Germination of *Argania spinosa*: Overcoming genetic and physical barriers of Morocco's infamous seed

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2017 SPAN- Morocco
2018 UMD University Honors Capstone Project

FSSP 5960 and FSSP 5970
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Abstract:

Argania spinosa (argan tree) is one of the oldest species of trees on the planet today, with individual trees living hundreds of years. Its vital role in providing food for animals, slowing desertification and providing precious cosmetic oil, make studying the tree of vital importance for the Moroccan economy and environment. The argan tree is secluded to growing in the southwest region of Morocco at the edge of the Sahara Desert. However, due to the rapid increase in the price of the oil its seeds produce and the expanding desert, this fragile and ancient tree's future is being threatened. Preservation efforts are being made, but first research is needed to know what threats the tree is facing and to what extent. The beginning of the tree's life if a very good place to start. We know that germination is being challenged, but by what factors?

This investigation took to a germination experiment testing the best conditions of argan seeds by comparing size, temperature and specific trees. No significant results were found from these comparisons. Seeds were infected by fungus and insects. Oil composition was compared between trees and found significant color differences, but not significant differences in fatty acid content. Also a cross-cultural comparison was realized for the differences in how Americans and Moroccans conduct research.

Literature Review and Introduction:

Argania spinosa (argan tree) is one of the oldest trees on the planet, with estimates of the tree being anywhere between 1.8- 65 million years old (Ruas, et. al, 2016). Since the middle ages, humans have had a long history of collecting necessities from the tree such as edible argan oil and argan wood for fires (Ruas, et. al, 2016). Humans today rely on the argan tree to provide an income through the oil it provides. They collect argan seeds, harvest the argan oil and sell the oil for a profit. Argan oil was originally used for cooking, and still is for some, but it has become a highly sought after commodity all over the world for cosmetic purposes. The argan tree is only found in one region of the world, on the northern edge of the Sahara Desert in Morocco and parts of Algeria. The tree has adapted especially well to live in this dry desert climate and is the only tree able to live in this area. Because of this, the tree is depended on by other species of life to provide shade, food and nutrients in the soil. It is also on the front line of battling desertification, which is the rapid expansion of the Sahara Desert through overuse of the land and climate change. The tree is able to do this by slowing soil erosion by having such a large root system. Studying the history, drought effects, germination and soil environment of this tree are important to understand before researching the tree further. Since the tree is so important to people and other forms of life in that region, studying these topics will allow a better understanding of the further research by all fields of science needed to predict the probable future of the argan tree.

History of the tree

The history of the argan tree is important to know, as it can apply to current research on the tree. The findings that historians, geologists, archaeologists and biologists make about the tree's history can be applied to learn that the argan tree has done in the past to help predict its future.

Alba-Sanchez, et. al used different software simulators and different climate history data to find migration patterns of the tree and predict where the tree could be in the future (Alba-Sanchez, et. al, 2015). During the last ice age thousands of years ago, the argan tree used to live in a more southern and coastal position than where they are found today (Alba-Sanchez, et. al, 2015). Over the thousands of years, the trees have been migrating more north and inland as the temperature of the earth has increased (Alba-Sanchez, et. al, 2015). The trees are able to migrate by dispersing their seeds and over time, natural selection due to warming temperatures has selected for the trees more and more inland (Alba-Sanchez, et. al, 2015). This seems plausible because in the ice age, the temperature of the earth was very cold. The ocean offers a more stable and warmer climate for the trees to survive verses the inland desert where climate is more likely to swing one way or the other. The researchers predicted two possible scenarios for the argan tree future based on two climate change predictions for the planet. The first is the sadly the more likely future. It is that climate change will continue at the rate it is happening today, and the trees will move south and coastal to be out of the harsher conditions of the desert (Alba-Sanchez, et. al, 2015). The second possibility is that climate change will slow, and the Argan trees will stay where they are or even migrate slightly more inland and up towards the Atlas Mountains (Alba-Sanchez, et. al, 2015). The researchers are credible. Alba-Sanchez and Nieto-Lugilde both hold a terminal degree in their fields and work at the Department of Botany at the University of Granada in Spain (Alba-Sanchez, et. al, 2015). Lopez-Saez also holds a terminal degree and works as a group investigative researcher in biology at the University of Madrid, Spain (Alba-Sanchez, et. al, 2015). Svenning holds a terminal degree as well and works at the section of ecoinformatics and biodiversity at the department of bioscience at Aarhus University in Denmark (Alba-Sanchez, et. al, 2015). Knowing that these researchers come from different departments at different established universities shows that their research is credible (Alba-Sanchez, et. al, 2015).

During the time when the argan tree was slowly migrating, humans in the region needed the argan tree to survive. Ruas, et. al found the earliest physical evidence for humans using the argan tree in the middle ages (Ruas, et. al, 2016). Through the work from an excavation site in Îgîlîz, Morocco, researchers found remains of burnt wood. They concluded that humans in the middle ages used the argan tree wood for fires (Ruas, et. al, 2016). This finding correlates to a previous article citing that the temperature of the region was quite cold, so if the trees were trying to get warmer, so were the humans (Alba-Sanchez, et. al, 2015) (Ruas, et. al, 2016). Researchers excavated tools from this time that are extremely similar to the tools used to make argan oil today (Ruas, et. al, 2016). They concluded that these people extracted the argan oil the same way as they do today (Ruas, et. al, 2016). Through this research, there are similarities and differences in how the argan tree has been used and how it is being used for human benefit. The argan oil is still extracted the same way. However, people today do not use the wood of the argan tree to burn for fires, unless the tree has died. People today also use the tree to feed their livestock, which there was no evidence for people in the middle ages doing this (Ruas, et. al, 2016). Based on this, the researchers say that people today use the argan tree more than people in the middle ages. I think most of these conclusions are valid based on the data and other research done in the field (Ruas, et. al, 2016) (Alba-Sanches, et. al, 2015). However, there needs to be more evidence to support the claim that people today use the argan tree more than people back in the middle ages did because the climates of today compared to eight hundred years ago could be vastly different (Ruas, et. al, 2016). This paper had a lot of researchers contributing to it, which makes it credible. Ruas, Ros and Andrianarinosy are archaeologists at the Museum of Natural History in Paris, France (Ruas, et. al, 2016). Terrai and Ivorra are evolutionary scientists at the University of Montpellier in France (Ruas, et. al, 2016). Ettahiri is an archaeologist from the Institute of Natural Sciences in Rabat, Morocco (Ruas, et. al, 2016). Fili is a faculty member for human sciences at the University of Al Jadida in El Jadida, Morocco (Ruas, et. al, 2016). And Staevel is a text archaeologist at the University of Paris Sorbonne IV campus (Ruas, et. al, 2016). This lengthy list of authors show that their combined fields produced this research and the results are credible.

