Increasing Soybean Germination Rate

Sophia Paul and Katie Gothard

Turpin High School

Increasing Soybean Germination Rate

Sophia Paul and Katie Gothard

Turpin High School

Outline

I. Abstract

II. Introduction to Soybean Germination

   A. Background of Germination, Molybdenum, Gibberellic Acid, Potassium Chloride, and Polyethylene Glycol

   B. Purpose

   C. Hypothesis

III. Materials: What is Needed

IV. Procedure: How to Germinate and Observe Soybean Seeds and Collect Data

V. Results of Seeds Germinated and Radicle Length

   A. Table of Percent Germinated and Statistics of Radicle Lengths

   B. Graph of Cumulative Germination and Radicle Length

   C. Written Results

VI. Conclusion: The Data Refuted the Hypothesis

VII. Discussion

   A. Explanation of Mold Growth and Radicle Growth

   B. Possible Changes and Ways to Improve the Procedure

   C. Future Experimentation: Protein Content and Resistance to Stinkbugs and Soybean Cyst Nematodes

VIII. Works Cited

Abstract

Seed germination begins with the rupturing of the seed coat and the emergence of the radicle, which will form the first roots of the seedling. Molybdenum is a catalyst for several enzymatic reactions in a cell, and potassium chloride is an essential macronutrient for plants. Gibberellic acid stimulates cell division, and polyethylene glycol lowers osmotic potential of cells. The experiment tested the germination rate of soybean seeds in molybdenum, potassium chloride, gibberellic acid, polyethylene glycol, and distilled water solutions. The hypothesis was that if gibberellic acid stimulates cell division and decreases dormancy, then a higher percent of the soybean seeds in the gibberellic acid solution will germinate than in the other solutions. 48 soybean seeds were germinated on cotton pads soaked in each solution for ten days. A similar percentage of seeds in the molybdenum, potassium chloride, gibberellic acid, and distilled water solutions germinated by the end of the duration of the experiment. Fewer seeds in the polyethylene glycol solution germinated, and the radicles of the germinated seeds in the polyethylene glycol solution were significantly shorter than the radicles of the seeds germinated in other solutions. The radicles of the seeds in the potassium chloride solution were longer than the radicles of seeds in the other solutions.

Introduction

Germination is the process during which a temporarily dormant, or quiescent, seed resumes growth and begins development from a seed to a maturing plant (Ashworth, n.d.). A portion of seeds never germinate, and remain dormant. The process begins with the removal of certain inhibitors that are responsible for keeping the seed in its dormant state (ibid) and lasts from the time the seed is planted to its emergence from the soil surface ("Soybean Production," n.d.). While the seed is dormant, it has extremely low moisture content, and it performs only the minimal chemical reactions that are necessary for it to stay alive ("Seed Science", n.d.). During imbibition, when the moisture is first taken up, the water content of the soybean (glycine max) seed increases from approximately thirteen to fifty percent ("Soybean Production," n.d.). Simply imbibing water does not determine its viability; certain seeds will remain dormant despite the presence of water ("Seed Science," n.d.).

After one to two days, the radicle will emerge from the seed coat and begin to grow to establish a primary root system ("Soybean Production," n.d.). The hypocotyl then grows, and the seedling plant grows up toward the soil surface (ibid). The cotyledons, in which soybean seeds store the energy required for germination, are part of the seed and will become the precursors to the seedlings first leaves (ibid). The energy of the seed is stored in the cotyledons, some of which is used for early growth of the seed ("Seed Science," n.d.). Soybeans follow epigeal germination, denoting that the cotyledons also function to help pull the seedling up from the ground during germination (ibid). The cotyledons begin photosynthesis, providing the seed with the energy to continue growth, but they drop off of the seedling once the plants first real leaves are grown.

