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Editor's Note

Although I have no way of knowing for sure, I suspect that *Homo sapiens* are one of the strangest creatures out there. Sure, there are limpets that can change their biological sex depending on whether or not they're swimming or stacked on top of one of their sisters (formerly brothers), and there are flowers which, in order to be pollinated, must engage insects a terpsichorean tumble that would make the Russian Ballet envious. But for all the odd and fascinating ways in which living things interact, humans still rank number one for strangeness because of one structure – our brains.

It's a wonderful organ, if little understood. Although some interesting new research points to some nifty ideas, we still don't know what something simple like a memory is actually made of, or where we can find one. Our gigantic cerebra allow us to think about all sorts of fascinating things and have dreams whose meanings we can only hope to understand. We can integrate huge amounts of knowledge and come to a logical decision, and we (or those of us who are good at baseball, anyway) can do projectile motion problems without touching a calculator.

But for all this, we can also make some pretty awful decisions, too. Because for every wonderful thing the brain can do, it can do ten things to trick us. A study* done several years back had participants try and memorize a number. Each person was given either a short number or a longer one. They were then told to go to another room for the next phase of the study. On the way, each participant was asked to choose a snack, and were presented with a nice healthy bowl of fruit salad or a luscious piece of chocolate cake. The vast majority of the people who were trying to remember a long number took the cake, while the short-number people took more fruit.

Why? We're not good at multitasking. Engage your logical mind with a task – memorizing a number – and the emotional part of your mind is left to do the work. So emotionally, we choose the cake because, well, cake. Perhaps there's even an evolutionary facet to this interesting experiment. Do we chose the cake because it is high in calories and ancient humans needed calories to survive? Interesting to speculate.

The point remains that when we are distracted by, say, smart phones, television ads and the like, we don't make very logical decisions. And modern humans are often very distracted. Extrapolating this to a global level, I'd wager that the scary number of climate-change deniers and anti-vaccination activists can be linked to this phenomenon. Our politicians, while perhaps not the evolutionary cream of the crop to begin with, are no doubt distracted by the large number of demands placed on them. Political ads try to encourage us to vote one way or another not usually with facts, but with emotion-based persuasion. And they work. Politically, many people often end up taking the proverbial cake.

So teaching our students what their brains can and can't do is important. Critical thinking has never been as important as it is now. The ability to realize when you're being manipulated consciously, or even when you're just too tired or hungry to make a good decision, is important. It's this fantastic intersection of science and society that makes what we do in the classroom every day so much more important.

D.M.S.
2015
Ithaca, NY

*You can read the full text of this fascinating paper here: <http://www.jstor.org/stable/10.1086/209563>

MAXIMIZING THE PERFORMANCE OF SURFACTANTS IN DISH SOAPS

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ABSTRACT

Surfactants are molecules that consist of a hydrophilic head and a hydrophobic tail. They are the primary component of soaps and detergents such as dish soap. Many products today are expensive because they have high concentrations of surfactants. Maximizing the performance of surfactants in even the least expensive dish soap would benefit consumers with cleaner dishes and people would be less likely to contract diseases spread by body fluids like saliva.

The objective of this study was to determine the concentrations and blends that specific surfactants would be most suitable for removing soil and grease from hard surfaces when added to a low-performing dish soap. Different surfactants were added to budget dish soaps to determine if they had significant impacts on characteristics such as foaming, viscosity, pH, interfacial tension, and cleaning.

The best blends and concentrations included 0.5% cationic co-surfactants, 1% cationic co-surfactant, and 0.5% cationic co-surfactant with 0.5% anionic surfactants. The first two blends were expected and produced a significant increase in foaming, viscosity, and cleaning. They were also measured to have a decreased interfacial tension. The last blend indicated that the two surfactants created a complex that enabled it to clean as good as, if not better, than the first two blends.

INTRODUCTION

A surfactant is a **surface active** agent: substances that have the ability to change the property of surfaces and interfaces where it is present (Rosen, 2000, p. 5). Interfaces refer to

boundaries between any two immiscible, or unable-to-mix, phases. Surfaces refer to boundaries where one substance is a gas (Rosen, 2000, p. 6). Surfactants are molecules that are structurally composed of a hydrophobic, water-repelling group, and a hydrophilic, water-attracting group as shown in Figure 1.1. When added to solvents, such as water, they distort the normal structure of the solvent and increase the free energy of the system. To decrease the free energy of the system, surfactants minimize the contact between water and hydrophobic groups by forming spherical structures called micelles or by migrating to surfaces in a process called adsorption (Rosen, 2000, p. 3).

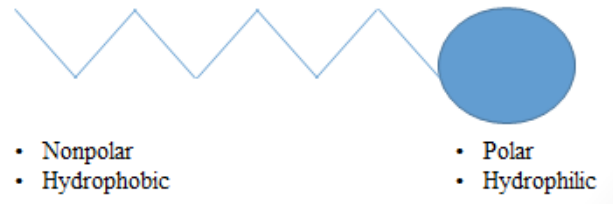


Fig 1.1. The structure of a surfactant includes a hydrophilic and hydrophobic end.

When a surfactant changes the properties of a solvent, the minimum amount of work needed to change it decreases. Interfacial free energy is the minimum amount of work required to create interfaces (Wang, 2008, p. 1238). The minimum work (W_{\min}) to increase an interfacial area (ΔA) is the product of the interfacial tension (γ_1) and the change in area (Rosen, 2000, p. 18).

The main application of surfactants is in the cleaning process. Cleaning itself takes up three types of energy: chemical, thermal, and mechanical. Since thermal energy involves temperature and mechanical energy involves the irritation and aggregation of substances on surfaces, the maximization of the cleaning performance of surfactants would increase the amount of chemical energy used so that the other two could decrease. Surfactants clean surfaces by attaching to the edge of a soil with similar polarity, reducing the contact angle with the soil,

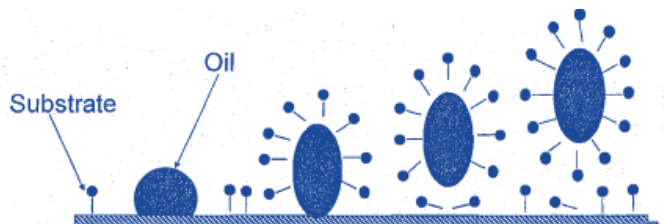


Figure 1.2. Oil becomes solubilized in water when it is lifted off of surfaces.

rolling the soil off the surface causing the soil to become solubilized in water (Rosen, 2000, p. 18). (Figure 1.2)

Interfacial tension is one of the characteristics that can be measured when a surfactant is in a solvent. It is a measure of dissimilarity between two substances. In the case of dish soap, the

lower the interfacial tension, the greater the similarity between the surfactant and the soil, which allows it to attach to the soil and roll it off the surface. Interfacial tension correlates with cleaning. Therefore, formulations that have low interfacial tension tend to clean surfaces better than those with high ones. Another characteristic of surfactants in solutions is viscosity: the resistance to flow. Foaming is another trait that is a viscoelastic property which occurs when a gas is introduced at the film of the solution where adsorption occurs (Rosen, 2000, p. 13). Although foaming and viscosity have no positive effect on the surfactant's ability to clean surfaces, they are important aspects to maximize because they are appealing to consumers.

The objective of this experiment was to maximize the performance of each surfactant in terms of its ability to foam, its viscosity, and its interfacial tension by combining different kinds

of surfactants. The type of surfactant depends upon the charge of the hydrophilic end. Cationic surfactants have a positive end. Anionic surfactants have a negative end. And nonionic surfactants have no charge on the hydrophilic end. The purpose of this experiment was to determine which concentrations of surfactants were most suitable to maximize the characteristics of the dish soap.

MATERIALS AND METHODS

A budget dish wash liquid and a premium dish wash liquid were purchased. An ingredient analysis was conducted by looking at the labels of the dish soap and analyzing the components. The independent variables were the concentrations of each surfactant added to the budget dish soap. The dependent variables were the characteristics of dish soaps such as foaming, cleaning, viscosity, and interfacial tension. The control groups were the negative and positive controls: a budget dish soap and a premium dish soap respectively. Controlled variables included same temperature and container for storage, same amount of each additive combined, and same machinery used to measure data.

Materials

The three surfactants to be tested: cationic co-surfactant, anionic alkyl ether sulfate, and nonionic surfactant were obtained. A balance, SITA Foam Tester, pH meter, Brookfield Viscometer, oven, and Interfacial Tensiometer were used. Canola oil, corn starch, olive oil, and lard were obtained to create a sample of soil for cleaning tests. Pipets, glass jars, sponges, porcelain plates, beakers, a camera, tap water, deionized water and a paintbrush was also used.

Creating Solutions

Blends were made for each surfactant by weight and by mixing it with the budget dish soap. The blends were each measured to be 100 g. with either 0.5%, 1.0%, 1.5%, or 2% of each surfactant by weight. The new blends were stored for a day to make sure the solution was stable and nothing fell out of the solution forming a solid.

Process of Cleaning

Each blend was then dissolved to make 1% solutions with tap water to imitate the setting of a kitchen. Forty milliliters of the solutions were placed in 150 ml. beakers. This was to control the height so that each sponge was soaked with the same amount of the blend. A sample of soil to imitate the soil found in kitchen sinks was made by combining 30 grams of canola oil, 40 grams of corn starch, 1 gram of black dye, 10 grams of extra virgin olive oil, and 20 grams of lard. The soil solution was painted in thin layers onto porcelain tiles and baked for an hour at

120°C. After the plates cooled, sponges of the same size and type were dipped into the beakers of two different solutions (blends) for 5 seconds by hand. A similar force was used to press the sponge up and down the porcelain tile and a picture was taken by the camera every two scrubs. This test was done four times for each set of blends and two were with the sponges of each blend on opposite hands to reduce any errors.

Testing Viscosity

Viscosity was measured using the Brookfield LVT Viscometer. (Figure 2.1) The machine was calibrated and the temperature remained constant. Each blend, located in its container, was placed on the base of the machine and the spindle was lowered into the solution. The same spindle size (12 millimeters in diameter) was used throughout the experiment. The speed of the spindle rotating was adjusted to measure a reading that did not produce an error. If the spindle spun too fast, an error resulted because the instrument could not measure viscosity, and the speed was decreased to record a measurable number. The data on the screen was recorded. After the spindle was screwed off and cleaned, it was put back on to test another blend.



Figure 2.1 A viscometer measure the resistance to flow of a solution.
(<http://www.scintek.com>)

Testing Foaming Ability

A solution containing 1% of the blend (by weight) with tap water was made to imitate the conditions of a kitchen. The blend to be tested was poured into the storage tank of the SITA Foam Tester. (Figure 2.2) The program on the computer was edited to set the parameters of the foaming test. The following parameters were used to conduct the foaming test:

- Sample Volume: 250 ml.
- Rotor RPM: 1000 R/min
- Stirring Time: 10 s
- Cleaning Mode: short
- Stir Count: 10



Figure 2.2 A SITA Foam Tester measure foam by spinning a rod at the bottom of a liquid and measuring the height of foam using sensors. (<http://www.elicomarketing.com>)

tested in a 1% solution with water was placed underneath the apparatus. The computer automatically executed a continuous growth of the volume of the bubble, calculated the volume of the bubble in real time in micro-milliliters and drew the calibration curve on the graph.

Foam Testers operate by rotating a rod in the liquid to be measured and using sensors to measure how much foam is created. In this case, the spindle was spun at a rate of 1000 rotations per minute for 10 seconds. The sensors detected the height of foam produced and the rod began rotating again. For each blend, 10 different measurements were taken. Data was saved in the form of excel sheets. The machine cleaned itself thoroughly between each measurement.

Testing Interfacial Tension

Surface tension is determined by fitting the shape of a drop of oil (using a camera) in a solution to be measured to the Young-Laplace equation which relates interfacial tension to drop shape. (Figure 2.3) The software, WDROP_2013, does this automatically. The needle was taken out and cleaned with ethyl alcohol. The syringe was filled with vegetable oil and placed in the machine. A small beaker of the blend to be

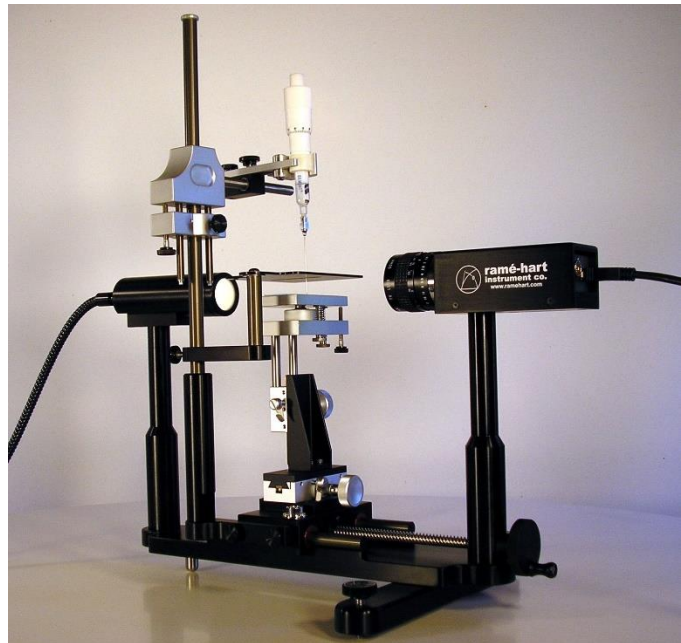


Figure 2.3 An interfacial tensiometer which uses the shape of a bubble or drop to measure interfacial tension. (<http://www.ramehart.com>)

RESULTS AND DISCUSSION

The best blends and concentrations included 0.5% cationic, 1% cationic co-surfactant, 0.5% cationic co-surfactant plus 0.5% anionic surfactant.

Viscosity

The most viscous blend was the 1% Anionic S1, 2% Anionic S2, and the combinations of Nonionic and Anionic S2. This is an expected result because these surfactants are anionic and therefore create more resistance to flow. The molecules of these surfactants tend to be larger with more branches of amides sticking off of the backbone. The viscosity differences after the Anionic S1 blend were minimal. These blends were mostly combinations. This shows that interacting surfactants create complexes which deteriorate viscosity but at the same time, has enough viscosity to be better than the negative control. Viscosity is a measure of resistance to flow so the higher the resistance, the higher the viscosity. (Figure 3)

Foaming

Foaming is a viscoelastic property that was measured using a SITA Foam Tester. The blend that produced the most foam was Premium formulation. The blends with the highest height of foam after the Premium were the combinations of the cationic and anionic surfactants. These would form more foam since they consist of a combination of oppositely charged surfactants and therefore would form more micelles. More micelles generated would contribute to an increase in the process of creating more foam. The foaming levelled off after the Anionic S1 most likely because the makeups of the blends were similar. The highest ranking blends for foaming included those with cationic surfactants and the combinations of anionic and cationic surfactants. Although foaming and viscosity are not important in terms of a soap's ability to clean, they are important aspects of soap that appeal to consumers. (Figure 4)

Cleaning

The cleaning tests indicate that the cationic co-surfactants cleaned the best. Over time, the soil on the plates was removed faster using sponges soaked with the cationic surfactants. The cationic co-surfactants used have a positively charged functional group allowing it to pick up organic, non-polar soil molecules better. The combinations of cationic and anionic surfactants also improved cleaning. The combined chemicals form micelles which trap soils effectively in the hydrophobic nonpolar regions of the molecule. The soil is wrapped around with the rest of the hydrophilic parts of the molecule and protected by the hydrophobic parts. Cleaning was thus indicated to be better for most of the blends when a surfactant was added to the budget dish soap. (Figure 5)

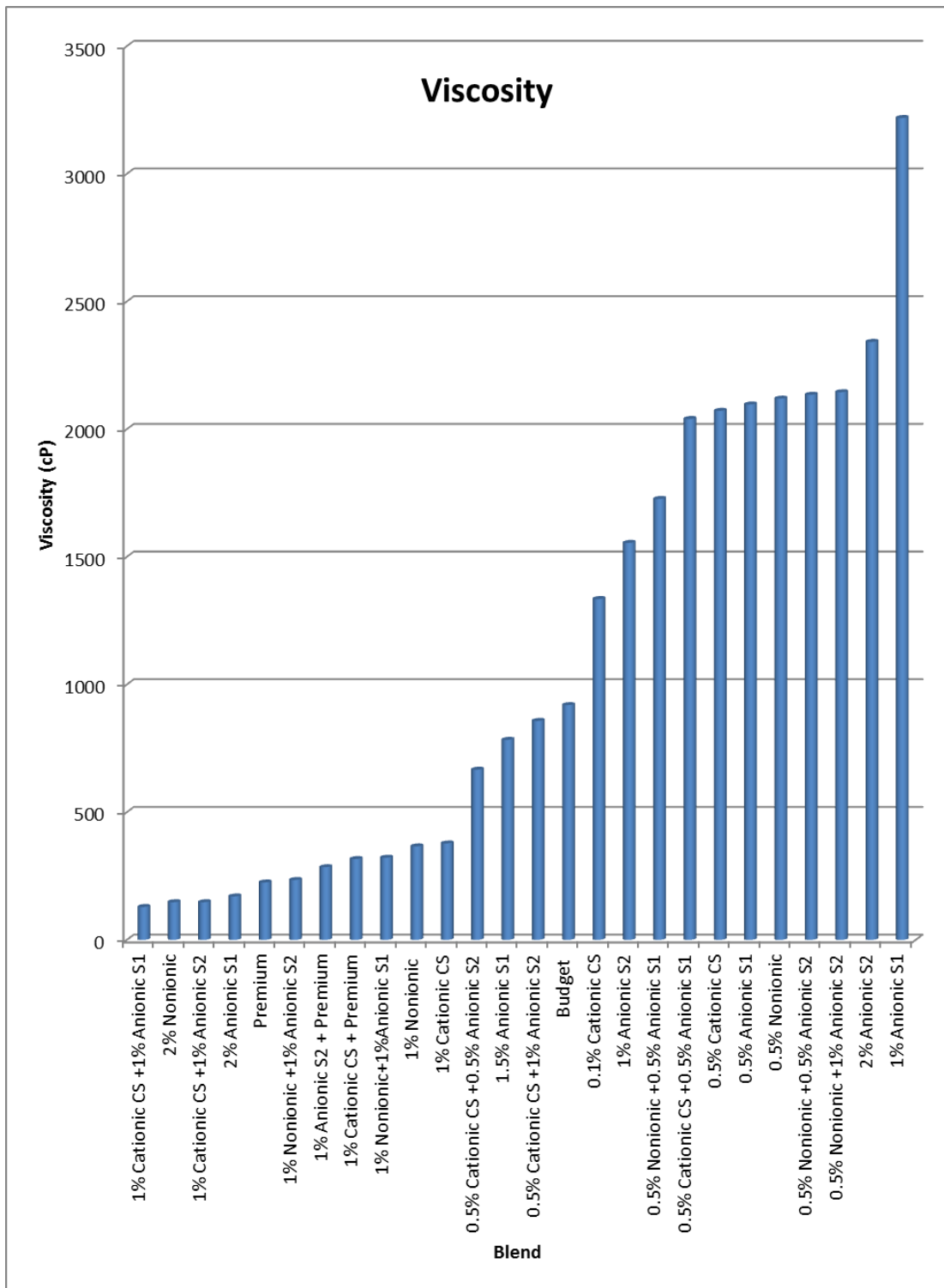


Figure 3: The results from the Viscosity tests show the viscosities of each blend measured in centipoise (cP) by an instrument that measures the force required to rotate a spindle at a specific rate.

