A Thesis Presented to

The Faculty of Alfred University

Improving Germination of *Allium tricoccum*: a Plant Threatened by Foraging Trends

Jacalyn DeValue

In Partial Fulfillment of

The Requirements for

The Alfred University Honors Program

May 15, 2013

Under the Supervision of:

Chair:

Dr. Cheryld Emmons

Committee Members:

Dr. Fred Beaudry

Dr. Michele Hluchy

ABSTRACT

Recent foraging trends in North America are endangering ramps, *Allium tricoccum*, which are a wild leek. Ramps germinate and grow more slowly than they are being foraged, which has led to a call for protection of the species in many states. In addition, they need a warm, moist period followed by a cold period to break out of dormancy. The goal was to find conditions for faster seed germination by scarring *Allium tricoccum* seeds with sulfuric acid and adjusting time in certain temperature environments. It was found that scarified seeds which spent 10 weeks in an environment of 20 degrees Celsius had percent germination similar to un-scarified seeds which received the same temperature treatment. Temperature and time were the important determining factors in seed germination for *Allium tricoccum*, not scarification. Further research may be done in the future.

INTRODUCTION

The decline in abundance of once common plant taxa is of concern to scientists all over the world. Many countries are seeing 10-15% of vascular plants on threatened or extinction lists (Norton, et. al., 1994). There are many different reasons, including habitat loss, competition, and loss of specialized pollinators (Norton, et. al., 1994). It is indisputable that human activity is in part responsible for these declines. Humans have cleared land for agriculture, introduced herbivores that over-graze, and over-harvested plant resources (Norton, et. al., 1994).

Plants are commonly overexploited for their medicinal and ornamental purposes. The American ginseng root, *Panax quinquefolius*, which grows wild throughout the eastern United States, is overexploited because it is thought to be an energy booster (Anonymous, 1998). Between its high commercial value, one root costing between $40 and $60, and the increase in harvesters, the American ginseng is on its way to extinction in seven states and threatened in nine others (Anonymous, 1998). In addition to ginseng, goldenseal, *Hydrastis canadensis*, is another overharvested herb. More than 60 million goldenseal plants are harvested each year and are not replaced (Dworkin, 1999). The Pacific Yew, *Taxus brevifola*, is also endangered in the United States. The drug taxol, extracted from the bark and needles of the Pacific Yew, is popular in treatments for cancers (Loyola-Vargas and Vázquez-Flota, 2006). However, because the tree produces such low quantities of taxol, a traditional isolation of 1 kilogram of the drug requires around 6.7 tons of Pacific Yew bark, or between 2,000 and 3,000 trees (Loyola-Vargas and Vázquez-Flota, 2006). The drug is in high demand but is extremely unsustainable to use, putting the tree on endangered species lists.

American herb consumption has risen at an average rate of 20 percent every year and many plants are at the point where they can no longer be picked in the wild (Dworkin, 1999). For many chefs aspiring to get ahead of the game, wild plants on the menu can play an important role in setting a restaurant apart (Strand and DiStefano, 2010). Plants like ramps and purslane are becoming as much a member of seasonal vegetables as tomatoes and corn, but in order to come out ahead, chefs have been utilizing more obscure plants such as toothwort, brown jug, pineapple weed, and licorice fern (Strand and DiStefano, 2010). For some chefs wild plants have acted as a sort of reset button, giving them a creative boost, allowing them to rediscover and reboot classic dishes (Strand and DiStefano, 2010). Chefs who favor this new trend often have their own foragers, such as Wild Gourmet Foods, a Vermont company that gathers more than 150 kinds of roots, greens, nuts, barks, and berries in a year (Strand and DiStefano, 2010). Foraging has become so popular among chefs and locavores, that professional forager Kerry

Clasby of Los Angeles now gets calls from big names in New York to send her products east (Food & Wine, 2010).

