

A note from The Hofshi Foundation:

We are pleased to post this paper on avocadosource.com. It is the 2003-2004 Science Project of Emily Whalen. Emily did very well on this project. On the actual paper she received 100%, a perfect score. She received a 98% on her oral presentation at school. From there she participated in the Pennsylvania Junior Academy of Sciences (oral presentation) for her region where she received first place with a perfect score (5 out of 5). She advanced to the state contest for PJAS, at which she received another first place (4 out of 5). She also participated in the Lehigh Valley Science and Engineering Fair where she was awarded first place in the Ninth grade botany division. She was subsequently invited to attend the Delaware Science and Engineering Fair.

Our congratulations to Emily!



Miocene Munch: The Avocado's Evolutionary Journey through a Prehistoric Gut

An experiment to investigate the co-evolution of avocados and prehistoric mammals

Emily Whalen Lehigh Valley, PA January 2004

Miocene Munch: The Avocado's Evolutionary Journey through a Prehistoric Gut

Background Information

The scientific name of the avocado is *Persea Americana*. This fruit originated in South and Central America (<http://www.accessscience.com>). Prehistoric mammals such as giant ground sloth, gomphotheres, and toxodons once roamed the same areas in which avocado trees grew. The thick, fleshy pulp of the avocado would have been enticing to these ancient mammals. Since the avocado was both available and tasty, it is probable that it was part of these ancient mammal's diets (Barlow, 2000).

Avocado growers around the world have found that avocados germinate more quickly if they are "roughed up" before planting (Witney, 2003). This process can include scarification (cutting of the sides, top, and/or bottom of the seed), heating of the seed, and the application of acids to the seed. It is not an accident that such treatments facilitate germination of avocado seeds (Arpaia, 2003). Researchers Dan Janzen and Paul Martin came up with the theory that avocados germinate well after receiving these treatments because these were the conditions in which avocados evolved tens of thousands of years ago. When prehistoric mammals ate avocado fruit, they swallowed the entire avocado, seed and all (just as a modern elephant would). Over the course of time, the avocado seed evolved in such a way that it would be able to survive – and even benefit from -- a trip through an ancient mammal's gut. Today, 13,000 years since prehistoric mammals became extinct, the avocado seed is an anachronism. Hungry ground sloth may be gone, but the avocado seed still retains its preference for a "chewed," warm, acidic preparation for germination (Barlow, 2000).

My experiment was designed to test that hypothesis through three different conditions that the avocados were subjected to (scarification, heat, and an acid bath in digestive enzymes). In other words, my experiment tested the effects of a (simulated) prehistoric mammal's digestive system on the germination of avocados (Arpaia, 2003). Digestion is the process of converting complex organic molecules to simpler ones that can pass through a cell membrane. The nutrients taken in by ancient mammals would have been too large to go into individual cells, though; so they would have been digested outside the cells during extracellular digestion. Extracellular digestion usually takes place in an animal's cavity. Enzymes in the cavity break down the food into smaller bits. Any undigested food (such as the seed) would have been expelled through the anus (Oham, 1994). Of course, prehistoric mammals don't exist today, but the logical place to look for information from which we can extrapolate conclusions about their digestive systems would be that of their closest living descendant: an elephant. An elephant's digestive system takes about 24 hours to complete a cycle, and its temperature is approximately 37 degrees Celsius. The pH of its digestive acids is approximately 3 (Shoshani, 2003).

Germination is a seed's development into a new plant. In order for the process to occur, the seed must be placed under the proper conditions so that the embryo (inside the seed) can be stimulated to grow. The embryo's growth involves the lengthening of the stalk-like hypocotyls, which forms an arch that pushes the upper part of the hypocotyls (the epicotyls) above the soil. The epicotyls forms the lower part of the stem once above ground. It also forms a pair of small leaves that opens early in germination. The bottom part of the hypocotyls is the radical, which will become the upper part of the primary root once the lengthening hypocotyls pushes it underground. The rest of the seed is composed of two fleshy cotyledons which act as food for the embryo. Throughout germination, these are shrinking as they are used by the growing embryo. Eventually they part to allow the epicotyls to come above the soil (Oham, 1994).

Purpose

The purpose of this experiment is twofold. Most importantly, the experiment seeks to determine if Janzen and Martin's hypothesis, that the avocado's evolution was influenced by prehistoric mammals, is correct. The other purpose is to find out under which of the applied conditions avocados germinate best.

