**4.08**	**2.40**	**0.08**	**0.07**	**100**	**5.41**	**1.05**	**4.98**	**10**
		StDev	0.23	0.02	0.21	0.07	0.03	0.02	0.05	0.26	0.09	0.09	0.01		0.70	0.60	2.85	
	CU1204	**Average**	**76.12**	**0.14**	**13.85**	**1.11**	**0.04**	**0.31**	**1.91**	**4.05**	**2.34**	**0.06**	**0.08**	**100**	**4.39**	**1.16**	**5.23**	**9**
		StDev	0.58	0.03	0.30	0.06	0.03	0.03	0.10	0.24	0.10	0.03	0.01		0.60	0.58	2.59	
	CU1205	**Average**	**76.23**	**0.13**	**13.79**	**1.07**	**0.06**	**0.32**	**1.77**	**4.13**	**2.37**	**0.07**	**0.07**	**100**	**4.53**	**1.11**	**5.20**	**8**
		StDev	0.36	0.06	0.23	0.04	0.03	0.01	0.05	0.28	0.07	0.03	0.01		0.68	0.59	2.75	
	CU1206	**Average**	**75.47**	**0.15**	**14.09**	**1.41**	**0.06**	**0.38**	**1.92**	**4.18**	**2.21**	**0.06**	**0.09**	**100**	**6.11**	**1.38**	**4.91**	**10**
		StDev	0.30	0.06	0.29	0.12	0.02	0.02	0.07	0.25	0.06	0.03	0.02		0.99	0.79	2.81	
	CU1207 (3-30-14)	**Average**	**75.38**	**0.11**	**14.39**	**1.20**	**0.03**	**0.39**	**1.91**	**4.19**	**2.26**	**0.07**	**0.09**	**100**	**6.17**	**1.19**	**4.84**	**9**
		StDev	0.36	0.02	0.11	0.06	0.02	0.02	0.10	0.38	0.06	0.03	0.03		0.80	0.72	2.93	
	CU1207 (10-31-12)	**Average**	**75.19**	**0.18**	**14.32**	**1.32**	**0.07**	**0.37**	**2.05**	**4.18**	**2.22**	**0.02**	**0.08**	**100**	**3.82**	**1.23**	**4.65**	**8**
		StDev	0.40	0.06	0.26	0.22	0.05	0.03	0.08	0.42	0.07	0.03	0.01		1.97	0.81	3.00	
	CU1208	**Average**	**75.78**	**0.14**	**14.29**	**1.19**	**0.05**	**0.36**	**1.90**	**3.88**	**2.26**	**0.075**	**0.10**	**100**	**5.44**	**1.55**	**6.14**	**14**
		StDev	0.55	0.02	0.21	0.11	0.03	0.02	0.09	0.48	0.05	0.039	0.04		1.08	0.12	0.48	
	CU1209	**Average**	**75.27**	**0.15**	**14.33**	**1.45**	**0.11**	**0.40**	**1.90**	**3.77**	**2.24**	**0.317**	**0.08**	**100**	**3.51**	**1.59**	**5.15**	**6**
		StDev	0.57	0.03	0.23	0.13	0.16	0.02	0.06	0.45	0.03	0.627	0.01		1.54	0.71	2.31	
	CU1210	**Average**	**75.59**	**0.14**	**14.19**	**1.29**	**0.03**	**0.36**	**1.82**	**4.12**	**2.33**	**0.048**	**0.09**	**100**	**6.92**	**1.65**	**6.45**	**13**
		StDev	0.28	0.02	0.09	0.06	0.02	0.02	0.10	0.30	0.09	0.026	0.02		0.99	0.07	0.24	
	CU1212	**Average**	**78.15**	**0.11**	**12.72**	**0.87**	**0.04**	**0.17**	**1.43**	**3.83**	**2.56**	**0.062**	**0.09**	**100**	**5.89**	**0.98**	**6.01**	**16**
		StDev	1.50	0.03	1.05	0.12	0.04	0.033	0.41	0.45	0.41	0.054	0.02		2.18	0.28	1.62	
	CU1213	**Average**	**76.29**	**0.10**	**13.67**	**1.07**	**0.04**	**0.31**	**1.84**	**4.14**	**2.40**	**0.08**	**0.08**	**100**	**6.27**	**1.38**	**6.54**	**12**
		StDev	2.07	0.03	1.38	0.13	0.03	0.04	0.74	0.50	0.33	0.09	0.02		2.43	0.48	2.26	
	CU1214	**Average**	**76.11**	**0.14**	**13.92**	**1.19**	**0.03**	**0.34**	**1.81**	**4.06**	**2.31**	**0.03**	**0.08**	**100**	**5.97**	**1.27**	**5.31**	**10**
		StDev	0.69	0.03	0.46	0.13	0.02	0.04	0.12	0.30	0.23	0.01	0.02		1.81	0.61	2.52	
	CU1215	**Average**	**76.07**	**0.13**	**13.80**	**1.16**	**0.02**	**0.33**	**1.71**	**4.20**	**2.43**	**0.06**	**0.10**	**100**	**6.39**	**1.06**	**4.74**	**5**
		StDev	0.44	0.01	0.22	0.04	0.01	0.04	0.11	0.04	0.08	0.03	0.05		1.31	0.73	3.24	
	CU1216	**Average**	**75.47**	**0.13**	**14.26**	**1.14**	**0.04**	**0.37**	**1.85**	**4.24**	**2.35**	**0.06**	**0.08**	**100**	**3.45**	**1.24**	**5.40**	**9**
		StDev	0.24	0.02	0.17	0.06	0.04	0.03	0.09	0.22	0.08	0.03	0.02		1.25	0.62	2.68	
	CU1217	**Average**	**75.32**	**0.14**	**14.21**	**1.25**	**0.05**	**0.37**	**1.87**	**4.48**	**2.17**	**0.08**	**0.08**	**100**	**4.08**	**1.35**	**5.54**	**5**
		StDev	0.58	0.03	0.28	0.10	0.02	0.03	0.15	0.14	0.05	0.04	0.01		0.65	0.67	2.72	
	CU1218	**Average**	**75.14**	**0.13**	**14.10**	**1.29**	**0.06**	**0.41**	**1.95**	**4.69**	**2.13**	**0.05**	**0.08**	**100**	**3.46**	**5.00**	**5.68**	**5**
		StDev	0.23	0.02	0.15	0.02	0.04	0.08	0.06	0.15	0.09	0.06	0.02		0.57	0.00	2.79	
	CU1219	**Average**	**75.20**	**0.14**	**14.27**	**1.39**	**0.04**	**0.39**	**1.94**	**4.32**	**2.16**	**0.07**	**0.08**	**100**	**5.69**	**1.43**	**5.18**	**4**
		StDev	0.17	0.07	0.27	0.14	0.07	0.04	0.11	0.23	0.11	0.02	0.03		1.04	0.81	2.91	

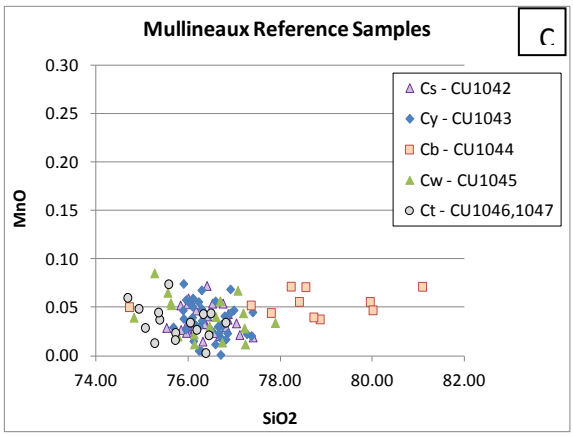
	Pub. ID	Sample Number		SiO2	TiO2	Al2O3	FeO	MnO	MgO	CaO	Na2O	K2O	P2O5	Cl	Total	H2O diff	FeO+ MgO	Na2O+ K2O	n
		CU1220	**Average**	**75.24**	**0.14**	**14.15**	**1.49**	**0.05**	**0.38**	**1.84**	**4.37**	**2.24**	**0.05**	**0.09**	**100**	**5.14**	**1.84**	**6.59**	**16**
			StDev	0.34	0.02	0.18	0.09	0.03	0.02	0.10	0.22	0.07	0.02	0.02		1.20	0.09	0.25	
		CU1221	**Average**	**76.39**	**0.15**	**13.75**	**1.18**	**0.01**	**0.34**	**1.62**	**4.12**	**2.34**	**0.05**	**0.07**	**100**	**5.26**	**1.41**	**6.00**	**13**
			StDev	0.66	0.01	0.38	0.02	0.03	0.03	0.25	0.33	0.14	0.02	0.02		0.74	0.41	1.75	
Owyhee River																			
		CU1292	**Average**	**76.50**	**0.10**	**13.68**	**0.96**	**0.04**	**0.27**	**1.69**	**4.21**	**2.45**	**0.06**	**0.07**	**100**	**5.42**	**1.18**	**6.37**	**22**
			StDev	0.38	0.01	0.24	0.05	0.01	0.02	0.06	0.32	0.08	0.02	0.01		1.19	0.26	1.42	
		CU1293	**Average**	**76.15**	**0.12**	**13.71**	**1.00**	**0.15**	**0.31**	**1.61**	**4.31**	**2.51**	**0.06**	**0.08**	**100**	**6.22**	**1.21**	**6.28**	**23**
			StDev	0.66	0.03	0.30	0.12	0.05	0.06	0.10	0.41	0.14	0.02	0.03		1.85	0.40	1.92	
		CU1294	**Average**	**76.28**	**0.12**	**13.68**	**1.05**	**0.04**	**0.29**	**1.58**	**4.25**	**2.61**	**0.04**	**0.07**	**100**	**5.29**	**1.28**	**6.55**	**21**
			StDev	0.45	0.03	0.23	0.12	0.01	0.07	0.07	0.33	0.14	0.02	0.02		1.02	0.34	1.50	

