F. Wilbur Gingrich Library
Special Collections Department
Albright College

Release of Senior Thesis

I hereby deliver, give, and transfer property, rights, interest in and legal rights thereto which I had, have, or may have concerning the Senior Honors Thesis described below to the Special Collections Department of the F. Wilbur Gingrich Library at Albright College as an unrestricted gift. While copyright privileges will remain with me, the author, all privileges to reproduce, disseminate, or otherwise preserve the Senior Honors Thesis are given to the Special Collections Department of the Gingrich Library. I place no restrictions on this gift and hereby indicate this by signing below.

Title: Ugandan Coffee (Coffea arabica and robusta) Agriculture: A Compassionate Holistic Perspective from Biochemistry to Ethnobotany

Signature of Author: David McCarrick Date: 4/21/16

Printed Name of Author: David McCarrick

Street Address: 3149 Lougheed Lane

City, State, Zip Code: Garnet Valley, PA 19060

Ugandan Coffee (*Coffea arabica* and *robusta*) Agriculture: A Compassionate Holistic Perspective from Biochemistry to Ethnobotany

David McCarrick

Candidate for the degree

Bachelor of Sciences

Submitted in partial fulfilment of the requirements for

College Honors

Departmental Distinction in Biology

Andrew Samuelsen, Ph.D.

Bryce Brylawski, Ph.D.

Pamela Artz, Ph.D.

<u>Ugandan Coffee (*Coffea arabica* and *robusta*) Agriculture: A Compassionate Holistic Perspective from Biochemistry to Ethnobotany</u>

As a science major, I use coffee all the time especially during finals week and in the writing of my honors thesis. Coffee is an important part of our daily lives in order to maintain our rather unsustainable work and sleep schedules. As a budding scientist and botanist, I have always been intrigued by the origins and intricacies of natural products particularly ones originating in plants. For this honors thesis I performed a thorough investigation of two plant species *Coffea arabica* and *Coffea robusta* Agriculture in a Ugandan context. I will start at the basic, but intricate level of caffeine biosynthesis and small molecule analysis. I will then delve into the biological aspects of coffee diseases and prevention. Finally, I will describe the ecological and environmental considerations for coffee agriculture and the ethnobotanical role of coffee in lives of Ugandan agriculturists.

<u>Coffee Biochemistry: Caffeine Biosynthesis and Small Molecule Analysis of Cup of Compassion Blends</u>

Coffee is a major agricultural product in tropical countries. The production of coffee has been linked to the development of low and middle-income countries' economies and the protection of the ecosystem through sustainable farming techniques. However, economic development and protection of ecosystems have often been in conflict. Greater economic development is usually directly related to market forces driving the production of greater yield sun-grown coffee and the use of pesticides in production of this sun-grown coffee. In contrast, shade-grown coffee often has environmental benefits by creating a plantation environment that is much closer to a natural forest ecosystem than a sun-grown coffee system. Shade-grown coffee can also incorporate various fruit trees leading to a polyculture in which the farmers have various sources of income compared to a sun-grown coffee system where the farmers is almost completely dependent upon the health and yield of the coffee tree.

In order to combat the problems associated with monoculture and economic distress, Cup of Compassion coffee (http://www.compassion-corps.com/cup-of-compassion-coffee/) hopes to create greater economic development combined with the protection of ecosystem services through their brands of coffee. They have created mixes of Liberian Robusta coffee with Ugandan and Kenyan Arabica coffee by buying coffee at fair prices and processing the coffee in the United States. All of their coffee is sourced from organic farms. However, they are not organic by choice. Coffee farmers, such as those in Western Uganda, want pesticides to combat certain diseases that affect crop yields (McCarrick, interview evidence collected in Uganda, summer 2015). Pesticides are expensive and **are therefore** not **used by** small coffee farms **like those in Western Uganda due to their cost**.

In order to promote economic development through coffee sales, the determination of the caffeine and aromatic compound content can be influential for consumers in terms of advertising the project. The evaluation of pesticide residues is also essential for consumers to authenticate that the coffee is organic even though it does not currently have an organic label. Once its authenticity is determined, the coffee can be marketed as organic and provide greater incentive for partner farmers to keep their organic practices.

The caffeine content of the various coffees offered by Cup of Compassion was determined by High Pressure Liquid Chromatography (HPLC) through caffeine peak area integration as compared to caffeine standards. In terms of results, the caffeine content should be mid-way between pure Arabica coffee and pure Robusta coffee as the grounds are a mixture of the two species. Robusta coffee is known to have about twice the caffeine content of Arabica coffee as the metabolic processes that generate caffeine in these coffee species differs (Perrois, *et al.*, 2015). The aromatic compounds and pesticide residues were evaluated through gas chromatography-mass spectroscopy (GC-MS). The intention is to use the technique of headspace, solid-phase microextraction (SPME) to introduce volatile organic compounds including caffeine, other volatile organic compounds and pesticide residues onto the GC-MS for analysis (Muller, *et al.*, 2014). There should be minimal pesticide residues. A comparison was done with conventional coffee grounds which are marketed with and without organic designation and will be further expanded with the inclusion of 100% Arabica.

In the evaluation of the headspace SPME technique, caffeine was not volatile enough to be detectable for Headspace GC-MS in the coffee samples. Caffeine was able to be detected in a saturated caffeine solution however this peak was too close to the baseline to be a determinant of caffeine content through this method. However, this technique worked well for volatiles in the coffee. The headspace SPME technique used was from Muller et al. The column oven temperatures were 50°C for 2 min, 20°C/min to 240°C, followed by an increase of 50°C/min to 310°C with a 2 min hold at 310°C. For obtaining accurate concentrations of caffeine and potential detection of pesticide residues, a direct immersion (DI) technique was used. The DI-SPME technique was used from the protocol of Tankewicz et al. The column oven temperatures were 50°C for 2 min, 25°C/min to 220°C, 2°C/min to 240°C, then 5°C/min to 270°C with a hold of 2 min. The temperature had an initial high ramp rate because compounds eluting during that time period were volatiles that were unimportant for this assay. Most pesticides that would be used in coffee agriculture such as imidacloprid used for green scale are not volatile and so are not conducive to headspace SPME approaches.