Ramps, *Allium tricoccum*, are a wild leek that grows during a short early-spring season in the woods from Georgia to Quebec (Sen, 2011). It has been hailed for decades as a nutritious spring vegetable and each year ramp festivals are held all over the south in its honor (Sen, 2011). It used to be that ramps would carpet the forest floor, but now foragers have to hike further into the woods to find a single ramp (Sen, 2011). Since the 1990s, ramps have grown in popularity, being featured in magazines, on menus in top restaurants, and fawned over in farmers' markets when they first arrive in April (Sen, 2011). With the local food movement in high gear, as well as a growing interest in wild and foraged plants, prices for ramp have gone up to $12 a pound in some areas (Sen, 2011). In 2004 the Great Smoky Mountains National Park in North Carolina and Tennessee banned ramp harvesting once a study in the area revealed that the only sustainable method for ramp harvesting was to take less than 10 percent once every 10 years (Rock, 2004). Ramp seeds take 6 to 18 months to germinate and the plants take 5 to 7 years to produce seeds, making sustainable harvesting an issue (Sen, 2011). How can we maintain ramp populations while keeping up with the demand from hungry locavores?

Cultivated herbs may be better than those found in the wild; they may have a higher concentration of the active ingredient for which pickers are looking (Dworkin, 1999). By learning to cultivate our own plants for food, we can get a more beneficial plant product as well as reduce overexploitation of wild species. Existing research tells us that ramps can be cultivated successfully for commercial purposes. It concluded that fall planting of seeds and a forest setting results in higher germination rates than spring planted seeds and an artificial shade structure or open field (Davis and Greenfield, 2002). They may also have a high rate of germination if grown in a laboratory setting at 20°C in complete darkness (Jones 1979). However, the main method of reproduction in wild populations of *Allium tricoccum* is asexual by which the bulb divides through lateral bud development rather than by creating new seedlings (Nault 1993).

The issue I will be addressing in this experiment is that of seed dormancy in *Allium tricoccum*. Seed dormancy is a mechanism that delays germination to make sure it occurs when conditions are suitable and the probability of survival and growth of the seedling is high. There are three types of dormancy: morphological, physical, and physiological (Fenner, 2005). With morphological dormancy, the seed's embryo is underdeveloped and needs time to grow or differentiate before it can germinate (Fenner, 2005). Physiological dormancy requires a chemical change in the seed (Fenner 2005). Morphological and physiological dormancies are related to the seed embryo and/ or the endosperm, whereas physical dormancy is related to the seed covering that protects the seed. Seeds with physical dormancy have an impenetrable seed coat that protects the embryo until the coat is broken and water may enter (Fenner, 2005). The seeds of *Allium tricoccum* were described in a study as having a thick, cartilaginous seed coat, which leads me to believe that acid scarification could be an effective means of breaking dormancy, which could be classified as physical dormancy (Jones 1979). Another method of breaking physical dormancy is to cut the seed coat with some sharp blade, but these seeds are so small that that is not a viable option. Stratification is a temperature treatment, usually meant to simulate a period of winter. The cold, wet period that comes from stratification may be another trigger for the seed's embryo to start growing. Stratification is a method of breaking dormancy that addresses seeds with morphological or physiological dormancies. Cold stratification is needed in *Allium tricoccum* to break shoot dormancy. A long enough period of stratification can act as an alarm for the seed. When the proper period is up, it usually means that germination is safe; the environment is ideal for plant growth.

What this research will explore is if it is indeed possible to treat *Allium tricoccum* seeds before sowing so that they will germinate faster. The seeds need a warm, wet period to break root dormancy and a cold, wet period to break shoot dormancy. I will vary the process of seed scarification by H_2SO_4 and the length and presence of warm and/or cold stratification to see if it is possible to speed the rate of germination for this plant.

METHODS

I collected seeds from a large population of *Allium tricoccum* in Almond, Allegany County, NY. In late summer into early fall, I searched the ground for dropped seeds and collected them in plastic tubes with screw-on tops. I scarified 225 seeds by soaking them in a solution of H_2SO_4 for 2 hours and rinsed them thoroughly with tap water. I placed 25 seeds each in nine sterilized petri dishes lined with two sterilized layers of filter paper. The seeds in my control group, which were not treated, were also placed in three petri dishes prepared in the same way. I watered all seeds with distilled water. I sterilized three clear plastic bins by spraying them with an ethanol solution and wiping them down with paper towels. The seeds that were going to get the warm treatment were put in one bin, the warm and cold in another, and the un-treated seeds in the third. To reduce the chances of mold spores getting in and to keep the dishes moist I put a piece of plastic wrap large enough to cover the top loosely over each bin. I placed these bins under fluorescent lights for a cycle of 14 hours of light and 10 hours of darkness at 20°C. The three petri dishes for the cold treatment were placed in a refrigerator with a fluorescent light also with a cycle of 14 hours of light and 10 hours of darkness and at 5°C.

