# Soybean Peroxidase

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**Abstract**

This study tested which type of soybean has the most peroxidase between genetically modified soybean strains, Bayer Credenz 3841 LibertyLink Soybeans and Asgrow Roundup Ready 2 AG03X7, and conventional soybean seeds. The function of peroxidase found in soybeans is to initiate the growth process when the plant is growing or injured. The hypothesis was that non-genetically modified seeds would have less peroxidase. This hypothesis was based on the results of my research last year. The procedure is to use three strains of soybean seed, two genetically modified and one non-genetically modified, to determine if there is differing levels of peroxidase. The hypothesis was proven correct because the results of this study shows that the non-genetically modified soybeans had more peroxidase than both of the genetically modified soybeans strains.

**Question**

Will there be a difference in the levels of peroxidase between the different strains of GMO (Liberty Link and Xtend) or non-GMO soybean seeds? Two different strains of genetically modified soybeans and one strain of non-genetically modified soybeans were provided for this project by The Ohio State University. The control of this experiment is the test without soybean extract.

**Hypothesis**

Last year's data using different soybean strains indicated that non-genetically modified soybeans have more peroxidase than genetically modified soybean seeds. Therefore, my hypothesis is that the Liberty Link and Xtend seeds will have less peroxidase than the conventional non-genetically modified seeds.

## Research

### Functions

Peroxidase is an enzyme in the seed coating that initiates the growth process when the plant is growing or injured. The chemical function of Soybean Peroxidase is to catalyze veratryl alcohol to veratraldehyde in the presence of hydrogen peroxide. Soybean Peroxidase has many different uses because soybean peroxidase is used to maintain a high thermostability. For example, it can also be used in medical tests, replace formaldehyde in adhesives and protective coatings. Another use of peroxidase has been the use of horseradish peroxidase in the cleansing of waste-water. However, since soybean peroxidase is so similar to horseradish peroxidase it can also be used in wastewater treatment resulting in a much cheaper method. (Soybeans: A Peroxidase Source for the Biotreatment of Effluents., 1956).

### Lignification

As Soybean Peroxidase catalyzes growth, the new cells contain more lignin than the previous cells. (Smith et al. 1994; Østergaard et al. 2000). Lignification is the process of turning a substance into wood or become woody through the formation and deposit of lignin in cell walls. Lignification occurs as a defense mechanism, making the new cells harder.

### Location

Soybean Peroxidase is found in the seed coatings of soybeans and the hulls, stems and trunk, of soybeans. It is found in the seed coatings to initiate growth and in the hulls to initiate repair.

### Classification

Seed coat soybean peroxidase belongs to class III of the plant peroxidase superfamily that includes the classical peroxidase, namely horseradish peroxidase. Soybean peroxidase must be stored at 2-8 C. (Soybeans; Gomez, Gomez, and Murcia)

### Bayer Credenz 3841 LibertyLink Soybeans

These seeds allow a different herbicide program to be used due to Liberty Link genes. These genes allow the plant to survive a crop treatment of LibertyLink Herbicide that kills the surrounding weeds. LibertyLink Herbicide is a fast acting herbicide that primarily kills grasses.

The primary ingredient in this herbicide is glufosinate-ammonium. According to Bayer agronomist, Marshall Beatty these soybeans average 65 bushels an acre in Missouri.

**Asgrow Roundup Ready 2 AG03X7**

These seeds average 70.5 bushels an acre. These seeds are also Roundup Ready resistant which means that they can survive a (Roundup Ready) glyphosate herbicide treatment to the crop.

**Gel Electrophoresis**

This technique is used to separate proteins by their size. This technique can be used in DNA insertion experiments because the gel demonstrates the structural differences in the resulting protein. An electrical current is used so that one side of the system is negative and the other positive. A buffer is also used in the system to maintain the pH of the system and to provide ions that carry a current. The proteins migrate across the gel with the smallest molecules moving the fastest and largest molecules moving the slowest. When the electrical current is removed the smallest molecules will be the farthest along the gel and the largest molecule will be the closest to the wells, or starting points.