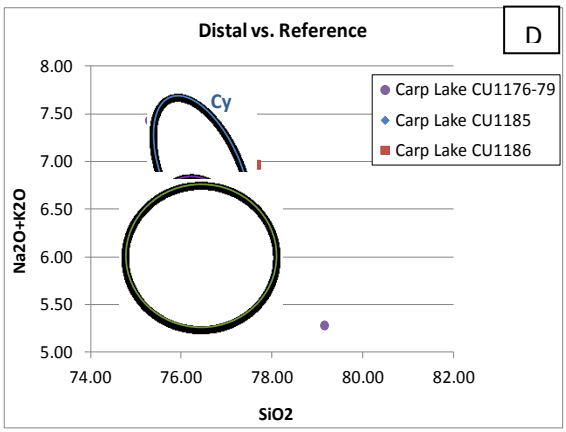
Results and Conclusions

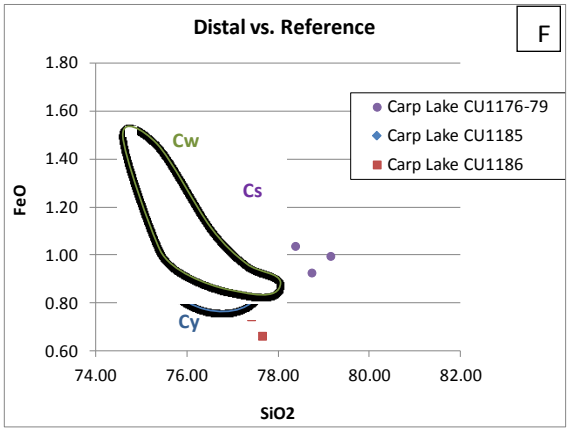
Using the ranges defined by Cy, Cw, and Cs reference samples,(Figure 3: A, B and C) some distal samples may be correlated to specific eruptions. A 35 cm layer at Carp Lake (CU1176-79) is likely Cy which may be the largest set C eruption (Figure 3: D and E). The much thinner, older CU1185 also has Cy-like chemistry and may correlate to a small eruption between Cw and Cy. The much older (~50 ka?) CU1186 has lower MgO than the others (Table 3) and may represent an eruption not yet identified at the volcano. Farther away at Summer Lake (Figure 3: F and G), a layer previously identified as Cy plots clearly in the field defined by the Cy reference sample as do two other samples from the Owyhee River. CU1293, however, has much greater MnO than any of the other samples, and may represent a previously un-described eruption (Figure 3: H).

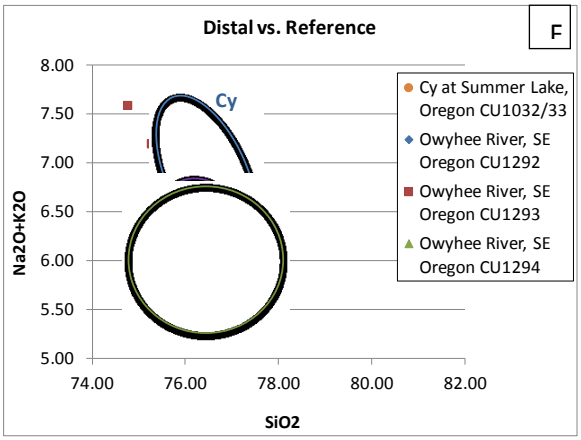
Figure 3: Plots Showing Chemical Composition of Several Analyzed Tephra Beds

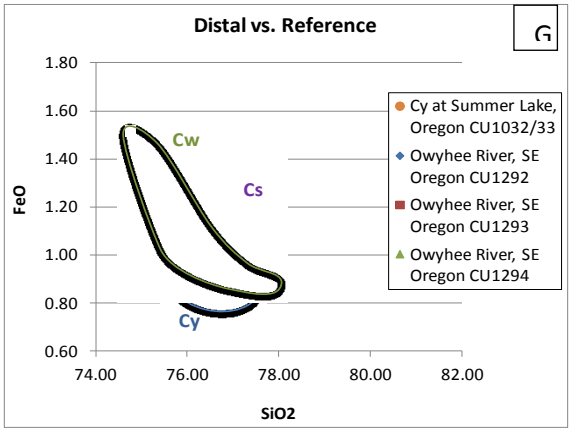


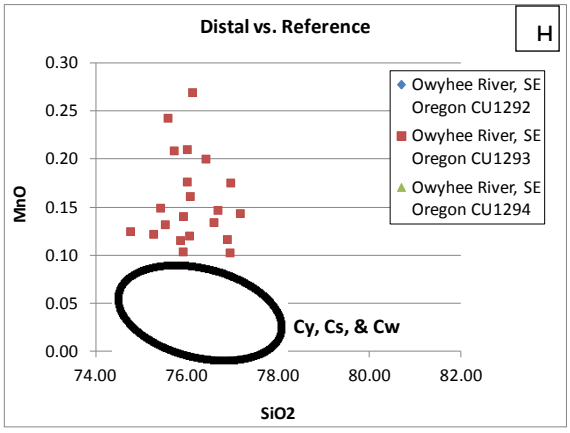












The rest of the data could be examined in a similar way to distinguish the different eruptions, better understand the eruptive history, and the distribution area for each eruption.

Some are not easily distinguishable so trace elemental data could be helpful. The mineral chemistry, in particular Fe-Ti oxides, could help distinguish eruptions. Fe-Ti oxide chemistry is sensitive to both the composition of the magma and the temperature. Eruption temperature can vary even when magma composition is the same. This has been used successfully at Mono Craters (Marcaida et al. 2014).

Acknowledgements

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Women Expatriates from Appalachia:
The Effects of Appalachian Culture on a Woman's Desire to Become an Expatriate

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Abstract

Strong family ties are a known obstacle for women when becoming expatriates. Since the importance of taking care of a family and working hard to support that family are main aspects of a woman's role in Appalachia, these strong family values and responsibilities may preclude Appalachian women more so than women in other parts of America when considering expatriate positions. This study examines the obstacles that Appalachian women face when contemplating becoming expatriates, and how they accommodate their relationships with their families once the positions are taken. It looks into the way that Appalachian culture influences women and how those influences reflect the women's feelings about working outside of the United States. The object of this study is to analyze these situations and reveal whether or not Appalachian culture truly does affect a woman's desire for and adjustment to expatriate positions.

Women Expatriates from Appalachia:
The Effects of Appalachian Culture on a Woman Becoming an Expatriate

This study includes research pertaining to women expatriates in general, what factors play a role in their decisions to become expatriates, and their adjustment to their new positions. With family ties being one of those factors, Appalachian women may be faced with larger barriers than women of other cultures when taking expatriate positions. In regards to Appalachian women becoming expatriates, this study also includes research about the woman's role in Appalachian culture.

The Problem

Appalachian culture traditionally places the woman in the position of caretaker in a family. She is expected to take care of her children, her significant other, and usually her parents and other family members, as well. Although times have changed, and women are now more active in the work force, they are still hesitant to move away from the Mountains for their careers. Why? Because they have an understood responsibility of looking after their families. And to uproot them from familiarity is usually frowned upon – especially by traditional, culture-rich Appalachian families. (Oberhouser 1995)

From studies about the role of the woman in a family, it has been concluded that these responsibilities do in fact take a toll on a woman's decision to become an expatriate. Since a woman's responsibility to take care of her family is considered significantly more important in Appalachian culture than in other cultures in America (Hall 1979), does that mean that women of Appalachia are faced with a larger burden of obstacles when deciding to become an expatriate? And if she does decide to move into an expatriate position, is it harder for her to adjust her relationship with her family than it is for other women across the U.S.?

Purpose of Study

The purpose of this study is to examine whether or not Appalachian women feel that they are placed in a higher position of responsibility for their families than other cultures in America, and whether or not these responsibilities profoundly affect their decisions to become expatriates. This study also aims to explore whether or not strong family ties greatly affect Appalachian women's adjustments to expatriate positions.

Review of Literature

To properly acquire all needed knowledge to research this topic, articles based on both business and Appalachian Studies subjects were read. These articles include the topics of the glass ceiling, international expatriates, the struggles that women face when balancing life and work, and the role of women in Appalachia.