Physical traits of the argan tree and how they react to drought conditions are necessary to know to further research the tree, its capabilities and could possibly lead to further knowledge of other trees that thrive in drought conditions. The current drought conditions for argan trees are normal and some thrive from. Thanks to climate change and overuse of land in the region, desertification is a threat to the region. As a result, if the desert keeps expanding and drought periods keep lengthening, the tree's future is questionable. This is why it is important to study the capabilities of the argan tree under artificial drought scenarios to better predict what might happen if these artificial conditions become reality.

The root system of the tree is a good place to start. Using Electrical Resistivity Imaging (ERI), Ain-Lhout et. al could look at the root systems of argan trees. ERI is a process that measures the ground's subsurface resistance distribution by collecting the data from a multielectrode resistivity meter. In addition to looking at the roots of argan trees, Ain-Lhout, et.

al wanted to know how the moisture of soil around the roots changed before, during and after rainfall to make conclusions about how the root system of the argan tree retrieves water and how much water it collects from the soil (Ain-Lhout, et. al, 2016). Knowing that argan trees have roots that spread far across, just below the surface of the soil, and roots that dive deep into the ground, the researchers predicted that the argan tree collected most of the water it needed from the surface roots right after rainfall (Ain-Lhout, et. al, 2016). After testing eight trees with ERI directly after rainfall, the evidence supported the hypothesis. Since the soil around the deep roots did not change, the researchers concluded that the tree uses these roots mainly for anchorage and to collect water if under extreme drought conditions (Ain-Lhout, et. al, 2016). These conclusions match the data collected and hint that it could match the data obtained from other trees that live in dry climates. However, the population size of the study was quite small with testing only eight trees. Further research in this field should be applied in extreme drought conditions to see if the deep roots do collect water from the soil. These researchers are from multiple departments, universities and countries. Ain- Lhout is a multi-department faculty member from the University of Ibn Zohr at the Taroudant campus in Morocco (Ain-Lhout, et. al, 2016). Boutaleb is from the department of Biology from the University of Ibn Zohr at the Agadir campus in Morocco (Ain-Lhout, et. al, 2016). Jauregui and Zunzunegui are from the Biology department at the University of Sevilla in Spain(Ain-Lhout, et. al, 2016). Knowing that the faculty members all hold terminal degrees in their respective fields, and from different universities, their work should be taken seriously as they have taken the time and resources to work and communicate together to get primary research questions answered.

Within the current region of argan trees, there are subtle differences in the physiological traits of them. Chakchar, et. al, wanted to test which trees may be better suited for the predicted future of drought. Four different regions were tested, two were from coastal regions and the other two were from inland regions (Chakhchar, et. al, 2016). This group of biologists and biochemists tested leaf water potential, stomatal conductance and many biochemical responses that act as a measuring tool for determining the stress level of a tree (Chakhchar, et. al, 2016). Stress in plants and trees comes when conditions are not ideal. The tree makes extra chemical signals that travel to other parts of the tree to communicate that conditions are not ideal. After the message is received, the tree responds in a number of different ways mostly shutting down non-essential processes to save energy. In argan trees, these chemical signals are superoxide dismutase, hydrogen peroxide, protein-bound carbonyls, and superoxide radical content (Chakhchar, et. al, 2016). These are the specific chemicals that the researchers measured (Chakhchar, et. al, 2016). After all these tests were done, it was concluded that argan trees from inland regions were better suited for survival through drought and had a healthier recovery after drought conditions were over (Chakhchar, et. al, 2016). This again ties into a previous study because the trees inland are being exposed to more variability in climates and need to be able to survive in them, whereas coastal trees are not used to the changes (Alba-Sanchez, et. al, 2015) (Chakhchar, et. al, 2016). There is a long list of researchers who helped produce this piece of literature. Chakchar, Lamoui, Wahbi, El Modafar and Aissam are all biotechnologists at the University of Cadi Ayyad in Marrakech, Morocco (Chakhchar, et. al, 2016). Ferradous is a forestry researcher who works for the state in Marrakech, Morocco (Chakhchar, et. al, 2016). Ibnsouda-Koraichi is a microbial biotechnologist at the University of Sidi Mohamed Ben Abdellah in Fes, Morocco (Chakhchar, et. al, 2016). Fiali-Maltouf is a Micro and Molecular biologist at the University of Mohammed V in Rabat, Morocco (Chakhchar, et. al, 2016). All these researchers have similar fields, but their paper is very specific and does not go in depth to other regions of science that they are not familiar with. Since there are so many researchers, with most holding terminal degrees, this piece of literature is credible because so many contributed to the final result (Chakhchar, et. al, 2016). Similar to the previous study, Bezzala, et. al, wanted to test how different regions of argan trees grow from sprout to one year with drought conditions in the soil (Chakhchar, et. al, 2016) (Bezzalla, et. al, 2017). The researchers compared two regions, inland and coastal. They let the seeds germinate under normal conditions for four weeks and then exposed them to experimental water amounts of 40%, 20% and 10% of field capacity, with 75% being normal, to simulate different drought conditions (Bezzalla, et. al, 2017). The seedlings were exposed to these conditions for twelve months, with measurements of shoot height, number of leaves, number of spines, length of taproot and root collar diameter, taken every three months (Bezzala, et. al, 2017). They to an undeniable conclusion that was the correlated with previous research: that inland trees are better suited to survive extreme drought conditions than were coastal trees (Chakhchar, et. al, 2016) (Bezzalla, et. al, 2017). The conclusions were excellent through the number of seedlings tested and the variety of measurement tests. Further research related to this would primarily be on inland trees, since these groups of researchers found inland trees being better suited for the likely future (Alba-Sanchez, et. al, 2015) (Chakhchar, et. al, 2016) (Bezzalla, et. al, 2017). The researchers themselves work at the accredited University of Oran, Algeria (Bezzalla, et. al, 2017). Three of the authors are from the biotechnology department and one is from the biology department (Bezzalla, et. al, 2017). They hold terminal degrees in their field and their research can be trusted.