**Procedure**

The soybean coatings were removed by placing seeds into distilled water and removed by hand. Seeds stayed in the water for about 10 minutes or until the soybean coating is loose. This was repeated on 200 grams of soybeans which is about 10 grams of soybean coatings. Once removed, soybean seed coatings were placed in a tube and frozen or placed in ice to prevent the denaturing of the soybean peroxidase.

Then, one gram of seed coatings was placed in a mortar with 9 mL of pH 7 buffer. The mixture was then ground until there are no fully formed soybean coatings remaining. The mixture was then placed in a centrifuge tube.

The centrifuge tube and its' contents were placed in a centrifuge and spun for 10 minutes. The soybean coating pieces were then pushed to the bottom of the tube. The liquid at the top of the centrifuge tube is soybean extract and will be removed. Two test tubes were prepared. In one test tube, 4 mL of pH 5 buffer and 1 mL soybean extract were placed. For the control, no soybean extract was added. 2 mL of hydrogen peroxide mixture and 1 mL of guaiacol mixture will be added to the second test tube.

Then, the two tubes were added together in a cuvette. First, the control was put into the spectrophotometer and tested for reference. Then, the second cuvette was added to the spectrophotometer to measure absorbance of photons. The mixture changed color due to the soybean peroxidase reacting to the hydrogen peroxide. The spectrophotometer measures the relative amount of peroxidase based on how much the color changes.

**Electrophoresis Gel**

The gel was created by mixing 1.3 grams of agarose with 100mL of proportioned reaction solution. Electrophoresis gels were used in this experiment to determine if there were multiple isoenzymes to peroxidase. Isoenzymes are two or more enzymes that have an identical function but different structure.

**Monobasic Potassium Phosphate**

Monopotassium phosphate buffer (KH2O4), was be made by putting 13.61 grams of 1 molar potassium phosphate into a volumetric flask per 100mL used. Then, the volume was brought up to 500 mL with distilled water.

**Diabasic Potassium Phosphate**

Dipotassium phosphate buffer (K2HO4), was made by putting 17.42 grams of 1 molar Dipotassium phosphate into a volumetric flask per 100 mL of H2O. Then, the volume was brought up to 500 mL with distilled water.

**pH 5 Buffer**

102.5 milliliters of 0.1 molar citric acid and 147.5 milliliters of 0.1 molar sodium citrate were put into a volumetric flask. The volume of the flask was then raised to 500 mL with distilled water.

**pH 7 Buffer**

61.5 mL of diabasic buffer and 38.5 of monobasic buffer were added together to make 100 mL of pH 7 buffer.

**Citric Acid Solution**

9.605 grams of Citric Acid was added to a 500mL volumetric flask and distilled water was added to the 500mL line to create a 0.1 molar solution of Citric Acid Solution.

**Sodium Citrate Buffer**

14.705 grams sodium citrate was added to a volumetric flask and the volume will be raised to 500 mL with distilled water.

**pH 5 Buffer**

Combine 20.5mL of 0.1 M citric acid solution  with 29.5mL of 0.1 M sodium citrate and 50mL distilled water.