Insch, McIntyre, and Napier (2008) examined the second layer of the glass ceiling, also known as the expatriate glass ceiling. The glass ceiling is the name of the invisible barrier that women and minorities face when attempting to move up in an organization. The authors of this article concluded that, in addition to the more well-known, first layer of the glass ceiling, a second layer also exists. This

layer is believed to prevent women executives from moving higher into management and, therefore, prevents them from moving into positions that offer or require foreign assignments. This prevention is due in part to the normal glass ceiling issues, such as gender stereotyping and the hesitance of moving women into top management positions, as well as other issues, such as family responsibilities and personal unwillingness of women to take higher positions. Whether or not a woman is from Appalachia, she will most likely face the obstacle of both layers of the glass ceiling when attempting to secure an international assignment.

Ma¨kela¨, Suutari, and Mayerhofer (2011) researched the conflicts and enrichment that women experience when participating in international assignments. The authors discussed how family roles and relationships have an impact on a woman's adjustment to expatriate positions. The article explains that, for women, family obligations can cause negative effects while on an assignment. For example, a woman's family may be unwilling to relocate to a new country, or, if a woman's family does relocate, the family may not adjust well. Another obvious obstacle of a woman's family ties while expatriating is being away from her family if they do not relocate with her on her assignment. These conflicts could put a mental strain on her, because, as a woman, her motherly role and sense of responsibility are very important, therefore, if these conflicts arise, she will have these personal problems to deal with, along with the stress and obligations of her expatriate position. This article stated that women who do not have such strong family relationships and obligations are more willing to become expatriates. From this article, it is evident that a woman's strong family ties are a large obstacle for her adjustment to an expatriate position, as well as hindering a woman's initial desire to even become an expatriate.

While reading about the obstacles that women, in general, face when becoming expatriates, some readings of Appalachian culture and of women's roles in Appalachia were also included in the research. This information helps to put the expected role of women in Appalachian culture into perspective, in relation to becoming international expatriates.

Oberhouser (1995) studied gendered regional geography, and about how and where Appalachian women are expected to work. This article states that the traditional expectations of Appalachian women in the household and the workplace have confined women to working in inferior positions in both of the aforementioned settings. In the article, a historical overview of Appalachian culture is given. It explains that, historically and traditionally, women in Appalachia have been expected to stay at home to look after the household and the family, while the husband is away at work. Sometimes, the women would sell homemade goods from the farm for profit. This was the women's first move into the workforce. In later years, women gradually moved into the workforce by working in entry-level positions and were normally expected to work in service positions, to assist workers in higher positions, which were usually obtained by men.

Lastly, in a unique interview, Jacquelyn Hall (1979) of the University of North Carolina at Chapel Hill spoke with Mary Robertson, an Appalachian woman who was active in the union movement, about her views on roles of women in Appalachia. Robertson was experienced and successful in overcoming the traditional roles of an Appalachian woman, and made an impact on the movement by being a leader and an innovator in the process. She surpassed the roles that she was expected to play as a woman of

Appalachia, and in an excerpt from her interview with Hall, she explained the reasoning and expectations for those roles. She spoke about the unusual contradiction between the way women in Appalachia take on somewhat dominant roles and the way they are still expected to serve their husbands. Robertson shared examples of men leaving the home for periods of time to work, and how the women were left at home to run the homestead and the farms, but as soon as the men returned, the women resumed their secondary, service roles to their husbands and their families. She spoke about how Appalachian women are expected to be strong and to be able to take care of themselves by performing traditionally male roles, but they are also expected to play motherly, servant roles, as well. Mary Robertson communicated, from first-hand experience, the roles and expectations of an Appalachian woman.

The articles reviewed for this research helped clarify the understanding of the two layers of the glass ceiling and the aspects of a female's experiences as an expatriate. It also brought forward the information describing what is expected of women in Appalachian culture. By collecting this information, it is easier to grasp the potentially higher level of difficulty for a woman from Appalachia to adjust to or take an expatriate position, in comparison to the difficulty for a woman from other parts of the United States.

Research Process

As a qualitative study rather than a quantitative study, the analyzed data were results from interviews with three women from the Appalachian region who were formerly expatriates. Questions asked regarded the women's family lives, in relation to their lives as expatriates, and how they felt that their culture has influenced the both of them. These questions were asked in order to determine how Appalachian culture affects a woman's desire to become and adjustment to becoming an expatriate.

Data Analysis

In the research process, three Appalachian women who were formerly expatriates were interviewed. The three women have a few things in common – they are each in the same age range, late 30s to early 40s, and, in their younger years, each of them never had a desire to relocate out of the country, but as they grew up and learned more about the world, they developed a desire to leave. But, despite their similarities, they each differed as well.

Subject 1, who worked in Singapore, Australia and Norway as an internal auditor for a global corporation, grew up having a strong family relationship. She is married with no children, and was alone on her expatriate journey. She did not decide to become an expatriate on her own; her job actually required her to become one. Although it was required, she still enjoyed her time as an expatriate, and she values the knowledge she acquired, and she attributes that appreciation to her upbringing in Appalachia. She believes that being raised in an Appalachian culture taught her to be respectful to others and to appreciate the differences that the world has to offer, since Appalachia is somewhat confined and is very traditional. Her upbringing definitely had a positive effect on her time as an expatriate.

Subject 2, who worked in Japan as an English teacher, and in the Czech Republic in an administrative position at an advertising company, had a very different experience. As a young girl, she was brought up in a somewhat traditional Appalachian family. The traditional gender roles were evident, and she had a strong relationship with her family during this time. But, with age, her relationship with her family completely diminished, pushing her to learn about the world on her own, therefore developing the desire to travel abroad. She was very eager to leave Appalachia and to see what the world had to offer. She took it upon herself to obtain an expatriate position, and she earned her education in Japan and Finland, as well. She is single with no children, and spent her expatriate journey alone. In regards to Appalachian culture and how her upbringing affected her in her expatriate positions, she felt that she was held back. She felt that the seclusion of Appalachia and the low amount of opportunities in the region, compared to the rest of the world, made an impact on her options to explore more of what the world had to offer. She had mixed feelings toward her background. She felt as if living in Appalachia gave her more of a desire to leave, because there were hardly any opportunities. Her relationship with her family gave her reason to leave, as well. But, after traveling and living abroad for a number of years, she has decided that she will someday return to Appalachia. She believes that living overseas and interacting with many different types of people have taught her to appreciate the simplicity of Appalachia.

Of the three, Subject 3 was the most traditional in her Appalachian background. Her expatriate path took her to Vienna, Austria, to work as a support coordinator for the company ABB. She was raised in a very traditional, Christian, tight-knit family and community and also held traditional motherly roles throughout her life, by taking care of her grandparents, parents, and her own child. In her younger years, she never saw herself leaving Appalachia, but as she grew older and learned more about the world, she developed a desire to explore and find new and different opportunities. After deciding to broaden her horizons, she was able to travel to Vienna, and was also accompanied by her husband. She had a very different experience than the other two, because she was not alone in her international assignment. Appalachian culture had a positive impact on Subject 3, in regards to her expatriate journey. She felt as if growing up in Appalachia helped her with her people skills, because Appalachian people are friendly and polite. It helped her in meeting new people in new places. It also, similarly to Subject 2, taught her to appreciate the simplicity of Appalachian culture.

Speaking with these three women revealed the many thoughts that go through an Appalachian woman's head when considering becoming an expatriate.

Conclusion

After conducting the necessary research and analyzing all data and information, it is evident that Appalachian culture does, in fact, have an effect on Appalachian women's adjustments to becoming expatriates, and on their desires to become expatriates, as well. There have been both positive and negative types of impact on these women, stemming from being raised in Appalachia. In some ways, their backgrounds helped them cope and adjust well, and in others, it held them back and made things more difficult. From speaking with these three women and learning about their experiences, it is easy to determine that it is likely that other women expatriates from Appalachia feel the same way about their

culture and how it has impacted their expatriate journeys. Whether it be positive or negative, Appalachian culture will, in some way, impact an Appalachian woman's expatriate experience.

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The Effect of Oleic Acid Concentration on Cholesterol Uptake in the Larval Zebrafish Enterocyte

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Abstract

High levels of cholesterol contribute to the development of diseases such as diabetes mellitus, heart disease, and atherosclerosis. The presence of fatty acids, specifically oleic acid, has been shown to facilitate the entry of dietary cholesterol into enterocyte cells in the intestine. *Danio rerio*, or zebrafish, is an excellent model organism for studying intestinal lipid and cholesterol absorption in vivo. Two potential mechanisms for cholesterol uptake in the presence of oleic acid into the enterocyte were proposed: a stoichiometric uptake or a signaling threshold. To differentiate between these two models, larval zebrafish were fed diets of 1000 μM, 100 μM, 10 μM, and 1 μM of oleic acid added to low fat diets with equal amounts of native and TopFluor-Chlolesterol (TF-Chl) available. After a 5.5 hour incubation, a lipid extraction was performed and the results were developed on a thin layer chromatography (TLC). The TLC plates were imaged with UV light and analyzed using the Gel Analyzer 2010 software to determine pixel intensity of the separation bands. To determine the quantity of TF-Chl that had been absorbed into enterocytes, the amount of esterified TF-Chl was quantified. Raw Volume results showed that 100 μM Oleic Acid had the highest uptake of esterified TF-Chl. The trend of uptake between 100 μM and 1 μM appears stoichiometric. However, finer intervals of oleic acid concentration between 0 μM and 1 μM are needed to fully support the stoichiometric model or rule out the signaling threshold hypothesis.