Molybdenum is a metal that acts as a catalyst for a variety of chemical reactions because of its importance in the regulation of certain enzymatic reactions ("Heart of the Matter," n.d.). It

has been found to be a key part of the enzyme nitrate reductase, which is responsible for the reaction that reduces nitrate to nitrite ("Molybdenum in Plant Nutrition," n.d.). Nitrite is then further reduced to ammonia, which is used by the cell in various reactions (Vijaykant, n.d.). Due to its key role in nitrogenous reactions, molybdenum deficiency mirrors nitrogen deficiency, causing reduced growth, abnormal leaf shapes, and pale and withering leaves ("Molybdenum in Plant Nutrition," n.d.). Molybdenum has also been found to be essential to the growth of legumes, as it is required by the root nodule bacteria (ibid). Furthermore, molybdenum deficiency has been most severe in legumes across America, particularly in soybeans (ibid). Though most soils have enough molybdenum, the metal reacts with acidic pH soils, and can then no longer be taken in by plants (ibid).

Gibberellic Acid is naturally produced in plants and by the fungus *Gibberella fujikuroi* (*Gibberellin synthesis*, 1978). It is a compound that, along with other gibberellins, stimulates cell division and leaf and stem growth (ibid). It also decreases dormancy rate of seeds (ibid). Gibberellins are prominent in agriculture; they are used to increase fruit size and yield and to decrease ageing in the rinds of fruit ("Gibberellic Acid," n.d.). Gibberellic is a growth regulator that is already present in plants, and seeds anabolize gibberellic acid during germination (ibid). Treatment of dormant seeds and seeds in adverse conditions, such as colder temperatures, with Gibberellic acid has improved germination rates and helped seeds overcome dormancy (ibid). A well-known response pathway of gibberellic acid is α-amylase synthesis; α-amylase is an enzyme necessary for the hydrolysis of starch (Sponsel, n.d.). Called modification, this process of this breakdown of starch occurs more quickly if the natural gibberellins of the seed are supplemented with gibberellic acid, because production of α-amylase increases (ibid).

Potassium is an important macronutrient for plants, along with nitrogen and phosphorous, and it is most commonly found in the form of potassium chloride (Pearson, n.d.). During germination, the chloride may be harmful to seeds; however, after the plant begins to grow, and soybean plants grow faster when treated with supplemental potassium chloride (ibid). Therefore, in order to avoid damage to germinating seeds, potassium chloride is often placed slightly away from the germinating seeds, and will be taken in when the plant is able to make use of it ("Potassium Chloride," 2014). In mature plants, potassium chloride is necessary for plants to maintain homeostasis and is involved in carbon dioxide fixation and transpiration, and a deficiency results in stomatal dysfunction ("Effect of Potassium Chloride", 2009). Stomata take in carbon dioxide and release small amounts of water, and when this part of the plant is not functioning correctly, the additional water loss becomes dangerous to the plant. Plants treated with potassium chloride have been shown to produce higher amounts of protein and are also more resistant to bacteria and rot (Pearson, n.d.).

Polyethylene glycol is a compound that is used to induce water stress in plants and to lower osmotic potential (Kumar, R. R., Karajol, K., & Naik, G., 2011). By decreasing the osmotic potential, polyethylene glycol reduces the roots' ability to take in water. Many plants with high levels of antioxidants are resistant to the water stress induced by polyethylene glycol because they are able to release electrolytes from the chloroplasts and mitochondria of their cells (ibid). Soybeans may be resistant to the water stress due to their high levels of the antioxidant isoflavone, which is able to neutralize free radicals ("Isoflavones," n.d.). Genistein, a particular isoflavone, acts as an antioxidant and enhances antioxidant enzyme activity (ibid).

The purpose of the experiment is to determine whether germinating soybean (glycine max) seeds in solutions of molybdenum, potassium chloride, gibberellic acid, and polyethylene glycol will increase the percentage of seeds germinated. The effects of these solutions on the growth of the radicles of the germinated seeds will also be studied at the end of the experiment.