**Hydrogen Peroxide Mixture**

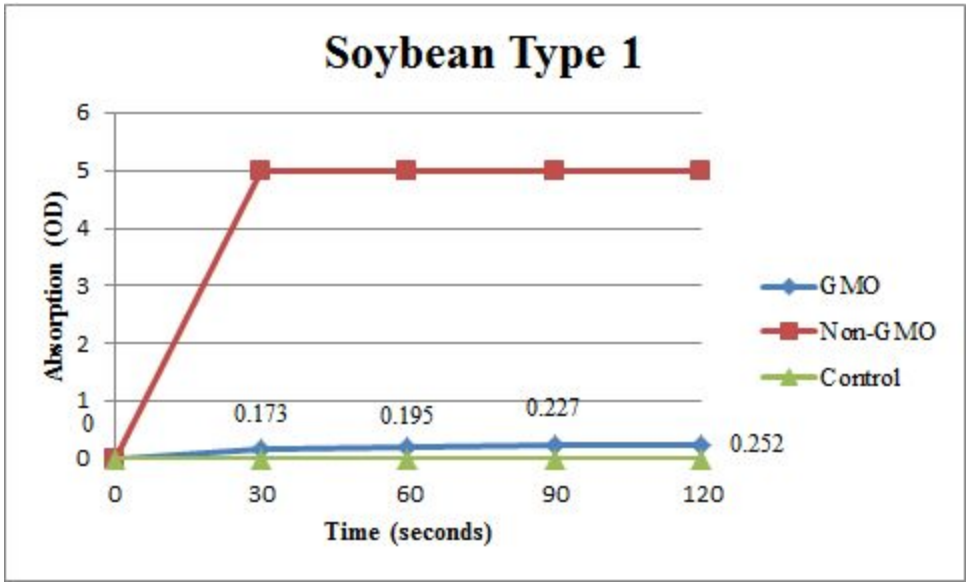
3.69 mL of hydrogen peroxide was put into a volumetric flask and the volume was raised to 500 mL with distilled water.