There are many ways future research could go, and more will be expanded on later in the paper. The biggest area of research that I believe would help expand this field based off these sources would be to look at how the inland trees germinate when in the presence of a drought.

This research could be crucial to the trees survival and future generations to understand at what conditions of drought the seeds can still germinate at, and which ones they cannot. The literature discussed here are all only a couple years old, which means research in this area is booming. It is expected that new directions of research that stem from these articles will branch out in a wide variety of subjects.

The future of the argan tree has many possibilities and can be predicted through research on the history, drought effects and germination. The argan tree history has been a very long one that includes slowly migrating inland over the years. It also includes providing for humans and other animals in the area; a trait that has not changed much in the last eight hundred years. The argan trees that are best suited physiologically for drought are those found inland. They are better at responding from drought and surviving through it. Germination is a critical step for a new argan tree in order to thrive. The research conducted discussed the benefits of soaking seeds before implanting to gain a better germination percentage. This literature review has gone over these topics for the purpose of leading to future research. The primary research question left to be answered is what is the minimum amount of water needed for an argan tree seed, found inland, to germinate and fully lead to a health sprout? The answer to this question could expand to other trees living in dry climates or give a better idea of argan tree survival if the climate does change like researchers predict.

Social Importance

The argan tree is central to some of Morocco's traditions and to their economy. The seeds feed grazing livestock, the oil is hand made by women and is used in cooking and cosmetically.

The picture above shows from left to right argan fruit, nut, nut casing and seed. The fruit falls naturally from the trees. The nut protects the seed. And the seed is what grows into a new Argan tree.

Argan fruit feeds goats. Traditionally, this is how argan oil making started. The goats would eat the fruit and excrete the nut. The Berber women would pick through the feces for the nuts, crack them open and get the seeds. Through the increase in domestication, more and more goats eat the fruit from the argan tree. Also, some Moroccans like to eat goat meat from goats that have eaten argan. They say they can taste the difference and it is far superior than goats that do not eat argan.

Argan oil is partially handmade by Berber women. In an interview with one of these women at an argan co-op, she said the process of making the oil is long and mostly mechanised (2017). There is one exception, getting the whole seed out of the nut casing. It requires a step of absolute perfection that machinery has not been able to match. Berber women are employed by many co-ops cracking the nuts open to get to the seeds. Most women will set the seed on a big flat rock, turn the nut on its side and crack it open by hammering down on the nut with another rock. This job opens doors to the women to enter Argan co-ops that can lead to daycare and learning how to read and write.

But, there have been rumors that maybe not all co-ops are not as beneficial as they sound. With the increase in popularity, more journalists are asking questions. Co-ops are run independently, so if they have a manager who treats the women well, working conditions are good. However, these journalists are asking questions about what happens if a co-op is managed by someone who does not treat their employees well. This has the potential to become a social problem.

This usage increase has effectively raised the price of seeds as well. Oil production requires a lot of seeds. To get one liter of oil, 2 kilos, about 4 and a half pounds of seeds are required (Anonymous, 2017). That is a lot of seeds! To put this into perspective, that is the number of seeds one argan tree produces in one year. One liter of oil per tree per year. The people who used to use the oil for cooking no longer use it as often because the price has increased so much (Anonymous, 2017). The increase in seed price also causes people to pick the seeds off the trees before they are ready to fall to the ground when they are ripe. This is problematic to the tree, but it does help those who need extra money. It is relatively easy to find argan trees, pick up their seeds and sell them to co-ops who then make the oil.

Argan oil is being used worldwide as a cosmetic product. It is being sold by itself, in shampoos, conditioners, lotions, gels and more. Just walking down any hair care aisle in any store in the U.S., you can tell that this oil is in many products.

The picture above shows a few of many cosmetic products that are making their way into and being sold in the U.S.

In 2014, the global usage of argan oil was 4,835.5 tons (*Argan Oil Market Analysis By Application (Cosmetics, Food & Medical) And Segment Forecasts To 2022*, 2015). That same year, the market was worth just over 100 million U.S. dollars (*Argan Oil Market Analysis By Application (Cosmetics, Food & Medical) And Segment Forecasts To 2022*, 2015). This report was generated in late 2015 and it projects a massive market growth. By the year 2022, they expect that the market will be worth 600 million U.S. dollars (*Argan Oil Market Analysis By Application (Cosmetics, Food & Medical) And Segment Forecasts To 2022*, 2015). A massive growth! This is mostly driven by new reports of the fatty acids and vitamins in the oil being great for skin and hair. Specifically antioxidant tocopherols, lupeol, omega 3 & omega 6 fatty acids, butyrospermol, triterpenoids and beta amyrine have been identified in the oil and thought to contribute to the changes in skin and hair (*Argan Oil Market Analysis By Application (Cosmetics, Food & Medical) And Segment Forecasts To 2022*, 2015). But the oil is also used in food and for medical reasons. Both of these reasons are increasing in popularity and also driving the market forward (*Argan Oil Market Analysis By Application (Cosmetics, Food & Medical) And Segment Forecasts To 2022*, 2015).

Threats to the Tree

As was mentioned earlier, the increase in price of argan oil has proved to be problematic to the tree. Main problems that face the tree are goats, desertification, people and infections.

The picture above shows goats climbing an Argan tree to eat the fruit. You can see the goat herder sitting in the background while his goats feast off the tree.

By Moroccan law, it is illegal to let goats climb trees to eat the argan fruit. However, many nomads who heard goats have no other way of feeding their livestock besides walking them through fields of argan trees. This law is difficult to enforce especially to the people who do not understand that the goats are hurting the trees. The penalty for breaking the law is to pay a fine, but many nomads who are breaking the law do not have the money to pay the fine (anonymous, 2017).