If gibberellic acid stimulates cell division and decreases dormancy, then a higher percent of the soybean seeds in the gibberellic acid solution will germinate than in the other solutions.

Materials

- 240 Organic Soy Bean Envy seeds from David's Garden Seeds
- Polyethylene Glycol (only active ingredient in MiraLax)
- Molybdenum (Molybdenum 500 by Douglas Labs)
- Potassium Chloride (NOW Foods Potassium Chloride Powder)
- Gibberellic Acid (SuperGrow Gibberellic Acid Starter Kit)
- Distilled Water
- Glass bowls
- Cotton pads
- Camera

Procedure

1. Obtain all materials.
2. Create solutions using 5 gallons of distilled water. In one gallon, dissolve 7 scoops (.53 g) of Gibberellic Acid to create a 25 ppm solution. In the second gallon, dissolve 17 g (1 serving size) of Miralax. In the third, dissolve 1 capsule (1 serving size) of Molybdenum. In the fourth, dissolve ⅛ teaspoon (1 serving size) of Potassium Chloride. The fifth gallon of distilled water will be used as a control.
3. Soak 48 seeds in 1 cup of the Gibberellic Acid solution for 5 hours. Repeat for all solutions.
4. Soak 10 cotton pads with 20 mL of Gibberellic Acid solution. Cover the bottom of 2 glass bowls with these cotton pads. Place 24 seeds that were soaked in the Gibberellic Acid solution on top of these pads. Repeat for all solutions, using 10 glass bowls total.
5. Place the glass bowls on a windowsill, making sure to label each set of bowls with their respective solution name. Cover each bowl with a dry tissue to prevent evaporation of the solutions.
6. Add 10 mL of solution to each set of seeds every morning.
7. Document the germination and growth rate photographically each day at 6 pm. Record all observations in a journal. The seed will be counted as germinated at first emergence of the radical from the seed coat.
8. On day 5 of experimentation, replace the cotton pads in each setup and soak them with 20 mL of their respective solution.
9. Each day, place germinated seeds in one bowl to separate them from the non-germinated seeds. This prevents spreading of mold.

10. Continue experimentation for 10 days.
11. At the end of experimentation, record the length of the radicles of all germinated seeds for each solution. Use this data to calculate the mean and median radicle lengths as well as the standard deviation.
12. Calculate the percentage of germinated seeds for all solutions.

    Seeds germinated/48 * 100 = percent of seeds germinated.
13. Create graphs of the results of cumulative seed germination and for mean height of the radicles of the seeds. Draw conclusions about effectiveness of each solution.