The caffeine content varied across the different coffee varieties with blends such as Justice Java, Liberia Light and Promise Perk having the greatest caffeine content. These blends have the same ratio of 1 part of robusta to 2 parts arabica. The lower caffeine content of Pearl of Africa is indicative of the robusta:arabica ratio of 1:3 instead of 1:2. One anomalous part of the research involved the 100% robusta blend created through the same processes. It showed a lower caffeine content than the Liberian blends of robusta and arabica. This might be due to an improper brewing process. A 100% Arabica blend was not tested during the ACRE, but will be tested during the semester to complete the research along with more 100% robusta.

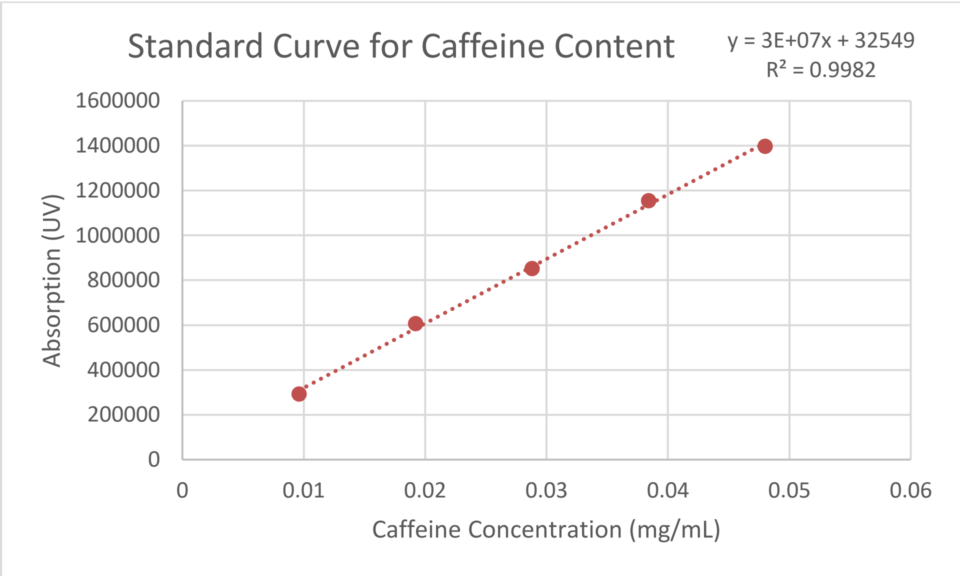

Figure 1: Standard Curve for Caffeine Concentration using HPLC

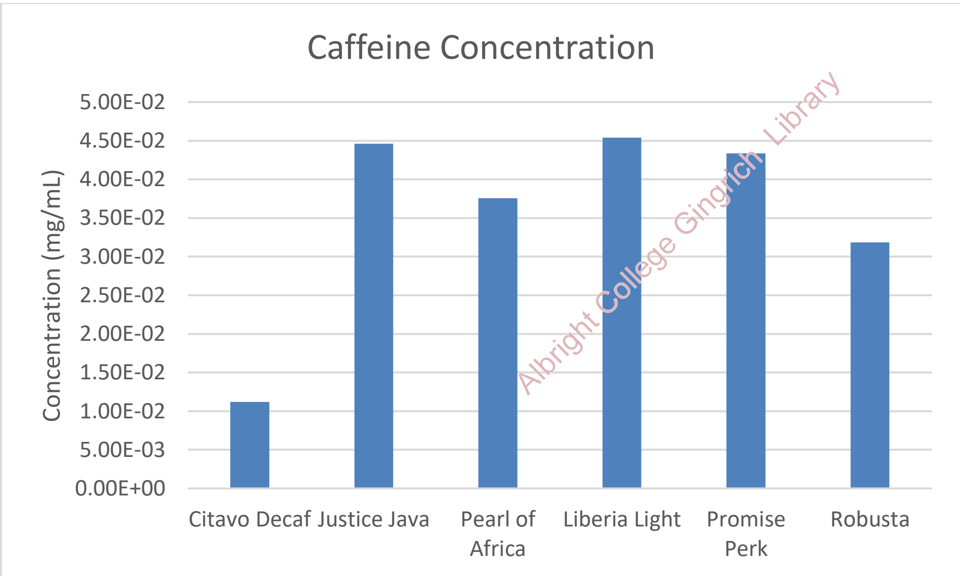

Figure 2: Caffeine Concentration through HPLC

The aromatic compounds in the coffee were mostly furan derivatives. The three most prominent furan derivatives were 2-furanmethanol, 2-furanmethanol, acetate and 2-Furancarboxaldehyde. The headspace technique showed that the promise perk and justice java had largest amount of 2-Furanmethanol and 2-Furanmethanol, acetate. The amounts of both of these aromatic compounds were linked because the varieties had the exact ranking compared to the other varieties for each compound. The 2-Furancarboxaldehyde was different in that the robusta and the citavo decaf variety had much more of this compound than the other blends.