Hypothesis

If I simulate the effects of a prehistoric mammal's digestive system on avocado seeds before planting, then a greater percentage of avocados will germinate – and at a faster rate.

Materials

114 avocado seeds 114 plastic cups soil knife grater oven or heating pads digestive enzyme pills food processor water pH strips

Procedure

Groups and variables within the experiment:

Group A (control group):

Intact and untreated avocado seeds

Group B:

Avocado seeds which have been scarified to simulate the chewing of a large prehistoric creature –

notches cut 1/8 of an inch deep on each of four sides, from the lower third to the tip of the seed;

seed tops scraped with a grater (see diagrams of scarification in Data section)

Group C:

Avocado seeds that have been heated to 37 degrees Celsius for 24 hours to simulate the

temperature conditions during passage through a prehistoric creature's gut

Group D:

Avocado seeds that have been soaked in digestive enzyme pills, ground to powder and dissolved in water, to simulate the digestive juices of a prehistoric creature

Group E:

Avocado seeds that have been scarified and heated

Group F

Avocado seeds that have been heated and soaked in digestive enzyme pills, ground to powder and dissolved in water

Group G:

Avocado seeds that have been scarified and soaked in digestive enzyme pills, ground to powder and dissolved in water

Group H

Avocado seeds that have been scarified, heated, and soaked in digestive enzyme pills, ground to powder and dissolved in water

Independent variable:

The cuts made in some avocados, the temperatures some avocados will be exposed to, and the digestive enzyme pills, ground to powder and dissolved in water, applied to some avocados Dependent variable:

The percentage of avocados that germinate in each group and the rate of their germination Controlled variables:

The plastic cups, the potting soil, the amount of soil put into each cup, the amount and frequency of the watering of the seeds, the conditions the seeds are placed in (amount of sunlight, temperature), the avocados, the knife and grater used to cut the avocados, the source and temperature of heat used to warm the avocados, the digestive enzyme acid bath given to the avocados

Steps:

1. Label all cups with group letter and number.

2. Fill all cups with $\frac{1}{2}$ cup of potting soil.

3. Wash all avocado seeds and remove skins.

4. With knife, scarify avocados in groups B, E, G, and H by making notches ¹/₄ of an inch deep on each of four sides, using the knife to etch an "x" on the top, and using grater to scrape the top (see diagram of scarification in Data).

5. Place digestive enzyme pills ("Superzymes") in food processor and grind until powder is fine.

6. Dissolve powder in water.

7. Use pH strips to ensure pH is approximately 3.

8. Soak avocados in groups D, F, G, and H in solution for 24 hours.

9. Heat seeds in groups C, E, F, and H to 37 degrees Celsius in oven for 24 hours (for seed groups that are also to be soaked in acid, soak and heat simultaneously).

10. After all seeds have been prepared, plant in appropriate seed cups.

11. Ensure 1/3 of the seed is above soil (see diagram of planting).

12. Water seeds, two tablespoons every other day, to keep soil moist.

13. Observe changes daily and record in scientific journal.

Data

The following three figures represent a single table, in three parts, of observations made on the changes in the planted seeds over the course of 74 days. The first four columns identify the cup number and whether each cup was scarified, heated, or soaked in acid. The rest of the columns specify the date on which observations were made, day 1 being the day the seed was planted.

One of the observations made was for the presence of mold appearing on the top of the seed. During the experiment, some of the seeds developed mold. It was interesting to observe that none of the seeds soaked in acid developed mold. Other observations were made for cracks, cracks with embryos, large embryos, and partially or fully germinated seeds. These were the

observable changes in the seeds. There is also one other minor factor I observed, that being the root. I didn't want to disturb the other seeds by pulling them out of the soil, so was not able to compare root growth of all the seeds. In one seed cup I was able to observe, through the transparent cup, a root curled around the bottom of the cup. See Figure 4 for more details on the terms used.