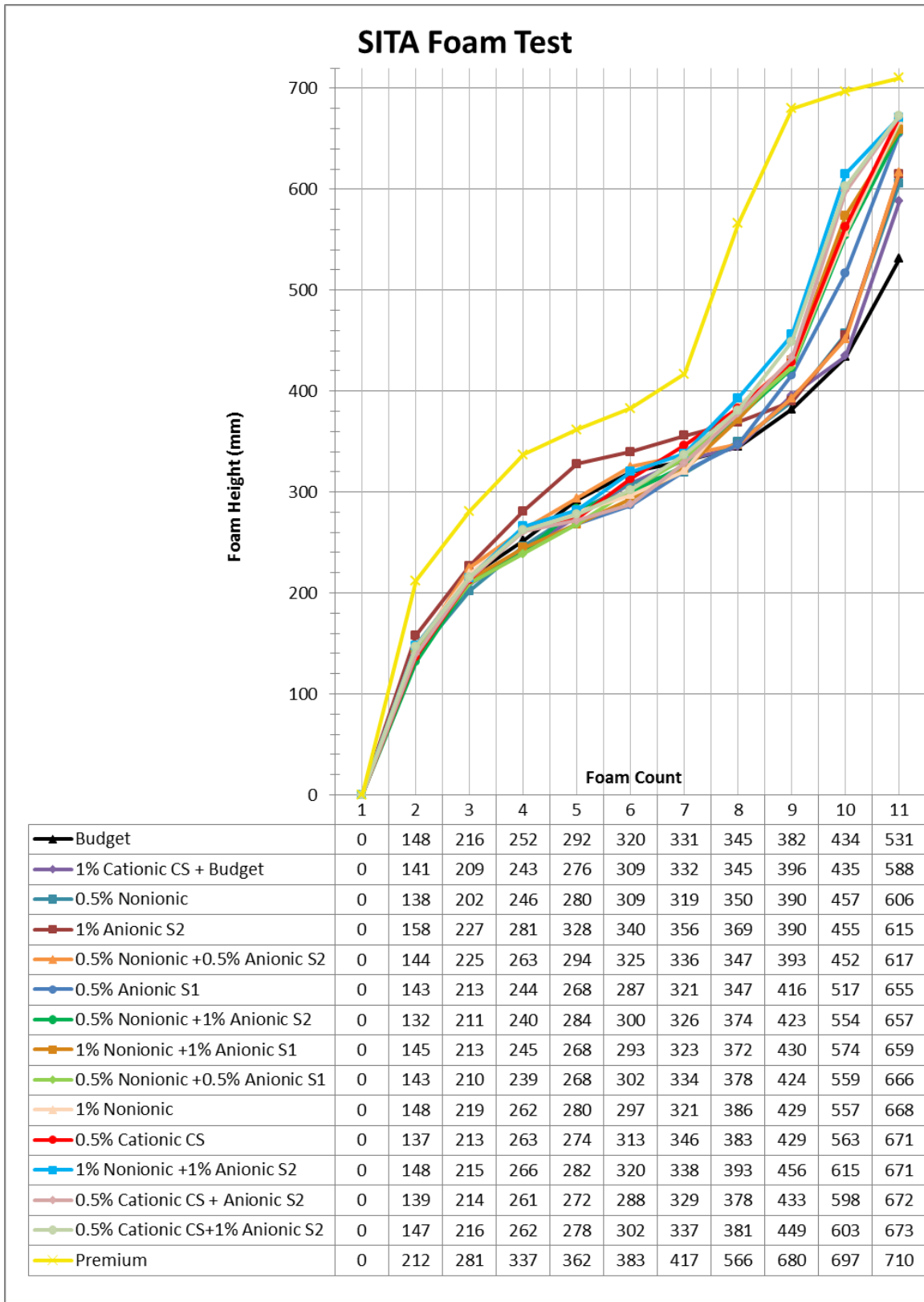


Figure 4: The results from the Foaming tests show the heights of foam created by each blend when a blade was spun with the parameters stated in *Materials and Methods*.

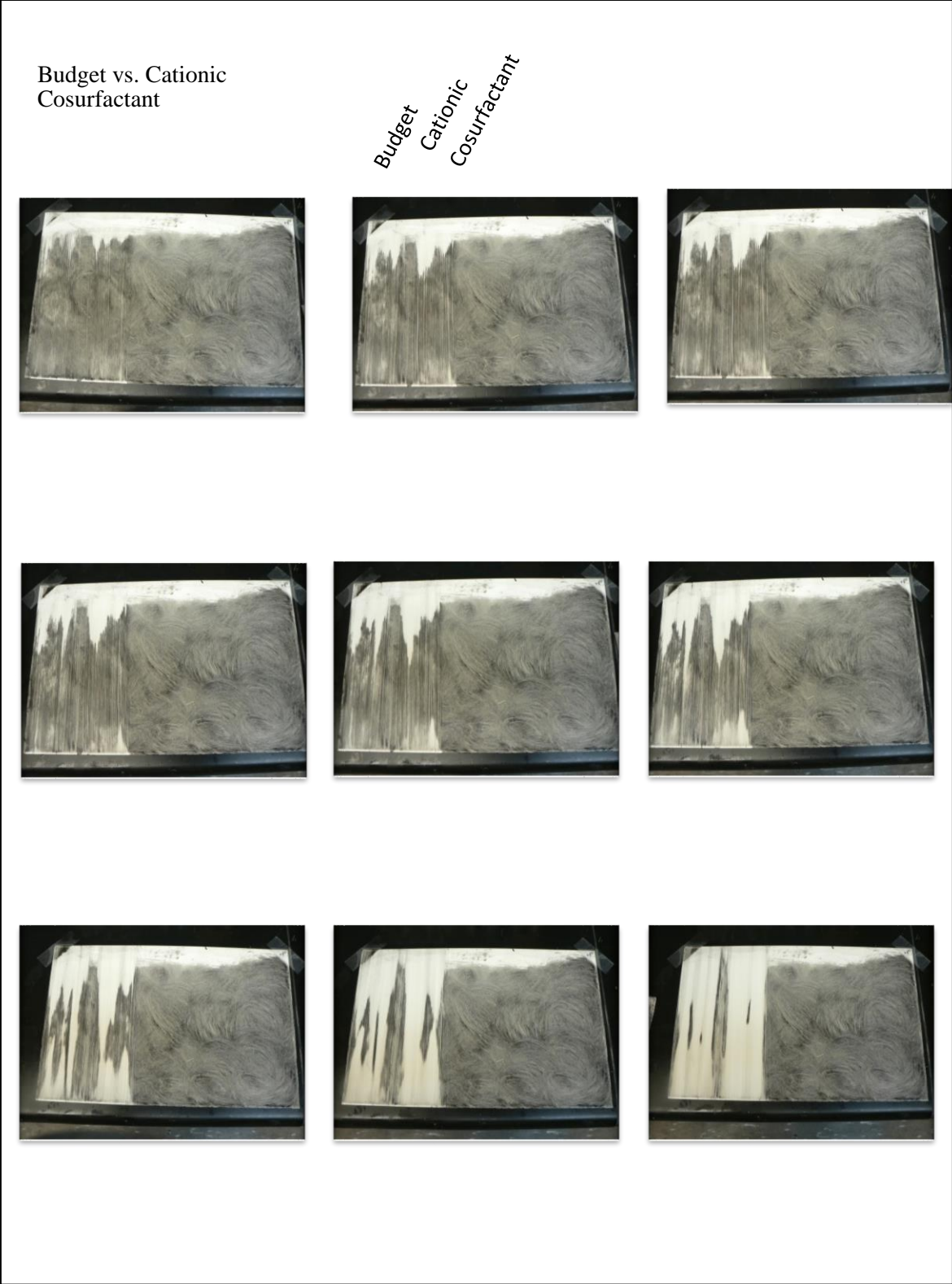


Figure 5: The results of the cleaning tests show photographs of the changes on the ceramic plates after every two scrubs of budget dish soap (on left) and cationic co-surfactant blend (on right).

Interfacial Tension

For the cationic co-surfactant and combination blends, interfacial tension decreased over a time span of 300 seconds. Interfacial tension directly correlates to the performance of cleaning. The lowered interfacial tension mirrors that of the improved cleaning and provides as scientific evidence. There is a significant difference in the interfacial tensions between the budget dish soap and the 1% cationic co-surfactant according to the 2-Sample T-Test.

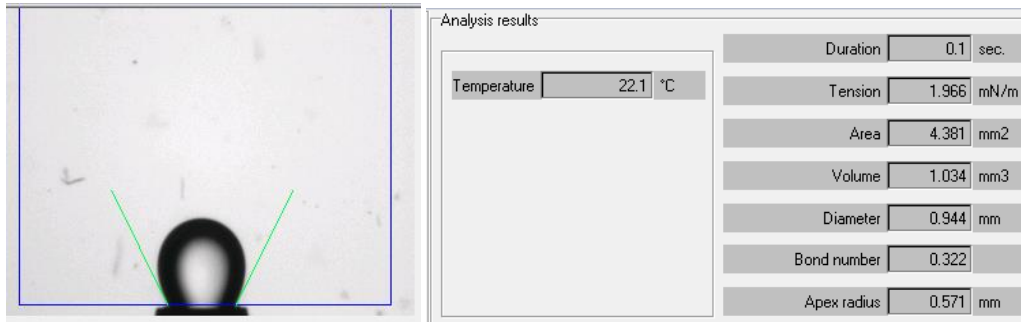


Figure 6: The screen on the program WDROP shows the bubble formation of oil in the blend - in this case the 1% Cationic Co-surfactant.

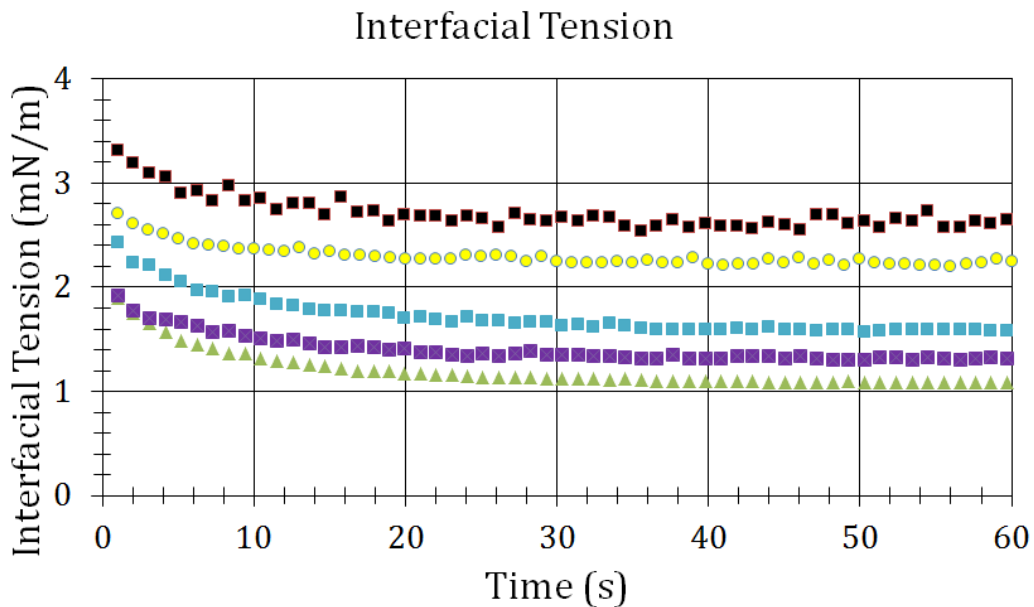


Figure 7: The graph shows the interfacial tension (milli-Newtons per meter) of five formulations over time.

- Interfacial Tension
- Premium
 - Budget
 - ▲ 1 % Cationic
 - 0.5 % Cationic + 1% Anionic
 - 1 % Cationic + 1 % Anionic

CONCLUSIONS

This research tested the effects of different additives on the performance of a dish soap. The experiments were carried out to maximize the performance of a dish soap in terms of viscosity, foaming, interfacial tension, and cleaning. Dish soaps with a high viscosity and foam build appeal to consumers more and dish soaps with low interfacial tension work better at cleaning surfaces. This research indicated that cationic surfactants at 1% and 0.5% added to a budget dish soap maximized its performance. Combinations of an anionic surfactant and cationic co-surfactant also showed enhanced performance. The combination of cationic and anionic surfactants most likely created complexes which increased the generation of micelles leading to an increased foaming ability. The complexes also made the formulation thicker because many more large molecular structures resisted flow. The formation of complexes also maximizes the formulation's ability to clean by attaching their hydrophobic ends to the nonpolar soil and ripping it off the surface because their hydrophilic ends are attracted to water. Since the formulations created work better with lower concentrations of surfactants than the Premium formulation, dish soap can be made cheaper than it used to. This research allows companies to create an enhanced product that is cheaply made. More people would have access to hand dish wash formulations that work better, have fewer chemicals, and cost less. Future research could include testing the effects of various additives on specific types of soils. It could also include testing different formulations on different surfaces (e.g. cloth, metals, and porcelain).

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A STUDY OF FUEL MOISTURE AND THE VEGETATION CYCLE OF THE PLANT CHAMISE CHAPARRAL (*ADENOSTOMA FASCICULATUM*) IN CALIFORNIA

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ABSTRACT

Fuel moisture is a major component to forest fire behavior and is used as an important factor in determining fire danger. The purpose of this study was to investigate the relationship between fuel moisture and the phenological stages throughout the life cycle of the plant Chamise Chaparral (*Adenostoma fasciculatum*), a densely growing coniferous shrub found in chaparral shrublands in California. Fuel moisture from Elk Creek, Sequoia National Park, was compared to data from other sites in California obtained through the National Fuel Moisture Database and old (one or more years old) and new growth (current year's growth) samples were also compared. Phenology refers to the study of the influence of climate on cyclical biological events such as growth or flowering. If during a particular phenological stage the fuel moisture can be determined, the intensity of forest fires during different seasons could be predicted allowing for better fire prevention and firefighting preparation. The results showed that fuel moisture varied based on its location because fuel moisture samples were statistically different among sites. A correlation existed between old and new growth in the new growth stages, meaning that in mid-spring when Chamise begins to grow, the fuel moisture will be higher resulting in less intense forest fires when compared to the rest of the year.

INTRODUCTION

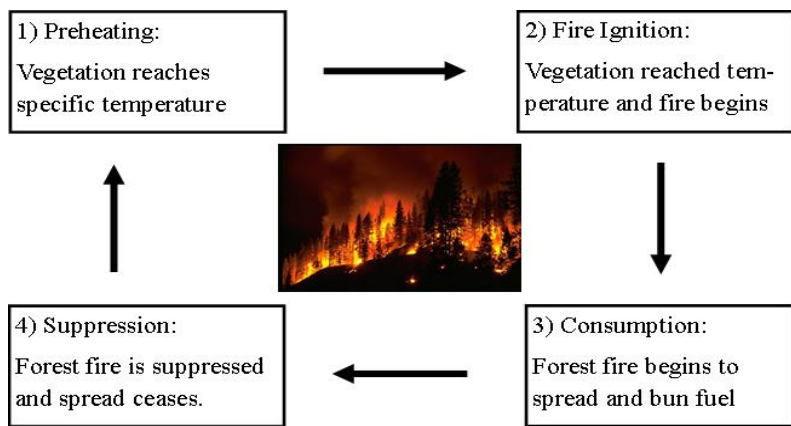
The United States has averaged 4 million acres of land burned due to forest fires each year from 1995 through 2003 and that average has increased by 3 million acres since 2005 until now (www.mnn.com). Before 2000, the total suppression cost of forest fires never reached over 1 billion dollars, but since 2010, the total suppression cost has averaged 1.63 billion dollars per year (<http://www.nifc.gov>). One of the major contributors to the severity of fires is the reduction of moisture in vegetation (Nelson, 2001). The reduction in moisture content is mainly due to relative humidity (Schroeder, 1970); the lower the relative humidity, the more moisture that can evaporate from vegetation. The amount of moisture in vegetation relative to the dry biomass is called fuel moisture. Fuel moisture is affected by factors such as temperature, wind, humidity,

rainfall and the type of vegetation. Fire danger can increase greatly if the fuel moisture of the vegetation in the area is low (Dennison, et al., 2003). The purpose of this study was to [1] determine if there is a difference between *old* and *new growth*, in a given area of California [2] compare fuel moisture among various sites in California and [3] determine if there is a relationship between the amount of moisture in vegetation and the phenological phase of the vegetation.

One way fuel moisture affects fire behavior is by reducing fuel consumption or slowing the rate of burning (Nelson, 2001). The rate at which a fire burns can be calculated by dividing the mass of fuel consumed by unit area of ground by the time required to burn the fuel in the unit area (Nelson, 2001). Higher fuel moisture will decrease the rate of fuel consumption, thus decreasing the amount of vegetation burned in a given time.

Forest fires burn in stages. The stages of a forest fire are the events that occur before, during, and after forest fires such as preheating and ignition. One of the first stages of the combustion process is preheating. It is during this process that heat is absorbed by the vegetation and moisture is evaporated into the atmosphere (Nelson, 2001). The greater amount of fuel moisture, the longer the preheating period will be until the temperature for ignition is reached (Nelson, 2001). Ignition is the beginning of the fire, which requires a temperature from 200°C-300°C in order to start burning regardless of fuel moisture content. Since the higher fuel moisture causes a longer preheating period, the ignition stage would be prolonged (Albini, et al., 1995).

Another way fuel moisture has an effect on fire behavior is that moisture reduces flame temperature, because the heat that the fire produces has to not only heat the vegetation, but also the moisture contained within the plant (Nelson, 2001). The water vapor in the air also decreases the oxygen available to the flames. The reduction in temperature usually results in the production of char (partially burned) rather than the production of volatiles (completely burned) (Nelson, 2001).



The third effect of fuel moisture on fire behavior is that it lengthens the particle residence time or the time in which the fire remains on a layer of fuel, which is what a fire burns such as vegetation, during combustion (Albini, et al., 1995). Experimental studies have shown that in order for fuel combustion to occur, it needs the heat from radiative energy (difference between energy from sun received by the Earth and the energy radiated back into space) and convective heat transfer (Albini, et al., 1995). Meaning that, heat from solar radiation will contribute to the ignition of a forest fire. Increased fuel moisture will reduce the amount of solar radiation that

reaches the vegetation, which in turn increases the length of time the fire will remain in an area of fuel and reduce the probability of a fire spreading.

In this study, data was used from a widely dispersed and densely populated plant found in California, called Chamise Chaparral (*Adenostoma fasciculatum*). Chamise is a dense coniferous shrub that covers 7,300,000 acres of California and is part of the rose family (Keeley, 1985). Chamise is a good indicator of seasonal changes and fuel moisture because it covers a large area of mountain slopes in central and southern California (McPherson, 1969) and fires associated with this species occur frequently. Chamise is a highly flammable plant because it has a low moisture content. Fires are very common among Chamise plants, especially during the summer months and during droughts (Keeley, 1985).

The prediction of how a fire will spread if Chamise Chaparral is burning is limited because current fire spread models were not designed for this type of live fuel and there is a limited amount of experimental data to develop and test with these models (Weise, et al., 2005). Since fire spread cannot be predicted while Chamise Chaparral is burning, fire managers, who are conducting controlled burns, typically begin a forest fire in the spring months when the fuel moisture is significantly higher compared to the other months in the year (Weise, et al. 2005).