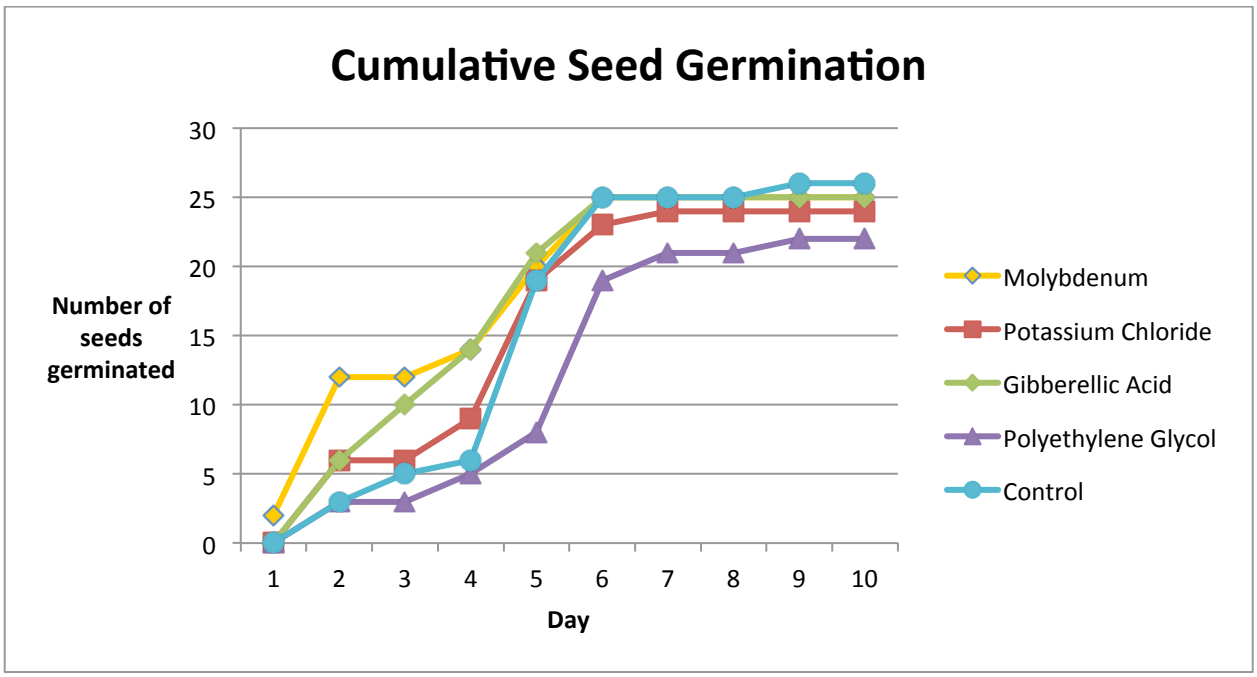
Results

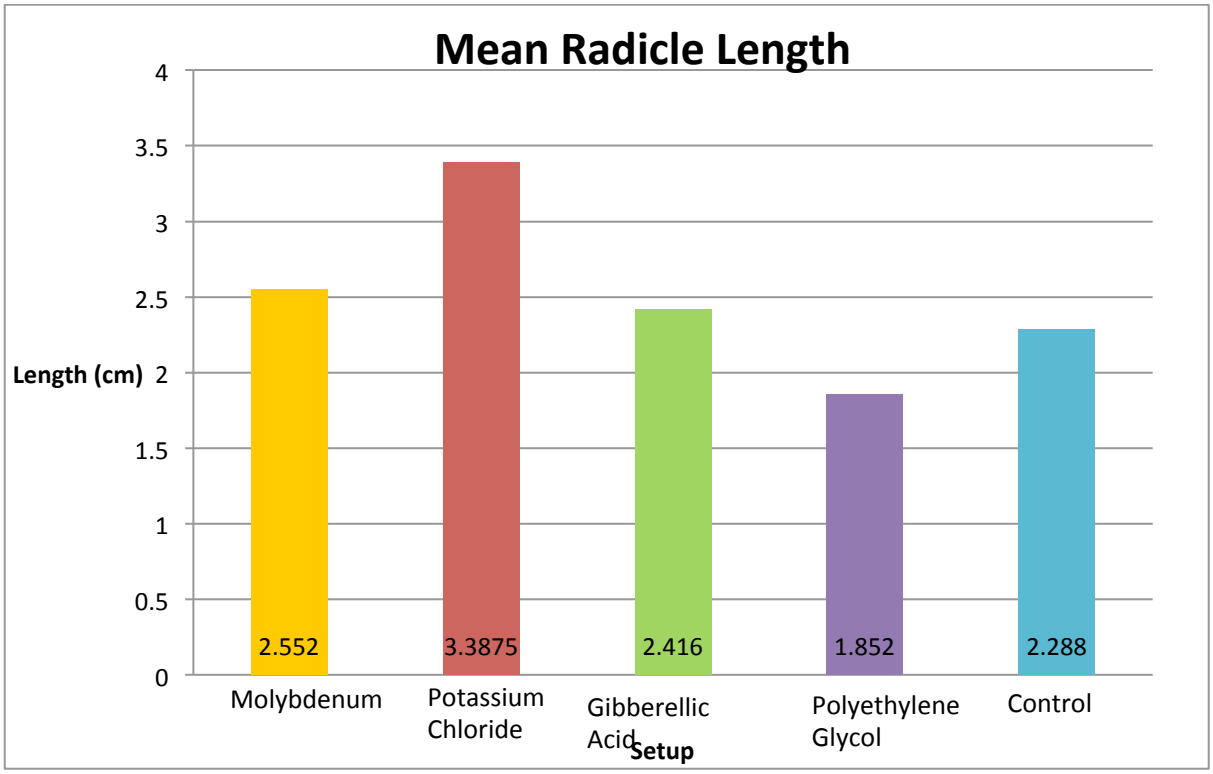
Percent of Seeds Germinated

Control	Polyethylene Glycol	Gibberellic Acid	Potassium Chloride	Molybdenum
54.16%	45.83%	52.08%	50%	52.08%

Statistics of Radicle Length at End of Experimentation

Solution	Mean Length (cm)	Standard Deviation (cm)	Median Length (cm)
Control	2.288	1.87	1.6
Polyethylene Glycol	1.852	.956	1.8
Gibberellic Acid	2.416	1.36	2.5
Potassium Chloride	3.388	2.57	2.75
Molybdenum	2.552	1.57	2.2


Cumulative Seed Germination
Number of seeds germinated
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
Day
Molybdenum
Potassium Chloride
Gibberellic Acid
Polyethylene Glycol
Control


Mean Radicle Length
Length (cm)
4
3.5
3
2.5
2
1.5
1
0.5
0
2.552
3.3875
2.416
1.852
2.288
Molybdenum
Potassium Chloride
Gibberellic Acid
Polyethylene Glycol
Control
Setup

Control: 26 of the original 48 seeds (54.16%) germinated during the 10 day duration of the experiment. Of the germinated radicles, the mean length was 2.288 cm, of which the standard deviation was 1.87 cm. The median radicle length was 1.6 cm.

Polyethylene Glycol: 22 of the original 48 seeds (45.83%) germinated during the experiment. Of the germinated radicles, the mean length was 1.852 cm. Most of the radicles were short, and the standard deviation was lower than the rest at .956 cm. The median radicle length was 1.8 cm.