The Effects of Oleic Acid Concentration on Cholesterol Absorption in the Larval Zebrafish Enterocyte

Introduction

The United States has seen a surge in diseases caused by the Western diet over the last 30 years (Cordain et al., 2005). Lipid disorders, also known as dylipidemias, and high cholesterol are contributing factors that have led to the increasing occurrences of diseases such as diabetes mellitus, heart disease, and atherosclerosis. Hyperglycemia causes high levels of triglycerides and lipoprotein abnormalities (Kreisberg, 1998). Cardiovascular disease accounts for nearly 40% of all deaths in the United States (Cordain, et al., 2005). High levels of absorption of dietary cholesterol increase blood levels of low density lipoprotein-cholesterol (LDLs) can form plaques leading to atherosclerosis. Athlerosclerosis is a disease caused by the hardening of arteries by plaques (Boden, 2004). High LDL levels can also lead to strokes, heart attack, and other serious health problems (Qi, 2008). Nearly one third of adults in the United States are obese, which increases their risk for many diseases and early death (Baskin, 2005). Rates of these conditions have increased with changes in the Western diet.

Since the agricultural revolution, Western diets have been characterized by processed foods that have different nutritional values than those diets previous generations. Low amounts of dietary fiber, vitamins, minerals, and high levels of dietary acid are some examples of nutritional changes (Cordain et al., 2005). One critical change brought on by the Western diet is the amount and types of fatty acids consumed and their frequency. Cattle in the United States are rapidly fattened and contain meat that contains more triglycerides in the muscle tissue than free range animals. The cattle also exhibit higher amounts of saturated fatty acids, those fatty acids known to cause heart disease (Cordain et al., 2005). Dietary cholesterol comes from meat sources, which are a staple in the American diet. These changes in the way Americans' foods are processed are one of the causes for increasing rates of disease. The Western diet contains high levels of fats and cholesterol, much of which can be absorbed readily by enterocyte cells in the small intestine. When increasing amounts of cholesterol are fed without fatty acids present, increases in LDL levels increase far less than in diets of both fatty acids and cholesterol (Woollett, 1994).

Increasing our understanding of the mechanisms by which dietary cholesterol is absorbed can aid in the development of drugs to decrease cholesterol levels and treat dyslipidemias.

Though high levels of cholesterol are associated with disease, the body needs cholesterol and is able to produce as much as it needs. Cholesterol and its metabolites serve several vital purposes in the body, including formation of steroid hormones and integration into cell membranes (van der Wulp, 2012). In, for instance, cell membranes, cholesterol molecules prevent diffusion and reduce cell membrane fluidity (Vucic, 2015) There are several forms of cholesterol within the body, including cholesteryl esters found in VLDLs and steroidogenic tissues (Chang, 2009). Cholesteryl esters are found in low levels in the body, and high levels of cholesteryl esters in macrophages are characteristic of early atherosclerosis (Chang, 2009). Free cholesterol that is absorbed from the diet is esterified upon uptake, as free cholesterol is a toxic molecule. The Walters lab has shown that absorption of cholesterol is facilitated by the presence of dietary fatty acids, particularly oleic acid.

Dietary fatty acids are ingested in the form of triglycerides (TGs), which cannot be absorbed by the enterocyte cells of the intestine until they are broken down by pancreatic lipases. The three fatty acids attached to the glycerol backbone of TGs exist as either saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids. It is the free fatty acid form that is taken into the enterocyte cells. Diets of saturated fatty acids result in higher levels of LDL-cholesterol in the blood than diets of unsaturated fatty acids (Woollett, 1994). Long chain monounsaturated fatty acids have been shown by the Walters lab to increase cholesterol absorption. Examples of long chain fatty acids include oleic acid and linoleic acid, which are both eighteen carbon chain in length and prevalent in the American diet (Cordain et al., 2005).Acyl-coenzyme A cholesterol acyltransferases (ACAT) proteins use these same long-chain fatty acids to modify dietary cholesterol into cholesteryl esters (Chang, 2009).

Enterocytes are the polarized cells that line the intestine and absorb dietary lipids at their luminal brush border. The zebrafish enterocyte is very similar to those of mammals (Anderson, 2011). When

dietary lipids are digested, lipases such as pancreatic lipases break down TGs into free fatty acids and monoglycerides (Semova, 2012). Bile salts then incorporate fatty acids into micelles, which allow the otherwise non-polar fatty acid molecules to move from the aqueous phase of the intestinal lumen to the polar water layer of the enterocyte brush border (Tso, 2004). Micelle formation is also critical for transportation of cholesterol molecules to the enterocyte (Tso, 2004). The average American ingests 300-500 mg of cholesterol per day, and about half of those molecules are absorbed (van der Wulp, 2012). The effects of gut microbiota on absorption of dietary lipids may be unclear, but the zebrafish displays similar gut microbiota to humans, thus making studies of lipid metabolism in zebrafish more relevant to human health (Semova, 2012). Gut microbiota play a role in lipid metabolism, as zebrafish raised germ-free showed less fluorescent fatty acid absorption and lipid droplets present than fish that had gut microbiota (Carmody, 2012).

The larval zebrafish is an excellent model organism to study the effects of dietary fatty acid concentration on cholesterol absorption *in vivo*. Zebrafish studies incorporate whole body effects on absorption, including pancreatic lipases, gut microbiota, and bile salts (Semova, 2012). These factors are not replicated in *in vitro* cell culture studies. Other advantages to using the zebrafish larvae include 80% genomic similarity to humans, conserved gastrointestinal anatomy and biochemical reactions, and optical transparency (Anderson, 2011). Zebrafish deplete their yolk and are ready for exogenous feeding by 6 days post fertilization (dpf). Cholesterol transport proteins such as Niemann-Pick C 1-like-1 (NPC1L1) are found both in the human intestine and in the zebrafish intestine. Zebrafish larvae are optically transparent for thirteen dpf, as shown in Figure 1. This allows researchers to visualize development and dietary processes as shown in studies by the Walters lab. These characteristics are useful because they allow for better study of the mechanisms by which existing drugs function and for development of new drugs.

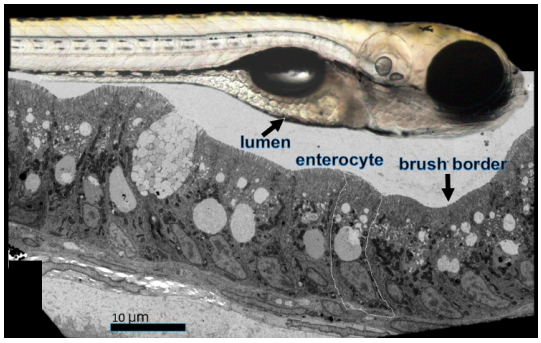

Figure 1: Zebrafish as a model organism for dietary studies.

Figure 1: An optically transparent zebrafish larvae at 6 dpf. Below the zebrafish larvae is a TEM image of the larvae small intestine, labeled with the enterocyte (outlined in white), brush border, and intestinal lumen. Many organs of the larvae are developed and visible through the body wall, including the swim bladder, eye, heart, ear, and intestine.

Current drug therapies for dyslipidemias include ezetimibe, statins, and orlistat. NPC1L1 is the target of ezetimibe drugs which inhibit cholesterol absorption in the enterocytes (Garcia-Calvo, 2005). This was shown originally in studies of mice models with NPC1L1 deficiency that showed significantly reduced cholesterol absorption no further decrease in absorption with ezetimibe treatment (Garcia-Calvo, 2005). Statins work to decrease LDL levels of plasma cholesterol by lowering synthesis of cholesterol throughout the body (Vucic, 2015). Orlistat causes lipid malabsorption, and has been shown to aid in weight loss, decrease risk for diabetes, and decrease blood pressure (Drew, 2007).

Existing drug therapies have some drawbacks, however. Some drugs that inhibit fat absorption, such as orlistat, may also decrease uptake of fat soluble vitamins like vitamin D (Drew, 2007). Statins, though very beneficial for patients with hypercholesterolemia, have adverse effects including lower hepatic function and increased risk for type 2 diabetes (Vucic, 2015). Lowering lipid levels in type 2 diabetic individuals greatly reduces their risk of coronary heart disease and heart attacks (Kreisberg, 1998). There is still a need to understand lipid and cholesterol absorption better in order to improve drug therapies.

Feeding assays performed in larval zebrafish can allow researchers to better understand the cell biology processes of lipid metabolism. To differentiate dietary cholesterol from cholesterol molecules already present in the zebrafish, feeding assays can be performed using a modified cholesterol molecule

with a fluorescent analog called Top-Fluor Cholesterol (TF-Chl). Studies from the Walters lab show that TF-Chl is metabolized similarly to native cholesterol. Structures of both TF-Chl and native cholesterol are shown in Figure 2. Furthermore, dietary cholesterol that has been absorbed can be distinguished from dietary cholesterol still present in the intestinal lumen by visualization techniques or lipid extraction and thin layer chromatography (TLC) as shown in Figure 3 (Ho, 2004). My research used larval zebrafish, TF-Chl and TLC analysis to better understand how long chain fatty acids mediate cholesterol absorption.