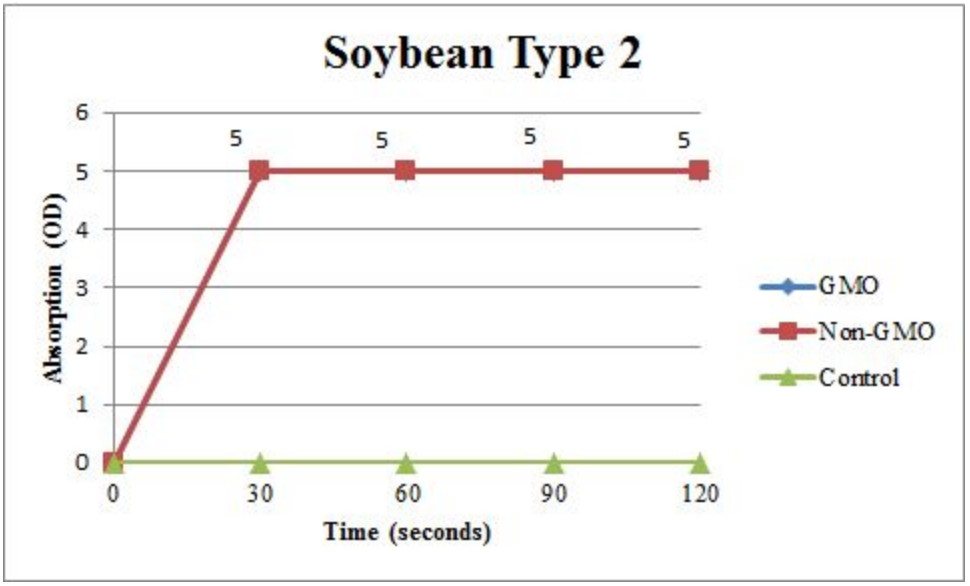
**Guaiacol Mixture**

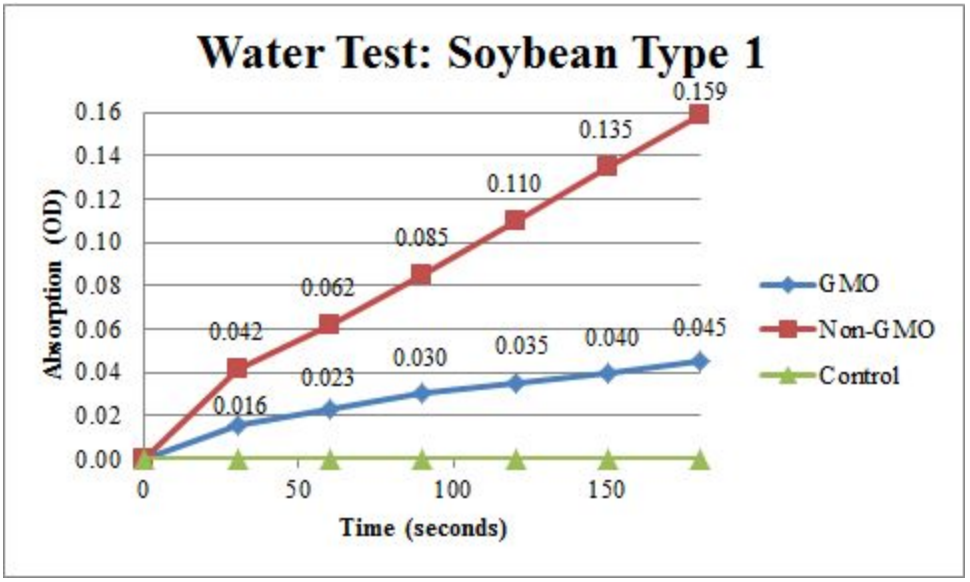
0.343 mL of guaiacol was added to 125 mL of isopropyl.