Because of problems with mold, I sterilized more petri dishes with two layers of filter paper and separated the seeds that had already started to sprout from the others and transferred those to the new dishes. The rest of the seeds I put in one small beaker per petri dish. I bleached these seeds by filling the beaker to above the seed line with a solution of 5 mL bleach and 95mL of sterile distilled water. I left them in this solution for 15 minutes then rinsed them all three times with more sterile distilled water. I then placed these seeds in the new petri dishes. After 4, 10, and 14 weeks I counted how many seeds had sprouted out of the 25 in the petri dishes marked to be checked that week. After 4 weeks in 20°C, I moved the seeds that would get the warm and cold treatment into the refrigerator at 5°C for another 4 weeks, after which I did the first germination count for that treatment. For the seeds that were at 5°C I left them out in the 20°C environment for seven days before doing the count. For results see Figure 2.

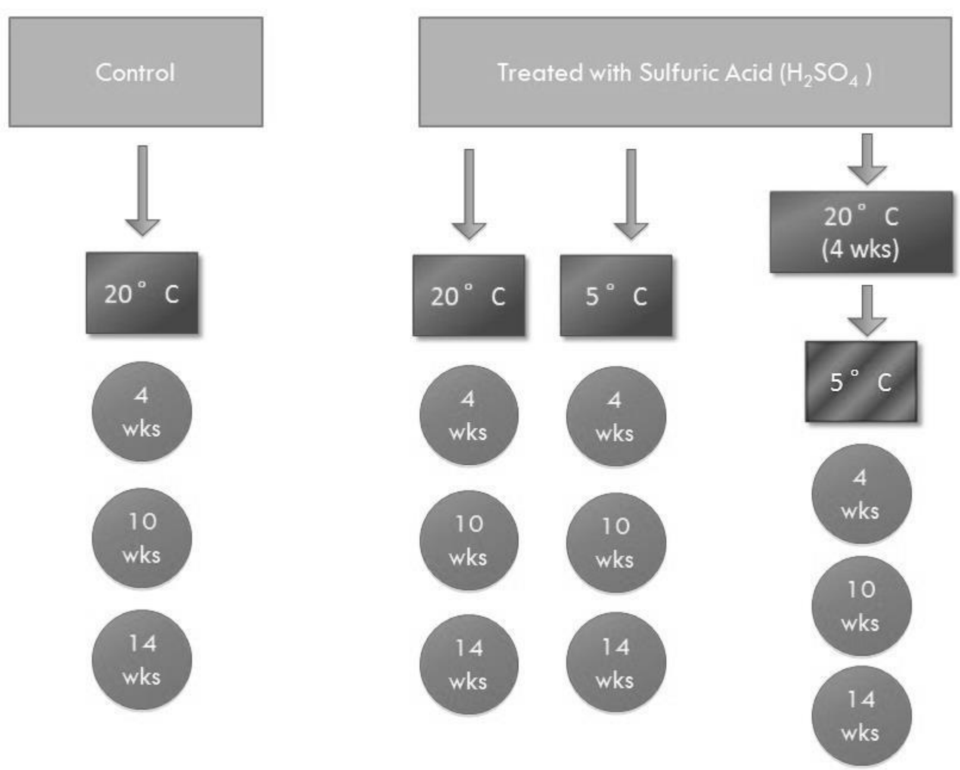

Figure 1. Diagram of my experimental setup.