Figure 2: Cholesterol Types

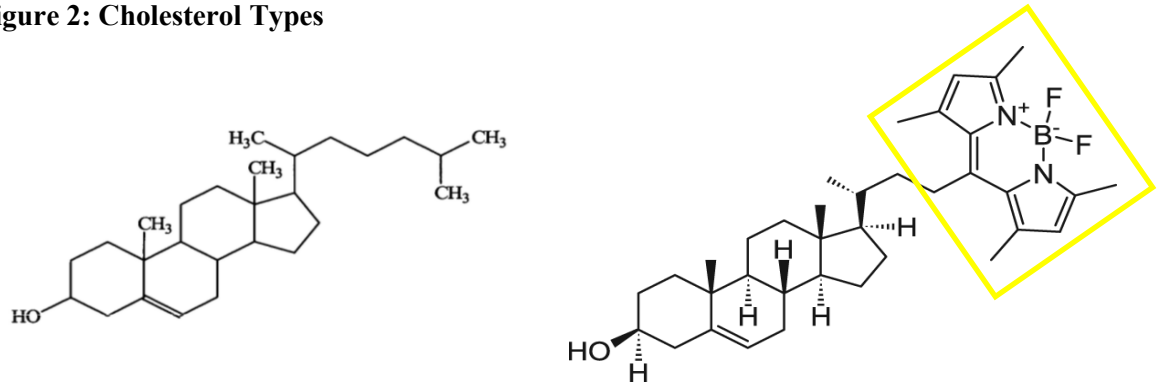

Figure 2: Forms of cholesterol used in the feeding experiments. The molecule on the left represents native cholesterol, the form found in egg yolk, whereas the molecule on the right represents TopFluor Cholesterol, a type of cholesterol that contains a fluorescent analog. The yellow box outlines the position of the fluorophore.

Figure 3: Models for Expected Results for Both Hypotheses

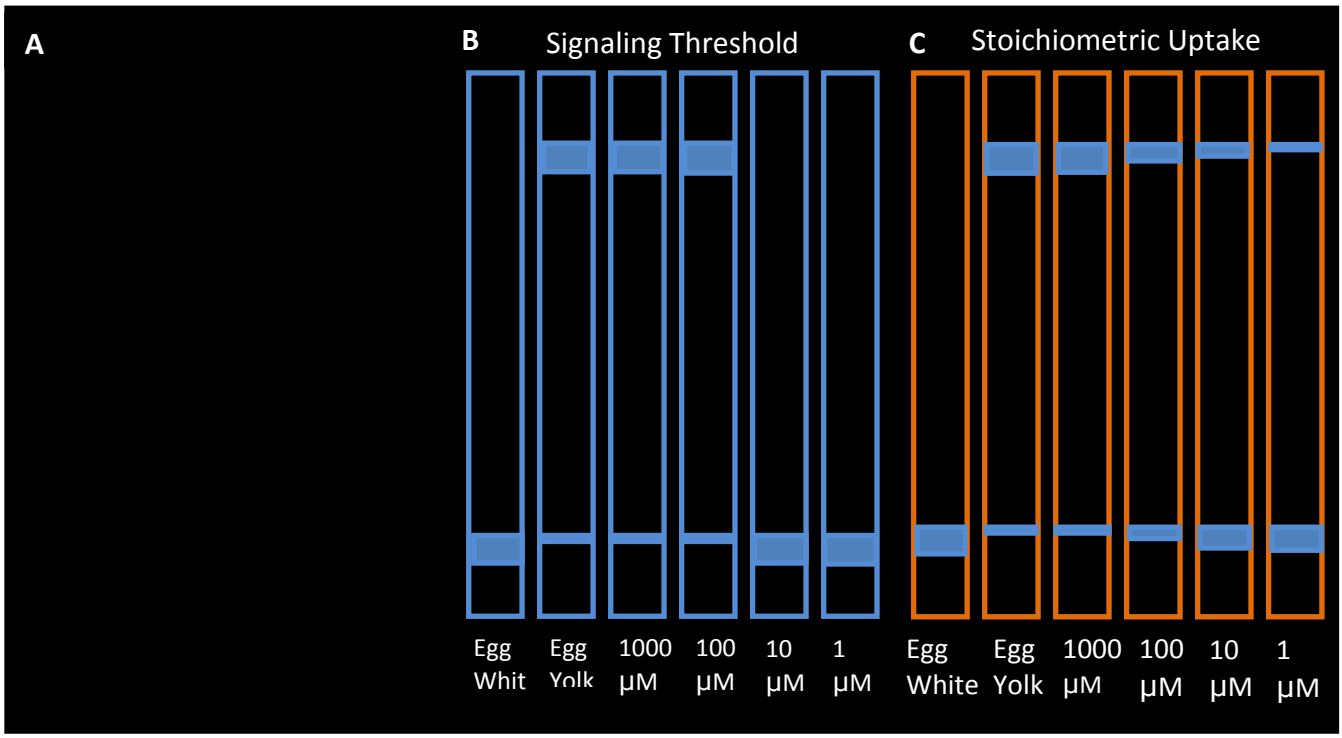

Figure 3: Comparing the expected results of the two hypotheses shows a threshold for absorption or linear relationship. A) This figure depicts the two hypotheses in a line graph. In this example, the signaling threshold is at 1 µM oleic acid. At this concentration, cholesterol absorption is maximized and sustained for higher concentrations of fatty acid. In the stoichiometric hypothesis, the concentration of esterified TopFluor Cholesterol increases as the concentration of oleic acid present increases. B) Example TLC plate for the signaling hypothesis is shown. The higher bands represent esterified TopFluor Cholesterol, whereas the lower bands represent un-esterified TopFluor Cholesterol that was not absorbed in the enterocyte and was present in the intestinal lumen. In this example of the signaling mechanism, cholesterol absorption beginning somewhere between 10 and 100 µM and being maximized and sustained at higher values of oleic acid. C) The example TLC plate for the stoichiometric or one to one absorption shows that greater amounts of esterified cholesterol (shown in the higher band) are present with higher concentrations of oleic acid.

Currently, we understand that the presence of dietary fatty acids is necessary for cholesterol absorption and that long chain fatty acids are preferential for cholesterol uptake. However, the cellular mechanism by which fatty acids mediate cholesterol absorption remains to be elucidated. One proposed mechanism for absorption is a signaling pathway, whereby long chain fatty acids act as a signaling molecule for the intracellular machinery for cholesterol uptake to translocate in the enterocyte to the luminal brush border. In this model, cholesterol absorption will begin and be maximized at the threshold and sustained at higher levels. Alternatively, the stoichiometric model states that for every molecule of cholesterol absorbed, a molecule of fatty acid must also be absorbed.

Materials and Methods

Adult zebrafish were marbled using mating cages six days prior to the feeding experiment. Embryos were harvested from mating cages, placed into petri dishes and treated with methylene blue in embryo media (EM) on the first dpf to prevent degradation from parasites. Larvae were raised in an incubator at 28.5°C and EM was refreshed each day. On the day of the feeding experiment, larvae were viewed under a microscope to ensure that their yolk was depleted and their intestines were well developed for exogenous feeding. Then, 15 larvae were placed in wells in a 12 well plate in 500 μL of EM.

Larval zebrafish were fed diets as described in Table 1. Egg white served as the negative control because egg whites are high in protein with no fatty acids. Since there are no fatty acids to induce the translocation of the NPC-1L1 cholesterol transport protein to the luminal brush border, minimal cholesterol should be taken into the cell. The positive control, egg yolk, is high in many fatty acids and cholesterol, which should facilitate absorption of TF-Chl. The experimental values of oleic acid tested were 1,000 μM OA, 100 μM OA, 10 μM OA, and 1 μM OA which reflects ten-fold dilutions. The experimental diets were prepared in bovine serum albumin, which contains no fatty acids. Native cholesterol was added to each of the dietary conditions to reflect the amount that is present in egg yolk.

Table 1: Oleic acid feeding conditions

	10% Egg White	10% Egg Yolk	2% BSA	TopFluor Cholesterol	C 18:1 1000 uM	C 18:1 100 uM	C 18:1 10 uM	C 18:1 1 uM	HEPES Buffer	Chl Stock
Egg White	500 uL	----------	----------	20 uL	----------	----------	----------	----------	50 uL	20 uL
Egg Yolk	----------	500 uL	----------	20 uL	----------	----------	----------	----------	50 uL	20 uL
1000 uM C18:1	----------	----------	500 uL	20 uL	10 uL	----------	----------	----------	50 uL	20 uL
100 uM C18:1	----------	----------	500 uL	20 uL	----------	10 uL	----------	----------	50 uL	20 uL
10 uM C18:1	----------	----------	500 uL	20 uL	----------	----------	10 uL	----------	50 uL	20 uL
1 uM C18:1	----------	----------	500 uL	20 uL	----------	----------	----------	10 uL	50 uL	20 uL

Table 1: Oleic acid dietary conditions for cholesterol absorption experiment. Egg white was used as the negative control, egg yolk is the positive control for cholesterol absorption. Experimental values of oleic acid include 1000 µM, 100 µM, 10 µM, and 1 µM. Equivalent amounts of both native and TopFluor Cholesterol were added to all conditions.