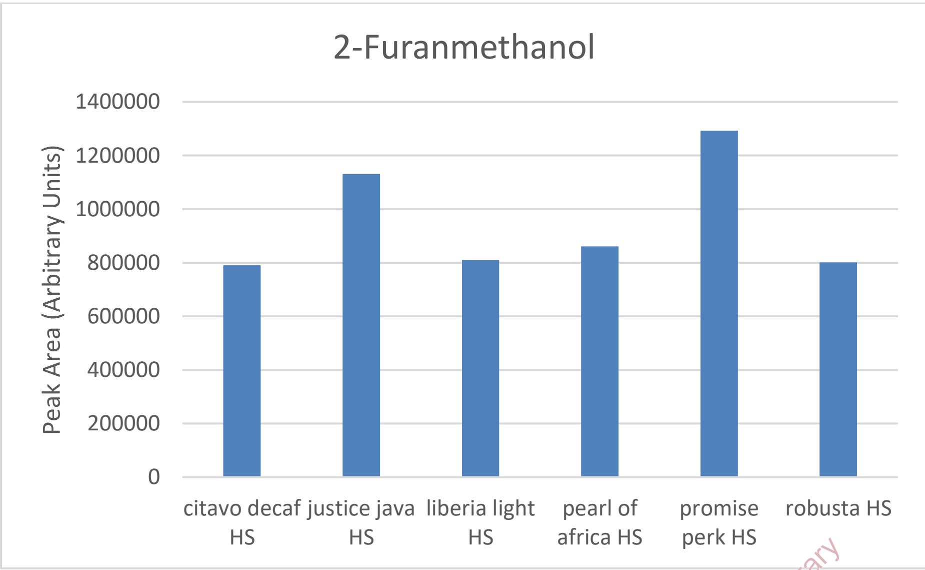

Figure 3: 2-Furanmethanol Concentration found through Headspace-SPME

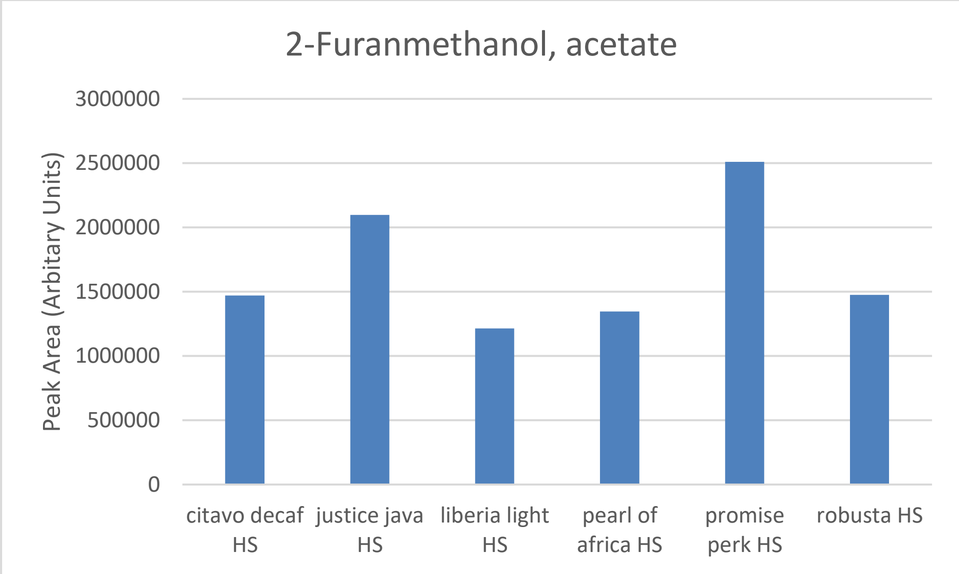

Figure 4: 2-Furanmethanol, acetate Concentration found through Headspace-SPME

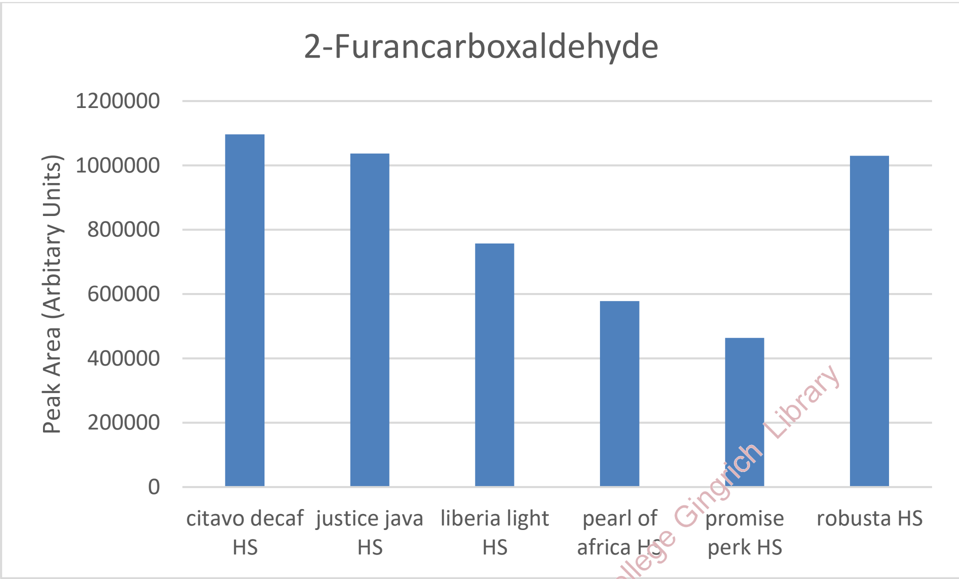

Figure 5: 2-Furancarboxaldehyde Concentration found through Headspace-SPME

Dr. Artz and I tested for pesticide residues using direct immersion solid phase microextraction. The direct immersion technique was also able to detect caffeine concentration as shown in Figure 6. The caffeine peak area found through the direct immersion technique can be directly correlated to the caffeine peak area derived from HPLC as seen in Figure 7. This can be used to get the concentrations of the volatile compounds. This concentration can then be correlated to the volatiles in the direct immersion technique compared to the headspace technique as shown in Figure 8 and 9. The 2-furancarboxaldehyde did not correlate well due to the small area of the peak being contaminated with an unknown substance of similar boiling point. This compound was seen with a rightward broadening of a peak on the gas chromatograph with a high molecular weight on the corresponding mass spectrum. In contrast to the headspace SPME, the 2-furancarboxaldehyde found through the direct immersion technique detected much more of the compound in the Pearl of Africa and Liberia Light blend. Another technique to quantify the volatile compounds was the use of an internal standard such as triphenylphosphate. There were no visible pesticide residues in any of the samples with most of the compounds eluting during the 240°C-270°C range other than those originating from the SPME fiber itself or caffeine.