RESULTS

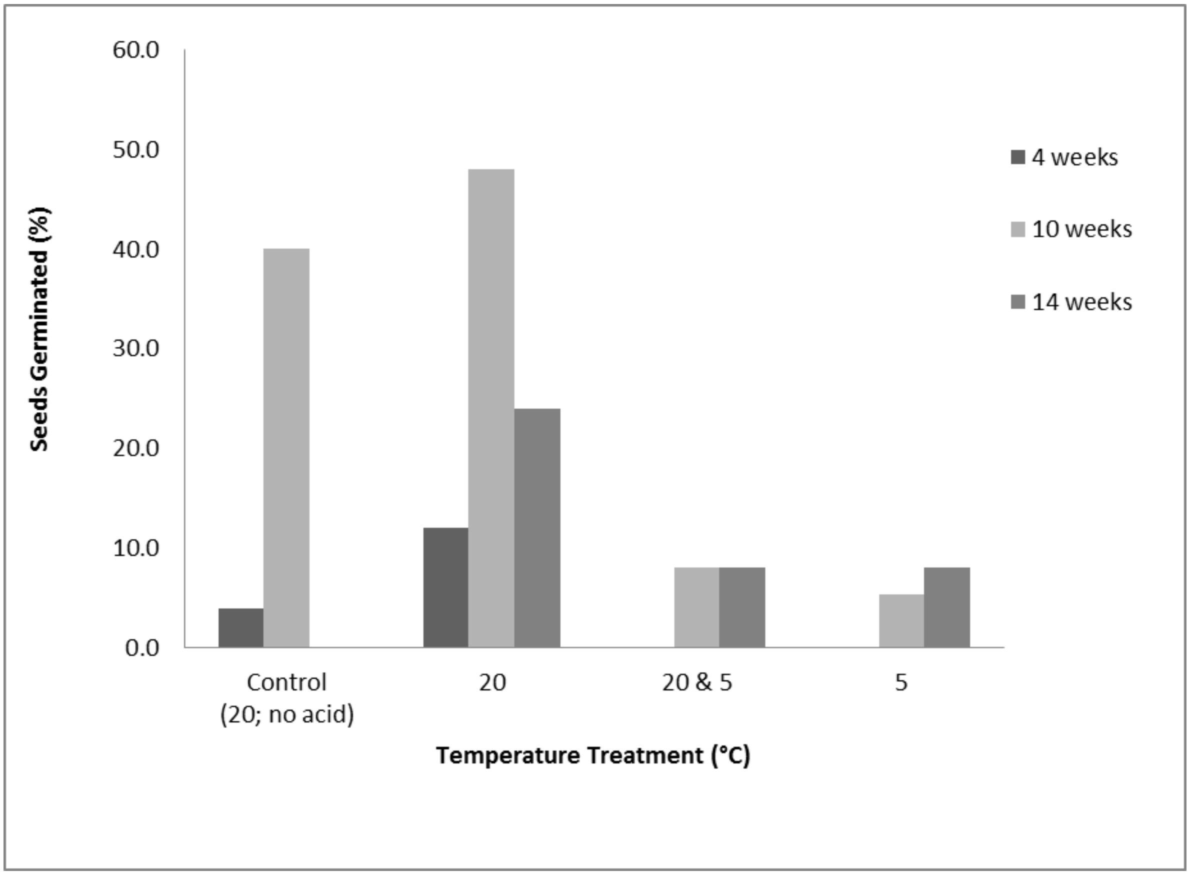

Figure 2. Percent of seeds germinated in each treatment. All treatment groups were treated with H_2SO_4 prior to temperature treatment.

Figure 3. Roots growing from germinating seeds.

The petri dish with seeds that were treated with sulfuric acid and left in an environment of 20°C for 10 weeks had the highest percent germination (48%) (Figure 2). This is 3 times that of the seeds left for 4 weeks (12%). Even the seeds left in 20°C for 4 weeks (12%) had 3 times higher germination than untreated seeds left in at the same temperature for the same amount of time (4%). Cold treatment delayed and reduced germination; no seeds had germinated at 4 weeks, and fewer after 10 and 14 weeks as compared to seeds treated with 20°C.

The majority of seeds sprouted between 4 and 10 weeks at 20°C with or without scarification. The un-scarified seeds from another study had roots that emerged after 40-45 days which is about the same period of time (Jones, 1979). I am led to believe that presence of sulfuric acid (H_2SO_4) had no significant effect on percent germination. Time and temperature, not scarification, seem to be the determining factors in percent of seeds that germinated. Although the seeds were scarified they still needed proper stratification for germination.