cup	cut	heat	acid	day 6	day 8	day 10	day 13	day 16	day 20	day 23
al	no	no	no							
a2	no	no	no							
a3	no	no	no							
a4	no	no	no							
a5	no	no	no							
аб	no	no	no						crack	
a7	no	no	no							
a8	no	no	no							
a9	no	no	no		mold					
a10	no	no	no							
a11	no	no	no							
a12	no	no	no							
a13	no	no	no							
a14	no	no	no							
b1	yes	no	no							
b2	yes	no	no		mold					
b3	yes	no	no							
b4	yes	no	no		mold					
b5	yes	no	no		mold					
b6	yes	no	no							
b7	yes	no	no							
b8	yes	no	no							
b9	yes	no	no							
b10	yes	no	no		mold					
b11	yes	no	no							
b12	yes	no	no							
b13	yes	no	no							
b14	yes	no	no							
c1	no	yes	no							
c2	no	yes	no							crack
c3	no	yes	no							
c4	no	yes	no							
c5	no	yes	no							
c6	no	yes	no							
c7	no	yes	no		mold					
c8	no	yes	no							
c9	no	yes	no							
c10	no	yes	no							

Figure 1. Table of Observations Part 1.

		t.								
	cut	heat	acic	1 (1 0	1 10	1 10	1 16	1 20	1 22
cup				day 6	day 8	day 10	day 13	day 16	day 20	day 23
c12	no	ves	no							
c13	no	ves	no							
c14	no	ves	no							
d1	no	no	yes							
d2	no	no	yes							
d3	no	no	yes							
d4	no	no	yes							
d5	no	no	yes							
d6	no	no	yes				crack			
d7	no	no	yes							
d8	no	no	yes							
d9	no	no	yes							
d10	no	no	yes							
d12	no	no	yes				crack			
d13	no	no	ves				Clack			
d14	no	no	ves							
e1	ves	ves	no							
e2	yes	yes	no							
e3	yes	yes	no							
e4	yes	yes	no							
e5	yes	yes	no					crack		
e6	yes	yes	no							
e7	yes	yes	no							
<u>e8</u>	yes	yes	no							
e9	yes	yes	no							
e10	yes	yes	no							
e12	yes	yes	no							
e13	ves	ves	no							
e14	ves	ves	no							
f1	no	yes	yes							
f2	no	yes	yes							
f3	no	yes	yes							
f4	no	yes	yes							
f5	no	yes	yes							
f6	no	yes	yes							
t7	no	yes	yes							
18	no	yes	yes							
19 f10	no	yes	yes							
f110	no	yes	yes							
f12	no	yes ves	ves							
f13	no	ves	ves							
f14	no	ves	ves							
g1	yes	no	yes							
g2	yes	no	yes			crack				
g3	yes	no	yes							
g4	yes	no	yes							
g5	yes	no	yes							
g6	yes	no	yes							
g7	yes	no	yes							
g8	yes	no	yes							

cup	cut	heat	acid	day 6	day 8	day 10	day 13	day 16	day 20	day 23
g9	yes	no	yes			crack				
g10	yes	no	yes							
g11	yes	no	yes							
g12	yes	no	yes							
g13	yes	no	yes							
g14	yes	no	yes							
h1	yes	yes	yes							
h2	yes	yes	yes							
h3	yes	yes	yes			crack				
h4	yes	yes	yes							
h5	yes	yes	yes							
h6	yes	yes	yes			crack				
h7	yes	yes	yes							
h8	yes	yes	yes							
h9	yes	yes	yes							
h10	yes	yes	yes							
h11	yes	yes	yes							
h12	yes	yes	yes							
h13	yes	yes	yes							
h14	yes	yes	yes							

Figure 2. Table of Observations Part 2.

cup	day 18	day 21	day 28	day 31	day 36
al					
a2					
a3					
a4				crack	
a5				crack	
a6				embryo	
a7				crack	
a8				crack	
a9					
a10					
a11					
a12					
a13					
a14					
b1				crack	
b2					
b3				crack	
b4				crack	
b5					
b6				crack	
b7				crack	
b8					
b9					
b10					
b11			crack	embryo	
b12				crack	
b13					
b14			crack	embryo	

cup	day 18	day 21	day 28	day 31	day 36
c1					
c2				embryo	
c3					
c4					
c5					
c6					
c7					
c8					
c9				crack	
c10					
c11				crack	
c12					
c13				crack	
c14					
d1	crack	embryo			embryo
d2		crack			crack
d3		crack			crack
d4		crack			crack
d5		crack			crack
d6		embryo			embryo
d7		crack			embryo
d8		crack			crack
d9		crack			crack
d10		crack			crack
d11		crack			crack
d12		embryo			embryo
d13		crack			crack
d14		crack			crack
e1				crack	
e2				crack	
e3				crack	
e4				crack	
e5				embryo	
e6				crack	
e7				crack	
e8				crack	
e9				crack	
e10				crack	
e11				crack	
e12				crack	
e13		1		crack	
e14		1		crack	
f1		1		1	
f2		crack			crack
f3					
f4	1	crack			crack
f5					
f6	1				
f7					
f8		1	1	1	1
f9					
f10		1		ł	1
f11		1		ł	
f12	1	1		1	1
f13		crack			crack
f14		CIACK			CIACK
σ1		crack			crack
σ ²		embryo			embryo
g∠	L	emoryo		1	emoryo