Vegetation phenology, the study of reoccurring vegetation cycles and their connection to the environment, plays an important role on Earth, especially when it comes to global environmental climate change, water resources and atmospheric chemistry (White, et al., 1997). Some key phenological phases include; greenup, the beginning of photosynthetic activity; maturity, when the maximum growth is completed; and dormancy, when physiological activity nears zero (Zhang, 2003). If the amount of fuel moisture differs during different stages of a plant's phenology then the intensity of a fire during certain times of the year may be predicted, and therefore allows for more preparation for the suppression of fires. However, there are other factors that may affect how forest fires burn such as if there was a drought, wind, and the amount of vegetation in the area.

There have not been extensive studies done for California on the relationship between fuel moisture and phenology. A study was done with species of plants in the Mediterranean area and how seasonal weather variations and phenology affect fuel moisture content and ignitability of Mediterranean plant species (Pellizzaro, 2007). The study was done in North/North-Western Sardinia, Italy. The plants were found to be moderately flammable in the spring, when the plants were re-sprouting and flowering (Pellizzaro, 2007).

Fuel moisture and phenology data for this research were obtained from undigitized data charts in logbooks in PDF format from Sequoia and Kings Canyon National Park. The data was collected from 2001-2014 and had not previously been analyzed. We digitized and analyzed the data using Excel spreadsheets. We hypothesized that there would be a difference between *old* and *new growth* samples and fuel moisture would not vary upon its location. We also hypothesized that a relationship would exist between fuel moisture and phenology.

MATERIALS AND METHODS

Data Collection

Fuel moisture and phenology data were obtained in PDF format from a fire ecologist at Sequoia and Kings Canyon National Parks (Fig. 1). The data was collected for 14 years from the Elk Creek area (39.36°N, 122.32°W). The data was digitized into Microsoft Excel spreadsheets (Fig. 2). Separate sections for *old growth* and *new growth* were created and gross wet weight, gross dry weight, can weight, dry weight, weight loss, and percent moisture were transferred as they were written on the PDF files. The data was then placed into one larger table with all of the same information for data analysis. Weather and phenology data were also digitized into Microsoft Excel Sheets (Fig. 3).

Other fuel moisture data from different national parks in California were obtained through the National Fuel Moisture Database (NFMD) (www.wfas.net/), (Fig. 4). This data was then entered into a separate spreadsheet from the fuel moisture data in Microsoft Excel.

Table 1. Example of fuel moisture organized into charts based on phenological stage. Different colors represent different years.

| ripe | Old 1 | Old 2 | Old 3 | New 1 | New 2 | New 3 |
|------|-------|-------|-------|-------|-------|-------|
| 68.3 | 57.1 | 67.6 | 74.4 | 68.6 | 69.8 | |
| 45.6 | 49.1 | 55.6 | 63.6 | 55.3 | 61.5 | |
| 47.8 | 53.6 | 44.8 | 56.0 | 53.8 | 52.1 | |
| 47.4 | 50.0 | 53.3 | 56.1 | 50.0 | 57.4 | |
| 55.0 | 41.9 | 48.7 | 54.3 | 53.6 | 52.3 | |
| 50.0 | 50.0 | 50.0 | 52.0 | 58.3 | 48.0 | |
| 70.2 | 47.4 | 56.8 | 74.2 | 71.4 | 61.1 | |
| 56.0 | 53.6 | 60.0 | 64.4 | 66.7 | 62.5 | |
| 52.3 | 50.0 | 54.1 | 61.3 | 67.4 | 75.0 | |
| 52.9 | 51.1 | 53.3 | 63.4 | 66.0 | 67.7 | |
| 44.4 | 46.7 | 49.2 | 66.7 | 62.0 | 64.0 | |
| 80.0 | 87.1 | 61.5 | 78.0 | 100.0 | 93.1 | |
| 58.8 | 70.6 | 55.6 | 80.0 | 88.6 | 65.1 | |
| 62.1 | 54.5 | 57.1 | 63.0 | 75.0 | 64.9 | |
| 52.6 | 56.0 | 61.9 | 76.5 | 59.3 | 72.2 | |
| 80.0 | 88.9 | 90.5 | 108.7 | 114.3 | 123.1 | |
| 61.0 | 62.0 | 50.0 | 67.0 | 67.0 | 65.0 | |
| 61.0 | 56.0 | 62.0 | 58.0 | 60.0 | 55.0 | |
| 50.0 | 60.0 | 42.0 | 46.0 | 52.0 | 50.0 | |

| Location: Elk Creek | | | | Area: Ash Mtn (NPS-SEKI-FMO) | | | | Type(s): Live | | | |
|--------------------------|-------------|---------|-------|--------------------------------------|--------------------|--------------------|--------------------|---------------------|--------------------|------------------------|------|
| Collection Record | | | | Moisture Determination Record | | | | | | | |
| Date: 7-1-05 | | | | Time in Oven: 1640 | | | | Date: 7-1 | | | |
| Time: 1620 | | | | Time out of Oven: 1075 | | | | Date: 7/3/05 | | | |
| By: | | | | | | | | | | | |
| # | sample type | Species | can # | A Gross wet | B Weight dry | C Can Weight | D dry weight | E weight loss | F % moisture | G sample average | |
| 1 | Old 1 | ADFA | | 155 | 123 | 107 | 0 | 0 | #DIV/0! | | 73% |
| 2 | Old 2 | | | 204 | 168 | 117 | 0 | 0 | #DIV/0! | | |
| 3 | Old 3 | | | 179 | 148 | 105 | 0 | 0 | #DIV/0! | | |
| 4 | New 1 | | | 157 | 128 | 104 | 0 | 0 | #DIV/0! | | |
| 5 | New 2 | | | 177 | 134 | 102 | 0 | 0 | #DIV/0! | | 123% |
| 6 | New 3 | | | 188 | 143 | 103 | 0 | 0 | #DIV/0! | | |

| | | |
|---|---------------------------|-----|
| Topographic Features: | Weather: | |
| Elevation: 2100 | 99 Dry Bulb | AVG |
| Aspect: S SE | 71 Wet Bulb | 96 |
| Shading: | 26 Relative Humidity | |
| Slope %: 17% | 2 % Cloud Cover | |
| <input type="checkbox"/> Upper 1/3 | 2-6 Wind Speed | |
| <input type="checkbox"/> Middle 1/3 | 26 Wind Direction | |
| <input checked="" type="checkbox"/> Lower 1/3 | 4 10 Hr Sticks (if known) | |
| <input type="checkbox"/> Ridge Top | | |
| <input type="checkbox"/> Saddle | | |
| <input type="checkbox"/> Valley | | |
| <input type="checkbox"/> Canyon | | |

ADFA = Adenostoma faciculatum "chamise"

File Name: elkcrf070105
Data entered by: Liz Carver

New Growth: DONE
Flowering: END
Fruit:

Fig. 1. Original data from Sequoia and Kings Canyon National Park National Park

| Location: Elk Creek Area: Ash Mountain Type: Live Year: 2005 | | Topographic Features | | Moisture Determination Record | | | |
|---|--|----------------------|------|-------------------------------|--------------|-----------|------------------|
| | | Aspect | S SE | Date | Time in Oven | Date | Time out of Oven |
| | | Elevational | 2100 | 5/17/2005 | 1215 | 5/18/2005 | 1840 |
| | | Shading | 0 | 5/31/2005 | 1530 | 6/2/2005 | 1245 |
| | | Slope | 17 | 6/15/2005 | 1450 | 6/17/2005 | 1430 |
| | | Upper 1/3 | | 7/1/2005 | 1640 | 7/3/2005 | 1045 |
| | | Middle 1/3 | | 7/15/2005 | 1630 | 7/16/2005 | 1845 |
| | | Lower 1/3 | x | 8/5/2005 | 1100 | 8/6/2005 | 1105 |
| | | Ridgetop | | 8/17/2005 | 1430 | 8/18/2005 | 1500 |
| | | Saddle | | 8/31/2005 | 1600 | N/A | N/A |
| | | Valley | | 10/2/2005 | 1125 | 10/3/2005 | 1230 |
| | | Canyon | | | | | |

| Collection Record | | Old Growth 1 | | | | | |
|-------------------|------|------------------|------------------|------------|------------|-------------|------------|
| Date | Time | Gross Wet Weight | Gross Dry Weight | Can Weight | Dry Weight | Weight Loss | % Moisture |
| 5/17/2005 | 1130 | 129.0 | 119.0 | 104.0 | 15.0 | 10.0 | 66.7 |
| 5/31/2005 | 1500 | 144.0 | 127.0 | 104.0 | 23.0 | 17.0 | 73.9 |
| 6/15/2005 | 1415 | 164.0 | 135.0 | 104.0 | 31.0 | 29.0 | 93.5 |
| 7/1/2005 | 1620 | 155.0 | 123.0 | 104.0 | 19.0 | 32.0 | 168.4 |
| 7/15/2005 | 1545 | 183.0 | 148.0 | 103.0 | 45.0 | 35.0 | 77.8 |
| 8/5/2005 | 1020 | 156.0 | N/A | N/A | N/A | 156.0 | N/A |
| 8/17/2005 | 1345 | 160.0 | 137.0 | 103.0 | 34.0 | 23.0 | 67.6 |
| 8/31/2005 | 1530 | 140.0 | 126.0 | 104.0 | 22.0 | 14.0 | 63.6 |
| 10/2/2005 | 1045 | 159.0 | 140.0 | 104.0 | 36.0 | 19.0 | 52.8 |

Fig.2. Data from Sequoia and Kings Canyon National Park digitized into Microsoft

| Date | Dry Bul | Wet Bul | Relative Humidity (%) | % Cloud Cover | Wind Speed (mph) | Wind Direction | 10 Hr Stick(if know) | New Growth | Flowering | Fruit |
|-----------|---------|---------|-----------------------|---------------|------------------|----------------|----------------------|------------|-----------|-------|
| 5/17/2005 | 66 | 58 | 63 | 5 | 1 to 2 (8) | SW | N/A | N/A | N/A | N/A |
| 5/31/2005 | 86 | 70 | 46 | 0 | 3 to 5 | ups | 8.5 | N/A | N/A | N/A |
| 6/15/2005 | 91 | 67 | 34 | 0 | 5 | 3 | 7 | N/A | N/A | none |
| 7/1/2005 | 99 | 71 | 26 | 2 | 2 to 6 | SW | 4 | done | ended | N/A |
| 7/15/2005 | 83 | 72 | 60 | 75 | 0 to 2 | up canyon | 5 | N/A | ending | none |
| 8/5/2005 | 93 | 61 | 14 | 20 | 3 | SW | 58 | N/A | N/A | N/A |
| 8/17/2005 | 97 | 72 | 30 | 35 | 2 | ups | 6 | N/A | N/A | N/A |
| 8/31/2005 | 96 | 67 | 22 | 0 | 2 to 5 | SW | 4.25 | N/A | N/A | N/A |
| 10/2/2005 | 75 | 61 | N/A | 0 | 1 to 3 | SW | 4 | N/A | N/A | N/A |

Fig.3. Weather and phenology data from Sequoia and Kings Canyon National Park digitized into Excel

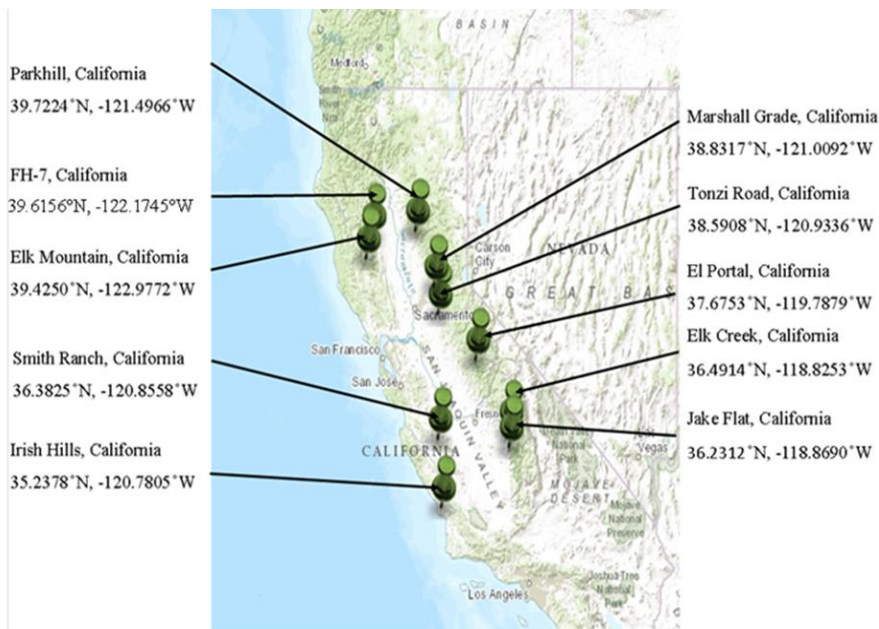


Figure 4. Map of the data locations used from California obtained from the National Fuel Moisture Database

Data Analysis

Old Growth Fuel Moisture vs. New Growth Fuel Moisture

After the Sequoia and Kings National Park data (2001-2014) was entered into Microsoft Excel, the fuel moisture was calculated by:

- 1) “Gross Dry Weight” – “Can Weight”^{*} = “Dry Weight”
- 2) “Gross Wet Weight” – “Gross Dry Weight” = “Weight Loss”
- 3) (“Weight Loss” / “Dry Weight”) x 100 = “% Moisture”

*Can Weight= the weight of the plastic container into which the live vegetation is placed during sampling in the field.

When all of the fuel moisture percentages were determined, the fuel moisture was categorized by phenology. Since the fuel moisture and phenology data were separated on the data charts, the phenology was transferred and matched by date to the corresponding fuel moisture percentage. When all of the phenological descriptions are paired with its appropriate fuel moisture by date, the data was organized into broader categories by phenology (Table 1). Three main stages of phenology are new growth, which is sub-divided into starting (the plant has just begun to grow), continuing (the plant is growing leaves, etc.), complete (all growth has come to a stop), and none (there is no new growth). The next stage is flowering, which is sub-divided into starting (flowers are beginning to grow), peaking (the flowers are done/almost done growing), drying (beginning stages of the flowers’ decline), none (no flowers are present), and declining (the flowers are dying). The last stage of phenology is fruit, which is sub-divided into starting (growth of fruit has started), ripe (the fruit has peaked), fallen (fruit is falling off the plant), and none (no fruit is present).

A One-way Analysis of Variance [ANOVA] test was used to test the null hypothesis that the samples within a particular stage came from populations with the same mean value. If the data among the samples within a stage were not statistically different then the data was grouped and an unpaired Student t-test was used to test the null hypothesis that the *old growth* compared to the *new growth* within the phenological stages was not statistically different.

A Comparison of fuel moisture among sites in California

Fuel moisture data was obtained from various sites throughout California that were selected from the National Fuel Moisture Database (www.wfas.net/). The data was compared among all of the sites. The fuel moisture data for each site went from 2002 to 2014. The mean and standard error were calculated for the months with the highest fuel moisture (March, April, and May) and lowest fuel moisture (August, September, and October). Using this data, two graphs were made for the highest fuel moisture months for *old* and *new growth*.

Additional graphs were created for the lowest fuel moisture months. Error bars represented the standard error on the graphs (Fig. 6-9).

Paired Student t-tests were used for each combination of locations in California (Table 2).

Table 2. P-values for all comparisons of sites in California for *old growth*; Yellow=p-values < 0.05

| Locations | El Portal | Elk Creek | Elk Mountain | FH-7 | Irish Hills | Jake Flat | Marshall Grade | Parkhill | Smith Ranch | Tonzi Road |
|----------------|-----------|-----------|--------------|------|-------------|-----------|----------------|----------|-------------|------------|
| El Portal | | 0.31 | 0.17 | 0.41 | 0.24 | 0.05 | 0.46 | 0.03 | 0.00 | 0.07 |
| Elk Creek | 0.31 | | 0.14 | 0.54 | 0.91 | 0.02 | 0.21 | 0.59 | 0.11 | 0.45 |
| Elk Mountain | 0.17 | 0.14 | | 0.04 | 0.04 | 0.00 | 0.01 | 0.93 | 0.97 | 0.03 |
| FH-7 | 0.41 | 0.54 | 0.04 | | 0.67 | 0.02 | 0.12 | 0.02 | 0.09 | 0.17 |
| Irish Hills | 0.24 | 0.91 | 0.04 | 0.67 | | 0.02 | 0.05 | 0.02 | 0.08 | 0.07 |
| Jake Flat | 0.05 | 0.02 | 0.00 | 0.02 | 0.02 | | 0.35 | 0.00 | 0.00 | 0.61 |
| Marshall Grade | 0.46 | 0.21 | 0.01 | 0.12 | 0.05 | 0.35 | | 0.00 | 0.00 | 0.78 |
| Parkhill | 0.03 | 0.59 | 0.93 | 0.02 | 0.02 | 0.00 | 0.00 | | 0.85 | 0.01 |
| Smith Ranch | 0.00 | 0.11 | 0.97 | 0.09 | 0.08 | 0.00 | 0.00 | 0.85 | | 0.00 |
| Tonzi Road | 0.07 | 0.45 | 0.03 | 0.17 | 0.07 | 0.61 | 0.78 | 0.01 | 0.00 | |

Relationship between Fuel Moisture and Phenology

The same fuel moisture tables used for comparing the *old* and *new growth* samples were used for this analysis. For each subdivisions of the phenology (continuing, complete, etc.) the average was taken for the *old growth* and *new growth*. Each phenology subdivision is listed in Table 3.

The numbers that represented the phenology were the x-values on the graph. The y-values were the means of the fuel moistures for the three samples in each phenological stage. To analyze the data, scatter plots were created and the correlation coefficient was determined to see if there was a relationship between fuel moisture and phenology (Fig. 10-12).

Table 3. Each phenology stage and its subdivision.

| New growth | Fruit | Flowering |
|------------|------------|-----------|
| Starting | Presenting | Starting |
| Continuing | Ripe | Peaking |
| Complete | Fallen | Drying |
| None | None | None |
| | | Declining |

RESULTS

Old Growth Fuel Moisture vs. New Growth Fuel Moisture

The ANOVA tests indicated that there was no statistically significant difference among the three samples for the stages: new growth, flowering and fruit (Table 4). Therefore, we were confident about grouping the data for the comparison of *old* and *new growth* for the subdivisions.

Table 4 shows that in all cases, except for the “none” phenology for both new growth and flowering, there was a significant difference between *old growth* and *new growth* fuel moisture. Figure 5 shows the means of *old* and *new growth* for the flowering stages.