However goats are not the only animals eating the fruits from the argan trees. Dromedary camels also like to snack on the fruits.

The picture above shows the Dromedary camels, or otherwise known as Moroccan camels. The camels do less damage to the trees when compared to goats since they do not have to climb up the tree to reach the tallest fruits. But their eating habits still pose a threat to the tree by pulling off fruit before it is ready to fall naturally.

With the increase in climate change, the weather patterns in the desert are causing the expansion of the Sahara desert. This means that the winds are stronger, the soil is more sandy and holds less moisture, there is less water in rainfall and the temperature is higher. All these conditions are not good for the tree. As previously stated, the argan tree can withstand some of these conditions, but a combination of all of them at the rate the desert is growing, desertification is a threat to the argan (Alba-Sanchez, et. al, 2015).

Infections by insects and fungi are another problem facing the argan tree. Researchers A. Imoulan, A. Alaoui and A. El Meziane were interested in identifying certain fungi in the regions of argan forests (2011). They wanted to see if time of year and location varied how common fungi were found in the samples and *Ceratitis capitata,* the Mediterranean fruit fly (A. Imoulan, et. al, 2011). They baited the fungus in the soil samples they collected from different regions in Morocco. They were able to do this by using the *Galleria* method (A. Imoulan, et. al, 2011). The researchers found from all their samples, 91.62% of their samples had fungus growing in it (A. Imoulan, et. al, 2011). The researchers found three different species of fungus; *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces lilacinus* (A. Imoulan, et. al, 2011). *B. bassiana* was found the most, making an appearance in 90.64% of the soil samples that had fungus in it (A. Imoulan, et. al, 2011). *M. anisopliae* and *P. lilacinus* were found less frequently in 15.27% and 1.48% of the soil samples that had fungus in them respectively (A. Imoulan, et. al, 2011). The researchers did more experiments to test the pathogenicity of *B. bassiana* to *C. capitata.* They found that 86.44% of the tested fungus isolates were virulent to the fly (A. Imoulan, et. al, 2011). What this means is Morocco's most common soil fungus is very virulent to the common fruit fly. More research is needed to investigate this relationship further. The researchers are reliable because they all have their Ph.D. They have also all worked with soil and fungus for a prolonged period of time. A. Imoulan was at University from 2006-2011 studying different fungi in the soils of the argan forests. This paper is important for the argan tree field of research because there are many threats facing the tree. The more we can learn about the argan trees environment, the better we can understand some of these threats. It is also important to the rest of the agricultural field since soil conditions affect all plants, no matter what they are.

Basic Physiology of Germination

Germination is defined as the development of a plant from a seed or spore. There are three basic steps to all seed germination, including argan germination. The first is the outside coat of the seed absorbs water. This event signals to the seed that growing conditions are favorable, and the seed will continue to germinate. The second step is the embryo will start to consume the stored food reserves of starch. The embryo will convert the starch to glucose, cellular respiration will occur and the embryo will grow, bursting open the seed coat. The third step is that the root emerges out of the seed and starts growing downward to start anchoring the plant. Using gravity, the seed is oriented so it knows which way is up and which is down. This is important for the seed to grow up and out of the soil to find sunlight as fast as possible.

Germination is the process of a seed going to a seedling, or sprout. Studying argan germination is critical in determining the future success of the tree, since it is the first major step of survival. Many challenges face the argan tree in terms of fertility so if a tree is fertile and makes seeds, biologists want to know the best conditions for the seed to germinate and survive.

Biology researchers from Algiers, Algeria tested argan seeds from two different regions in Algeria. Like other studies, one was coastal, Mostaganem, the other was inland, Tindouf (Zohra, et. al, 2014). They tested how sterilization and soaking affect the success rate of germination (Zohra, et. al, 2014). They collected the seeds, sterilized them to prevent microbial contamination, soaked them in water for variable amounts of time (72, 96, and 120 hours), then grew the seeds in variable temperatures (30, 28, and 25^{o}C) to simulate drought conditions (Zohra, et. al, 2014). The researchers measured germination rate, lengths of roots and stems, and number of leaves and thorns (Zohra, et. al, 2014). Based on other research, they unsurprisingly found most that the Tindouf, inland, seeds germinated better across all variable temperatures and soaking periods with the exception of 72 hours at 30^{o}C (Zohra, et. al, 2014).

This correlates with the other researchers that inland argan trees and seeds are better acquainted to survive in drought conditions (Alba-Sanchez, et. al, 2015) (Chakhchar, et. al, 2016) (Bezzalla, et. al, 2017) (Zohra, et. al, 2014). Something new that they found was that soaking the seeds increased germination when compared to the control of not soaking and just watering normal amounts (Zohra, et. al, 2014). This technic along with sterilization, could help the survival of argan trees when trying to grow more in the wild (Zohra, et. al, 2014). The researchers used a variety of measurements, but one thing that future research should lead to is drought stress with germination. This experiment tested some similarities to drought, but it would be good to do something like Bezzalla, et. al did and test drought as a percentage rather than a time (Bezzalla, et. al, 2017) (Zohra, et. al, 2014). The researchers who conducted these experiments are all from different universities in Algeria (Zohra, et. al, 2014). Zohra is from the biology department at Houari Boumediene University of Sciences and Technology (U.S.T.H.B), El Alia campus (Zohra, et. al, 2014). Ali is a plant biologist at the University of Mascara, Algeria and Moulay is a plant physiologist from the University of Oran, Algeria (Zohra, et. al, 2014). These experts vary in their knowledge and have terminal degrees in their fields, so their research can be trusted.

Literature Review Conclusion

Comparing all the research together, the future of the argan trees has many options. The inland argan trees could move more inland if climate change slows or stops or they could move towards the coast if climate change continues (Alba-Sanchez, et. al, 2015). Multiple researchers recommended that future research be done on the inland trees, since they have better genes for survival of drought (Chakhchar, et. al, 2016) (Bezzalla, et. al, 2017). The impact humans have on the argan tree is an expanding field of research and could greatly impact the future of the tree. The more research that is done on the argan tree, the better.