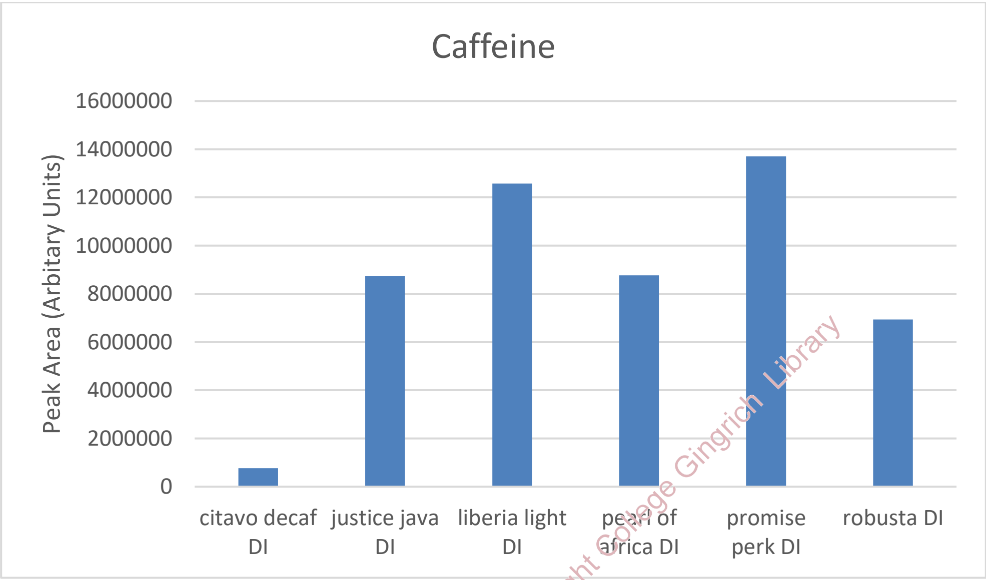

Figure 6: Caffeine Peak Area found through Direct Immersion-SPME GC-MS

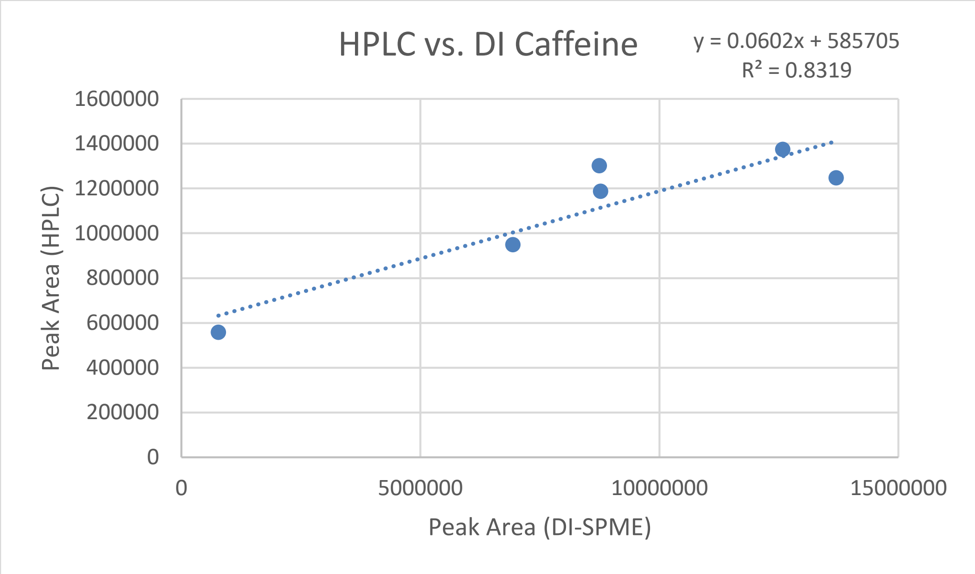

Figure 7: HPLC vs. Direct Immersion Caffeine Concentration

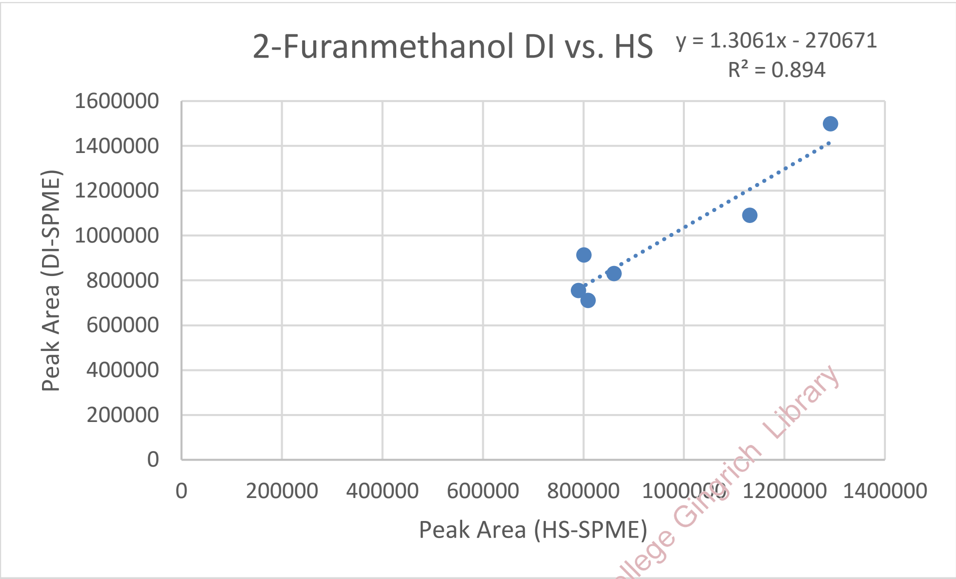

Figure 8: Direct Immersion vs. Headspace SPME GC-MS of 2-Furanmethanol

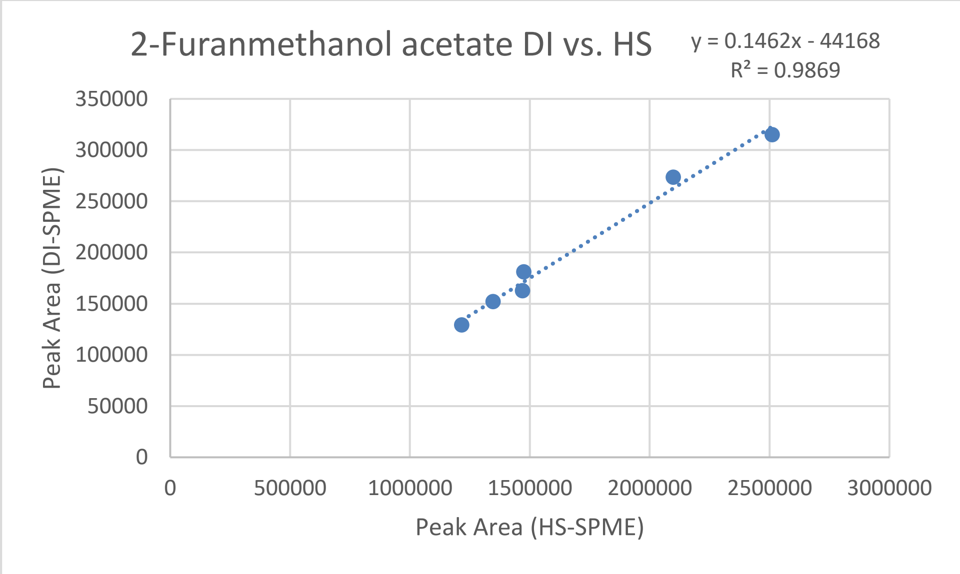

Figure 9: Direct Immersion vs. Headspace SPME GC-MS of 2-Furanmethanol, acetate

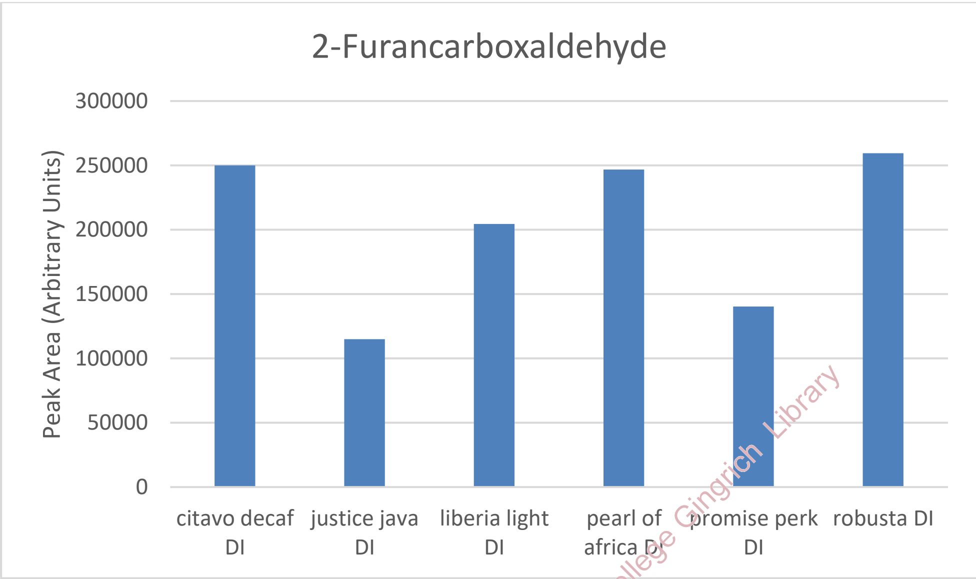

Figure 10: 2-Furancarboxaldehyde Peak Area with Direct Immersion-SPME GC-MS

These furans are indicative of various Maillard reactions of proteins and hexose sugars and are sometimes indicated to produce negative health effects. One possible Maillard reaction can occur when hexoses are reduced and 1-deoxyosone and 3-deoxyosone are produced. These products are then produced aldotetrose and other intermediates and then further converted through dehydration to 3-furanone. The possible carcinogenic capacity of these furans was found by the International Agency for Research on Cancer in its white paper looking at a range of compounds (IARC, 1995). This Maillard reaction resulting in potentially cancer-causing furan usually occurs during the roasting process. When the process is longer, the furans are more likely to be generated. One study found that if dicarbonyl trapping agents, antioxidants and reducing agents are added to coffee then the production of furan was reduced (Zheng et al 2015). Many of these agents such as caffeic acid and chlorogenic acid are actually commonly found in coffee. Ideally, these agents could be engineered into plants to have increased expression, however the flavor profile would be modified as these antioxidants tend to produce an off flavor while the furans produce the characteristic nutty flavors that are desired in coffee. As far as known, no genetic engineering increasing these acids has been done with coffee plants