Gibberellic Acid: 25 of the original 48 seeds (52.08%) germinated during the experiment. The mean of the germinated radicles was 2.146 cm, and the standard deviation from the mean was 1.36 cm. The median length of the radicles was 2.5 cm.

Potassium chloride: 24 of the original 48 (50%) seeds germinated. The mean length of germinated radicles was 3.388 cm and the standard deviation was 2.57 cm. The median length of the radicles was 2.75 cm.

Molybdenum: 25 of the original 48 (52.08%) seeds germinated. The mean length of the radicles of the seeds was 2.552 cm, with a standard deviation of 1.57 cm. The median radicle length was 2.2 cm.

Conclusion

The results refuted the hypothesis. Though the seeds in the gibberellic acid solution had high early germination rates compared to the other solutions, they were outpaced by seeds in the molybdenum solution on the first three days. The seeds in the gibberellic acid then germinated at a rate similar to the seeds in the control, potassium chloride, and molybdenum solutions for the rest of the duration of the experiment. The radicles of seeds in the potassium chloride solution showed the greatest growth, as demonstrated by the high mean and median length of the radicles. The radicles of the seeds in the polyethylene glycol solution were the shortest, as shown by the low mean and median radicle length. The low standard deviation of the radicle length for seeds in the polyethylene glycol solution indicates that there was low variability in radicle length, so the radicles were consistently short compared to the lengths of radicles of seeds in other solutions.