cup	day 18	day 21	day 28	day 31	day 36
g3		crack			crack
g4		crack			crack
g5		crack			crack
g6		crack			embryo
g7		crack			crack
g8		crack			crack
g9		embryo			embryo
g10	crack	embryo			embryo
g11		crack			crack
g12	crack	crack			crack
g13		crack			crack
g14		crack			embryo
h1		crack			crack
h2		crack			embryo
h3		embryo			embryo
h4		crack			embryo
h5		crack			embryo
h6		embryo			embryo
h7		crack			crack
h8	crack	embryo			embryo
h9		crack			crack
h10		crack			embryo
h11		crack			crack
h12		crack			embryo
h13		crack			crack
h14		crack			crack

Figure 3. Table of Observations Part 3.

cup	day 46	day 46	day 47	day 57	day 64	day 74
a1	embryo			near		near
a2	crack			crack		crack
a3		mold				
a4	crack	mold		embryo		embryo
a5	crack			crack		crack
a6	embryo	mold		embryo		embryo
a7	crack			crack		crack
a8	crack	mold		crack		crack
a9		mold				crack
a10	crack			crack		crack
a11				crack		crack
a12						crack
a13	crack			crack		crack
a14						
b1	crack			crack		crack
b2		mold				
b3	partial			germinate		germinate
b4	crack	mold		crack		crack
b5						
b6	embryo			partial		partial
b7	crack			crack		crack
b8						
b9						
b10						
b11	embryo			embryo		near
b12	crack			crack		crack

cup	day 46	day 46	day 47	day 57	day 64	day 74
b13	-	-				crack
b14	embryo			embryo		embryo
c1	embryo			partial		partial
c2	embryo			near		near
c3	crack			crack		crack
c4	crack			crack		crack
c5						
c6	crack			partial		germinate
c7				1		
c8				partial		partial
c9	crack			crack		crack
c10						
c11	crack			crack		crack
c12						
c13	crack			crack		crack
c14						
d1			embryo		embryo	near
d2			crack		crack	crack
d3			crack		crack	crack
d4			embryo		embryo	embryo
d5			embryo		near	near
d6			embryo		embryo	embryo
d7			embryo		embryo	embryo
d8			crack		crack	near
00 d0			crack		crack	crack
d10			crack		crack	crack
d11			crack		crack	crack
d12			embryo		ombryo	nartial
d12			crack		crack	ombryo
d14			crack		crack	crack
01 01	orack		CIACK	crack	CIACK	crack
02	crack			crack		crack
e2	araak			araak		orack
e5	crack			orack		orack
-64	crack			crack		ombauo
e5	emoryo	-		emoryo		embryo
-7	crack			crack		emolyo
e/	crack			crack		crack
-0	embryo			partial		partial
e9	embryo			partial		partial
e10	crack			crack		crack
e11	partial			germinate		germinate
e12	crack			crack		сгаск
e13	embryo		+	embryo		embryo
e14	crack	_		crack		crack
11		_	- <u>,</u>		,	crack
<u>f2</u>			embryo	-	embryo	embryo
t3					-	
f4			crack		crack	crack
f5			crack		near	partial
f6			crack		near	partial
f7						
f8						
f9						
f10						
f11						
f12			crack		near	partial
f13			crack		crack	crack
f14						

cup	day 46	day 46	day 47	day 57	day 64	day 74
g1			partial		germinate	germinate
g2			embryo		embryo	embryo
g3			crack		embryo	embryo
g4			partial		partial	germinate
g5			embryo		near	near
g6			partial		germinate	germinate
g7			crack		embryo	near
g8			crack		embryo	near
g9			near		near	near
g10			embryo		near	near
g11			partial		partial	germinate
g12			partial		germinate	germinate
g13			near		partial	partial
g14			near		near	near
h1			crack		embryo	embryo
h2			near		near	near
h3			embryo		partial	germinate
h4			near		partial	partial
h5			embryo		embryo	embryo
h6			near		near	near
h7			partial		partial	germinate
h8			partial		partial	partial
h9			crack		embryo	embryo
h10			partial		germinate	germinate
h11			crack		near	near
h12			near		near	near
h13			near		partial	germinate
h14			crack		embryo	embryo

Figure 4. Key to figures 1, 2, 6, and 7.