Many people are concerned about caffeine content. Previously mentioned data has quantified the amount of caffeine in these blends. The difference in caffeine expression between arabica and robusta beans is due to the regulation of the caffeine biosynthesis genes. The caffeine biosynthetic pathway starts with xanthosine. This is primarily derived from degradation of adenine or guanine nucleotides or produced *de novo* with inosine monophosphate as an intermediate for all of these processes. The xanthosine is converted into 7-methylxanthosine by 7-methylxanthosine synthase (EC 2.1.1.158) which is a type of N-methyl transferase. The 7-methylxanthosine is then converted into 7-methylxanthine through *N*-methyl nucleosidase (EC 3.2.2.25). The 7-methylxanthine is then converted to theobromine. Theobromine is known as a common flavor component of chocolate and was first isolated in chocolate (Theobroma is the genus of cacao). The theobromine is then converted to caffeine. The 7-methylxanthine to theobromine and the conversion of theobromine to caffeine is usually a concerted mechanism involved SAM-dependent *N*-methyltransferases. The primary method that researchers have used to reduce caffeine content in vivo is to knock-out N-methyltransferases (Ashihara *et al*. 2008). Most research has gone into knocking out the 7-methylxanthosine transferase. This has been accomplished with both RNA interference and the production of antisense RNAs that result in the gene's RNA to be degraded (Ashihara *et al*. 2006). The use of these decaffeinated varietals could be an important part of worldwide coffee production without the use of decaffeination processes. This could be especially important in robusta coffee if the genes could be downregulated without silencing them, then robusta coffee could have equal caffeine content.