Discussion

A similar percentage of seeds germinated in the molybdenum, potassium chloride, gibberellic acid, and control solutions. The radicles of the seeds germinated in the potassium chloride solution were longer than the radicles of seeds in other solutions. A lower percentage of the seeds in the polyethylene glycol solution germinated, and the radicles of these seeds were consistently shorter than radicles of seeds in the other solutions. During experimentation, seeds in each bowl began to rot and grow what looks like a white and gray mold. Seeds in the polyethylene glycol solution had the highest amount of mold growth, and certain seeds in each bowl had turned a dark brownish purple. In addition, areas of the cotton pads had turned from white to pink in each setup; discoloration was strongest in the cotton pads of the polyethylene glycol.

A white, fluffy fungus often grows on seeds when they are germinated in high-humidity conditions (Rhoades, n.d.). Though it is often mistaken for a mold, this is a fungus that spreads and eventually kills seedlings (ibid). The 10 mL of water that the seeds were given each day in attrition to the paper towel covering that preserved the high humidity most likely encouraged the growth of this fungus.

The pink discoloration of the cotton could have been due to two separate causes. *S. marcescens* is a bacterium that often the cause of discoloration on fabrics, such as the cotton pads that the seeds were germinated on (Madison, 2011). Commonly referred to as “pink mold,” it grows in warm, damp, dark conditions (ibid). Another possible cause of the discoloration is the pathogen *C. kikuchii* (“Soybean Diseases,” 2009). The pathogen primarily affects soybean seeds, causing a pink to dark purple coloration of the seed coat (ibid). The presence of this pathogen

would explain the discoloration of certain seeds. The coloration of the seeds may have stained the cotton pads as well.

The effectiveness of the potassium chloride solution in terms of radicle growth can be explained by the nutrients that it provides the seed with. Potassium is a vital macronutrient for the growth of plants, and chloride is thought to be a micronutrient important in plant growth as well ("Plant Nutrition," n.d.). When plants are grown hydroponically, it is essential to supply the plant with macronutrients, such as potassium, in the water ("Plant Nutrients," n.d.). Since this vital macronutrient was not supplied to the other seeds, the seeds germinated in the potassium chloride solution had the advantage of access to the nutrient, and therefore grew faster. Molybdenum is only used in minute quantities by the plant (ibid), and gibberellic acid, though in low quantities, is already produced in the seed.

The seeds in the polyethylene glycol solution had a lower percentage of seeds germinated, and were consistently shorter. The drought stress induced by the polyethylene glycol inhibited the ability of the seeds to take in the nutrients needed. Water stress is detrimental to plant growth and significantly limits crop yield (ibid). The water stress, combined with the lack of available nutrients, led to the low rate of germination of the seeds in the polyethylene glycol solution and to the low growth rate of the radicles of the seeds.

There are many possible changes to the procedure. An immediate change that would be implemented if the procedure was to be repeated would be to lower the amount of water given to the seeds each day. This would lower the humidity of the environment of the seeds and would thereby discourage mold growth. Using a higher number of seeds would also increase the reliability of the results. The duration of the experiment could also be extended in order to measure the effect of the solutions on growth rate of the soybean seedlings rather than just

germination rate. Furthermore, the seeds may grow differently when germinated in soil rather than on cotton pads. With access to the nutrients in the soil, changes in germination and growth rate would truly be caused by the added nutrients of the solutions, rather than being affected by deficiencies of the cotton pads. However, the seeds would no longer be visible for documentation of emergence of the radicle from the seed coat.

There are many possibilities for future experimentation. With sufficient equipment to measure protein content, it would be interesting to measure the effect of these solutions on the protein content of the seeds grown in the solutions. Due to the widespread use of soybeans as sources of protein, increasing the protein content of soybeans would be very relevant to soybean production and marketing. Another possibility would be to test the effects of the solutions on the ability of the plants grown in different solutions to resist common plagues to soybeans, such as stinkbugs or soybean cyst nematodes.