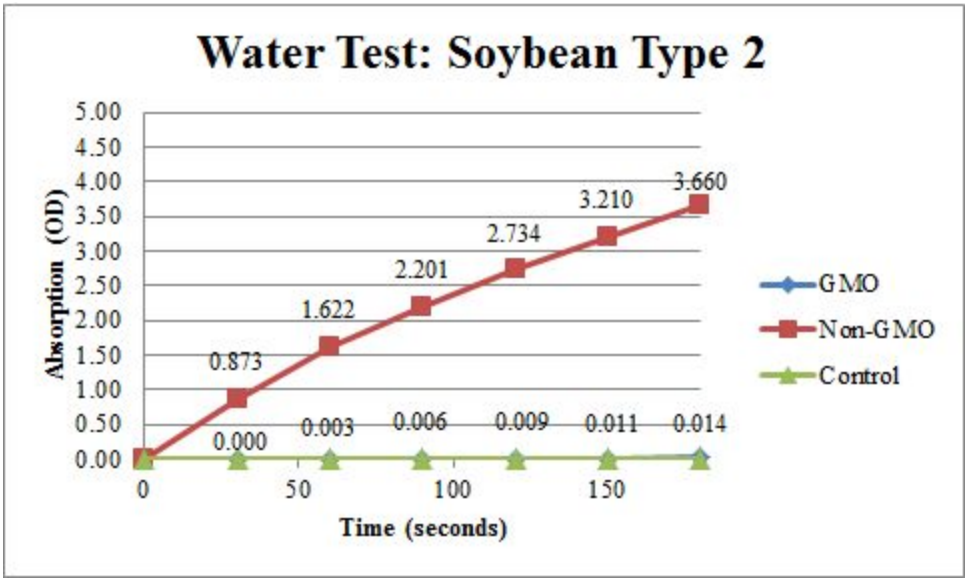
**Results19**

**2016-2017**

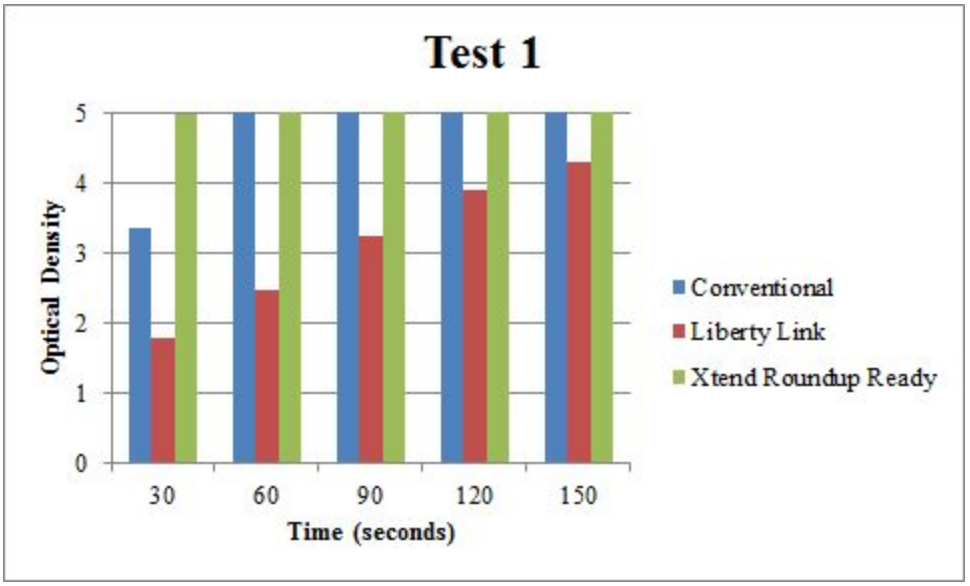


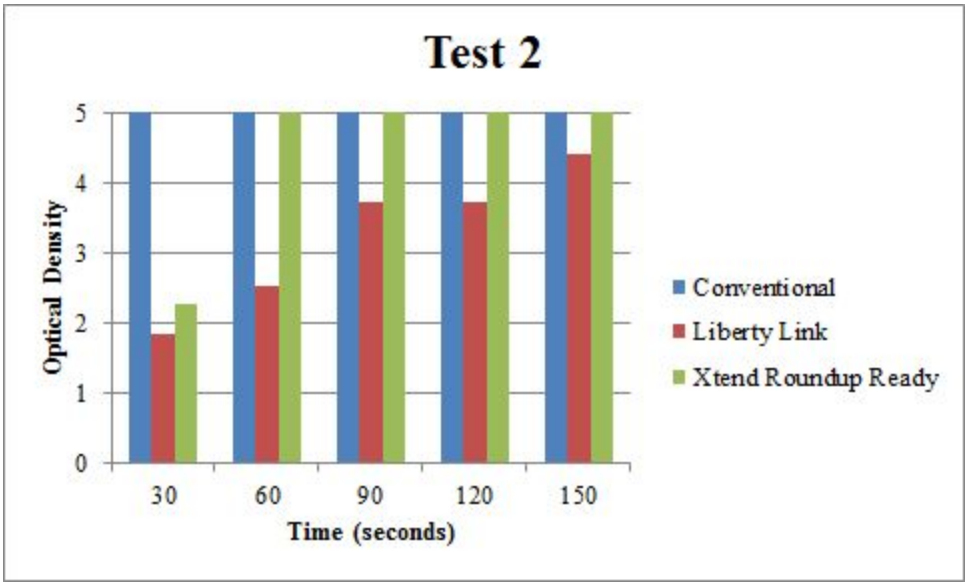



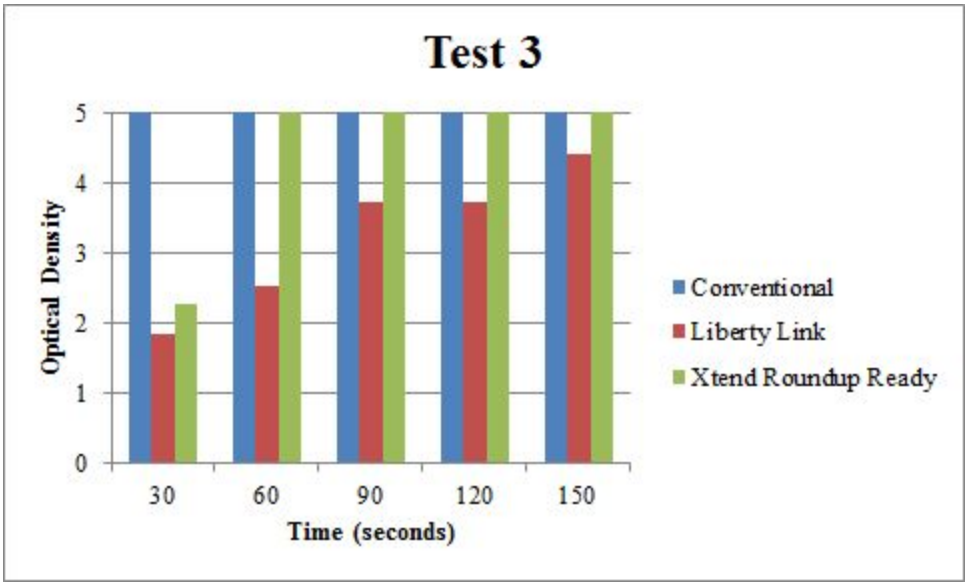

Water Test: Soybean Type 1
Absorption (OD)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
50
100
150
Time (seconds)
0.042
0.062