The Conducted Experiment

While in Morocco for 8 weeks, germination of the argan tree was studied in a controlled lab experiment. Germination was considered in progress if the embryo sprout protruded from the outer seed coat. This leads to the actual experiment that was conducted. For it, we asked what factors help germination? And sub questions of, which temperature is best for optimal germination rates? To help answer this question, there were two temperatures the seeds grew in,

30 °C or 26°C. Wha

germination? Added onto the end of this experiment, was the question of the quality of argan oil from different trees. Answers to these questions would be partially answered by an experiment done in conjunction with the National Institute for Agricultural Research- Agadir. The experiments were done in controlled lab settings and used seeds from trees on the controlled outdoor farm.

Materials and Methods:

Seed collection

Argan fruits were collected from the National Institute for Agricultural Research- Agadir farm. Two different trees' fruits were picked off the ground, T1L1 and T3L6. The pulp was removed by hand, then the nuts were cracked open using the traditional rock tapping method that the Berber women use. Whole healthy seeds were placed in bags separated by tree. T1L1 contained 113 seeds and T3L6 contained 136 seeds.

Preparation of seeds

The experiment was started 5 days later to make sure that the Muslim Holiday of Eid did not impact the integrity of the experiment by the labs being closed.

Petri dishes (9.5 cm diameter) were sanitized and labeled. Filter papers were collected along with tweezers, 4 L of distilled water, and bleach solution (17% bleach/ hypochlorite). The tools were sanitized in a pressure pot for 20 min of 121 ℃ water at 1 Bar.

The seeds were inspected to make sure they were healthy. This entailed making sure there were no cracks in the seed, the entire seed was intact and there was no discoloration. The seeds were measured by mass (g), width, length and height (mm) at their largest parts. Density of the seeds were approximated and sorted either large of small density, and by the tree they came from.

Seeds were cleaned to try to avoid contamination from fungi or bacteria. All work done was between two lit bunsen burners to kill airborne contaminants. Seeds were rinsed with bleach solution for 3 min, three times. Then seeds were rinsed with distilled water for 3 min, three times. Seeds were placed in petri dishes lined with filter paper. 9 seeds per dish. 1 mL of distilled water was added to each dish and then sealed with micropore tape. Dishes were labeled with the name, date, tree, size and temperature of growing condition.

Germination

The seeds were grown for 72 hours in their respective temperature incubators and then checked for germination rate purposes. The few seeds that were infected with black fungi were rinsed with 2% bleach solution for 3 min and washed with distilled water for 1 min, and the filter paper of the dish was changed. Each dish was watered with 1 mL of distilled water and placed back in the incubators.

Five days later, the number of germinating seeds were counted. Once again, there were diseased seeds. Dead seeds were counted and rid of. The living seeds were sanitized by the original cleaning methods and placed in clean petri dishes with clean filter papers. 1 mL of distilled water was added, petri dishes were sealed and placed back in their respective incubators.

One week later, seeds were once again contaminated. Results were recorded.

Oil Extraction

This protocol was adapted from Dr. Aabd's paper (2013). New seeds were gathered from the same two trees at the farm. They were obtained by the original previously noted methods. 10g of each tree type of seeds were crushed using mortar and pestle into a fine paste. Paste was placed in an incubator at 30 ℃ overnight. Th

180 mL of Hexane was loaded at the bottom of the apparatus. The hot plate was set at 150 ℃ and the cool water running through was between 12-40 ℃. The apparatus ran u

concentration was level to the filter. The hexane was recycled and repeated 3-4 more times. The oil was then filtered from the extracts. Then the oil was separated from the hexane. This whole process was done for each tree and the differences in oil quality and fatty acid analysis by gas chromatography was done. The gas chromatography was analyzed by using a Agilent 6890 gas chromatography apparatus with a split injector and a flame ionization detector. 1 µL of each tree oil sample was placed on a BPX70 capillary column at a 1:50 split ratio with a helium carrier gas flow of 1.5 ml/min at a temperature of 175 ℃ during separation and a detector temperature of 220 ℃.

Data:

Figure 1: Seed germination set-up. Argan seeds (shown above) were placed in petri dishes with watered filter paper and sealed off.

Figure 2: Germinating seeds. Image shows an unidentified fungus growing on germinating seeds 7 days into the experiment.

Figure 3: Germinating seeds and larval state of an insect. Image shows an unidentified insect at larval stage infecting germinating seeds 10 days into the experiment.