Coffee Biology: A Taxonomic Overview and Disease Considerations

Coffee is a tall shrub or small tree that is commonly grown on hillsides in tropical regions. The arabica coffee is commonly grown in sub-montane regions while the robusta coffee can be grown in lower altitude regions. The leaves are evergreen and the tree usually has 2-3 fruiting seasons in which the flowers are pollinated by various midges. The fruit which is called a cherry is initially green and then ripens to a dark red. Inside the fruit, there is a papery parchment region surrounding the greenish colored bean. The bean only develops its characteristic brown color upon roasting and the associated Maillard reactions.

There are various diseases associated with coffee production. The main problems experienced are various fungal diseases affecting the leaves and the coffee cherry. In the survey, 13% of people surveyed noted coffee leaf rust causing a problem in their crops. Coffee leaf rust is caused by the fungus *Hemileia vastatrix*. One study focused on the use of anti-fungals in promoting normal leaf function. They monitored normal leaf function through the evaluation of photosystem II quantum efficiency. They found that the use of the anti-fungals epoxiconazole and pyraclostrobin were able to maintain quantum efficiency compared to the rust-free plants (Honorato *et al*. 2015).

However, Compassion Corps would like to keep their coffee sources organic, so the use of the fungicides epoxiconazole and pyraclostrobin would not be advisable. In another study, coffee plants were inoculated with the fungus again and cell autofluorescence was monitored to see the reaction of plant cells to the fungal infection. This autofluorescence was a way to monitor the hypersensitive reaction in which the cells localized around the inoculation died through peroxidase activity. The production of peroxides through the peroxidase activity of the cell leads to cell lysis near the infection site which can then affect the fungus itself and increase the lignification of the cells around the infection site. This is similar to cellular apoptosis found in animal cells in which localized cell death can provide a protective function for the rest of the cell (Silva *et al*. 2008). This peroxidase activity can then eventually lead to disease-resistant coffee varietals that can then express more of these peroxidases in times of stress and potentially have a more localized cell death due to more precise signaling in response to the fungal infection.

In addition to focusing on the fungal infection and the plant, there are also environmental factors that can contribute to increased fungal resistance. Another non-pathogenic fungus called *Lecanicillium lecanii* specifically attacks coffee rust. This fungal species is also entomogenous which means that the fungus can both live off of other fungi and inside insect species. This fungus is pathogenic is scale insects including the coffee scale, *Coccus viridus* which also is a major disease contributor in coffee farming (Jackson *et al.* 2012). If this fungus was able to be used as a type of organic spray that would be an ideal alternative to conventional pesticides.

Coffee Ecology: A Review of Entomological Herbivory and Pollinators in Coffee Agriculture

Coffee cherry borers are one of the main entomological concerns in coffee agriculture and can have a devastating effect on crop yield. For the 60% of participants that noted insect trouble with their crop, 78% of them noted various cherry borers that affected their yields. These borers commonly cause spotting on the leaves along with hollowing out the coffee cherries. In addition to coffee cherry borers, there are numerous stem borers that affect coffee production. In the study, for the participants that noted insects as a major trouble in their agriculture, 67% of those farmers noted that they have problems with various stem borers including “coffee aphids”. There are numerous coffee borers including the true coffee borer**:** *Hypothenemus hampei*. Many people referred to insects that have black with white spots. Since *Hypothenemus hampei* is an all-black insect, their description could be referring to the insect *Xylosandrus compactus* which does not drill directly enter the endosperm of the coffee cherry. One research article focused on possible insect predators that would attack the coffee borer *Xylosandrus compactus*. The insect predator found was a formicid ant *Phagiolepis* sp. The study was actually conducted in Uganda. The region of Fort Portal which is close to Kasese did not have any detectable formicid ants, however the region of Kyenjojo was found to have the ant. The actual region of Kasese was not monitored (Egonyu 2015). *Hypothenemus hampei* could still be a threat as well. These can be controlled through parasitoids. The abundance of these predatory species can probably be increased with increased use of forest trees in a polycultural landscape.

Pollinators are an important part of coffee farming because coffee is not self-fertilizing. One study used a statistical model to evaluate the pollination value provided by stingless bees and honeybees in coffee farms in the highlands of Columbia. They found that the distance from continuous forest negatively affected the amount of honeybees ($F=52.94$, d.f.=5, $P=0.002$) visiting the flowers while the amount of stingless bees ($F=6.02$, d.f.=11, $P=0.034$) and the diversity of pollinators ($F=10.82$, d.f.= 5, $P=0.030$) increased with greater proximity to the forest (Bravo-Monroy *et al*. 2015). The honeybee abundance was not quantified in Uganda, but honey production did not seem to play a major role in the lives of coffee farmers as none noted during their interviews that they produced marketable honey. Therefore, their reliance on outside pollinators is extremely important for adequate fruit set. Furthermore, given the origin of coffee in Ethiopia and undomesticated coffee relatives living in Ugandan forest, the pollinator fauna is evolutionarily adapted to coffee species. When native trees are established in coffee farms, this would then enable these pollinators to thrive on coffee farms and help increase fruit set. Some of these species such as parasitic wasps could then help with aforementioned cherry and stem borers.