To prepare the high fat diet, an emulsion of 10% egg yolk was made by adding 1 mL of egg yolk to 9 mL of embryo media in a sterile 15 mL conical tube. The mixture was force pipetted for five minutes with a glass Pasteur pipette to create liposomes. Once mixed into the final diet, this would yield a final concentration of 5% egg yolk. For the low fat and variable diets, an emulsion of 10% egg white was made by adding 1 mL of egg white to 9 mL of embryo media in a sterile 15 mL conical tube. The mixture was force pipetted for five minutes with a Pasteur pipette to create liposomes. 2x diets were prepared as described in Table 1 in 2 mL Eppendorf tubes. The zebrafish larvae were transferred into a 12 well plate, with 15 larvae per well, in an initial volume of 500 µL of embryo media to dilute the 2x diets.

After a 5.5 hour incubation with the diet, the larvae were washed five times with embryo media to remove the diet conditions. Zebrafish were visualized under fluorescence microscopy to determine that they had eaten the dietary conditions (Figure 4). However, it cannot be determined by imaging if the TF-Chl had been taken into the enterocytes. To separate the lipids, a modified Bligh and Dyer lipid extraction was performed as described in Appendix A. The extracted lipids were dried in a speedvac for two hours

on day of extraction and stored at -80° Celsius. The results of the lipid extraction were reconstituted in chloroform on the day of the TLC, and prepared so that each lane represented a sample of six larvae. The samples were then developed on a TLC to determine the effects of each concentration on the uptake of cholesterol.

Figure 4: Visualization of fluorescent cholesterol in the larval zebrafish

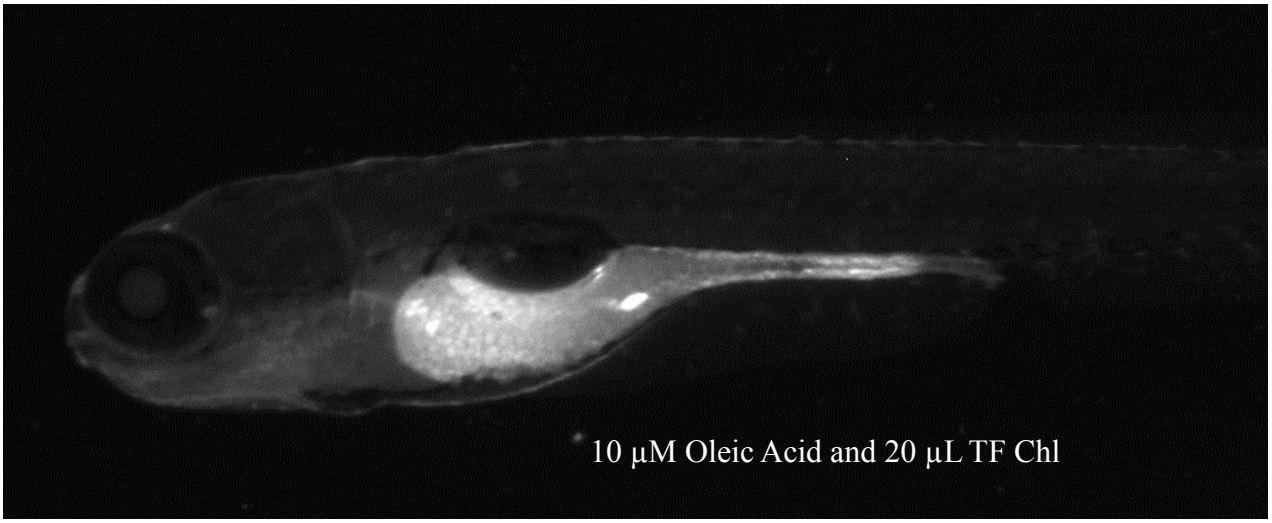

Figure 4: Fluorescent cholesterol in the zebrafish intestine. Fish representative from N3 trial with 10 µM oleic acid and 20 µL TopFluor Cholesterol diet.

Results

Three TLC plates were developed with the following conditions: N1 and N2, N3 and N4, and standards for calibration of the unproven solvent system. Each of the TLC plates were imaged with UV lighting to reveal fluorescence, then sprayed with primulin to reveal other lipids or sterols in the lane that do not fluoresce, and the images were analyzed using Gel Analyzer 2010 software. Primulin permanently stains lipids by forming a non-covalent bond with the lipids on the TLC plate and creating a complex that is detectable by UV light. This technique was done after an initial image of the plate, because the primulin-lipid complex may alter or destroy the fluorophore of the TF-Chl molecule. The primulin stain was applied to the plates as described in Appendix A.

Figure 5: Thin Layer Chromatography Analysis

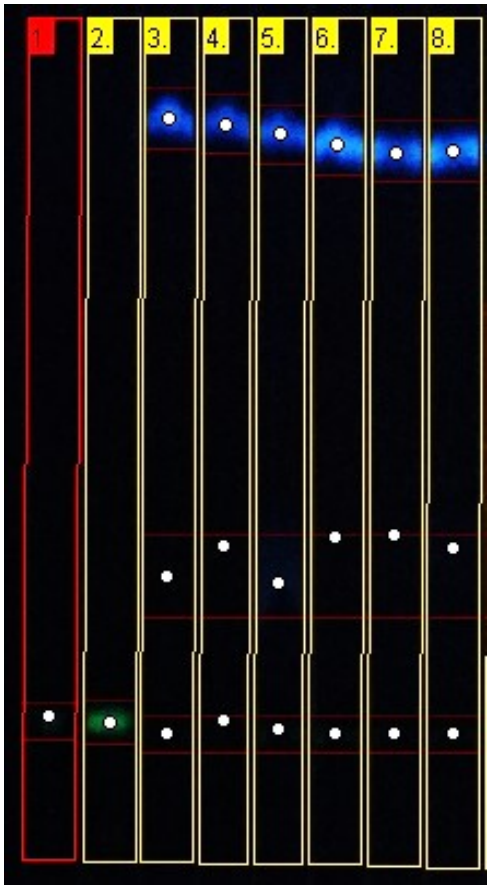

Figure 5: TLC analysis of trial N3. The upper bands contain esterified TF-Chl that had been taken into the enterocyte. The lower lanes represent unesterified TF-Chl and other lipids. The contents of each lane are as follows: 1) 1 µl TopFluor Cholesterol, 2) 5 µl TopFluor Cholesterol, 3) Egg White, 4) Egg Yolk, 5) 1000 µM oleic acid, 6) 100 µM oleic acid, 7) 10 µM oleic acid, 8) 1 µM oleic acid.

To quantify the amount of TF-Chl in each lane, boxes of equal size were drawn around the esterified TF-Chl bands. Gel Analyzer software then determined the pixel intensity for each band.

Statistical analysis was done by finding the Pearson correlation coefficient or r value. To calculate, the sum of the OA concentrations times the pixel intensity value is divided by the sum of the OA concentrations squared times the pixel intensity values squared for each trial. If the value is close to 1, it shows a strong positive correlation, whereas a value close to -1 shows a strong negative correlation. The r values are shown in Table 2. The range in these r values is due to the range of OA values tested, the few trials that were able to be performed, and statistical outliers.

Trial	R Value	P Value
N1	-0.761	0.064 (should be under 0.005)
N2	0.0639	0.322 (should be under 0.013)
N3	-0.4602	0.789 (should be under 0.023)
N4	-0.896	0.196 (should be under 0.015)

Table 2: Statistical Analysis

Table 2: Statistical analysis using Pearson correlation coefficients and P value.

Conclusions

100 μM OA showed the highest uptake of TF-Chl. In 10 μM and 1 μM, the uptake of cholesterol seems to plateau. The highest concentration of OA (1000 μM) was expected to have the highest uptake of TF-Chl, but this may have been too high of a concentration of fatty acid and may have oversaturated the cellular machinery for cholesterol uptake. This trend does not seem to correspond with the initial hypotheses, but it shows that between 100 μM and 1000 μM the fatty acid will compete with cholesterol for absorption. However, we are able to conclude that the mechanism for OA mediated cholesterol absorption does start at or below 1 μM OA.

The positive control, egg yolk, had 20 μL of native cholesterol added which was incorrect. The 20 μL of native cholesterol added to each diet was equivalent to twice the amount found in the 500 μL of 10% egg yolk. Since this was added to the egg yolk condition, it was likely supersaturated with native cholesterol. This could have prevented the uptake of TopFluor Cholesterol, which we see in our analysis. In future trials, 10 μL of the cholesterol solution should be added to each diet except the egg yolk condition.

The negative control, egg white, was expected to have little to no absorption. However, we see that TF-Chl was taken into the enterocytes. This may be due to the incubation period. In the experiment

previously conducted by Dr. James Walters, the zebrafish were fed the diets in a three hour incubation followed by immediate lipid extraction. This experiment had a four hour incubation followed by a rinse of the dietary conditions and a one and a half hour wait before the lipid extraction. This would have allowed the cells to take in cholesterol without the presence of dietary fatty acid by recruitment of intracellular fatty acids or other cholesterol uptake mechanisms.

Due to the unavailability of a chemical, a novel solvent system was used to separate the lipids in the TLC. This solvent system of 80 mL petroleum ether, 15 mL ethyl acetate, and 0.5 mL acetic acid was calibrated with the Kates' 3 solvent system of 80 mL petroleum ether, 20 mL diethyl ether, and 1 mL acetic acid solvent system as shown in Table 3. This shows that the lipids separated in a similar fashion in the novel solvent system as the proven Kates' 3 solvent system.