Works Cited

Ashworth, W. & Little, C. E. Germination (environmental studies). In *Science online*. Retrieved from http://www.fofweb.com/activelink2.asp?ItemID=WE40&SID=5&iPin=envrnstud1392& Sin gleRecord=True

The Effect of Potassium Chloride on Plants. (2009, December 24). Retrieved October 1, 2014, from http://www.ehow.com/facts_5794666_effect-potassium-chloride-plants.html

Gibberellic Acid. (n.d.). *J. L. Hudson, Seedsman*. Retrieved October 1, 2014, from http://www.jlhudsonseeds.net/GibberellicAcid.htm

Gibberellin synthesis ends long quest. (1978). *Science News*, *114*(26), 437.

Hamayun, M., Khan, S., Shinwari, Z., Khan, A., Ahmad, N., & Lee, I. (2009). Effect of Polyethylene Glycol Induced Drought Stress on Physio-hormonal Attributes of Soybean. *Pak. J. Bot., 42(2)*, 977-986. Retrieved October 17, 2014.

Isoflavones as antioxidant. (n.d.). *Isoflavones.info*. Retrieved October 1, 2014, from http://www.isoflavones.info/antioxidant.php

Kumar, R. R., Karajol, K., & Naik, G. (2011). Effect of Polyethylene Glycol Induced Water Stress on Physiological and Biochemical Responses in Pigeonpea (Cajanus cajan L. Millsp.) . *Recent Research in Science and Technology*, *3(1)*(2076-5061), 148-152. Retrieved October 1, 2014, from http://recent-science.com/index.php/rrst/article/view File/6561/3356

Madison, L. (2011, September 13). Pink Mold Stain Removal From Cotton. Retrieved October 17, 2014, from http://www.ehow.com/info_12058234_pink-mold-stain-removal-cotton.html

Pearson, C. (n.d.). The Effect of Potassium Chloride on Plants. Retrieved October 1, 2014, from http://www.gardenguides.com/130060-effect-potassium-chloride-plants.html

Plant Nutrients. (n.d.). Retrieved October 17, 2014, from http://www.smart-fertilizer.com/ articles/plant-nutrients

Plant Nutrition. (n.d.). Retrieved October 17, 2014, from http://ag.arizona.edu/hydroponictomato es/nutritio.htm

Potassium chloride. (2014). [ebook] International Plant Nutrition Institute. Available at: http://www.ipni.net/publication/nss.nsf/0/8FBD66599EAB433F852579AF00741710/$FI LE/NSS-03%20PotassiumChloride.pdf [Accessed 1 Oct. 2014].

Rhoades, H. (n.d.). Preventing White, Fluffy Fungus On Seed Starting Soil. Retrieved October

17, 2014, from http://www.gardeningknowhow.com/garden-how-to/seeds/preventing-white-fluffy-fungus-on-seed-starting-soil.htm

Seed Science: Seed Germination. (n.d.). *Corn and Soybean Digest*. Retrieved October 5, 2014, from http://cornandsoybeandigest.com/seed/seed-science-seed-germination?page=2

Soybean Diseases – Purple Seed Stain. (2009, September 28). Retrieved October 17, 2014, from http://extension.udel.edu/kentagextension/2009/09/28/soybean-diseases-purple-seed-stain/

Soybean Production Planting: Growing and Harvesting Soybeans. (n.d.). *NSRL : About Soy: Soybean Production*. Retrieved October 1, 2014, from http://www.nsrl.uiuc.edu/aboutsoy/production02.html

Sponsel, V. (n.d.). Topic 20.2: Commercial Uses of Gibberellins. *Plant Physiology Online: Commercial Uses of Gibberellins*. Retrieved October 1, 2014, from ://5e.plantphys.net/article.php?id=372

Vijaykant, A. (n.d.). Complete information on nitrogen assimilation in plants. *Preserve Articles*. Retrieved October 1, 2014, from http://www.preservearticles.com/201012251621/nitrogen-assimilation-in-plants.html