Coffee Ethnobotany: The Intertwined Lives of Coffee Farmers, Coffee and Environment

Stereotypes about emerging economies usually fall under two categories of oversimplification. They either state that they are doing great and do not require any assistance or they are doing terrible and need international aid to pull them out of their misery. The first oversimplification causes people not to give charitably or help volunteer in emerging economies because they think that the people can pull through all of their problems. This view negates the knock-down effects still happening from the colonial periods pre-World War 2. For one example, the Berlin conference divided Africa among the colonial powers without consultation of indigenous peoples because they were officially subjugated. These divisions eventually created the modern African nation-states which are usually rife with internal conflict due to ethnic divisions which have led to decreased productivity. The second oversimplification negates the ability of indigenous peoples to be able to pull themselves out of poverty with various schemes whether agricultural commensalism, increased trade with other communities and increased education. Each of these schemes can be helped with international aid but international charity workers need to realize that their partners are equal to them in terms of planning and division of labor.

Compassion Corps has decided to help the farmers of Liberia and Western Uganda through the aid of newly found agricultural cooperatives, creation of stable trade relations between Compassion Corps and coffee farmers and helping with training of the farmers themselves and the education of their children. A new cooperative was formed in Western Uganda called Namhuga Joint Cooperative Federation (NJCF). It intends to provide training to the farmers in correct terrace building and correct shading of coffee. The pamphlets associated with this honors thesis are intended to be used for some these trainings. Compassion Corps has created trade relations by buying Arabica coffee from farmers at higher price than in Kasese. Many of the middlemen in Kasese adulterate the Arabica coffee given to them and give the farmers a lower price per pound. These farmers who have been working with coffee for an average of 22.6 years decided to stop going through the middleman. Compassion Corps recognizes that the coffee grown by these farmers is quality so they pay a high price for it. In developing this coffee trade, Compassion Corps is helping people pay for school fees for their children as most schools are private because of the increased profit from coffee.

During the survey involving coffee farmers, the farmers noted numerous environmental and various resource problems. 26.7% of participants in the survey stated that they wanted more trainings. In addition, 73.3% of participants in the survey stated that they wanted more tools. In addition, 20% of farmers wanted more taplines. Taplines are sheets that are used to dry coffee. A portion of participants (13.3%) cited that they needed pesticides. This is interesting because they currently do not have access to pesticides because of cost. In order to create an organic label, the people from Compassion Corps have to work to provide sustainable organic solutions such as those mentioned in previous sections for coffee insects and rusts. Additionally, 13.3% of participants also noted that they wanted help with school fees which might be something that Compassion Corps has pursued. However, many people (50% of participants) noted that they used their supplemental income for school fees. 21.4% of participants also noted that they used their income to buy medicine.

In addition to physical resources, many farmers noted various environmental concerns in their farming. Many of the parents of these farmers also worked with coffee (86.7%) and their farms seemed to be varied in terms of different crops. Many of them worked with cassava (78.6%), bananas (64.3%) and beans (64.3%) as staple crops. Yams (28.6%), Maize (28.6%), Peanuts (14.3%) and Potatoes (14.3%) were also used as well. When the farmers asked about other sources of income other than coffee, 20% of them noted that coffee was their only cash crop and relied on it for their income. These farms definitely need to diversify to avert the potential disasters with monoculture. Also, only 26.7% of participants mentioned that they used fruit trees such as mangos or bananas to supplement their income. This statistic might undershoot the real amount of fruit trees because some fruit trees might not be brought to market and only used for the household. Even so, increased fruit tree usage might have a real beneficial effect. Some of these positive effects were quantified with a banana and coffee study based in Uganda. While the coffee yields did not significantly change between the monocrop and the intercrop, the banana yield greatly increased when intercropped with the coffee (Asten 2011)

Climate change will become more and more of an issue in all types of agriculture. One of the problems associated with anthropogenic climatic change is the decrease in precipitation across various tropical biomes. Tropical agriculture will be affected by this drying by drought-related injuries to crop plants and crop yield decrease. One way to combat the drying effects of climate change on tropical coffee agriculture is the use of shade agroecosystem. One study found that with 60-80% shade cover, soil evaporation rates decreased significantly by 41% compared to the low shade cover (10-30%). Even with less drastic shade cover (30-65%), soil moisture was maintained during the tropical dry season (Lin 2009). This study was conducted in the Mexican highlands and the precipitation of Chiapas and Kasese is approximately equivalent. This means that the shade cover advantages to crop yields can be translated to the Ugandan context. A third of the participants have experienced problems with land fertility and weather which is some cases is exacerbated by full sun coffee agriculture and the water-related problems associated with it. Much of the coffee growing land around Kasese is very steep which means that many of the farmers have major problems with erosion. When I traversed up to a farm on one of the hills, it was clearly evident that some of the soil had washed away at least on the track to the farm.

In addition to soil erosion, deforestation was clearly evident with hillsides devoid of trees and replace with cassava or sun coffee crops. One study decided to investigate the differences between shade grown coffee and primary forest in Ethiopia which is relatively close to Uganda. It found that the amount of bird species present in the shade grown coffee environment was approximately equal while some of the forest specialists and understory insectivores were more abundant in the little-disturbed forest. This study highlighted the fact that primary forest is still important to protect, but shade grown coffee plantations can be effective buffer and provide necessary habitat for many species of birds. In addition, *Coffea arabica* has its origin in Ethiopia so a shade grown coffee is even more effective as a "bird-friendly" coffee compared to the other similarly grown coffee in other areas (Buechley *et al*. 2015). This gives more impetus for farmers in Uganda and other coffee-growing region in the Afromontane region of Africa to grow coffee under native tree species. In addition to helping with native bird populations, the creation of shade grown coffee plantations also can help with other members of the ecosystem including lizards and insects. One study found that the abundances of 7 different orders of insects increased in the shade grown context compared to full sun in Puerto Rico. Also, the abundance of certain native lizards called anoles increased for certain species while other anoles decreased with increasing forest cover. This is probably related to the niches that these species fulfill with certain species capitalizing on sunnier or shadier environments (Borkhataria *et al*. 2012). These adaptations can happen in the Ugandan environment with more habitat for native fruit bats, insects and other vertebrates.