Table 3: TLC Solvent System Comparison

Rf Values Comparison Between Solvent Systems			
Standards:	Unproven Solvent System	Kates' 3 Solvent System	Kates' 3 Expected Value
Fatty Acids	0.79	0.518	0.39
Methyl Ester	0.065	0.057	
Cholesterol	0.563	0.625	0.19
Cholesteryl Oleate	0.032	0.024	
BODIPY C-12	0.767	0.749	
Top-Fluor Cholesterol	0.762	0.735	

Table 3: Kates' 3 solvent system compared to unproven solvent system against expected book value.

Future Directions

We are able to conclude in this experiment that OA mediated cholesterol absorption begins at under 1 μM OA, so future experiments should test a range under 1 μM, with values such as 0.2 μM, 0.4 μM, 0.6 μM, and 0.8 μM OA. This range would be chosen because the values are uniform distances apart, as opposed to the ten-fold dilutions which left large gaps between data points. Additionally, the incubation time should be reduced to three hours and the lipid extraction should immediately follow the removal of the dietary conditions. The value of native cholesterol in the egg yolk condition was the standard for native cholesterol in the other diets, so native cholesterol should not be added to the egg yolk condition. This should decrease the competition for absorption that may have caused the negative control to have higher levels of esterified TF-Chl than expected.

Appendix A

Modified Bligh & Dyer Lipid Extraction

1. Transfer larvae to a sterile 15 mL conical tube
2. To disrupt larvae, sonicate 1 mL of larvae EM for 1 minute in 4 groups of 15 second pulses. Between pulses, cool tube in wet ice bath.
3. Immediately add 1 mL of pre-mixed chloroform and methanol (1:2)
4. Repeat sonication and cooling procedure as described in step 1
5. Add 3 mL of pre-mixed chloroform and methanol (1:2) 1 mL at a time, rinsing off the sonication tip with the solvent and collecting the rinse into the conical tube.
6. Secure cap on conical tube and vortex solution for 3 minutes
7. Allow tube to sit for 15 minutes on the benchtop to settle
8. Add 1.34 mL chloroform and vortex for 3 minutes
9. Add 1.34 mL nanopure water and vortex for 3 minutes
10. Centrifuge at 5,000 rpm for 10 minutes
11. Prepare 4 newly labeled 1.5 mL Eppendorf tubes by poking small holes in the top with a needle to allow for evaporation
12. Use a glass Pasteur pipette to remove the organic layer and add to the Eppendorf tubes
13. Place tubes in speedvac and evaporate the extraction at room temperature until dry
14. Reconstitute samples in chloroform in the fume hood
15. Combine samples into a new 1.5 mL conical tube, rinsing new tubes with 20 µL of 100% chloroform and adding to final tube
16. Concentrate by evaporation in speedvac to 100 µL

Thin Layer Chromatography

1. Dried down samples should be reconstituted in chloroform in the fume hood
2. Each sample should represent six larvae
   Example: With 15 larvae per condition, the solution should be brought up to 200 µL and 80 µL should be loaded into the lane to represent 6 larvae
3. Using a micropipette, samples were dotted on to lanes of a TLC plate
4. After the plate is loaded, prepare the solvent system of 80 mL petroleum ether, 15 mL ethyl acetate, and 0.5 mL of acetic acid
5. Pour the solvent system into the glass TLC chamber
6. Place the TLC plate on a rack with a piece of blotting paper behind it to ensure that the chamber is humid
7. Lower the TLC evenly into the solvent, observing that the solvent travels up the lanes evenly
8. Place the lid on the TLC chamber and allow the solvent to migrate up until it reaches half an inch from the top of the plate
9. Remove the TLC plate and allow it to dry in the fume hood

10. Images of the TLC plates are to be taken on black backgrounds to improve contrast in UV light

Primulin Staining Protocol

1. Prepare the fume hood by hanging paper to catch excess spray and place an eight inch tall box in the fume hood for elevation of the TLC plate from the fume hood floor
2. Weigh out 0.01 grams of primulin
3. Add 0.01 grams of primulin to a mixture of 16 mL acetone to 4 mL distilled water and mix. This will give you 5% primulin in 80/20 acetone/distilled water, which is only enough solution to stain one 12 inch square TLC plate.
4. In the fume hood, spray this solution evenly on the dry TLC plate by running air into a spray atomizer and gently applying in even, careful lines across the entire TLC plate
5. View TLC plate under 365 nm UV light to show lipids. They will now fluoresce. Previously fluorescent molecules, such as BODIPY C-12 or TopFluor Cholesterol may be destroyed, so be certain to view the plate with 365 nm UV light prior to using the primulin spray.

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Absorption of Bisphenol A Affects Growth of Radish (Raphanus sativus) Plants

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ABSTRACT

Thermal receipt paper contains the highest level of bisphenol A (BPA), ranging from 3 to 22 mg/g, and contributes to more than 98% of exposure to the environment. When leached into the environment, BPA poses a potential threat to plant life. Knowledge of the potential effect of BPA on plant growth is limited. This study aims to determine the effect of BPA leached from thermal receipt paper into soil in which Radish (Raphanus sativus) seedlings are grown. In this study, the effect of three different types of paper products, thermal receipt paper containing BPA, BPA-free thermal receipt paper, and Whatman 1 filter paper, were tested. Radish seeds were grown in soil exposed to three experimental paper products for a period of seven days. There was no statistical difference between the control group and the BPA-free thermal paper group or the control group and the Whatman 1 filter paper group for the duration of the study. The BPA thermal paper group was statistically different from the control group on days 5 (t-value=3.4192, p-value=0.0057), 6 (t-value=6.1044, p-value= 0.0001), and 7(t-value=4.4549, p-value=0.0010). The root length of the BPA group was also statistically different from that of the control group (t-value=2.9193, p-value=0.0140).

INTRODUCTION

Bisphenol A (BPA) is a common organic chemical used in the synthesis of polycarbonate plastics and epoxy resins (1). BPA is also present in the manufacturing of paper products such as thermal receipt paper, magazines, mailing envelopes, and many other paper products (2). It was found that thermal receipt paper contains the highest level of BPA, ranging from 3 to 22 mg/g, among paper products and contributes to more than 98% of exposure to the environment. Thermal receipt paper consists of two layers, a standard paper layer and a thermal sensitive layer that consist of BPA, thermochromic dye, and a solvent. When the stylus of a printer heats the thermal sensitive layer above the melting point of the solvent, BPA acts as a weakly acidic developer that interacts with thermochromic dye to produce color on the paper.

There are several routes BPA can be leached into the environment where it poses a potential risk to the environment. BPA can be leached into air, water, and/or soil by various means including pollution by manufacturing plants or from littering. Exposure of BPA to the environment can pose a potential risk to many organisms, in particular plant organisms. The half-life of BPA is estimated to be between 3 – 6 days in river water and studies have shown it can remain unchanged in sediment and ground water for up to 70 days (1). However, when leached into the environment, sediment can act as a reservoir trapping BPA. BPA leached into the soil and water can potentially effect plant seed germination and growth. The U.S. Environmental Agency does not classify BPA as a bio-accumulative substance in aquatic ecosystems and concentrations below 100 μg/L are considered to have no effect on aquatic ecosystems (3).

It is known that plants can metabolize BPA. However, there are limited studies that conclude the effect of BPA on plant growth. The levels at which BPA can be metabolized without effect on plant growth is unknown. The mechanism for BPA metabolism in plants is known as glycosylation (6). In one study, tobacco BY-2 cells suspended in culture were subject to BPA (4). The concentration of BPA in solution with the cells decreased until it was undetectable 2.5 hours after application. It was found that

BPA was metabolized into four metabolites within the cell. To test this even further, [^{14}C] BPA was supplied to tobacco seedlings. It was found that the radioactive carbon labeled BPA was absorbed by the roots, metabolized to β-glucoside, and then transported to the leaves for storage. In a second study, BPA was absorbed by water convolvulus, a vegetable, and was undetectable within the plant one week later (5). Therefore, the plant was able to metabolize BPA. This experiment not only looks at the importance of BPA absorption and effect on plants, but also points to the significance of bioaccumulation of BPA within plants that are consumed by humans. In a third study, soybean seedlings were treated with BPA at a concentration of 1.5, 7.0, 12.0, 17.2, and 50 mg/L (7). At a low BPA concentration (1.5 mg/L), indices of plant growth seemed to increase when compared to the control. However, as the concentration of BPA increased, the indices of plant growth decreased. Soybean seedlings treated with 1.5 mg/L of BPA had an increase in root growth while seedlings treated with higher concentrations had a negative effect (8).

This study aims to determine the effect of BPA found in thermal receipt paper on Radish (Raphanus sativus) seedlings growth in soil. It is hypothesized that thermal receipt paper containing BPA will affect the growth of the seedlings when compared to BPA-free thermal receipt paper. Thermal receipt paper from the soil will also be analyzed using Gas Chromatography (GC) to determine the concentration of BPA leached in to the soil from the BPA containing thermal receipt paper.