Germinated seeds only grew roots; shoots did not emerge. This is supported by research on *Allium tricoccum* germination where roots emerged but shoots stayed within the seed coat (Jones, 1979). The same study did not scarify their seeds or give them a cold treatment and kept them in the dark. Germination was 100% for seeds in these conditions, better than the germination rates for any of my seeds which were all kept in the light. The presence of absence of light also seems to be a deciding factor for seed germination. Proper stratification for breaking root dormancy was approximately 4-10 weeks or 40-45 days in 20°C, but I did not have cold stratification afterward, especially not enough to break shoot dormancy.

DISCUSSION

Seeds that were scarified with sulfuric acid and placed in an environment of 20°C for 10 weeks germinated the best with 48% of the seeds germinated. This was even better than the control seeds which, at 10 weeks, had only 40% germination. Still, the majority of seeds that germinated did so at 20°C with or without scarification. Seeds that also received a cold treatment or were only treated in the cold had less success with germination than the control group. I collected seeds from a shaded, woody area with well-draining soil, which is where other research suggests are good natural growth conditions for *Allium tricoccum* (Greenfield and Davis, 2001). It was found that germination of ramps was greatest for fall-sewn seeds and that that planting in a forest setting is better than under artificial shade or in an open field (Davis and Greenfield, 2002). My seeds were taken from plants that came from these optimal forest conditions, thus making the seeds a good fit for my experiment. Past research says that soil moisture is important; I kept my seeds moist, but occasionally they were dry when I was unable to have access to them (Greenfield and Davis, 2001).

I also collected and examined information regarding American ginseng which has a lot in common with ramps. From Maine through Georgia and the Carolinas, American ginseng also grows on slopes in well-draining soil and has seeds that are slow to germinate. It grows naturally in dense shade and when cultivated light needs to be reduced to about a quarter of its regular intensity (Stockberger, 1928). Ginseng is another plant that has seeds which require a long stratification with a warm and cold treatment (Davis 2003). One can plant seeds, but small ginseng roots can more easily be grown by propagating from transplanted roots. Transplanting speeds time from planting to harvest and ensures a more uniform stand of ginseng (Davis 2003). The disadvantage of this is that ginseng roots cost much more than the seeds. *Allium tricoccum* may be easier to come by and cheaper than ginseng and germination and growth of the two seem so similar that techniques for propagation of American ginseng can be used with ramps. A Canadian study regarding transplants of *Allium tricoccum* showed low initial mortality rates with minimal transplantation shock, as long as they were not seedlings (Vasseur 1994). The study showed that ramps might be flexible enough to grow under various environmental conditions, such as open agricultural fields because of its tolerance to full sunlight (Vasseur 1994). Seedling emergence rate was mostly affected by soil moisture, whereas light did not have a significant affect in survival and growth (Vasseur 1994). It seems to grow well anywhere as long as there is enough moisture in the soil.

In growing a plot of *Allium tricoccum*, it is important to be aware of viability of the population after harvesting. Sustainable harvest means that plant products can be harvested forever from a limited area with little impact to the populations being harvested (Rock, 2004). The average recovery time from a 25% harvest is approximately 22 years, meaning harvesting is not sustainable except at very small levels (Rock, 2004). In this study they harvested only once in a 5-year time period, which is not realistic for actual harvesting frequency. Still, they deduced that the sustainable harvest level of a population of *Allium tricoccum* to be 10% or less every 10 years (Rock, 2004). This ensures approximately an 85% probability that the population will recover by the time of the next harvest. However, the researchers removed plants of all sizes whereas harvesters are more likely to collect larger plants, likely having a more negative effect on the recovery of a population. This may not leave enough plants to propagate through bulb division, the main mode of population growth for *Allium tricoccum* (Rock, 2004). The extinction threshold of the species, at least in Quebec, is 140 to 480 plants. The minimum viable population is approximately 300-1030 plants (Nantel 1996). These are the numbers of plants that need to be in a population for it to survive. Bulb division as a method of population growth is backed by Nault whose study found that clonal development of already-established ramets is the best and most important method for population growth. In the end, germination rate had nearly no effect on population growth rate (Nault 1993).