0.085
0.110
0.135
0.159
0.016
0.023
0.030
0.035
0.040
0.045
GMO
Non-GMO
Control

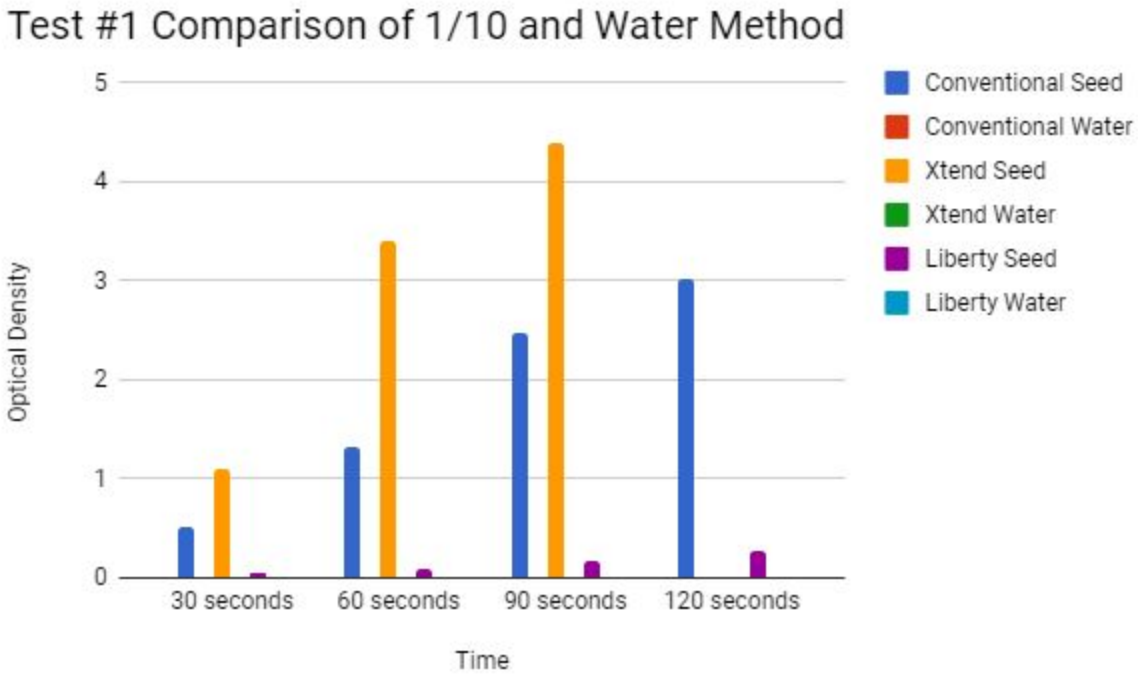

Water Test: Soybean Type 2
Absorption (OD)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0
50
100
150
Time (seconds)
0.873
1.622
2.201
2.734
3.210
3.660
0.000
0.003
0.006
0.009
0.011
0.014
GMO
Non-GMO
Control