Table 4. ANOVA test probabilities for each fuel type in the three phenological stages. Also shown are the t-test probabilities between *old* and *new growth*.

| | | ANOVA | | T Test |
|------------|------------|-------|------|-------------|
| Phenology | | Old | New | Old vs. New |
| New Growth | Continuing | 0.39 | 0.74 | 6.6E-12 * |
| | Complete | 0.44 | 0.39 | 0.03 * |
| | None | 0.12 | 0.53 | 0.39 |
| | Starting | 0.95 | 0.65 | 0.0002 * |
| Flowering | Starting | 0.97 | 0.92 | 3.2E-14 * |
| | Peaking | 0.85 | 0.88 | 1.9E-13 * |
| | Drying | 0.92 | 0.99 | 3.4E-07 * |
| | None | 0.28 | 0.78 | 0.43 |
| | Declining | 0.53 | 0.69 | 0.03 * |
| Fruit | Presenting | 0.52 | 0.47 | 2.7E-09 * |
| | Ripe | 0.95 | 0.94 | 0.0002 * |
| | Fallen | 0.81 | 0.65 | 0.004 * |
| | None | 0.46 | 0.68 | 2.6E-14 * |

*data is statistically significant (p<.05)

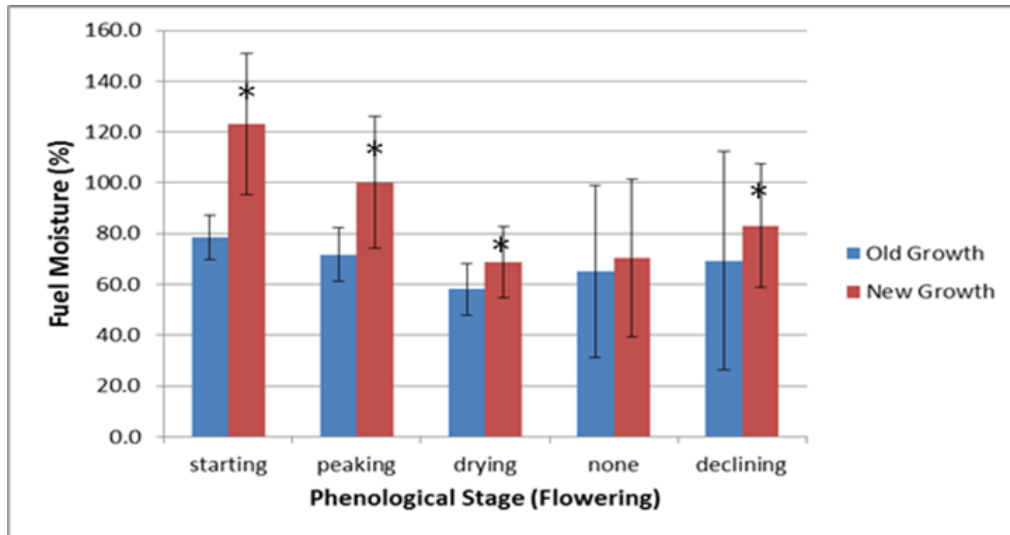


Figure 5. Example of a graph of means of *old* and *new growth* with standard error bars. * = Statistical difference between *old* and *new growth*, (p-value ≤ 0.05%)

A Comparison of Elk Creek fuel moisture to other sites in California

The graph for the spring months of *new growth* indicated that Marshall Grade had the highest mean fuel moisture and Elk Mountain had the lowest (Fig 6).

Similar to the spring months, Marshall Grade had one of the highest mean fuel moistures along with Irish Hills and Tonzi Road for fall months of *new growth*, while Smith Ranch had the lowest (Fig 7).

Graphs were also made for *old growth* for the fall and spring months. Marshall Grade still had one of the higher mean fuel

moisture, but Tonzi Road had the highest. Similar to the spring months for *new growth*, Elk Mountain had the lowest mean fuel moisture for *old growth* (Fig 8).

The mean for Marshall Grade decreased, and it is not one of the higher means consistent with data in Figures 6, 7 and 8. During the fall months for *old growth*, Jake Flat and FH-7 had

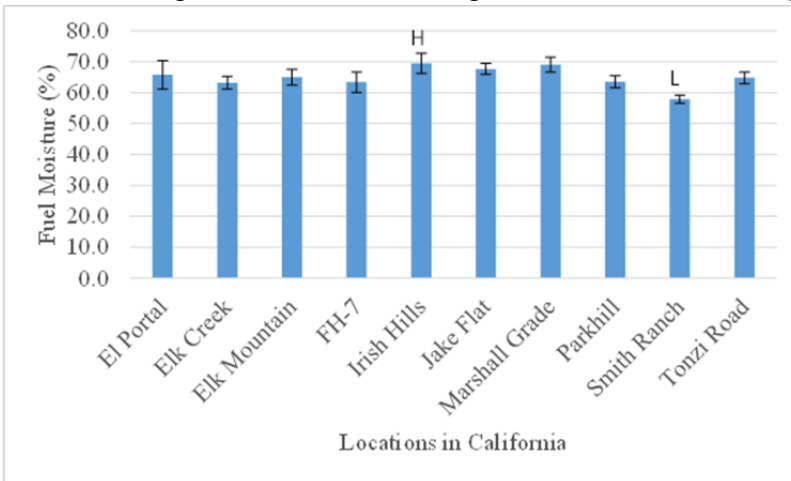


Figure 7. *New growth* means for August, September and October, H=Highest, L=Lowest

the highest mean fuel moisture. Smith Ranch had the lowest, comparable to the fall for *new growth* (Fig 9).

Paired Student t-tests were done to test the null hypothesis that the fuel moisture of different locations for both *old* and *new growth* was statistically different.

Table 2 indicated that for 28 out of the 46 possible combinations, there was no statistical difference between the two locations. Every location had at least one difference with another. The location that had the lowest amount of statistical differences with any other was Elk Creek, with only one instance with Jake Flat. Jake Flat and Parkhill had the most differences compared to any other location for *old growth* as shown in Table 2.

Table 5 shows that out of the 46 combinations of locations, 33 of them were statistically different. The only location that was statistically the same to every other location was Irish Hills. For *new growth*, Marshall Grade, Parkhill, Smith Ranch and Tonzi Road had the most differences with other locations.

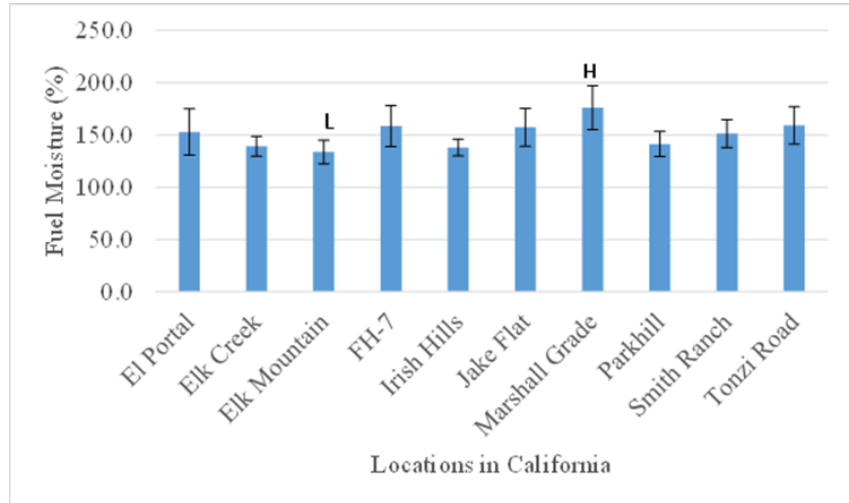


Figure 6. *New growth* means for March, April and May, H=Highest, L=Lowest

Relationship between Fuel Moisture and Phenology

The means of all of the samples were graphed along with their corresponding phenological stage based on Table 3.

Figure 10 indicates that the fuel moisture for “starting” phenology in new growth is higher than that of all other subdivisions, with greater differences between the “starting” subdivision and “complete” and “none” subdivisions. The lowest fuel moisture by phenological subdivision of *old growth* was “complete” and for *new growth* the lowest was “none”.



Figure 8. *Old growth* means for March, April, and May, H=Highest, L=Lowest

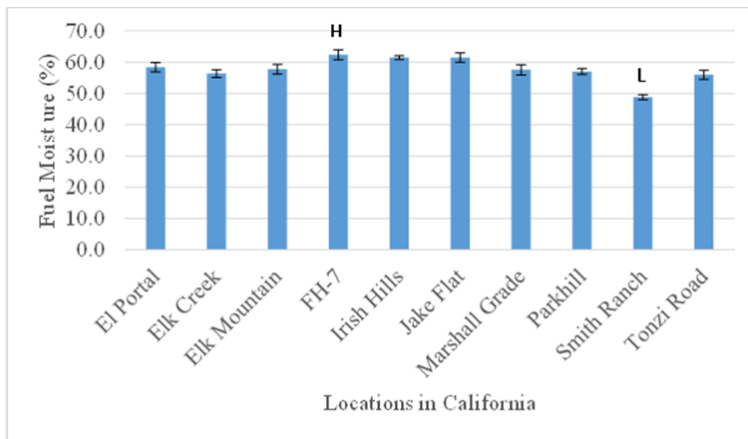


Figure 9. *Old growth* means for August, September and October; H=Highest, L=Lowest

“complete” and for *new growth* the lowest was “none”.

The phenological subdivision of “presenting” has the highest mean fuel moisture for both the *old* and *new growth* graphs for fruit. In both graphs “none” has the second greatest fuel moisture percentage and the fuel moisture increases greatly when the “fallen” stage has ended.

“Fallen” has the lowest fuel moisture in both graphs and the fuel moisture decreases from “ripe” when it’s changing into the “fallen” stage as seen in Figure 11.

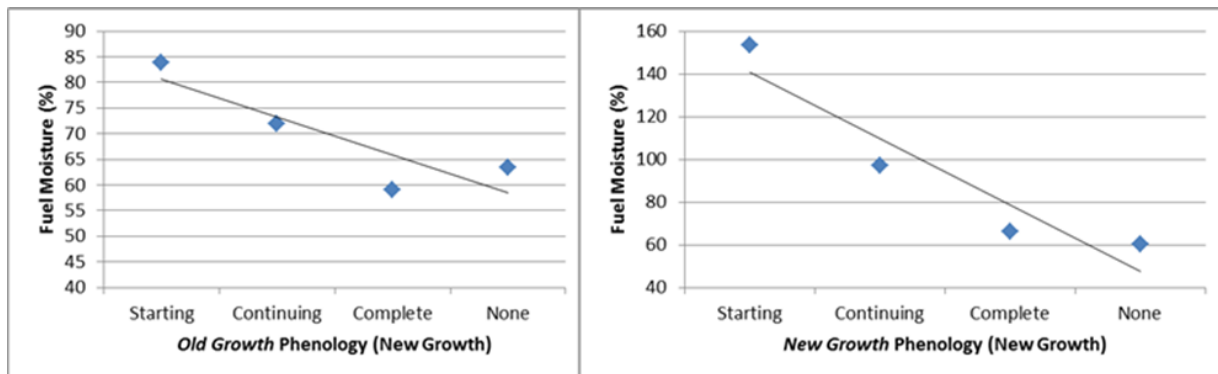


Figure 10. Relationship between the mean fuel moisture mean to the phenological subdivisions of new growth. Fuel mean moisture= Average amount of water in vegetation. Phenological stage= stage in life cycle of Chamise

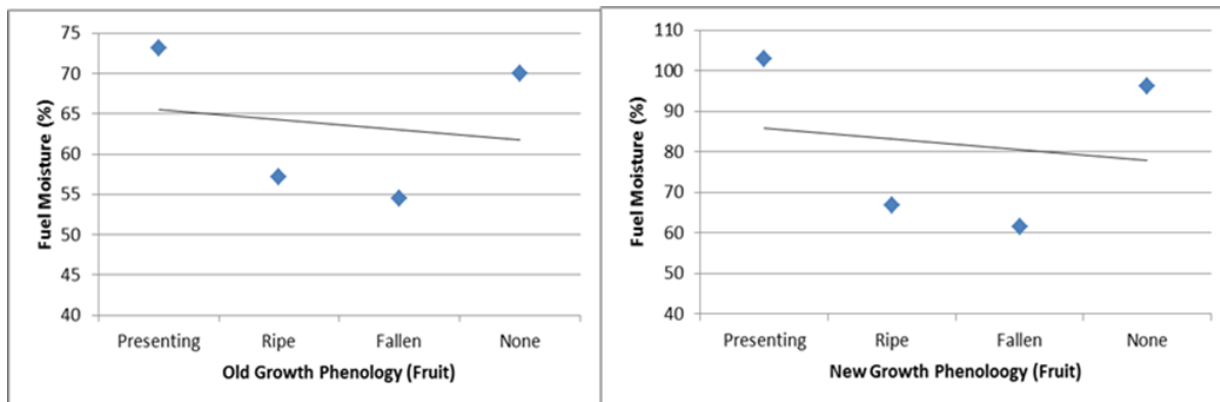


Figure 11. Relationship between the fuel moisture mean to the phenological stages of fruit. Fuel mean moisture = Average amount of water in vegetation. Phenological stage= stage in life cycle of Chamise

“Starting” had the highest mean fuel moisture and “drying” had the lowest mean for flowering. As the “peaking” stage arrived, the fuel moisture decreased more in the *new growth* graph than the *old growth* graph. Also the increase in fuel moisture from “drying” to the “none” stage increased much more in *old growth* graph than in the *new growth* (Fig 12). Correlation coefficient values were calculated (Table 6);

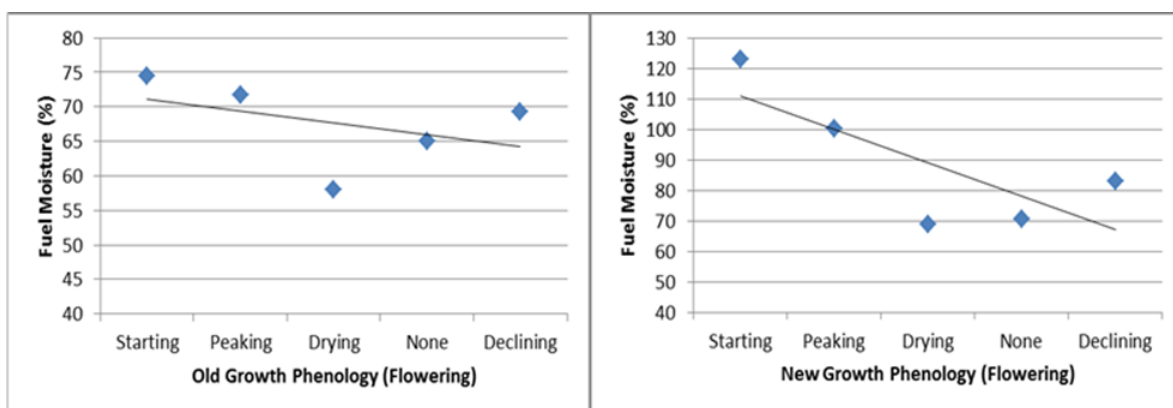


Figure 12 Relationship between the fuel moisture mean to the phenological stage for flowering. Fuel mean moisture = Average amount of water in vegetation. Phenological stage= stage in life cycle of Chamise

Two of the stronger correlations are between the *old growth* and *new growth* fuel moisture and phenology for the new growth stages. A weak correlation was seen between *new growth* fuel moisture and its phenology in the flowering subdivision; however the *old growth* for flowering did not show any correlation. The *old/new growth* fuel moistures had no correlation with the phenology in the fruit subdivision as well.

Table 6. Correlation coefficient values for the relationship between phenology and fuel moisture.

| | Old | New |
|------------|--------|--------|
| New Growth | -0.874 | -0.940 |
| Fruit | -0.172 | -0.161 |
| Flowering | -0.416 | -0.762 |

DISCUSSION

One of the primary goals of this study was to determine if there was a relationship between fuel moisture and phenology. If a relationship did exist between the two, then forest fires would occur at varying intensities as vegetation goes through different stages of its life. If the fire intensity of a potential fire could be predicted using the phenology of vegetation, then more preparation could be made in anticipation of a fire, because the speed at which fires spread could be predicted. Based on our results, fuel moisture had a strong correlation with the *old* and *new growth* of the new growth stages and a weaker correlation for the *new growth* of the flowering stage. This means that when Chamise Chaparral starts growing in early to mid-spring, the fuel moisture will be higher than at other points during the year. During this time, the intensity of forest fires will be less than they would be during the rest of the year. Chamise generally blooms from late spring to early summer in both a diverse vegetative environment and a Chamise only environment (Stohlgren, et. al., 1984). However, when in a diverse environment, Chamise is still the dominant plant. During this time, the fuel moisture decreases, so the intensity of forest fires will increase due to the lack of moisture. This study corroborates Pellizzaro's (2007) study involving Mediterranean plants. Because there have not been extensive studies done in California on the relationship between fuel moisture and phenology, future research could entail studying the same relationship with other species found in California.

Our original hypothesis was that the fuel moisture of Chamise Chaparral would not vary relative to its location. However, the data showed that the fuel moisture was different throughout the sites we observed in California. The only instance when there was no difference in fuel moisture based on location was the *new growth* in Irish Hills. For *old growth*, Elk Creek had the fewest number of differences in fuel moisture compared to other locations. The only location where there was a difference was at Jake Flat, which happened to be the location geographically closest to Elk Creek. Although our graphs show overlapping error bars, this could be due to difficulty in obtaining a consistent sample size for the different areas. We further hypothesized that rainfall, relative humidity and elevation could be key factors for the difference in fuel moisture. More field research would be required to test that hypothesis.

Another aspect of this study was to determine if the fuel moisture of *old* and *new growth* samples were the same or different. To do this we needed to see if the samples themselves (*old/new*) were the same. Since they were comparable, the samples were juxtaposed. From our data, it was obvious that *new growth* moisture was always higher than the *old growth*. Because they are different and the *new growth* was higher than the *old*, fires could potentially burn Chamise at different rates since the plant itself has different fuel moistures.

Our results support that during different seasons of the year, forest fires will burn at different intensities. These intensities can be predicted based on the phenological stage of Chamise Chaparral and that the fuel moisture for Chamise varies by location. Data for fuel moisture has been used in making strategic decisions when it comes to igniting prescribed forest fires and has been used ever since the 1940s in order to calculate fire danger (Weise, et al. 1998).

Results from this study can potentially be used to help determine the intensity of forest fires, as fuel moisture continues to be an important part of determining fire danger, not only in California but in other parts of the United States and many other countries. This can be used as a baseline for understanding how a changing climate would affect vegetation as well as potential changes in fires and their effects.

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ALLIUM SATIVUM (GARLIC) IS AN INHIBITOR OF HMGB1-STIMULATED INFLAMMATION IN RAW 264.7 CELLS (MOUSE CELLS) WITH POTENTIAL THERAPEUTIC EFFECTS IN THE TREATMENT OF SEPSIS

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ABSTRACT

Sepsis is a serious global, public health problem that causes 225,000 deaths annually in the United States, and there are currently no vaccines or drugs to treat sepsis. The pathogenesis of sepsis involves a hyper-response by the body to an infection of the blood. This hyper-response is caused by the excessive release of pro-inflammatory cytokines. A relatively new pro-inflammatory cytokine named HMGB1 has been a target for the treatment of sepsis since its release coincides with the death of septic patients. Garlic extract has been shown in multiple studies to be able to reduce LPS-(Lipopolysaccharide, an endotoxin) stimulated inflammation. Due to garlic extract's anti-inflammatory effects it was chosen as a possible inhibitor of HMGB1. Garlic extract, trypsinized garlic extract (only reduced TNF), and boiled garlic extract successfully inhibited HMGB1 inflammation by reducing the levels of TNF (Pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine). Also, garlic extract and boiled garlic extract increased the levels of IL-6 (anti and pro-inflammatory cytokine) and IL-1 β (Pro-inflammation cytokine). These four cytokines were significant; since, the previously shown relationships between four would demonstrate the anti-inflammatory response cause by garlic extract. Additionally, Garlic extract, trypsinized garlic extract, and boiled garlic extract inhibited NF-kB activation at NLS p50. Furthermore, the anti-inflammatory molecule/molecules in the garlic extract were heat stable and not proteins. These results demonstrate that garlic extract inhibits HMGB1 inflammation and therefore could be tested as a novel treatment for sepsis.