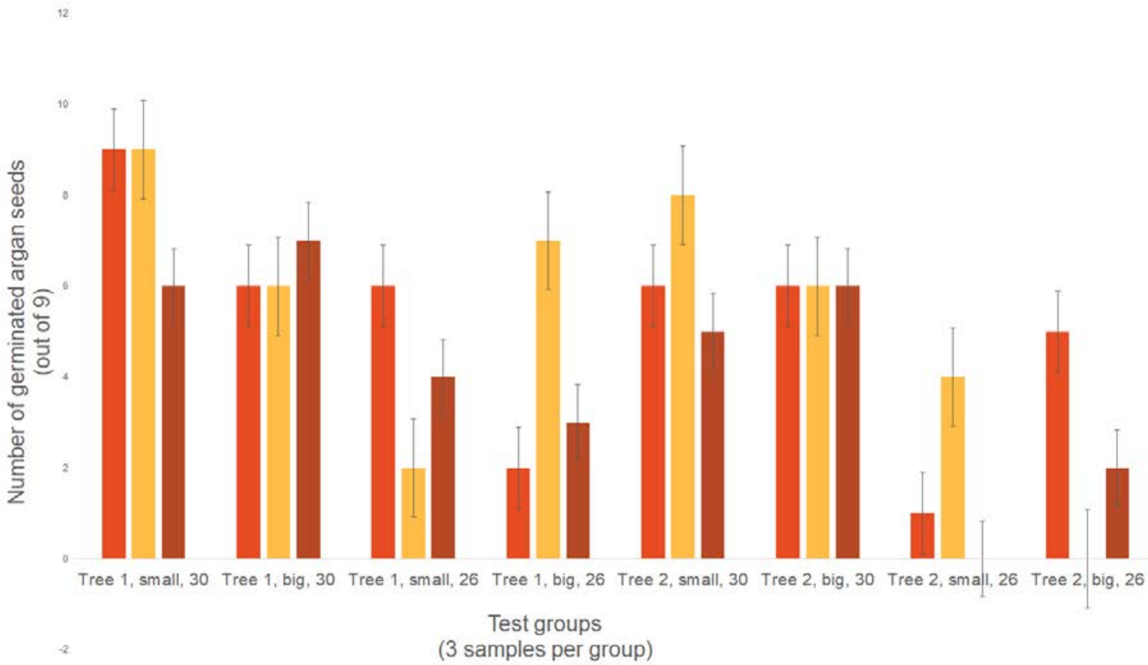

Figure 4 shows a graphical comparison of all the germinating seeds in their different classifications and environmental treatments. Different colors in each test group bundle represent a different petri dish. Each test group had 3 petri dishes.

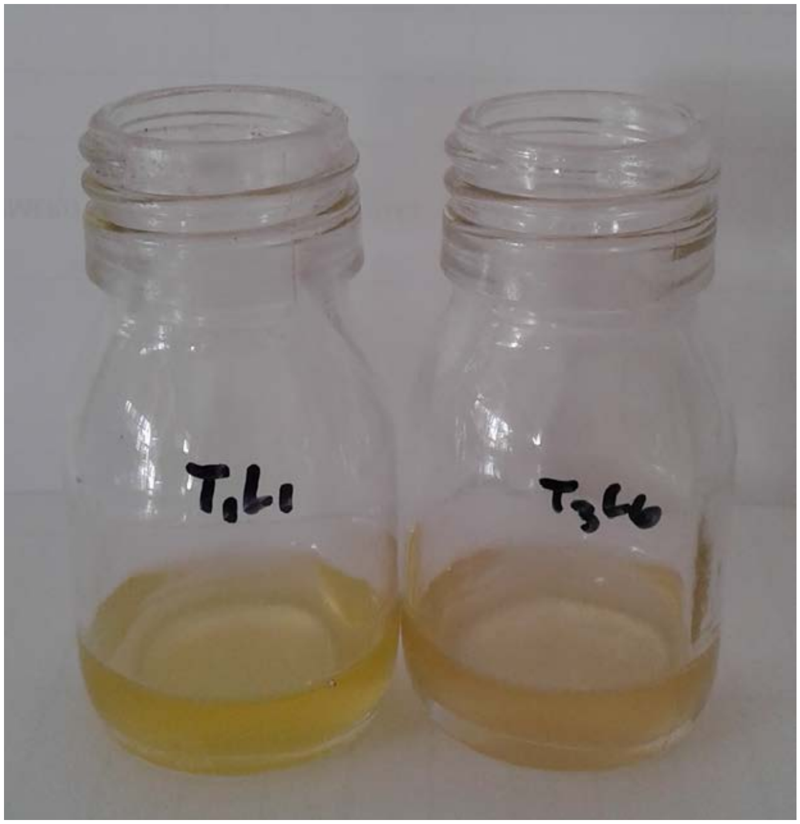

Figure 5: Extracted argan oil. Image shows a color difference between the two trees tested. T1L1 is yellow and T3L6 is drab brown.

	T1L1	T3L6
Oil weight	144.71g	129.87g
Fatty acid content in oil	45.55%	46.34%

Figure 6 shows the final weight and fatty acid content in the extracted oil from the T1L1 and T3L6 trees that were tested in germination.

Discussion:

Germination results

The first result was that the experiment did not yield any statistically significant results. Figure 4 shows this graphically. All of the error bars overlap with another error bar when we compared size, temperature and specific tree. A possible reason for this could be that there were not enough seeds to make these comparisons. Another reason could be that the temperatures, sizes and trees we tested really do not make a big difference in germination results. A repeat of this experiment with more seeds would need to be done to make these possible claims stronger.

Unexpected results:

The results from the germination experiment did not go as planned for a couple of reasons. What we wanted to have happen was compare the two temperatures of germinating seeds, compare the sizes of the seeds and compare some of the genetics of the two different trees we used. This did not happen because of infection, Ede and time limitations.

The first unexpected result was from the infections that plagued the germinating seeds. The argan nut protects the seed from a lot of unwanted infections, but due to time constraints, we only used the seed and not the seed within the nut. This change made the seed more permeable to infection. Figures 2 and 3 show the seeds being infected. However, we took great care with trying to keep the seeds from getting contaminated. We worked between bunsen burners to rid of airborne contaminants, we worked with sterile tools, we cleaned the seeds before they started germinating, we cleaned entire petri dishes of seeds at the first sight of contamination and sealed thee petri dishes while the seeds grew.

Some possible reasons why these seeds kept getting infected are because we could not work in a fume hood. There were not enough fume hoods in the lab for everyone to use, so I did not want to impose on the other researchers' experiments. Another reason could be that other agricultural experiments were happening with bacteria and insects. There was a couple experiments being conducted with *Ceratitis capitata*, the Mediterranean fruit fly, and tomato plants. It is possible that some of those studied species worked their way into my lab and infected my seeds. However, this is just one of many possible insects that could have affected my seeds (figure 3). There is no way to confirm that these flies were the culprit of contamination just by looking at the picture (figure 3). Further testing would need to be done to see if these Mediterranean fruit flies did infect my seeds. It also cannot be said definitively that the other experiments contaminated my seeds. It is possible that the larva were already inside the seeds when they were gathered and the bleach solution did not kill them. Further testing would need to be done to see from where these bugs came from.

Figure 2 also shows some germinating seeds and some non germinating seeds. It looks like the seeds that are not germinating are more infected than the seeds that are not germinating. It is possible that these more infected seeds carried the fungus into the petri dish, then the fungus grew and infected the germinating seeds. A possible culprit of the fungus from figure 2 could be *Beauveria bassiana.* This was the most common fungus found from all the soil samples in the A. Imoulan et. al paper (2011). I think *B. bassiana* infected the seeds because they have a strong relationship to the fruit flies that I think could maybe be the insects that also infected the seeds (A. Imoulan, et. al, 2011). Also this fungus is the most common in Morocco, so chances are pretty good that this is the identity of the fungus pictured in figure 2. Of course we cannot definitively say this without further testing, but this possible identity is a good educated guess.