**2017-2018**

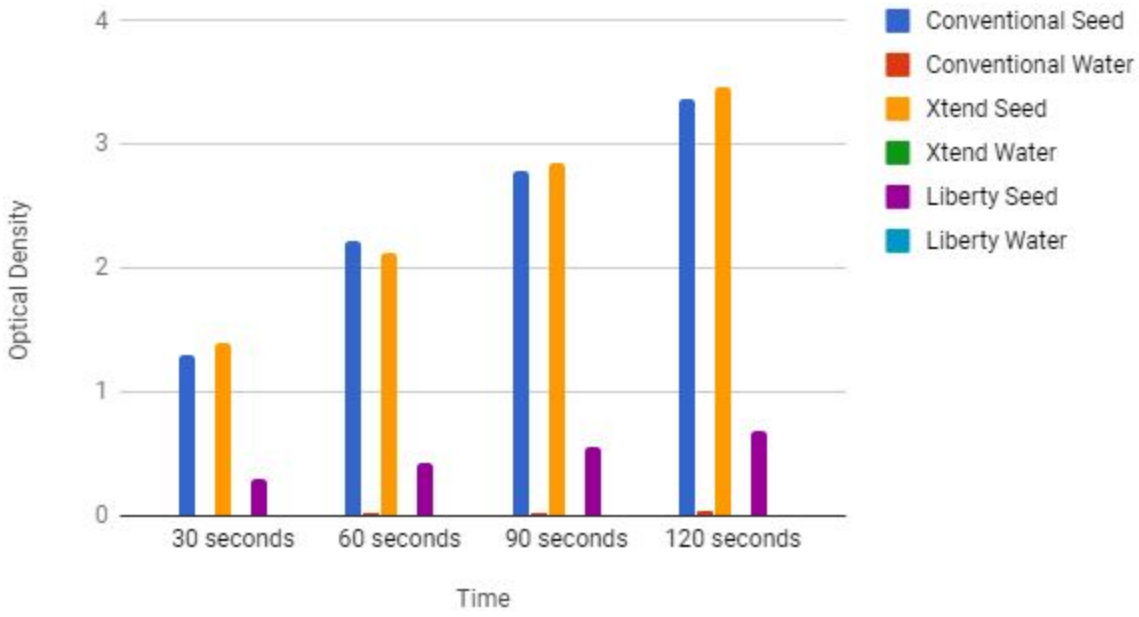

Test 1
Optical Density
5
4
3
2
1
0
30
60
90
120
150
Time (seconds)
Conventional
Liberty Link
Xtend Roundup Ready


Test 2
Optical Density
5
4
3
2
1
0
30
60
90
120
150
Time (seconds)
Conventional
Liberty Link
Xtend Roundup Ready