INTRODUCTION

In April 2012, Rory Staunton, a sixth grader from Queens, suffered a minor cut while playing basketball. Nobody, not even the doctors at NYU Langone Medical Center expected what happened next; Rory was taken to the emergency room after experiencing severe discomfort in his abdominal region. He was sent home after being diagnosed with a stomachache. Days later Rory died. He died from the complications of sepsis (Dwyer, 2012).

The death of Rory Staunton galvanized nationwide awareness of sepsis and the effort to stop sepsis, as Rory's case is not an isolated incidence. About 750,000 people in the U.S. each year get sepsis, and about 225,000 of them die from it (Wang et al., 2004). The condition is an infection of the bloodstream, and it can arise from a number of infections that attack the body, such as meningitis, pneumonia and infections of the skin or bladder, to name a few. The blood poisoning is caused not by the infections themselves, but by the body's hyper-response (to release excessive amounts of pro-inflammatory cytokines) to those infections, when it releases a barrage of chemicals that can lead to organ failure (Mayo Clinic, 2014). The lethality of sepsis is apparent considering it is the third leading cause of death in the ICU (Wang et al., 2004). Unfortunately, even with the best antibiotics and supportive care, a third of these patients will die and no current drugs are able to treat the infection.

In recent years, research involving the inhibition of pro-inflammatory cytokines to treat sepsis has been conducted. Cytokines are small molecules that allow signaling between cells, their interactions control chemotaxis, cellular growth, and cytotoxicity (Heath, 2002). Additionally, cytokines regulate the immune system and the body's inflammatory response (Heath, 2002). This makes them a target for treatment of inflammatory diseases. The cytokines predominantly targeted in sepsis studies are tumor necrosis factor (TNF) and interleukin 1 (IL-1). Inhibition of TNF and IL-1 was shown to be very successful in improving sepsis survival rates in animal models. Despite success in animal models, clinical trials failed to improve survival rates in septic patients (Wang, 1999). The reason for this is the early release of TNF and IL-1 during sepsis; TNF and IL-1 are released at peak levels 1 to 2 hours into sepsis (Wang et al., 2001). This early release creates a very small therapeutic window and does not address downstream cytokines that cause death. A late mediator of sepsis has been discovered and identified, high mobility group box 1 (HMGB1) protein. HMGB1 was originally thought to be a nuclear DNA-binding protein. It was believed that HMGB1 functioned only as a cofactor in transcription regulation, but it was later discovered that HMGB1 has many different roles including being an intercellular messenger and a pro-inflammatory cytokine when released extracellularly. HMGB1 release reaches its peak at about 20 hours after the infection (Wang et al., 1999) and it was also discovered that HMGB1 release coincides with death from sepsis (Yang et al., 2013). In recent years it has been shown that anti-HMGB1 treatment has been successful in mice models of sepsis; studies have shown treating for HMGB1 significantly increases survival rates in mice suffering from sepsis induced by bacterial toxin LPS (Wang et al., 1999). These facts make HMGB1 a very intriguing therapeutic target, since inhibiting HMGB1 would create a substantially larger window for the treatment of sepsis and theoretically eliminate the mediator of death from sepsis. For these reasons, my project aimed at finding a better treatment for sepsis and using *Allium sativum* (Garlic) as a possible natural inhibitor of HMGB1.

Garlic is a known anti-microbial and anti-inflammatory substance (Keiss et al., 2003). Garlic also has many active compounds including allicin and thiocremonone. Thiocremonone is a sulfur based compound that has been shown to inhibit nuclear factor-kB (NF-kB) activity when RAW 264.7 cells were stimulated with lipopolysaccharide (LPS), an exogenous toxin from the

cell wall of bacteria (Ok Ban et al., 2009). In other studies garlic has been demonstrated to significantly reduce production of TNF, IL-1 α , interleukin 8 (IL-8), interferon-gamma (IFN- γ) and interleukin 2 (IL-2) in peripheral blood mononuclear cells (PBMC's) when stimulated with LPS (Hodge et al., 2002). Garlic's inhibition of LPS stimulated NF-kB activity and cytokines, has led me to hypothesize that garlic could possibly also inhibit HMGB1, leading to improved treatment for sepsis.

MATERIALS AND METHODS

Materials

LPS (E.coli. 0111:B4) and toluene (99.8%) were purchased from Sigma (St. Louis, MO, USA). Trypsin-EDTA (0.5%), Dulbecco's Modified Eagle Medium (DMEM, Fetal Bovine Serum (FBS), Penicillin-Streptomycin (P/S), and Opti-MEM (OP) were purchased from Gibco by Life Technologies (Grand Island, NY, USA). Raw 264.7 cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Garlic was purchased at Waldbaums (Island Park, NY, USA). Mouse TNF enzyme-linked immunosorbent assay (ELISA) kits were purchased from eBioscience (San Diego, CA, USA). Mouse IL-6, IL-10, and IL-1 β ELISA kits were purchased from R&D Systems (Pittsburgh, PA, USA). NF-kB ELISA kit and Nuclear Extract Kit were purchased from Active Motif (Carlsbad, CA, USA). Bio-Rad Protein Assay Dye Reagent was purchased from Bio-Rad Laboratories Ltd (Hemel Hempstead, UK). HMGB1 was made in the lab according to standard protocol (Li J et al., 2004).

Cell Culture and Treatment

Murine macrophage-like RAW 264.7 cells were cultured in DMEM, FBS and P/S. Cells were cultured in sterile 96 and 6 well plates purchased from Fisher Scientific (Waltham, MA, USA). The various extracts were added to individual wells using a micropipette.

Cytokine Measurements

TNF, IL-6, IL-10, IL-1 β released in the supernatants of RAW 264.7 cell cultures were measured using commercially available ELISA kits according to the instructions of the Manufacturer (eBioscience and R&D Systems).

Production of Nuclear Extract

RAW 264.7 cells cultured in a six well plate were aspirated of their medium and frozen overnight. The next morning, 3 mL of cold PBS/Phosphatase inhibitor was added to each well. The cells were removed from their wells using a cell scraper. Once removed the cells were

transferred to pre-chilled 15 mL conical tubes. The cells were then centrifuged for 5 minutes at 200 x g at 4 degrees Celsius. The supernatant was discarded and the cell pellet was kept on ice. The pellet was then gently resuspended in 500 microliters 1x hypotonic buffer. The cells were transferred from the 15 mL conical tube to pre-chilled microcentrifuge tubes. The cells were incubated on ice for 15 minutes. 25 microliters of detergent were added to the cells and the cells were vortexed at the highest setting for 10 seconds. The cells were then centrifuged for 30 seconds at 14,000 x g at 4 degrees Celsius. The supernatant (cytoplasmic fraction) was transferred to pre-chilled microcentrifuge tubes and stored at -80 degrees Celsius. The pellet was then resuspended in 50 microliters of complete Lysis Buffer and vortexed for 10 seconds at the highest setting. The resuspended pellet was incubated for 30 minutes on a rocker set to 150 RPM's on ice. After incubation the resuspended pellet was vortexed for 30 seconds at the highest setting. The resuspended pellet was then centrifuged for 10 minutes at 14,000 x g at 4 degrees Celsius. The supernatant (nuclear fraction) was transferred to a pre-chilled microcentrifuge tube and stored at -80 Celsius till use. The remaining pellet and reagents were discarded.

NF- κ B Measurements

NF- κ B in the nuclear extract was measured by a commercially available ELISA kit according to the instructions of the manufacturer (Active Motif, Carlsbad, CA, USA).

General Protein Measurements

Protein levels in the cell wells were measured using a commercially available Protein Assay Dye Reagent according to the instructions of the manufacturer (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK).

Garlic Extraction

10 g of fresh garlic was chopped and mixed with 20 mL of toluene. The mixture was incubated overnight on a rocker. The mixture was filtered through Whatman no.1 filter paper. 10 mL of sterile water was added to the remaining mixture. The mixture was stirred at room temperature for 24 hours. The aqueous phase was separated from the organic phase of the mixture. The aqueous phase of the mixture was sterile filtered and aliquots were made. Store at -20 degrees Celsius (Rasmussen et al., 2004).

Preparation of Boiled Garlic Extract

An aliquot of garlic extract was thawed and boiled for 10 minutes at the highest setting possible. The boiled garlic extract was allowed to cool to room temperature and then centrifuged quickly (pulsed) at 8,000 RPM.

Preparation of Trypsinized Garlic Extract

Aliquot of garlic extract was thawed and 1.0% trypsin-EDTA (0.5%) was added to the aliquot. It was incubated overnight (16 hours) at room temperature.

Statistical Analysis

Data is presented as means +SEM. Differences between groups were determined by a Two Tailed Student t test. P values less than 0.05 were considered significant.

RESULTS

TNF Release by RAW 264.7 Cells is Inhibited by Garlic Extract, Boiled Garlic Extract and Trypsinized Garlic Extract

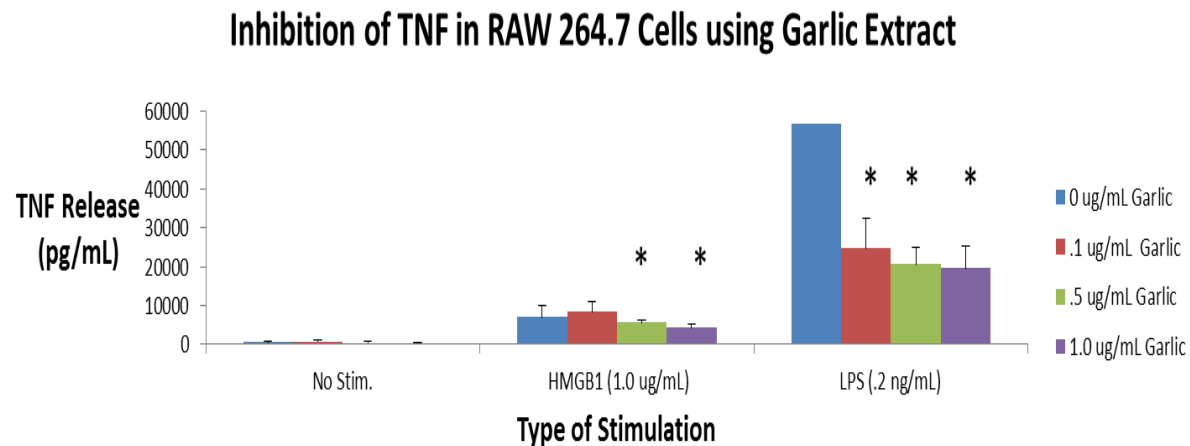


Figure 1. RAW 264.7 cells were stimulated overnight with HMGB1 and LPS, they were treated with various concentrations of garlic extract. Garlic extract inhibited TNF release for both HMGB1 and LPS. N=27. *P<0.05 versus HMGB1 or LPS alone as shown by a 2 Tailed Student T-Test.

TNF release was stimulated using HMGB1 or LPS. LPS stimulated TNF release acted as a control, since previous studies showed that garlic extract inhibited LPS stimulated TNF

release (Hodge et al., 2002). HMGB1 was used to stimulate the cells and the levels of other cytokines were measured to show the inhibition of HMGB1. Like other studies LPS stimulated TNF release was inhibited by garlic extract and HMGB1 stimulated TNF release was also inhibited by the garlic extract (Figure 1).

It was found that HMGB1 stimulated TNF release significantly decreased at $\geq .5$ ug/mL garlic extract in dose dependent manner. The dose dependence of the garlic extract is promising, since it implies the garlic extract is inhibiting TNF release through a mechanism.

The inhibitory effects of boiled and trypsinized garlic on HGMB1 and LPS stimulated TNF release were also tested. The purpose of boiling and adding trypsin was to see if the garlic extract would lose its anti-inflammatory properties when the proteins in the extract were denatured. It was discovered that the boiled and trypsinized garlic extract still inhibited HMGB1 and LPS stimulated TNF release (Figure 2).

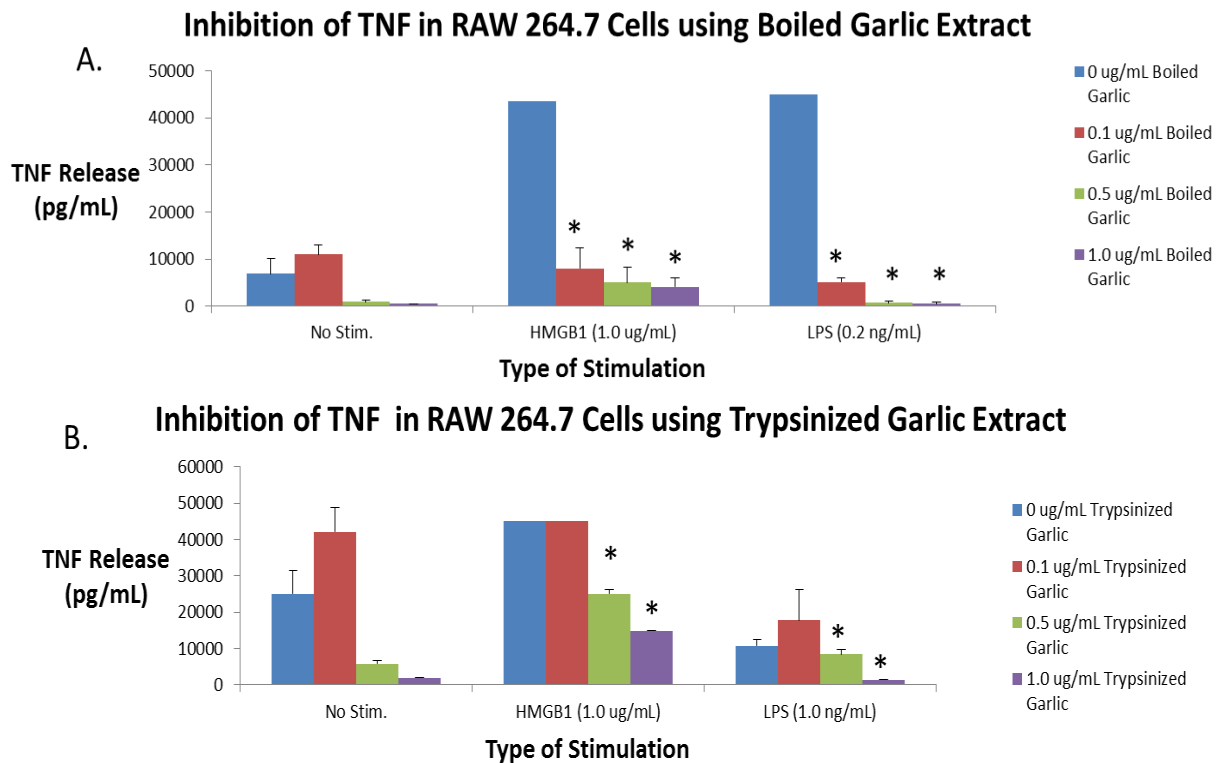


Figure 2. A: RAW 264.7 cells were stimulated overnight with HMGB1 and LPS; they were treated with various concentrations of boiled garlic extract. Boiled garlic extract significantly inhibited TNF release for both HMGB1 and LPS. N=9. *P<0.05 versus HMGB1 or LPS alone. B: RAW 264.7 cells were stimulated overnight with HMGB1 or LPS; they were treated with various concentrations of trypsinized garlic extract. Trypsinized garlic extract significantly inhibited TNF release for both HMGB1 and LPS in a dose dependent manner. N=9. *P<0.05 versus HMGB1 or LPS alone.

Boiled garlic extract significantly inhibited HMGB1 and LPS stimulated TNF release at concentrations ≥ 0.1 ug/mL of boiled garlic extract. It was also found that at ≥ 0.5 ug/mL of

boiled garlic extract inhibited TNF release below baseline levels. Trypsinized garlic extract also significantly inhibited HMGB1 and LPS stimulated TNF release at concentrations ≥ 0.5 ug/mL of trypsinized garlic extract.

IL-1 β Release by RAW 264.7 Cells is Increased by Garlic Extract

IL-1 β release was stimulated using HMGB1 and LPS. It was found that HMGB1 did not stimulate IL-1 β (Data not shown). In previous works, LPS stimulated IL-1 β release was inhibited by garlic extract (Keiss et al., 2003). In my study it was found that garlic extract does not inhibit IL-1 β , but it increases significantly at concentrations ≥ 1.0 ug/mL of garlic extract (Figure 3).

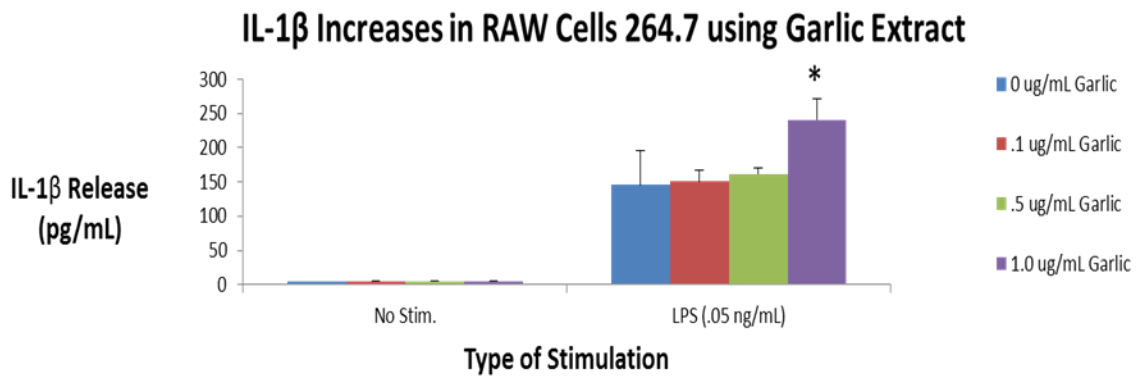


Figure 3. RAW 264.7 cells were stimulated overnight with LPS and treated with various concentrations of garlic extract. Garlic extract significantly increased IL-1 β release. N=15. *P<0.05 versus LPS alone.

IL-1 β Release by RAW 264.7 Cells is not Affected by Boiled Garlic Extract

In this experiment IL-1 β release was stimulated using HMGB1 and LPS. As in the previous experiment HMGB1 did not stimulate IL-1 β release. It was found that boiled garlic extract had no effect on LPS stimulated IL-1 β release at any concentrations of boiled garlic (Figure 4).