MATERIALS AND METHODS

Growth of Radish Seeds

A total of twenty radish seeds were randomly distributed in groups of four among five Dixie cups for each of three experimental groups and one control group. Therefore a total of eighty radish seeds were used. Seeds were planted in Dixie cups in a mixture of soil and thermal receipt paper or filter paper. For experimental group one, four radish seeds were planted in a mixture of 0.3 g BPA containing thermal receipt paper and 1.7 g of Miracle Grow potting soil. A total of five replicates were prepared for group one. For experimental group two, four radish seeds were planted in a mixture of 0.3 g BPA-free thermal

receipt paper and 1.7 g of Miracle Grow potting soil. A total of five replicates were prepared for group two. For experimental group three, four radish seeds were planted in a mixture of 0.3 g Whatman 1 filter paper and 1.7 g of Miracle Grow potting soil. A total of five replicates were prepared for group three. The control group was prepared in the same manner as the experimental groups; however, no paper product was added to the soil. Each Dixie cup was watered with 3.0 mL of Athen's Town tap water daily for seven days. The stem length for all radish plants was recorded daily, with the exception of days 3 and 4. Days 3 and 4 occurred on the weekend and no measurements were recorded. On day 7, all radish plants were uprooted. The radicle length and stem length for each plant within each group was recorded. Statistical analysis was performed to determine if there was a statistical difference among the three groups.

Gas Chromatography Analysis

The procedure above was repeated, however, no radish seeds were grown in the soil. One Dixie cup filled with the soil-paper mixture was taken from each group, excluding the filter paper experimental group, and prepared for GC analysis. To prepare soil samples for GC analysis, all paper products were removed from the soil. The soil was then placed in a 200 mL Erlenmeyer flask. A total volume of 50 mL of 99.8% methanol (CAS: 67-56-1, Sigma Aldrich) was added to each flask. The soil was allowed to soak in methanol overnight. The soil samples were then filtered using gravity filtration. Filtered samples were labeled and saved for GC analysis. A BPA standard was prepared in methanol. A total mass of 0.0500 g of bisphenol A (CAS: 80-05-7, Sigma Aldrich) was dissolved in 10 mL of 99.8% methanol. The molarity of the BPA standard was calculated to be 0.022 M. The GC instrument was prepared. Agilent 6850 GC Gas Chromatography instrument was used under the following operating parameters: carrier gas was helium, flow rate of gas was 20mL/ min, injection temperature of 275°C, column temperature of 240°C, detector temperature of 310°C, and sample injection volume of 1 μL.

RESULTS

For the current study, radish seeds were grown in a mixture of potting soil and paper product to determine the effect of BPA on radish plant growth. One control group and three different experimental groups were established. The experimental groups were BPA containing thermal receipt paper, BPA-free thermal receipt paper, and Whatman 1 filter paper. Radish plants were observed on day four of growing. Stem height was recorded from day 3 to day 7. On day 7, the radish plants were uprooted and radicle and stem height were recorded. A statistical test was performed to determine if there was a significant difference between the control group and each experimental group. Table 1 displays the statistical data of stem height for all three experimental groups individually compared to the control group for day 4 through 7. There was no statistical difference between the control and any of the three treatment groups for day 4. For day 5, there was no significant difference between the control and the filter paper or BPA-free groups. However, there was a slight significant difference between the control and the BPA group (t-value=3.4192, p-value=0.0057). The same trend is seen for day 6 and 7 in which there is no statistical difference between the control and the two groups, filter paper and BPA-free. However, for both days there is a statistical difference between the control and the BPA group. On day 6, the statistical p-value was calculated to be 0.0001 for the comparison of the control to the BPA group (t-value=6.1044). On day 7, the statistical p-value was calculated to be 0.001 for the comparison of the control to the BPA group (t-value=4.4549). On day 7, all plants were uprooted and the root length was measured. There was no statistical difference between the stem height of the control group and the two groups, filter paper and BPA-free paper. However, there was a statistical difference between the stem height of the control and the BPA group (t-value=4.4549, p-value=0.0010). There was also a statistical difference between the root length of the control group and that of the BPA group (t-value=2.9193, p-value=0.0140). Figure 1 through 4 in the appendix display actual pictures taken of the radish plants after being uprooted on day 7. It can be seen from comparing the pictures that plants grown in soil with BPA containing thermal receipt paper have shorter roots and stems when compared to the other groups.

Day	Stem Height of Experimental Group Compared to Stem Height of Control Group	T-Value	Calculated P-Value
4	Filter paper	1.9754	0.0738
	BPA	1.0042	0.3369
	BPA-Free	0.5122	0.6187
5	Filter paper	0.5556	0.5896
	BPA	3.4192	**0.0057**
	BPA-Free	1.0853	0.3010
6	Filter paper	0.5134	0.6178
	BPA	6.1044	**0.0001**
	BPA-Free	1.4355	0.1790
7	Filter paper	0.3440	0.7373
	BPA	4.4549	**0.0010**
	BPA-Free	0.9752	0.3504

Table 1. The statistical data for the stem height of all three experimental groups individually compared to the stem height of the control are listed. A student's unpaired T-test was performed to determine if there was a significant difference between each of the experimental groups and the control.

Day	Root Length of Experimental Group Compared to Root Length of Control Group	T-Value	P-Value
7	Filter paper	0.6005	0.5604
	BPA	2.9193	**0.0140**
	BPA-Free	0.4005	0.6963

Table 2. The statistical data for root length of all three experimental groups individually compared to the root length of the control are listed. A student's unpaired T-test was performed to determine if there was a significant difference between each of the experimental groups and the control.

To analyze the BPA present within the soil samples, a soil analysis was attempted using gas chromatography. A prepared BPA standard with a concentration of 0.022 M displayed a peak with a retention time of 10.141 minutes. Upon analyzing the soil extracts, no BPA peak was observed for any of the groups sampled: BPA, BPA-free, and control. Each sample was run multiple times, but no BPA peak was observed for any sample.

DISCUSSION

The content of BPA in thermal receipt paper poses a potential risk when thermal receipt paper is discarded into to the environment. Plants have the ability to metabolize BPA in certain concentration. However, BPA in high concentrations can cause a decrease in germination of seeds and growth of plants (9). In this study, radish seeds were exposed to BPA containing thermal receipt paper and BPA-free thermal receipt paper to determine if BPA effects the growth of radish plants.

From this study, it was concluded that exposure to BPA containing thermal receipt significantly impacted the growth of radish plants and could pose a threat to environmental plants. The experimental group with BPA containing thermal receipt paper displayed a significant reduction in stem length, root length, root hair composition, and lateral root growth when compared to the remaining three groups. The BPA group was statistically different from the control group on days 5 (t-value=3.4192, p-value=0.0057), 6 (t-value=6.1044, p-value= 0.0001), and 7(t-value=4.4549, p-value=0.0010). On day 7, the radish plants were uprooted and stem height along with root length was recorded. The root length of the BPA group was also statistically different from that of the control group (t-value=2.9193, p-value=0.0140). There was not statistical difference between stem height and root length of the two groups, BPA-free and filter paper, when compared to the control. Therefore, it was concluded that the chemical BPA found in BPA containing thermal receipt paper affected the growth of radish seeds when grown in a paper-soil mixture. Although it was hypothesized that BPA was the main chemical in thermal receipt paper that effects radish growth, this effect could be due to other chemical components of the thermal receipt paper. However, this

study did not support the effects of any other chemical other than BPA because the BPA group was the only group that was statistically different from the control group. A GC analysis of the soil was completed in hopes of determining the concentration of BPA within the soil that caused these effects. However, no conclusive data was gathered from GC analysis of the soil. A BPA standard displayed a peak on the chromatogram at a retention time of 10.141 minutes. However, samples from the BPA containing thermal receipt paper soil mixture did not display a peak at this retention time. Therefore, no conclusions were drawn concerning the concentration of BPA within the soil. This could be due to the method of BPA extraction from the soil. BPA was extracted by soaking the soil in 99.8% methanol overnight. In a future study, BPA extraction could be purified using column chromatography. This would yield a pure BPA sample that could be easily detected by GC analysis as there are no other chemicals or compounds present that would cause unwanted peaks on the chromatogram. The chemical composition of thermal receipt paper should be considered in future studies.

CONCLUSIONS

From this study, it was concluded that BPA containing thermal receipt paper greatly impacts the growth of radish plants. This is observable both statistically (table 1 and 2) and visually (fig. 2). The effect of BPA, from BPA containing thermal receipt paper, on plants grown in this study proves that BPA could have a potentially harmful effect on plants within the environment. Therefore, further studies should be conducted to determine the concentration of BPA that is harmful to the environment.

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APPENDIX

Figure 1. Four groups of radish seeds, one control group and three experimental groups were grown in the lab. From left to right the groups are: Filter paper, BPA thermal receipt paper, BPA-free thermal receipt paper, and control (no paper).

Figure 2. The root length and stem height of all four groups are compared. As seen from the picture, the radish seeds exposed to BPA thermal receipt paper have a shorter stem and root as compared to radish plants from the remaining three groups.

Figure 3. Radish plants grown in soil mixed with BPA-free thermal receipt paper are shown. As seen from the picture, the radish plants have variable measure of plant indices.

Figure 4. Radish plants grown in soil mixed with BPA containing thermal receipt paper are shown. As seen from the picture, the majority of radish plants have discernably smaller measures of plant indices as compared to the other groups.