Another study looking at potential carbon storage also saw another possible benefit to shade grown coffee. They found that shade coffee farms can incorporate 50-62% of the above-ground biomass of forests. This can then serve to mitigate carbon (Tadesse *et al*. 2014). In respect to trees used, various pruned legume tree species can actually increase soil organic carbon stocks more than unpruned timber trees (Noponen *et al*. 2013). However, this would be potentially would be disadvantageous for farmers because the timber trees can provide an income to the farmer instead of the legume trees. Nevertheless, legume trees such as Inga species in Central America have been used for various annual crops such as maize to increase their yield and prevent erosion. The non-profit Inga foundation that has implemented this agrarian system in Honduras has not currently found an equivalent for African agriculture.

An agronomic ecosystem is something that could be potentially be implemented in each of these farms with a little planning. The upper slopes which tend to be rockier and also on a steeper incline should be forested to prevent erosion. Firewood usage and potentially timber production could be used on lower portions of this forest boundary. Firewood usage has already been a part of their land usage, so appropriate firewood take would be an essential part of education for the farmers. The middle part should include all of the crops for the family including cassava and the farm animals. 33% of farmers already have various animals. The coffee should then be interspersed on the lower slopes and the upper ones. On the upper slopes, the forest trees should be allowed to mingle with the coffee plants to create a shade coffee farm. This would include the back tree (*Ficus thonningi*) which is actually a tree that was mentioned and is currently used to stop erosion. This and other trees should be used to line the path downslope to prevent erosion that way and possibly help on a larger scale to prevent the losses caused by frequent floods in the region. On the lower slopes, the coffee trees should be intermingled with various fruit trees such as banana to mutually increase their yield. With the fruit trees confined on the lower slopes, it also allows for a more natural environment on the upper slopes. The erosion of the slopes can also be stopped by terracing which is widely known but takes manpower to be implemented in Uganda. All of these solutions are manageable, but require hard-working individuals working with locals to more fully assess current problems, recognize solutions and achieve sustainable development in the Kasese, Uganda.

Literature Cited

Ashihara H., Zheng X-Q., Katahira R., Morimoto M., Ogita, S., Sano H. 2006. Caffeine biosynthesis and adenine metabolism in transgenic *Coffea canephora* plants with reduced expression of *N*-methyltransferase genes. Phytochemistry 67: 882-886

Ashihara H., Sano H., Crozier A. 2008. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. Phytochemistry 69: 841-856.

van Asten P.J.A., Wairegi L.W.I., Mukasa D., Uringi N.O. 2011. Agronomic and economic benefits of coffee-banana intercropping in Uganda's smallholder farming system. Agricultural System 104: 326-334.

Borkhataria R.R., Collazo J.A., Groom M.J. 2012. Species abundance and potential biological control services in shade vs. sun coffee in Puerto Rico. Agriculture, Ecosystems and Environment. 151: 1-5

Bravo-Monroy L., Tzanopoulos J., Potts S.G., 2015. Ecological and social drivers of coffee pollination in Santander, Colombia. Agriculture, Ecosystems and Environment. 211: 145-154

Buechley E.R., Sekercioglu C.H., Atickem A., Gebremichael G., Ndungu J.K., Mahamued B.A., Beyene T., Mekonnen T., Lens L. 2015. Importance of Ethiopian shade coffee farms for forest bird conservation. Biological Conservation. 188: 50-60

Egonyu J.P., Baguma J., Ogari I., Ahumuza G., Kyamanywa S., Kucel P., Kagezi G.H., Erbaugh M., Phiri N., Ritchie B.J., Wagoire W.W. 2015. The formicid ant, *Plagiolepis* sp., as a predator of the coffee twig borer, *Xylosandrus compactus*. Biological Control. 91: 42-46

Honorato Junior J., Zambolim L., Aucique-Perez C.E., Resende R.S., Rodrigues F.A. 2015. Photosynthetic and antioxidative alternation in coffee leaves caused by epoxiconazole and pyraclostrobin sprays and *Hemileia vastatriz* infection. Pesticide Biochemistry and Physiology. 123: 31-39

IARC (Internation Agency for Research on Cancer) 1995. Monographs on the evaluation of carcinogenic risk to humans: Dry cleaning, some chlorinated solvents and other industrial chemicals. *Lyon France* 63 (pp. 393-407)

Jackson D., Skillman J., Vandermeer J. 2012. Indirect biological control of the coffee leaf rust, *Hemileia vastatriz*, by the entomogenous fungus *Lecanicillium lecanii* in a complex coffee agroecosystem. Biological Control. 61: 89-97

Lin B.B. 2010. The role of agroforestry in reducing water loss through soil evaporation and crop transpiration in coffee agroecosystems. Agricultural and Forest Meterology. 150: 510-518

Muller, C., *et al.* 2014. Determination of caffeine, myosmine, and nicotine in chocolate by headspace solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry. J. Food Sci. 79(2): T251-255

Noponen M.R.A., Healey J.R., Soto G., Haggar J.P. 2013. Sink or source—The potential of coffee agroforestry systems to sequester atmospheric CO_2 into soil organic carbon. Agriculture, Ecosystems and Environment. 175: 60-68

Perrois, C., *et al*. 2015. Differential regulation of caffeine metabolism in *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta). Planta. 241(1): 179-191.

Silva M.C., Guerra-Guimaraes L., Loureiro A., Nicole M.R. 2008. Involvement of peroxidases in the coffee resistance to orange rust (*Hemileia vastatrix*). Physiological and Molecular Plant Pathology. 72: 29-38

Tadesse G., Zavaleta E., Shennan C. 2014. Effects of land-use changes on woody species distribution and above-ground carbon storage of forest-coffee systems. Agriculture, Ecosystems and Environment. 197: 21-30

Tankiewicz, M., *et al*. 2013. Multi-residue method for the determination of 16 recently used pesticides from various chemical groups in aqueous samples by using DI-SPME coupled with GC-MS. Talanta. 107:1-10

Zheng, L.W., Chung, H., Kim, Y-S. 2015. Effects of dicarbonyl trapping agents, antioxidants, and reducing agents on the formation of furan and other volatile components in canned-coffee model system. Food Research International. 75: 328-336.