Effect of Boiled Garlic Extract on IL-1 β in RAW 264.7 Cells

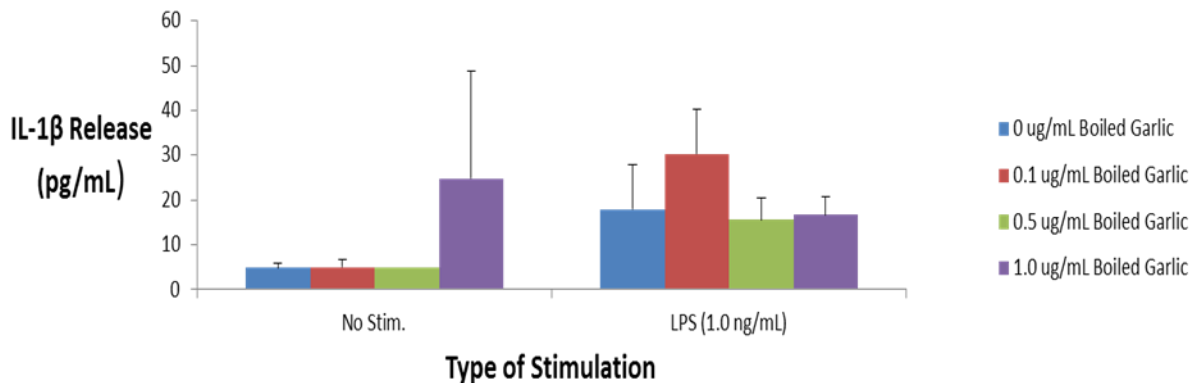
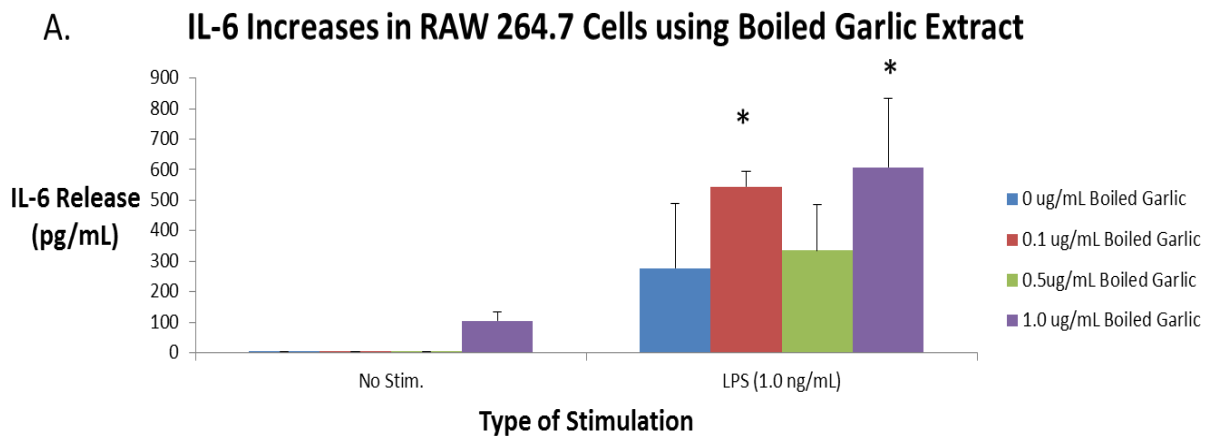


Figure 4. RAW 264.7 cells were stimulated overnight with LPS; they were treated with various concentrations of boiled garlic extract. Boiled Garlic extract did not affect IL-1 β release for LPS. N=3. *P<0.05 versus LPS alone.

IL-6 Release by RAW 264.7 Cells is Increased by Garlic Extract and Boiled Garlic Extract

In previous studies, LPS stimulated IL-6 released was inhibited by garlic extract (Hodge et al., 2002). In my experiment HMGB1 and LPS were used to stimulate IL-6 release in RAW 264.7 cells. Like IL-1 β , IL-6 was not stimulated by HMGB1, but when RAW 264.7 cells were stimulated with LPS and treated with garlic extract or boiled garlic extract IL-6 release increased (Figure 5).



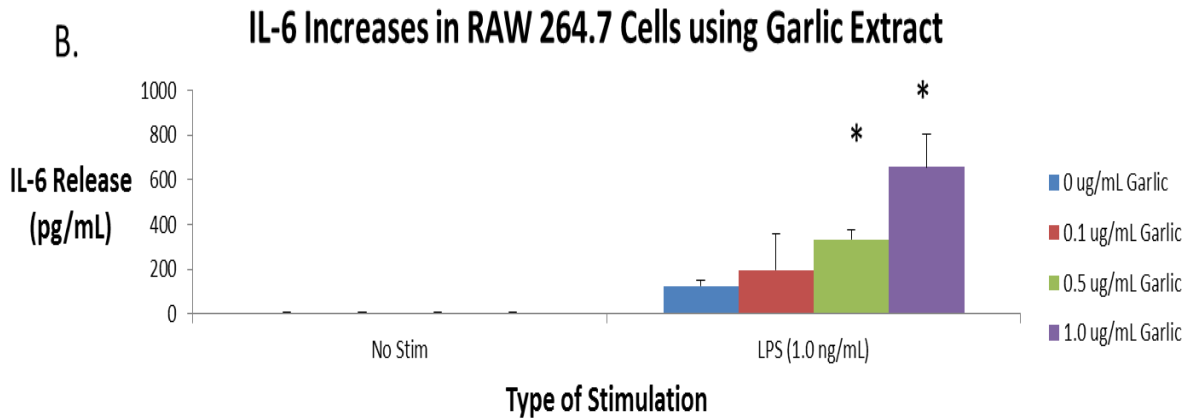
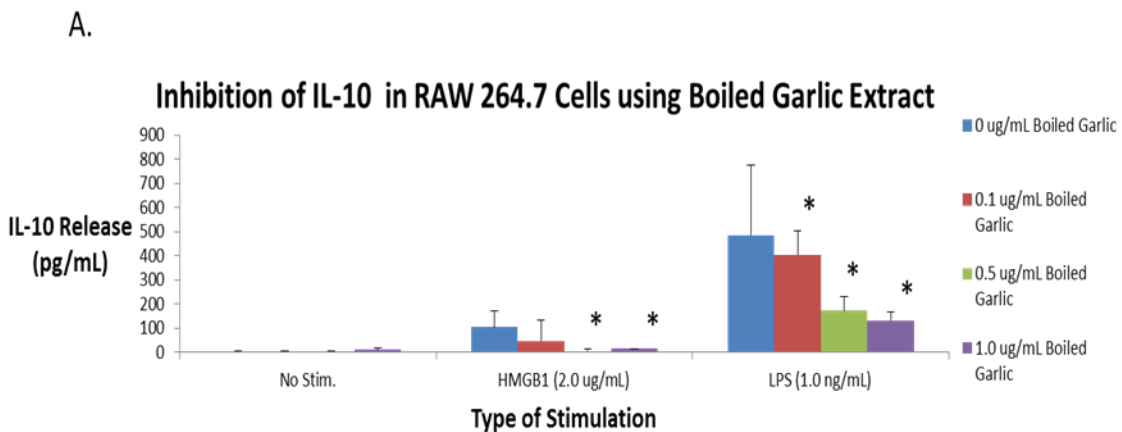


Figure 5. A: RAW 264.7 cells were stimulated overnight with LPS; they were treated with various concentrations of boiled garlic extract. Boiled garlic extract significantly increased IL-6 release when stimulated with LPS. N=6. *P<0.05 versus LPS alone. B: RAW 264.7 cells were stimulated overnight with LPS and treated with various concentrations of garlic extract. Garlic extract significantly increased IL-6 release in a dose dependent manner. N=12. *P<0.05 versus LPS alone.

It was discovered that garlic extract causes a significant increase in LPS stimulated IL-6 release at concentrations ≥ 0.5 ug/mL garlic extract. It was also found that boiled garlic extract causes a significant increase in LPS stimulated IL-6 release at 0.1 ug/mL boiled garlic extract and 1.0 ug/mL boiled garlic extract.

IL-10 Release by RAW 264.7 Cells is Inhibited by Garlic Extract and Boiled Garlic Extract

In previous experiments, LPS stimulated IL-10 levels were found to significantly decrease when BALB/c mice were treated with garlic extract (Ghazanfari et al., 2000). Furthermore, other studies showed that LPS stimulated IL-10 in peripheral blood mononuclear



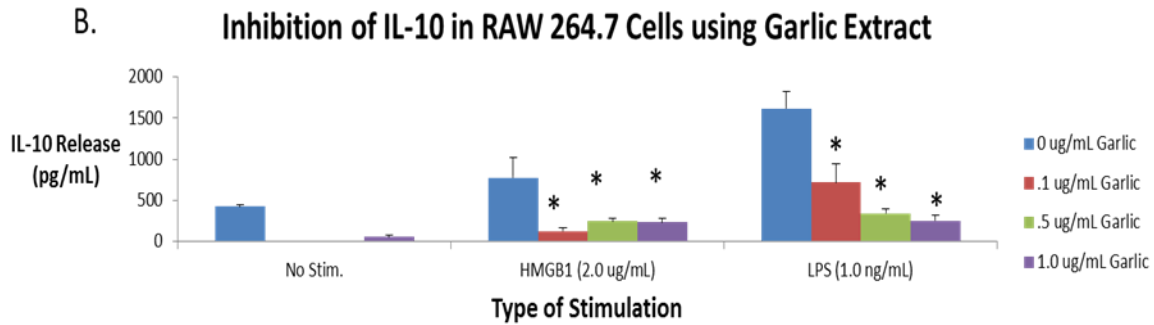


Figure 6. A: RAW 264.7 cells were stimulated overnight with HMGB1 or LPS; they were treated with various concentrations of boiled garlic extract. Boiled garlic extract significantly inhibits HMGB1 or LPS induced IL-10 release. N=6. *P<0.05 versus HMGB1 or LPS alone. B: RAW 264.7 cells were stimulated overnight with HMGB1 or LPS and treated with various concentrations of garlic extract. Garlic extract significantly inhibited HMGB1 and LPS induced IL-10 release. N=9. *P<0.05 versus HMGB1 or LPS alone.

cells (PBMC's) increased when they were treated with garlic extract (Hodge et al., 2002). In my experiment RAW 264.7 cells were stimulated with HMGB1 and LPS. It was found that treatment of the cells with garlic extract and boiled garlic extract inhibited both HMGB1 and LPS stimulated IL-10 release (Figure 6).

The inhibition of LPS stimulated IL-10 release for both garlic extract and boiled garlic extract was significant at ≥ 0.1 ug/mL. Moreover, inhibition of HMGB1 stimulated IL-10 release for garlic extract was significant at ≥ 0.1 ug/mL. Lastly, HMGB1 stimulated IL-10 release was significantly inhibited by boiled garlic extract at ≥ 0.5 ug/mL.

Inhibition of NF-kB Activity in RAW 264.7 cells using Garlic Extract, Boiled Garlic Extract and Trypsinized Garlic Extract

Previous studies have shown that NF-kB activity is inhibited by garlic extract (Ok Ban et al., 2009). NF-kB is a very important transcription factor in inflammatory diseases since; it regulates many pro-inflammatory cytokines including TNF, IL-1, IL-6 and IL-12 (Oeckinghaus et al., 2009). Inhibition of NF-kB would be very significant due to the fact, that various key cytokines would be inhibited. In my study RAW 264.7 cells were stimulated with HMGB1 and various types of garlic including garlic extract, boiled garlic extract, and trypsinized garlic extract. After stimulating, the nuclear content of the cells was extracted and tested for active NF-kB expression at nuclear localization sequences (NLS) p50. It was found that HMGB1 stimulated NF-kB activity was significantly inhibited by garlic extract, boiled garlic extract, and trypsinized garlic extract (Figure 7).

Inhibition of NF-kb Transcription Factor in HMGB1 Stimulated RAW 264.7 Cells Using Various Types of Garlic Extract

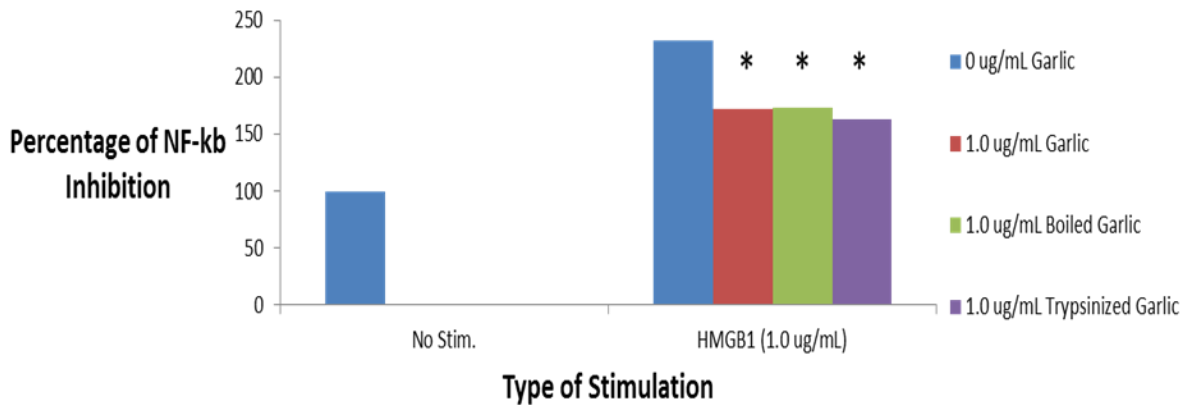


Figure 7. RAW 264.7 cells were stimulated for six hours with HMGB1; they were treated with various types of garlic extract. The various types of garlic extract significantly inhibited NF-kb activation when stimulated by HMGB1. N=4. *P<0.05 versus HMGB1 alone.

All types of garlic extract significantly inhibited NF-kB activity at 1.0 ug/mL. In addition, all types of garlic extract inhibited NF-kB activity about 50%; this inhibition is identical to TNF inhibition at 1.0 ug/mL of garlic extract. This correlation further proves that garlic extract inhibits cytokine release through a biological mechanism.

DISCUSSION

The addition of garlic extract to RAW 264.7 cells reduced HMGB1 and LPS-induced TNF release and caused a variety of significant changes in cytokines and NF-kB levels, revealing a potential therapeutic use in sepsis.

Various concentrations of boiled garlic extract and garlic extract increased LPS stimulated IL-6 and IL-1β levels in RAW 264.7 cells. The IL-6 results suggest that IL-6 is working through classic signaling, given its anti-inflammatory properties, during this experiment. The difference between the two types of signaling is that classical signaling leads to an anti-inflammatory response; trans-signaling leads to a pro-inflammatory immune response (Rose-John, 2012). Classic signaling is the activation of a membrane bound receptor, while trans-signaling is activation of a soluble receptor that attaches to a membrane. It is found that IL-10 down regulates IL-6 and IL-1β (Platzer et al., 1994). These relationships were confirmed in my results since, as IL-10 decreases IL-6 and IL-1β levels increase.

Additionally, multiple concentrations of boiled garlic extract and garlic extract decreased HMGB1 and LPS stimulated IL-10 levels. It has been demonstrated that TNF production up-

regulates IL-10 levels (Platzer et al., 1994). This previously described relationship was confirmed in my results since; as TNF levels decrease IL-10 levels also decrease.

NF- κ B is the key pro-inflammatory transcription factor since; it creates TNF, IL-1, IL-6 and IL-12. NF- κ B attaches to various nuclear localization sequences (NLS). The most common NLS for NF- κ B is p50 (Oeckinghaus et al., 2009). For this reason, NF- κ B levels were measured at the NLS p50. It was found that garlic extract, boiled garlic extract, and trypsinized garlic extract inhibited NF- κ B activity at NLS p50 by 50%. This inhibition is equal to garlic extract's inhibition of TNF levels, which was also 50%.

From the results that were gathered I determined that the inhibitory molecule/molecules are heat-stable and not a protein. The anti-inflammatory molecule/molecules in the garlic extract were shown to be heat stable, since the garlic extract did not lose its effect when boiled. In fact, the effect of the garlic extract was amplified when it was boiled. This increased effect can be seen in the TNF release, since boiled garlic extract at 1.0 μ g/mL return TNF levels to below the baseline; compared to the 50% inhibition seen with normal garlic extract. This is an extremely significant increase in inhibition. Moreover, it can be seen that the anti-inflammatory molecule/molecules in garlic extract are not proteins. The reason for this is that trypsin, a protease, rendered the proteins in the garlic extract inactive. In addition, proteins denature at high temperatures, so the boiled garlic extract results show that the anti-inflammatory molecule/molecules were not protein since the boiled garlic extract retained its anti-inflammatory affect. It could be concluded that the anti-inflammatory molecule/molecules in garlic extract could be an antagonist of Toll-like receptor 4 (TLR4). The reasoning behind this is that it is known that TLR4 binds with both HMGB1 and LPS (Wang, 1999); subsequently TLR4 also activates NF- κ B (Hoshino et al., 1999). These functions and mechanisms of TLR4 make a likely candidate of inhibition for the anti-inflammatory molecule/molecules in garlic extract.

CONCLUSIONS

Garlic extract was shown to act as an effective inhibitor of HMGB1. Garlic extract, boiled garlic extract, and trypsinized garlic extract were able to reduce HMGB1 and LPS stimulated cytokine release (TNF release, IL-10 release) and NF- κ B activity (as measured by p50 expression). Also, garlic extract and boiled garlic extract increased IL-1 β and IL-6 release. Additionally, my findings showed that the anti-inflammatory molecule/molecules in the garlic extract were heat stable and not proteins. This is evident since the boiled and trypsinized garlic extract both maintained their anti-inflammatory properties.

These results fulfill the aim of finding a better treatment for sepsis. The overall results are encouraging and prove that garlic extract has potent anti-HMGB1 effects, which have been validated in previous studies (Wang et al., 2004). Moreover, garlic extract also demonstrated to be non-toxic using a general protein assay (results not show). The showed that cells did not die because protein levels were similar in all experiment and control wells. This lack of toxicity and garlic extract's potent effects provide hope that garlic extract could be used as an effective

inhibitor of HMGB1 in animal models and it would lack the adverse side effects of a synthesized drug. If taken with antibiotics, garlic extract could have therapeutic potential for the treatment of sepsis and my ultimate aim is to save the lives of people like Rory.

FUTURE RESEARCH

The results of this project created many new directions for further research. First, the effects of dithiothreitol (DTT) on garlic extract could be tested. This would reveal whether or not the anti-inflammatory molecules in garlic could be reduced. Next, the garlic extract could be broken down into different molecules using chromatography, and then each individual molecule's anti-inflammatory effect would be tested. This would reveal which molecules are responsible for the anti-inflammatory effect of garlic extract. Lastly, the effect of garlic extract on the survival rate of septic mice could be tested. This would show if the garlic extract could be metabolized by an organism's body and be effective in preventing experimental induced sepsis in mice.

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THE EFFECTS OF GAMIFICATION ELEMENTS ON THE ENGAGEMENT AND COLLABORATION EFFORTS OF HIGH SCHOOL STUDENTS IN AN EDUCATIONAL CONTEXT

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ABSTRACT

Gamification is an incentive system that is under-studied and not truly understood. In this study, an online interface is used to measure the effects of gamification on the engagement and collaboration efforts of high school physics students, in preparation for a final exam. The ideas of engagement and collaboration are quantified for the purposes of measurement. The results show that gamification is a highly complex incentive; it is a strong motivator in the short-term but its effectiveness diminishes over time. The gamification elements also seemed to encourage engagement and discourage collaboration among the students.

INTRODUCTION

Incentive schemes increase motivation and engagement in an organization by relating compensation to productivity. They may also reduce turnover among good performers and are cost-effective due to savings that result from productivity improvements. Management usually assumes that money is the best motivating force, but oftentimes, it is the intangible and nonfinancial motivator that has the greatest impact (Uke and Coker, 2012).

Incentive systems are classified into three types: monetary, or cash, non-monetary tangible, such as restaurant coupons or vacation trips, and non-monetary intangible, like “employee of the week” recognition or positive performance reviews (Condly, 2003). In workplace history, the first two categories of incentives, monetary and non-monetary tangible, have been widely used by management. Non-monetary intangible systems, on the other hand, have been recently researched and tested by organizations. An example of a non-monetary intangible system, employee recognition, is arguably the most effective of all the available incentive systems because it offers a relatively low-cost but high-impact means to reward employees. This recognition could be done by holding annual dinners at which high-achievers or performers are celebrated, or by distributing certificates and gold nameplates for those who have earned them (Ude and Coker, 2012).