Word	Meaning
cut	scarified
heat	heated
acid	soaked in acid
root	the seed had on top, but a visible root at the bottom of the cup
mold	the seed developed mold
crack	the seed was cracked, but not enough to see the embryo
embryo	the seed had a crack with a visible embryo
near	the seed had a large green embryo that was near germination
partial	the seed was partially germinated, with a stalk that had emerged from
	the seed, but the leaves were not yet unfurled
germinate	the seed had fully germinated, with a fully emerged stalk and
	completely unfurled leaves

As the following two graphs show, in each group a different number of seeds partially or fully germinated by the end of the experiment. Groups G and H had the most seeds partially or fully

germinated by the end of the experiment with six seeds. Figure 8 shows what percentage of each group germinated (partially or fully).

Figure 5. Graph of seeds germinated in groups A, B, C, and E.



Figure 6. Graph of seeds germinated in groups D, F, G, and H.



The following two graphs show that, in each group, it took a varying number of days to reach a given condition. Some groups reached that condition faster than others. In general, groups B, G,

and H took the least amount of time to reach the various conditions. Refer to figure 3 for a definition of the words on the horizontal (x) axis.



Figure 7. Graph of average number of days to reach a specified condition in groups A, B, C, and E.

Figure 8. Graph of average number of days to reach a specified condition in groups D, F, G, and H.



Figure 9. Graph of percent of seeds germinated.



In each group, a different percent of the total seeds germinated. Groups G and H had the greatest percents at 43%. The average percent germinated is 24%.

Figure 10. Photograph of acid bath.



Figure 11. Photograph of scarification (notches).



Figure 12. Photograph of scarification (grated top and X mark).



Figure 13. Photograph of an avocado seed before germination.



Figure 14. Photograph of planting.



Figure 15. Photograph of two of the seeds with mold***



Figure 16. Picture of a seed without a crack.

There are cut lines (from a knife) on this avocado seed, but no crack has formed.



Figure 17. Photograph of a cracked seed without a visible embryo.**

Note that the natural crack (running almost horizontally) has appeared independently of the lines of scarification (running more vertically and forming an X near the upper portion of the photograph).



Figure 18. Photograph of a cracked seed with visible embryo.*

As in Figure 17, the natural crack (running horizontally) has appeared independently of the lines of scarification (running more vertically and forming an X across the top of the seed). The embryo has become enlarged but is still located at the bottom of the seed. It can be seen due to the width of the large natural crack which has formed across the top of the seed.



Figure 19. Picture of a nearly germinated seed.*

The embryo has grown up inside the seed from the bottom of the seed, but has not yet reached the top of the seed.



Figure 20. Picture of a partially germinated seed. The stalk has fully emerged, but leaves have not unfurled.



Figure 21. Picture of a fully germinated seed. The first leaves have unfurled.





Figure 22. Diagram of the parts of an avocado seed after germination.

Figure 23. Picture of a hungry ground sloth.



*arrow points to embryo in crack **arrow points to crack ***arrow points to mold

Analysis

The results of my experiment indicate that avocado seeds germinate best when they have been exposed to digestive conditions (or rather, when they have gone through the gut of a prehistoric mammal or the simulated effects thereof) before they are planted. Group H (the group that tested all three variables) and group G (the group that tested scarification and acid) yielded virtually identical results. Both had six germinated seeds which is 43% of the total 14 planted in each group. Though 43% of the seeds germinated in both groups, the seeds in group G germinated in approximately two fewer days, on average. In Figure 8, one can see that, though it took group H a equal or shorter amount of time than group G to crack, crack enough to see the embryo, and develop a large green embryo, group G took a shorter amount of time to germinate (partially or fully) than group H. Perhaps the heat, when paired with the scarification and acid bath, had some adverse effect on the avocado seeds. This hypothesis would be supported by the fact that group F (the group heated and soaked in acid) did not begin germinated by the end of the experiment. Perhaps this happened because in group F, the heat paired with the acid had an adverse effect on the seeds, but in group H, the scarification countered the heat and acid's adverse effect, making the seeds do better. This fact supports what I will talk about next, the most important single condition.