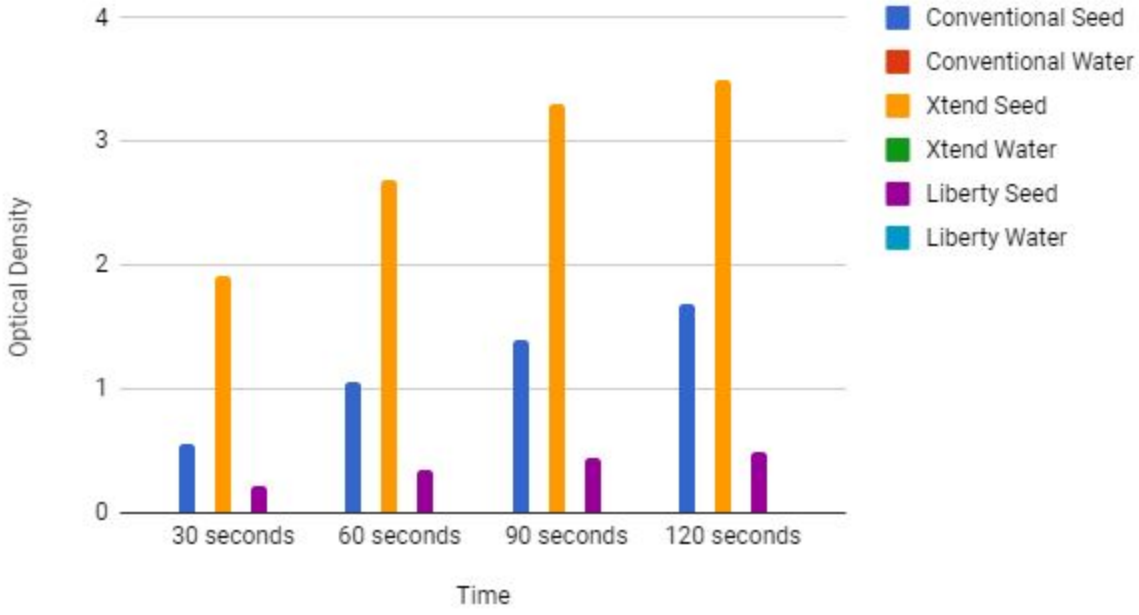

Test 3
Optical Density
0
1
2
3
4
5
30
60
90
120
150
Time (seconds)
Conventional
Liberty Link
Xtend Roundup Ready


Test #1 Comparison of 1/10 and Water Method
Optical Density
0
1
2
3
4
5
30 seconds
60 seconds
90 seconds
120 seconds
Time
Conventional Seed
Conventional Water
Xtend Seed
Xtend Water
Liberty Seed
Liberty Water

## Test #2 Comparison of 1/10 and Water Method




## Test #3 Comparison of 1/10 and Water Method



| P-Scores |
| --- |
| XvC 0.106 |
| LvC 0.042063 |
| LvX 0.0166 |

If the P-score is less than 0.05 the data is significantly different.

## Conclusion

Three recorded trials were completed of the original testing method. In all three trials the conventional seed had more peroxidase than the Liberty Link seed. It was unclear whether conventional seed or Xtend Roundup Ready seed have more peroxidase based solely on these tests. At 30 seconds on 2 of the 3 trials, the conventional seed has more peroxidase than the Asgrow Roundup Ready 2 AG03X7. However, past 30 seconds both the conventional and the Asgrow Roundup Ready 2 AG03X7 seeds have greater than 5.0 O.D. (Optical Density). Thus the difference in peroxidase levels could not be measured by the spectrophotometer because the spectrophotometer can only measure to 5.0 O.D. This testing method proved the hypothesis was correct because the conventional seed has more peroxidase than the Liberty Link seed. However, the results for the Roundup Ready seed were inconclusive. Due to this uncertainty, the procedure was modified using a water method and 1/10 of a mL of soybean extract. This series of three tests showed Xtend Seed to have to most peroxidase. To make sure this was statistically accurate, I compared p-scores of the results of the 1/10mL Method. The p-scores demonstrated that the difference in peroxidase between Xtend and Conventional Seed were not significantly different, while both Xtend and Conventional Seed were significantly different from Liberty Seed. Theoretically, plants with more peroxidase would be naturally resistant to pest attacks due to lignification and be able to recover from a pest attack faster.

### Future Research

If this experiment was repeated, the soybeans should also be grown and observed with a pest added to the environment to see which strains of soybean would recover faster. The tests should also be repeated, but due to time constraints, that could not be done in this experiment.
As this project is continued, the 1/10 mL method would continued to be utilized because this form of the original procedure provides the most accurate data.

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