Uke and Coker (2012) define motivation, the human force that incentive systems appeal to, as a cognitive decision making process through which goal-directed behavior is initiated, energized, directed, and maintained (Ude and Coker, 2012). Motivation begins with the realization that individuals have needs or expectations that they want to meet, which results in a driving force or behavior to accomplish the desired goals. Motives are usually classified as intrinsic or extrinsic, and create desires that are manifested in goal-oriented behavior, physiological responses, and self-reported feelings. Individuals moved to do something for the sake of the activity itself are said to be intrinsically motivated, while extrinsic motivation manifests in the form of prodding, pressure, rewards, or threats of punishment. Four types of extrinsic motivation are defined along a control/autonomy continuum: external regulation, introjected regulation, identified regulation, and integrated regulation. External regulation classifies a motive for external rewards or threats of punishment, while introjected regulation classifies a motive driven by internalized feelings of guilt, goodness, or pride that affected self-esteem and internalized by societal norms. Identified regulation classifies a motive based upon the importance of something to the individual, and integrated regulation coincides with a person's internal values and needs (Coleman, 2011).

Gamification is the use of game-play mechanics for non-game applications, and its main goal is to improve the engagement of users by using game-like techniques such as scoreboards and personalized fast feedback to make people feel more ownership and purpose when engaging with tasks. This mode of creating incentives desires to combine intrinsic with extrinsic motivation in order to raise motivation and engagement. Common gamification elements are levels, points, virtual goods, leaderboards and badges, which are all indications of progression (Muntean, 2011).

Gamification and its elements have backing in several psychological lenses. Gamification as a whole is supported by the self-determination perspective, which advocates that controlling and mastering a situation fulfills the key psychological needs for competence, autonomy, and relatedness, because any type of gamer seeks to control and master the game or gamified platform at hand. The specific impacts of badges and levels in a gamification platform can be explained by either the trait perspective, which sees the need for self-fulfillment, recognition, and affiliation as stable sources of motivation, or the cognitive perspective, which states that motivation depends on clear goals and a high value of consequences. Badges and levels achieve all of these needs, working as virtual status symbols, a means of group identification, and a means of setting and achieving short-term goals. Point systems, on the other hand, are based on the behaviorist learning psychological lense, which is centered on the belief that learning and motivation are based on reinforcement and punishment. Gaining or losing points is a form of reinforcement and punishment and is therefore an effective means to motivate, according to behaviorists (Hense).

This purpose of this study was to investigate the effects of gamification on collaboration efforts and engagement of high school students. The experiment sought to answer the following questions: 1) Does the implementation of gamification elements in an online class forum

promote student collaboration? 2) Does the implementation of gamification elements in an online class forum improve the quality of student responses? The hypothesis was that if gamification was used as an incentive, engagement and collaboration levels would increase.

MATERIALS AND METHODS

In this study, two classes of the same level and with the same teacher were used as test subjects. One class was the experimental group (30 students) and the other was the control group (42 students). The subjects were assigned anonymous user accounts, distinguished only by a code number to maintain anonymity. Each subject was also paired with another subject as a “partner.”

For the experiment, an online interface was used that allowed a participant to answer other participants’ questions, either briefly, by using a “Quick Help” mode, or in-depth, by using an “Extra Help” mode. Once a student response was posted it could be shared on a selected basis, rated, and commented. The experimenter posted review questions on this interface for the New York State physics Regents to all subjects two times a week for 3 weeks. As an incentive to continue checking the interface for ongoing information exchange, the teacher told both groups that if they showed a consistent, decent effort throughout the 3 weeks, 5 points would be

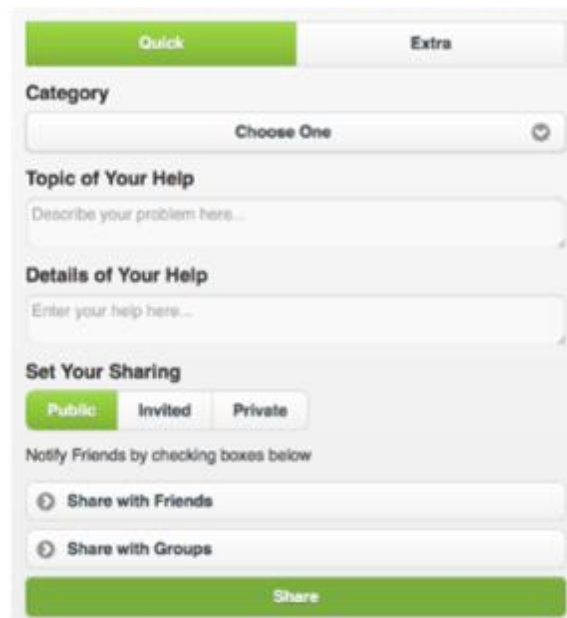


Figure 1: This image shows the online interface used for the experiment.

| Rank | Username |
|------|----------|
| 1 | 126114 |
| 1 | 126127 |
| 2 | 126113 |
| 3 | 126107 |
| 4 | 126103 |
| 5 | 126110 |
| 5 | 126124 |
| 6 | 126115 |
| 6 | 126119 |
| 6 | 126120 |
| 6 | 126125 |
| 7 | 126129 |

Figure 2: This image shows a sample leaderboard for the gamification group.

added to their lowest test grade. This consistent, decent effort was defined as an average of at least 3 points a week.

Participants submitted their response to the posted questions, sharing it only with the experimenter, their teacher, and their assigned “partner” (See Figure 1). During the same time period, each student could “review” their partner’s post by commenting, with the intention of helping the poster improve their response on the next assigned question.

The only distinction between the two groups was that the experimental group had gamification elements implemented. Each of the experimental group participants was shown individual point totals on a scoreboard, along with different achievement levels (See Figure 2). The student also received private feedback from the experimenter on how they were performing. The control group had no gamification feedback. Engagement was measured as the number of points the students receive, based on a set of rubrics (See Tables 1-3).

| Points | Possible Characteristics |
|--------|--|
| -1 | - Inappropriate or irrelevant response |
| 0 | - No response |
| 1 | - A very minimal response (ex: one word) |
| 2 | - Just a link - A very short response (ex: one sentence) - Nothing in the "Extra Help" tab |
| 3 | - Just answers the "what" - Simply copied and pasted (word for word) - No analysis or connections - Nothing in the "Extra Help" tab |
| 4 | - Detailed - Mostly copied and pasted (word for word), but there is some analysis - Copied and pasted, but not word for word; student attempted to make the answer his/her own - "Extra Help" tab is utilized - Not exemplary (a 5) |
| 5 | - Exemplary - Very detailed and analytical - Draws connections to other topics - Includes information from outside sources, but the majority is the student's own thinking - Can include links - Every field is completed, including "Extra Help" |

Table 1: This table is the rubric for the answer post.

| Points Earned | Possible Characteristics |
|---------------|---|
| -1 | - Irrelevant or inappropriate feedback |
| 0 | - No feedback |
| 1 | - Minimal feedback, such as "Good job" or "Ok" |
| 2 | - Good feedback, but it is not very detailed - Doesn't highlight both the positives and the negatives |
| 3 | - Very helpful, detailed feedback - Highlights the positives and negatives - Constructive criticism |

Table 2: This table shows the rubric for the peer-to-peer feedback.

| | |
|------------------------------------|-------------------------------|
| Participation 2 questions in a row | 2 points added to point total |
| Participation 3 questions in a row | 3 points added to point total |
| Participation 4 questions in a row | 4 points added to point total |
| Participation 5 questions in a row | 5 points added to point total |
| Participation 6 questions in a row | 6 points added to point total |

Table 3: This table shows the rubric for consistent participation.

RESULTS

The mean total number of points (counting 0 points) was slightly higher for the gamification group (3.9 vs. 3.2), but the mean total number of points (not counting 0 points) was higher for the control group (13.8 vs. 9.7). The median total number of points (not counting 0 points) was higher for the control group as well (11 vs. 5). However, none of these differences are statistically significant (See Figure 3). More students responded to at least one question in the gamification group than the control group (40% vs. 24%). This difference was statistically significant (See Figure 4).

The differences in number of responses between groups was minimal and not significant. However, the number of responses in each group over time showed interesting patterns. For the gamification group, the percentage of students who responded to each question decreased steadily with each passing question. The control group also descended overall, but less rapidly and consistently than the gamification group (See Table 4 and Figure 5).

| | Gamification | Control |
|--|---------------------|----------------|
| % of Students Who Responded to Question 1 | 20.0 | 16.7 |
| % of Students Who Responded to Question 2 | 16.7 | 11.9 |
| % of Students Who Responded to Question 3 | 16.7 | 9.5 |
| % of Students Who Responded to Question 4 | 13.3 | 11.9 |
| % of Students Who Responded to Question 5 | 6.7 | 9.5 |
| % of Students Who Responded to Question 6 | 6.7 | 11.9 |

Table 4: In the gamification group, the percentage of students who responded decreased steadily with each passing questions. This did not occur to such a noticeable extent in the control group.

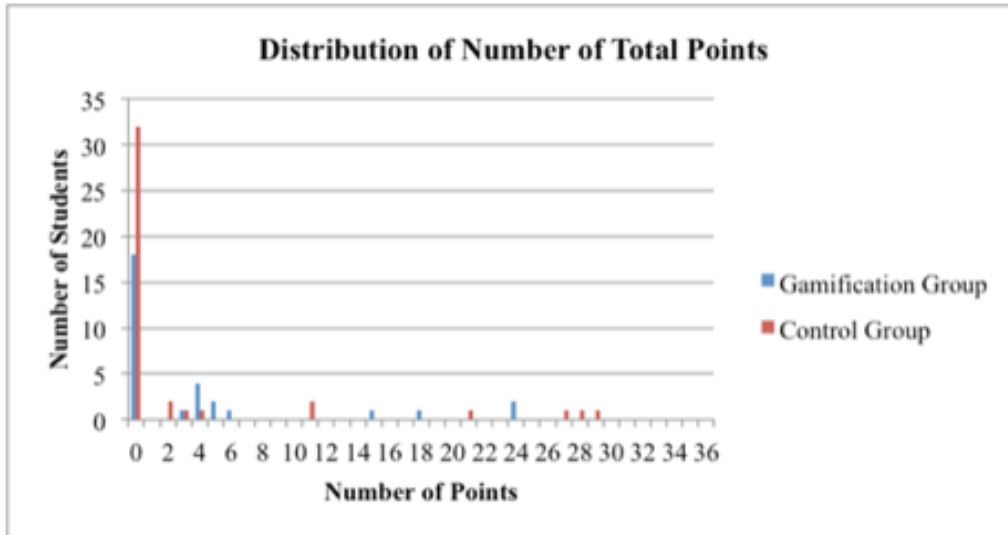


Figure 3: The distribution for the control group is wider than that of the gamification group. There is no statistically significant difference in total number of points for the two groups.

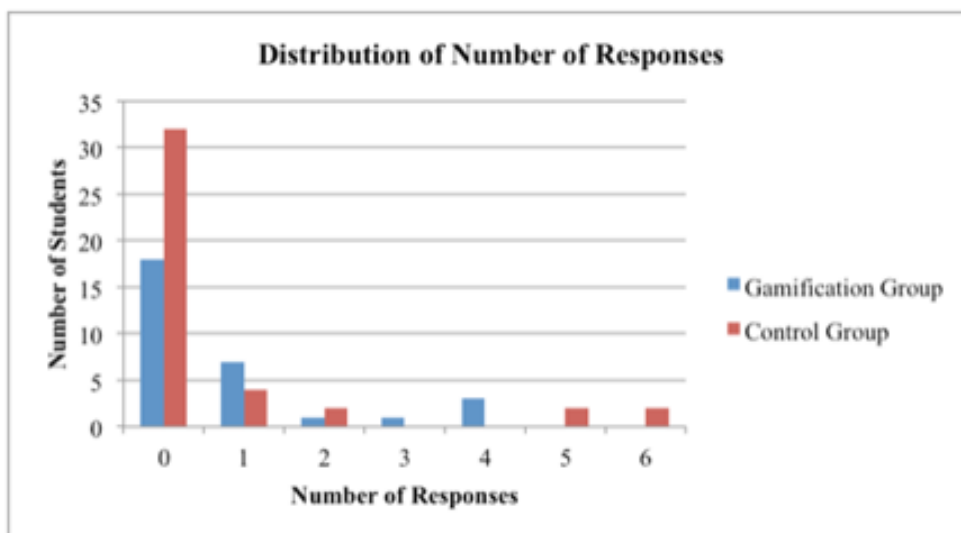


Figure 4: More students responded at least once in the gamification group than in the control group. Only in the control group did some students answer all of the questions. This difference is statistically significant.

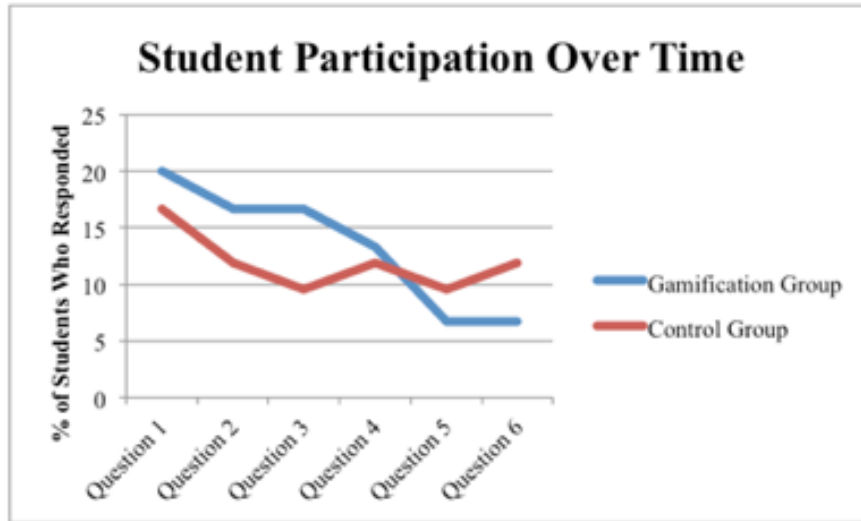


Figure 5: The gamification group had a more pronounced decline in student participation over time.

DISCUSSION

The results indicate that the non-monetary intangible incentive of gamification provides an initial burst of motivation. It was a form of introjected regulation, as participants were driven by feelings of goodness and pride reaffirmed by societal norms. However, this extrinsic motivation does not transfer to the long-term. As seen in Figure 4, a higher percentage of students responded at least once in the gamification group. However, as seen in Table 1, the percentage of students that responded to each question decreased greatly and consistently in the gamification group, with each passing question.

The trends show that the individuals in the gamification group that gave up after the first one or two questions were either at the top of the leaderboard or the bottom. The students at the top may have become overconfident in their ranking, while the students at the bottom may have lost their intrinsic motivation.

There were not as many fluctuations in the trends of the control group. The students in this group that answered the first question had *intrinsic* motivation, and naturally this motivation did not tend to decrease over time. Those who attempted the questions in the beginning had no reason to quit. Gamification, on the other hand, initially gave students a reason to participate, but it also gave them a reason to eventually quit, when their rankings and results were either highly favorable or unfavorable.

The very nature of gaming may explain these results. Games give users a sense of hope, excitement, and motivation at the start, but players can become quickly discouraged if they are not performing to what they believe is their highest potential. This feelings tend to be exacerbated if elements such as leaderboards and levels are part of the program, because individuals are directly compared to their peers and counterparts.

There were limitations to this study that could have biased the results, however.

While the study was designed to also measure collaboration efforts, none of the participants in the study chose to collaborate with their partner, leaving no data to analyze for that dependent variable. In addition, several students, for the first few questions, answered the questions from a username other than their assigned code number. I could not record their data for this reason, and their responses were ignored. If these students had participated from the proper account, the data could have been slightly different.

Further research needs to be conducted about gamification, perhaps both in schools and in the workplace. There is still limited understanding as to how gamification truly affects the mind, and if scientists can begin to develop a deeper understanding of this, gamification incentives could be used strategically to increase engagement, productivity, and motivation in any context.

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JOCKS VS. NERDS...IS THERE A DIFFERENCE?

AN ANALYSIS OF ATHLETES AND NON-ATHLETES WITH REGARDS TO GRADES AND ATTITUDE TOWARD SCHOOL

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ABSTRACT

According to the common definition of a “jock,” an athlete is one that cares more about athletics rather than academics. The purpose of this study was to test where there was a correlation with being an athlete and one’s grades, how one acts in class, and how one looks at academics and athletics in their community. The hypotheses were: 1) Athletes will report higher self-reported grades compared to non-athletes, 2) Non-athletes will cut class more often than athletes, 3) Athletes will study more each night compared to non-athletes, and 4) Athletes will put more time in to their classes to have a better chance to succeed compared to non-athletes. Some of the hypotheses were proven correct from my results. Athletes reported a higher GPA and they also tended to cut class more. Also, compared to female non-athletes, female athletes put more time into both getting good grades and studying. This study doesn’t prove the definition of “jock” wrong so much as it shows the definition to be oversimplified. In fact, being an athlete was associated with good academic outcomes for females, but not so much for males because they scored the lowest in question two and three of the hypotheses.

INTRODUCTION

Jock- Someone who puts more into their athletics rather than their academics. This suggests that as an athlete you don’t put a lot of time into academics. But is one who partakes in athletic competition really that bad in the classroom? A typical weekday for a student-athlete involves, at a minimum, four hours of intense training in addition to a full time academic schedule (Santucci, 2012). Student athletes have a lot of work both school and athletic related. They have to find a balance between both of those things in order to be successful in both aspects as a student and as an athlete (Yu, 2012).

Do the long hours of commitment to a sport day in and day out affect one’s ability to study and do well on tests? In one study, done in a rural high school in Canada, researchers found that all athletes scored higher than non-athlete on their final grade in all subjects, though not statistically significant. This study consisted of 134 high school students from grades 10-12,

including 52 athletes and 82 non-athletes. The study compared both the midterm grades and final grades of each subjects for athletes and non-athletes. A limitation of this study was that if a person was involved in more than one sport, they were eliminated from the experiment. The results show that athletes had higher midterm and final grades than non-athletes in all subjects except mathematics in the midterm grade. Even though the athletes had higher grades; the only subject that was statistically significantly higher was science in both the midterm and the final (Zaugg, 1998).

In another study, the same results were shown again but this time in Kansas, student athletes had a better final GPA then non-athletes. The researchers used academic data from the Kansas State Department of Education and athletic data from the KSHSAA master roster of students who participated in high school sports during the 2008-2009 school year. There were over 62,000 participants in this study due to the availability of their grades from Kansas State Department of Education. The participants included students from grade 9-12. The researchers used self-reported GPA scores to compare athletes and non-athletes. There results showed that 80.1% of athletes said they had a GPA of 3.0 or above and only 70.5% for non-athletes. For those who said they have a GPA of 3.5 or higher, 51.8% of athletes agreed with that statement while only 39.8% of non-athletes agreed (Lumpkin & Favor, 2012). In both these studies, even with the long hours that come with being an athlete, these students prevailed in academics as well.

The “Jock” name comes with a classroom effect as well. The idea of the jock suggests minimal participation in the classroom and that these students may not be taking meaningful courses that will help them in the job market. In a study conducted at the University of California, Berkley, athletes were found to lack motivation in the classroom because they focus most of their time on their athletics. This study consisted of 361 Division I athletes. The experimenters used a Likert scale instrument based on the self-worth theory. The results showed that athletes weren’t motivated to succeed in college academically but they tried to avoid failure in order to continue play on their respective teams (Covington, Simons, and Rheenen, 1999).