Allium tricoccum not only reproduces sexually, but also asexually; the bulb divides through lateral bud development (Nault 1993). A ramet of the plant has 1 to 3 leaves attached to a bulb, at the base of which stems from multiple growing seasons form a rhizome (Nault 1993). This means that *Allium tricoccum* could be propagated by breaking apart pieces of the rhizome to generate new plants. The terminal, or mother bulb, is larger while the lateral, or daughter bulbs are smaller and take 5-8 years to become independent (Nault 1993). However, this asexual reproduction would mean that each part broken off from the rhizome would be a clone of the original, creating a genetically homogenous population. This lack of variety in genetic material is dangerous, especially if a disease hits the population and none of the plants are equipped with a mutation or adaptation that would allow it to survive. Despite the lack of variety in genetic material that may result from asexual reproduction, it is still an option in restoring or cultivating *Allium* tricoccum populations.

Results from the experiment indicate that germination of *Allium tricoccum* seeds cannot be accelerated by scarification. Sulfuric acid does not break the type of dormancy that *Allium tricoccum* has. It does not matter whether or not the seeds are scarified; to properly break root dormancy seeds should be kept in an environment of 20°C for 4-10 weeks and to break shoot dormancy seeds need a long period of cold stratification. Propagation may be more effective by breaking apart bulbs and assisting the plant in asexual reproduction. For growing *Allium tricoccum* in the field, I would suggest artificial shade or shaded woody hills with moist, sandy soil. I would not start from a seedling but would transplant bulbs and grow new plant clusters from them. Clonal propagation is important in population growth of the species in the wild and I recommend using it for commercial or personal growing of *Allium tricoccum* as well.

For future research I would like to have more replicates for each treatment so that I can be certain that they work or do not work. I would reexamine cold stratification now that I know proper warm stratification is 4-10 weeks in an environment of 20°C. I would also like to do some comparisons between germination in light and darkness. Another option for future research is to use a hormone treatment to address seed dormancy to see if a hormone such as Gibberellic acid might break one of the two dormancies more effectively than sulfuric acid.

LITERATURE CITED

1. Anonymous. The root of the problem. Environment 40.7 (1998): 19-20.

2. Davis JM. 2003. Care and planting of ginseng seed and roots. North Carolina State University.

3. Davis JM, Greenfield J. 2002. Cultivating ramps: wild leeks of Appalachia. P. 449-452. In: J. Janick and A. Whipkey (eds.), Trends in new crops and new uses. ASHS Press, Alexandria, VA.

4. Dworkin N. Where have all the flowers gone? Vegetarian Times. 1999 Sep.

5. Fenner M, Thompson K. The Ecology of Seeds. Cambridge University Press. 2005 Feb 24.

6. Foraging: the next locavore fixation. Food & Wine. 2010 Jul.

7. Greenfield J, Davis J. CULTIVATION OF RAMPS (Allium tricoccum and A. burdickii). NC State University HIL-133 (2001) (accessed 04-20-2012 http://www.ces.ncsu.edu/hil/hil-133.html)

8. Jones, AG. A study of wild leek, and the recognition of *Allium burdickii* (Liliaceae). Systematic Botany. 1979;4(2): 29-43.

9. Loyola-Vargas VM, Vázquez-Flota F. Plant Cell Culture Protocols. Humana Press, 2006. eBook.

10. Nantel P, Gagnon D, and Nault A. Population viability analysis of American ginseng and wild leek harvested in stochastic environments. Conservation Biology, 1996; 10: 608–621.

11. Nault A, Gagnon D. Ramet demography of *Allium tricoccum*, a spring ephemeral, perennial forest herb. Journal of Ecology. 1993;81(1):101-119

12. Norton DA, Lord JM, Given DR, De Lange PJ. Over-collecting: an overlooked factor in the decline of plant taxa. Taxon. 1994;43(2):181-185.

13. Rock JH et al. Population recovery after harvesting. Biological Conservation. 2004

14. Sen I. When digging for ramps goes too deep. New York Times 2011 Apr 20; Sect. D:1

15. Stockberger WW. Ginseng Culture. Farmers' Bulletin. U.S. Department of Agriculture 1928;1184.

16. Strand O, DiStefano J. Chefs look for wild ingredients nobody else has. New York Times. 2010 Nov 24; Sect. D:1.

17. Vasseur L, Gagnon D. Survival and growth of *Allium tricoccum* AIT. Transplants in different habitats. Biological Conservation. 1994;68(2):107-114.