Towards the end of the experiment, it became obvious that one of the three individual conditions tested was more effective than the others. That one condition was scarification. Group B, the scarified-only group, was the first group to partially germinate with seed B-3 on day 46. Though group B has one fewer germinated (partially or fully) seed than group C (the just-heated group), it has one more than group D (the just-soaked-in-acid group). In the paired groups, the ones that include scarification did better than the ones without it. In group F (the group heated and soaked in acid), 21% are fully or partially germinated. In group E (the group scarified and heated), 21% have also fully or partially germinated, but in group G (the group scarified and soaked in acid) 43% fully or partially germinated. The group that tested heat, acid, and scarification (H) is doing the same as the group that tested scarification and acid (G), but the group that tested heat and acid (F) only has 21% fully or partially germinated where the other two have 43%, which is more than two times more. One surprising thing I learned about avocado seeds is that avocados have natural "fault lines" along which they crack. The X cut and side cuts I made with the knife did not seem to facilitate cracking, because the avocados would crack along

their natural "fault lines" anyway. Some of them would even begin the crack on the bottom, allowing the root to grow more, then slowly develop a crack on top. I discovered this one day (day 31) when I noticed one of the seeds (C-6) had a large crack along the bottom, but none along the top. I looked at the bottom and saw the root curled around the bottom of the cup.

One thing that this experiment definitely indicated was that it is better to do something to an avocado seed than to do nothing. The single variable groups (group B-just scarified; group Cjust heated; group D-just soaked in acid) had 14%, 21%, and 7% germinated respectively, and the control group had 0% germinated. Though the control group (A) had only 2 seeds without top cracks, and the groups just scarified (B), just heated (C), and just soaked in acid (D) each had at least 3 without top cracks. The single-variable groups did have at least one or more seeds with almost-germinated embryos and germinated embryos than the control group. The single variable groups may simply be slow in cracking, or they may have started cracking on the bottom. The second scenario is most likely, because groups B, C, and D were the groups in which I observed the few visible bottom cracks.

Fairly early in the experiment, some avocados began to develop mold, but not all of the avocados in all of the groups were affected by it. In fact none of the seeds in the groups soaked in acid and heated (F), soaked in acid and scarified (G), or soaked in acid, heated, and scarified (H) developed any mold at all. It must have been that the acid I soaked the seeds in before planting prevented the mold from growing. Perhaps this means that after avocados seeds had gone through a prehistoric mammal's digestive system and had been deposited back on the ground, that they were immune to mold and so could grow better. However, the mold doesn't seem to have had a particularly adverse effect on the avocados it grew on. In a few of the avocados, mold grew inside the crack and on top of the embryos. These embryos didn't seem to be germinating until towards the end of the experiment when a stalk emerged from the mold inside the crack of one of these seeds.

This experiment has led to some answers, but at the same time, to many surprises that present puzzling new questions.

Conclusion

Avocados grow best when exposed to the combination of scarification and acid. The best individual condition was scarification. This indicates that avocados are anachronisms to prehistoric times because the combination of scarification and acid tested simulated a prehistoric mammal's digestive system. This supports Janzen and Martin's hypothesis.

Practical Application

One major practical application of the results of this experiment would help avocado growers. The results might suggest techniques that could be used to make more avocado seeds germinate and grow faster. In this case, an avocado farmer would want to scarify, heat, and soak in acid all of his avocado seeds. This would increase the percentage of his crop that germinates and help them germinate faster.

For Further Investigation

This experiment was successful, but it also opened up many different possibilities for future experiments. First of all, if you had more time, you could test not only how these conditions affect germination, but how they affect the entire growth and development of the plant. The acid I used was one made of digestive enzymes, but you could design an experiment to test different types of acids. In my experiment, many of the seeds developed mold around the edges and sometimes even in the cracks. You could design an experiment to test the effect of the mold on germination. Another thing I discovered during germination was that every seed has a natural "fault line" that it cracks along. I expected the seeds to crack along the scarification lines. They didn't. They cracked along their own "fault line" (a visible line across the top of each seed). You could design an experiment in which you identified the "fault line" and scarified along that line to see if that would aid cracking/germination of the seed.

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