Most of the studies that looked at classroom participation in the classroom were done at the collegiate level, and this study was conducted at the high school level. Lately, in the news, there have been reports coming out about college athletes taking classes that don’t exist just so they can stay on the team. These students took classes that they never had to attend and their final consisted of essays that were a paragraph long. All these fake classes can be done at the collegiate level where people are looking the other way, but it cannot be done at the high school level where this study tested it.

The present study looked at high school students and student-athletes from the south shore of Long Island in New York and their GPAs, class participation, and athletic and academic values.

Hypotheses

- 1) Athletes have a higher self-reported GPA compared to non-athletes.
- 2) Non-athletes cut class more often than athletes.

- 3) Athletes study more each night compared to non-athletes.
- 4) Athletes will put more time in to their classes to have a better chance to succeed compared to athletes.

MATERIALS AND METHODS

Participants

Participants in this study were students at a south shore public high school on Long Island, NY. The participants were 186 male and female high school students of varying races of from 14 to 18 years old. Some participants were student athletes (n=104) and others were not (n=82). Out of the entire sample, 21% were male non-athletes, 31% were male athletes, 23% were female non-athletes, and 25% were female athletes.”

Instrument

The survey was a total of 34 questions. The first part, which everyone took, was 24 questions long. The survey was administered using a 5 point Likert scale, with responses ranging from 1 (“Strongly disagree”) to 5 (“Strongly agree”). Some questions were scored reversed, so each question was looked at differently. In this part, I asked the participants to provide me with their self-reported grades (“*What kind of grades do you usually get?*”), with responses ranging from 1 (mostly Fs) to 5 (mostly As). I also wanted to find out how these students felt about their community in regards to academics and athletics (“*Athletics are important in my community*”, “*Academics are important in my community.*”). I also wanted to find out how the students felt about school (“*It would really bother me to get a bad grade*”). The last 10 questions were used only for athletes to answer. Here, I wanted to find out what sports they play, if they wanted to continue with their sport career in the collegiate level (“*Do you expect to play a sport in college?*”), and how much time they dedicate to playing the sport each week (“*During your sport season, how many hours a week do you devote to athletics (including playing, practicing, etc.)?*”). Responses ranged from 1 (1 hour) to 5 (10 hours or more).

Procedures

Since all students are required to take English every year of high school, I contacted the English teachers through email and in person. The English teachers distributed and administered the surveys to the subjects. It took approximately from 5-10 minutes. Students were informed that their participation was voluntary and that their responses would be kept anonymous, that is, their identities would never be publicly associated with their responses. After the surveys were returned, I organized and analyzed the data in an Excel spreadsheet. Lastly, there statistical analysis on the data collected.

Data Analysis

After the surveys were completed, the data was analyzed and separated the data into three tests (Athletes vs. Non-Athlete, Male Athletes vs. Male Non-Athlete, and Female Athletes vs. Female No-Athletes). Then, I analyzed the results and determined if there was statistical significance. For analysis of the data test, I tested the average for a pair item of athletes and non-athletes and did a 2 sample t-test. Lastly, I compiled my data into graphics and tables.

RESULTS

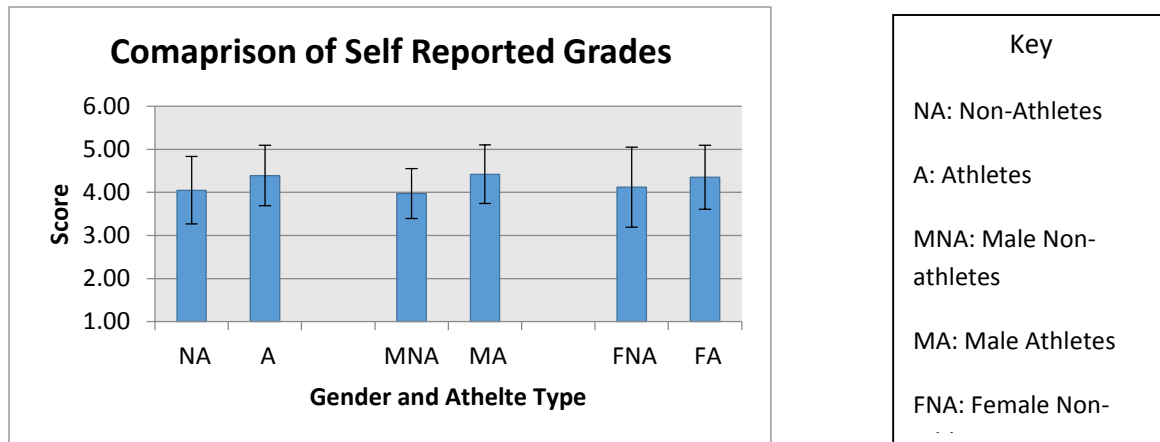


Figure 1.

Students were asked to report their usual grades, ranging from Fs (=1) to A's (=5). In a comparison of self-reported grades, the athletes (n=104) scored a mean of 4.39 and the non-athletes (n= 82) scored a mean of 4.05 ($p<.01$). Male athletes (n=58) scored a 4.42 while male non-athletes (n=39) scored a 3.97 ($p<.001$). The difference in the mean self-reported grade scores for female athletes (n=46) and female non-athletes (n=43) were not statistically significant. Male athletes reported the highest grades while male non-athletes reported the lowest (figure 1).

Students were asked how often they cut class, with responses ranging from “never” (=5) to “every day” (=1). Results were significantly different only for athletes and non-athletes as a whole. The athletes (n=104) scored higher with a mean self-reported score of 4.67 while the non-athletes (n=82) scored lower with a mean self-reported score 4.46 ($p<.05$) (figure 2).

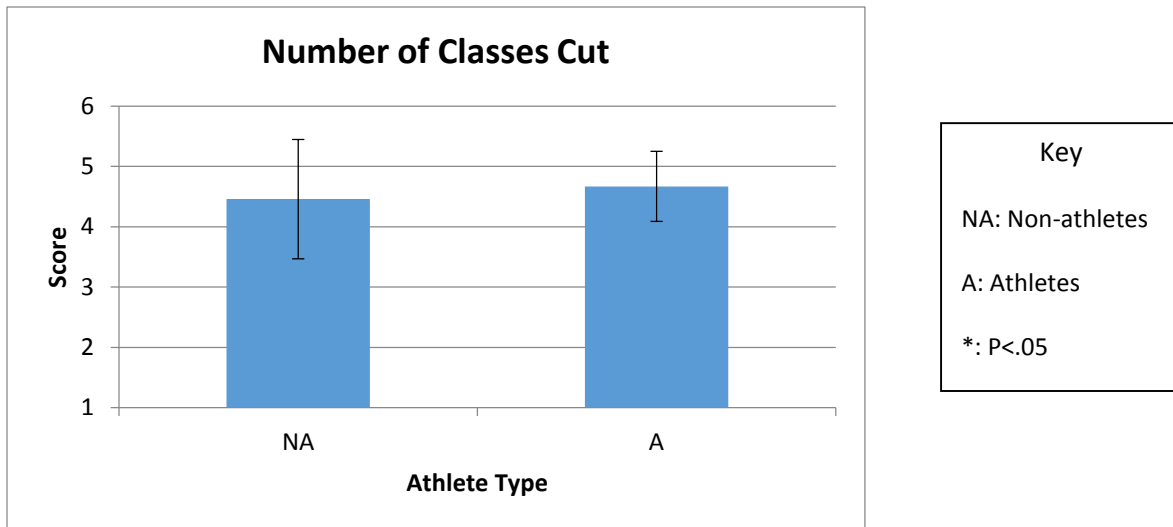


Figure 2.

To compute this score two items were averaged together: “*It is important for me to learn what is taught in my classes*” and “*I am willing to put in the time to earn great grades in my classes.*” Female-athletes (n=46) scored the highest with an average of 4.02, while male-athletes (n=58) scored the lowest with an average of 3.62. Male non-athletes (n=39) scored an average of 3.90. The difference between male-athletes and male non-athletes was statistically significant (p<.05) (figure 3).

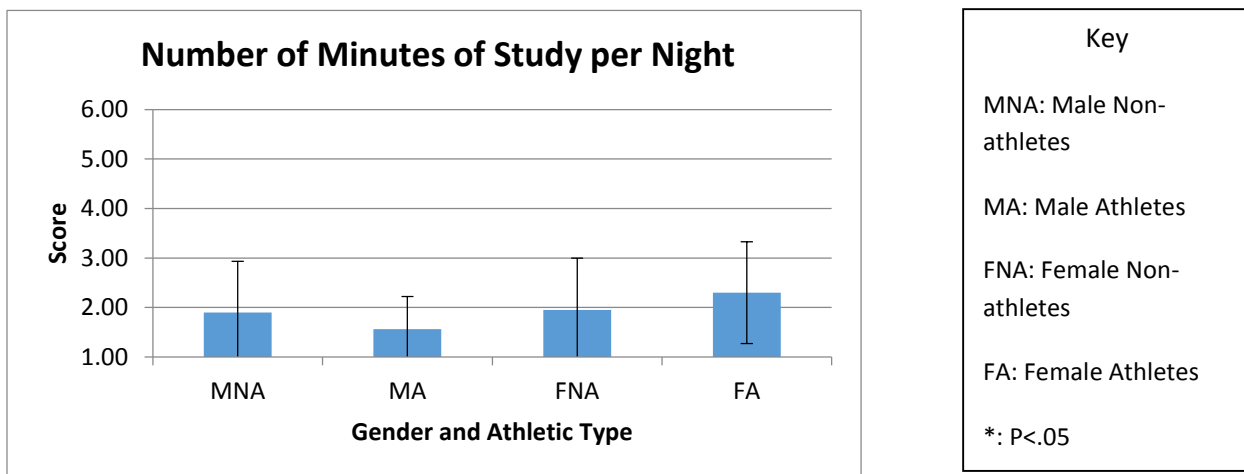


Figure 3.

In the scores presented here, the figures on the y axis correlate to: 1=0 minutes, 2=30 minutes, 3=60 minutes, 4=90 minutes and 5=120 minutes or greater. Female-athletes (n=46) scored the highest with an average of 2.30, while male-athletes (n=58) scored the lowest with an average of 1.56. Male non-athletes (n=39) scored an average of 1.90. Between male athletes and

male non-athletes ($p < .05$). Between female athletes ($N=46$) and female non-athletes ($n=43$) the p -value was not statistically significant ($p > .05$) but very close at $p = .0572$ (figure 4).

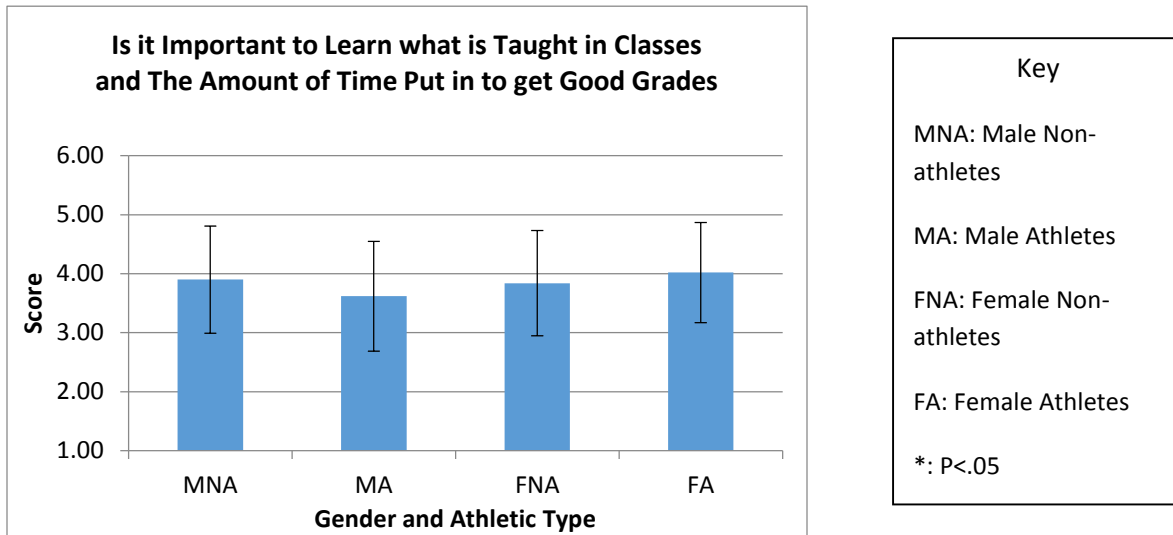


Figure 4.

CONCLUSIONS

The top finding in the experiment was that athletes self-report higher grades than non-athletes. According to figure #1, athletes reported statistically significantly higher grades than non-athletes ($p < .01$). But the most significant findings were the gender differences in the associations between sports participation and academic outcomes. For females, sport was associated with better academic performance (cutting fewer classes, more studying, etc.) and attitudes about school. My results correlate with previous studies because in this study, female athletes tend to have higher grades than non-athletes and in other studies; the same results were concluded (Maloney, Robert E. McCormick, 1993). For males, sport was actually correlated with worse performance and attitudes. My results also correlate with a previous study, because the experimenters (Covington, Simons, Rheenen) concluded that athletes lack motivation in the classroom.

Throughout the study I was faced with a few limitations that I had to fix. First, when I was limited to one high school. Due to my district being on the smaller side, we only have one high school and I couldn't go to different districts to conduct my survey because I was limited on time. Another limitation was the recruitment of English teachers. I had to contact each teacher individually and due to the limited time at the end of the year, a few teachers wouldn't distribute the survey to their students. Because my surveys were distributed by the teachers in each of their classes, my response rate was about 96%.

Future research that can be done from this study is to use all high schools in New York. If I had unlimited access to schools all over New York, the results would be more revealing. I

could compare the results to where one is from and how that can possibly affect the results. This could be very helpful because I could compare geographic locations and determine different results based on their location. More future research that can be done is taking it to the collegiate level, but I'd monitor what classes the athletes took so the data wouldn't be skewed.

ACKNOWLEDGMENTS

I would like to thank Rachel Koenigstein and David Shanker for all the help they provided me over my years in Science Research. Without their expertise and knowledge, and don't think I could be where I am today. But I would like to especially thank my mentor, Kathleen Miller, for the hard work and time she has put into helping me succeed. My project wouldn't be half as good as it is today without her guidance and help throughout this journal. She would write back to me the day I sent an email and in it would be all that I asked for. I couldn't have asked for a better mentor. Thanks to all that have been a part of this project and made it the best that it could be.

APPENDIX – SURVEY INSTRUMENT

This survey is completely voluntary. If you decide not to participate there will not be any negative consequences. You are not required to complete it, and you may drop out of the study at any time. Do not put your name anywhere on the questionnaire. This study will be kept confidential. There is a professional who you may speak to in your school if you feel uncomfortable while taking the questionnaire.

1) **What is your gender?**

Male Female

2) **Grade**

9th 10th 11th 12th

3) **What kind of grades do you usually get?**

a) Mostly As (90s) b) Mostly Bs (80s) c) Mostly Cs (70s) d) Mostly Ds (60s) e) Mostly Fs

4) **What is your highest score to date on each of the following standardized tests?**

(If you haven't taken one or more of these tests, leave that line blank.)

| | | | |
|--------------|----------------------------|--------|---------------------|
| PSAT: _____ | Fall | Winter | Spring (Circle One) |
| SAT: _____ | Fall | Winter | Spring (Circle One) |
| SATII: _____ | Subject(s) you took: _____ | | |
| ACT: _____ | Fall | Winter | Spring (Circle One) |

Please indicate how much you agree or disagree with the following statements.

- 5) **Athletics are important at my high school.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 6) **Academics are important at my high school.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 7) **Athletics are important in my community.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 8) **Academics are important in my community.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 9) **I am confident that I can achieve a high grade point average this year (85 or above).**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 10) **It is important for me to learn what is taught in my classes.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 11) **I am willing to put in the time to earn great grades in my classes.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 12) **I will be able to use what is taught in my courses in different aspects of life outside of school.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 13) **It would really bother me to get a bad grade.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 14) **It would really please me to get a good grade.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 15) **Student athletes get treated differently from non-athletes in my high school.**
a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree
- 16) **How often do you do your homework?**
a) Never b) Rarely c) Sometimes d) Most days e) Every day
- 17) **How often do you cut class?**
a) Never b) Rarely c) Sometimes d) Most days e) Every day
- 18) **How often do you monitor your grades online (PowerSchool) or with a teacher?**
a) Never b) Rarely c) Sometimes d) Most days e) Every day
- 19) **On average, how many minutes a night do you spend studying? (Choose the answer that comes closest.)**
a) 0 minutes b) 30 minutes c) 60 minutes d) 90 minutes e) 120 minutes or greater
- 20) **Regarding standardized testing, will you or do have a tutor? (ACT, SAT, PSAT)**
a) Yes b) No
- 21) **If you answered yes, how many times have you or will meet with your tutor?**
a) Once a month b) Twice a month c) Once a week d) Twice a week e) Three times a week
- 22) **Did you take any review classes for a standardized test?**
a) Yes b) No
- 23) **If you answered yes, how many times did your class meet?**

a) Once a Month b) Twice a month c) Once a week d) Twice a week e) Three times a week
 24) Are you participating in any school-sponsored sport/Travel (fall, winter, and/or spring season) this year?

No Yes

If you are NOT participating in a school-sponsored sport/Travel, the survey is now complete.

Thank you!

If you ARE participating in a school-sponsored sport, please continue to the next page.

25) Please select the sport (or sports) you are participating in this year (Check all that apply).

Fall Season

| Sport | School Team | Travel Team |
|---------------|-------------|-------------|
| Cross Country | | |
| Football | | |
| Soccer | | |
| Tennis | | |
| Swim | | |
| Cheerleading | | |
| Kickline | | |
| Volleyball | | |
| Field Hockey | | |

Winter Season

| Sport | School Team | Travel Team |
|--------------|-------------|-------------|
| Basketball | | |
| Bowling | | |
| Rifle | | |
| Winter Track | | |
| Wrestling | | |

Spring Season

| Sport | School Team | Travel Team |
|----------|-------------|-------------|
| Baseball | | |
| Golf | | |
| Lacrosse | | |
| Tennis | | |

| | | |
|------------------|--|--|
| Track | | |
| Softball | | |
| Badminton | | |

Other: _____

Please indicate how much you agree or disagree with the following statements.

26) Achieving a high level of performance in my sport is an important goal for me this year.

a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree

27) It is important to me to learn the skills and strategies taught by my coaches.

a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree

28) It is important for me to do better than other athletes in my sport.

a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree

29) The time I spend engaged in my sport is enjoyable to me.

a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree

30) I get more satisfaction from earning a 100 on a test than winning a game in my sport.

a) Strongly disagree b) Disagree c) No opinion d) Agree e) Strongly agree

31) During your sport season, how many hours a week do you devote to athletics (including playing, practicing, etc.)? (Choose the answer that comes closest.)

a) 1 hour b) 3 hours c) 5 hours d) 7 hours e) 10 hours or more

32) During your sport season, how many minutes a night do you devote to studying? (Choose the answer that comes closest.)

a) 0 minutes b) 30 minutes c) 60 minutes d) 90 minutes e) 120 minutes or more

33) When do you perform better academically in school – during your sport season, or off-season?

a) Much better in season b) A little better in season c) No difference d) A little better off season e) Much better off season

34) Do you expect to play a sport in college?

a) Definitely not b) Probably not c) Maybe d) Probably yes e) Definitely yes

The survey is now complete. Thank you!

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