Another reason the seeds were infected is because argan seeds could just more susceptible to disease. Some species are better at heading off infections than others. I could not find any research articles that made this claim, but it is possible that the argan seeds are just not as prepared to fight off insects, fungi, etc. Since there was no research to support this claim, future research could look into this question to find if argan seeds are more susceptible to disease than other tree species.

Ede is the Muslim holiday that takes place at the end of Ramadan. To make a simple comparison, it is kind of like their Christmas. Since Morocco is predominantly Muslim, the whole country shut down for Ede. Therefore the lab was closed those days too. Ede was also on a Monday and the lab is not open over the weekend. So there was a stretch of 4 days were I could not check my seeds for germination or infection. Maybe had I been able to get into the lab sometime in those 4 days, I could have prevented the severity of the infections and taken a better timeline of the germination schedule.

Time limitations was perhaps the biggest challenge we had. I was in Morocco for 8 weeks. I was in Agadir for 6 weeks, and only started working with people from the lab for four weeks. We had four weeks to draft up a protocol and finish the experiment. This is a super short amount of time to get the amount of research done that I wanted to. We ran out of time for the genetic analysis of the two trees I studied. Also I would have prefered to run another germination rate test, but there was not enough time for that either.

Oil extraction results

There is a distinct color difference between the two oils in figure 5. The T1L1 has a more yellow color while T3L6 has a more drab brown color. The difference in color is due to different fat and protein contents. The yellow color has a healthier content of fats and protein than does the drab color (Aabd, 2013). This is interesting because according to figure 6, T1L1 has less fatty acid in it than does T3L6. This difference is not statistically significant. It is also possible that the fatty acid analysis had an error to give a false number of contents in the oil.

Future research

Had I had the time in Morocco, I would have wanted to do more experiments related to argan germination. I would have wanted to do a germination experiment with the seeds inside the nut, a further genetic analysis of the trees, doing a more complete test on fatty acid content in the oils, testing a wider temperature range for germination, a test on tree and seed ages and testing for .

The first direction I would want to go is to try to decrease the amount of infections that plagued the seeds. This could maybe be done by keeping the seeds' protective nut around the seed and doing the germination rate test that way. This experiment would take much longer than the one I did, but I believe the infection rate would be lower due to the nut being around the seed.

The second experiment I would want to go would be to do a deeper genetic analysis of the two trees that I used. I would want to look at known genes of the trees and see if there is a difference in the health of the seeds/ the germination rates due to the different genetic properties. This could possibly lead to identification of problematic genes, or vital healthy genes in the two trees. Identifying these genes could possibly lead to expansions in biotechnology with the argan tree. Knowledge gained from these tests could lead to a mini-hand- genetically modified organism (GMO). By taking the trees that have known good/ bad genes and crossing them with each other, we have the possibility of creating stronger argan trees at a low budget. Testing them would take years to see if that tree physically expresses those genes, but it is a possible slow solution. This research could even go further is cosmetic companies are willing to pay for expensive GMO lab creations of argan seeds. Lab created GMOs are expensive, and the cosmetic industry is large and rich, so if they put money into research and development of argan GMOs, they could make a huge profit.

A third experiment I would like to conduct would be to analyze which fatty acids were present in the oil we extracted. This would tell us more about the environmental conditions impacting the tree. It is known that the climate, soil type, water amount/ components, etc. impact individual fatty acid contents (Aabd, 2013). By analyzing the fatty acids in the oil, we can adjust the environmental conditions of the germinating seed to yield a higher percentage of prefered and healthy fatty acids in the oil of the seeds it produces (Aabd, 2013).

A fourth and final experiment I would want to do it identifying the fungus and insects that infected the seeds from the original seeds. I would want to see if the insect was in fact what I thought it was, *Ceratitis capitata.* And if the fungus was *Beauveria bassiana.* I would need to work with the other researchers who were studying *C. capitata* and reach out to the researchers who study *B. bassiana* to see how they identify these species from other species. I would want to do this to then see what I can do for other argan germination tests to prevent infection from both these species.

Contribution to Field:

This experiment adds little actual findings to the field. However, it does raise more questions about germination of the argan seed that hopefully future research will help answer. The growing global trend of using argan oil is putting pressure on the argan forests of Morocco. This experiment has raised questions whose answers will help preserve these historic forests so that there will be enough oil to satisfy the global craving for it.

With the global rise in popularity, more people are starting to ask questions about the Berber women's working conditions, the preservation of the forests and other social and economic topics related to the argan tree. With this rise in awareness, maybe more people will become interested in investigating these topics. My research will bring some of this awareness to my home community and my friends.

Experimental Conclusion

This experiment was done to figure out what some problems *Argania spinosa* faces while germinating and to possibly figure out some conditions to make these problems less of a factor. While we did not find any significant results for what helps germination happen the fastest, we made one discovery. What we found was that seeds are easily infested by insects and fungi. Further research is needed to find which species are infecting the seeds, to find optimal conditions for germination rates and to generally make stronger claims about what conditions are best for total argan germination.

Cross- Culture Experience:

I spent over the two months in Morocco and I learned a lot different ways to conduct research, diversity, friendships with people of other cultures and America's obsession with consumer waste. Now that I am back in the U.S. I like taking time to reflect on what I have learned and how I can implement it better into my everyday life.

My time in Morocco was a dream come true. In certain ways, the research project was just an excuse for me to be there and to live and experience their rich culture. I have so many amazing memories from my time abroad. And some of those are from my research.

However, the research project was extremely eye opening to how Moroccans conduct research. Often times it frustrated me. The Moroccan view on planning is that the present is the most important thing and the future will be worried about in the future. The American view is that the future should always be planned and we are constantly looking ahead. So when I would email professors in Morocco, they would get to it… eventually. It took a couple emails to get an appointment, and then it would take a couple appointments to get a plan in place. I learned very quickly that this was a blessing in disguise. Once I had the appointment, my mentors and advisors had their undivided attention on me. Their willingness to help and not have appointments behind me blocking their minds, gave me a ton of wisdom and made me feel special.

I also learned that Moroccan labs do not have much, but they are more than willing to share. The things that I was used to working with in the labs were not present. Things like fume hoods, nuclease- free water, disposable pipettes and more were not there. At first it made me pity the researchers in Morocco because they did not have all the resources we have here. But after just one day at the labs, I learned they made the best of what they had and were very creative with their materials. For example, instead of buying insect jars, they would save money and use old clean peanut butter jars. When I came back to the States, I was overwhelmed with everything that is in our labs and if we even really needed it.

While working in the lab, I made many connections with people and learned bits of arabic and a lot about different ways Moroccans are devoted to Islam. The people in the lab loved teaching me about what they believe and why they believe it. And then they would ask what types of things I believed in and why. There was one really special day, I do not even remember what we were doing with my research, but we had like 10 people crammed into my little room talking about religion and languages. There was no judgment. None. They were so open to talking about religion and other people's beliefs. It was extremely refreshing having just come from the U.S. 6- months after a presidential election. I do not think I'll ever have an experience like that ever again.

While I was in Morocco, I gained a very special friendship with a wonderful Moroccan woman named Mimi (pictured above). She is a college student living in Agadir going to one of the top business schools in Morocco. We loved telling each other about our traditions, languages, religion, family, lives ect. Mimi would go out of her way to make sure that the other SPAN-ers and I were able to do something that we wanted to do! There was one day during Ramadan where her and her sister took us to the local bath house, or hammam, because we wanted to know what it was like to go to one! During Ramadan, Muslims do not eat or drink anything, so it was special that they took us to the extremely hot hammam. It was such a rich experience because it was a real hammam and not a touristy one that we would have gone to without Mimi and Hajar. There was another day when Mimi texted me wanting to hang out, but I was not feeling well. 10 minutes later I hear a knock at my door and it's Mimi and Hajar. They come in with, "gifts for [my] stomach." They put tea on the stove, unwrapped breads and hard candies they eat when they are not feeling well and told me I must eat these things if I wanted to feel better. It was so sweet. I felt loved and cared for. But the thing I will NEVER forget about Mimi was a conversation we had. I was telling her that I was stressed that I did not know what I wanted to do after I graduated. I told her I wanted to go to Pharmacy school, but I did not like chemistry/ was scared of the chemistry classes in school and that I did no have any experience to get into Pharmacy school. She looked at me in the eyes and said, "Becca. You are an amazing person and you can do anything you want to. I am sure with every fiber of my being that you will get into Pharmacy school." Well with Mimi cheering me on, I signed up to take the PCAT, and later applied and got into the University of Minnesota pharmacy school. Mimi changed my life, and I can not thank her enough for it.

Reverse culture shock with coming home was rough. I realized how wasteful Americans are with almost everything. In Morocco, everything had a place. Food didn't come super packaged in plastic, everyone recycled, you did not waste water on things like grass or water fountains, time was important to people and not wasted, and on and on. When I came home, I found myself angry by the fact that cucumbers were wrapped in plastic. It sounds silly, but I was not a happy camper in Aldi that day. Over time, I found things that I could do to waste less. I now bring my own reusable bags to the store and I keep the bags in my car so I don't forget. I bought some plants that I can use in my cooking and that also, "green up" my house. I take way shorter showers and do my best to recycle absolutely everything that I can. By doing these little things, I am more content with what I learned in Morocco and by applying little things like this to my life, the reverse culture shock was lessened.

We need to keep in mind that everyone is different. Everyone has different personal and cultural experiences that shape their values. Even if it feels like our values are the correct ones, they will feel just as passionately about their views. This, to me, is the most important. It may not provide peace of mind, but it will calm the emotions that may flare in response to something that goes against our own values. Having different values and beliefs is something that makes the world unique. We all have different experiences that motivate us to do, or not do, certain activities. We must try to keep in mind that differences are something that exist among all people. I imagine it will be difficult to do in an area of the world that is so different from our own, but with cultural preparation and a few other tips, we can respectfully coexist.

The biggest lesson that I learned and keep in mind daily is the idea of diversity and how to get along with others who have different experiences. We can try to understand their views at a deeper level by sincerely trying to understand why their values are what they are. This could be done through conversations with Moroccans about values. Maybe there are purposes for the way things are that are not obvious to us at first. We would not disagree or agree with them, just simply asking questions and explaining things that we are unfamiliar with. This will not change our core values, but it may provide a fuller picture that makes more sense than the glimpse we get otherwise. We should also keep in mind when we are abroad, is that we are living in their country. Arguing or disagreeing with Moroccans will not change their set cultural values, as it would not if the roles were reversed. If we strongly disagree with a value and have talked about it with a Moroccan, we should do our best to avoid it. This cannot be the case for some situations we may find ourselves in, but for the ones we can ignore, we should. If we keep constantly thinking about our differences, it will drive us mad! My last idea is to focus on shared values rather than differences. This will make our big differences seem smaller. It will make living side by side more enjoyable if you see more similarities than differences. This can again be done through conversations with Moroccans. For example, I know I will find similar values with Moroccans based on family. I can try to find more similarities about music, science, travel and other areas I have strong feelings about.

I learned many things while in Morocco and being home after my trip. SPAN gave me the opportunity to open my eyes to the world. And I can not wait to see what comes next.

Acknowledgments:

Special thanks to the National Institute of Agricultural Research- Agadir for letting me use their materials and space to conduct this experiment. Also to Dr. Jennifer Liang for her guidance in writing the report.

A very special thank you to Dr. Naima Aabd, Dr. Rachid Bouharroud and Merriam Koufan for guiding my research project during my short time in Morocco. They took me under their wing and gave me the knowledge and the materials to make this project happen. And to Dr. Ryan Goei and Dr. Dana Lindaman for helping me prepare for my time in Morocco, supporting me while there, and keeping in touch when we returned to the states.

I could not have done this without the amazing support from all these people.

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