Biodiesel Waste Products as Soil Amendments– Evaluation of Microbial, Biological, and Plant Toxicity

MBTC Project 3025

Thomas S. Soerens, Ph.D., P.E.

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The objectives of this research project were to evaluate the toxicity and growth effects of methanol-stripped glycerol from biodiesel waste on microbial, biological, and plant systems in soils. Three tests were used: 1. Activated sludge respiration inhibition test; 2. Earthworm toxicity; and 3. Plant toxicity.
Results of the respirometry studies suggest that there is no microbial inhibition due to the glycerol. Microbial activity was observed in a wide range of glycerol amounts applied to soils. In plant studies, there was inhibition of growth and germination with glycerol quantities above 1% by weight. Lower levels of glycerol did not appear to inhibit plant germination or growth and in fact appeared to be beneficial to growth. In worm assays, glycerol concentrations above 1% were fatal on contact with earthworms due the glycerol absorbing water and desiccating the worms. Worms survived when exposed to glycerol in lower concentrations.

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The author thanks Mark Kuss of Challenge Technologies for the use of the AER-200 Respirometer system and for assistance in setting up and troubleshooting the system.

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INTRODUCTION

Biodiesel is generally considered an environmentally friendly, "green" technology. However, there is a byproduct of biodiesel production, glycerin, which must be processed or disposed of. The disposal of this byproduct is a significant factor in the sustainability of the bioenergy chain. Biodiesel Magazine (Smith, 2008) states:

> "As relative newcomers to the industrial world, biodiesel producers, who are generally regarded as environmentally friendly, need to be good neighbors when it comes to properly disposing of byproducts. Although the scientific and regulatory communities have yet to agree on the toxicity of biodiesel byproducts, the industry should be prepared as the regulatory framework for the fledgling industry materializes."

The byproduct of biodiesel production is primarily glycerol, also called glycerin or glycerine, along with methanol, and a small amount of unused catalyst and soaps. The methanol can easily be stripped from the glycerol by separation or evaporation. The commonly used term glycerin can refer to the raw waste product with methanol or the refined product without methanol or other contaminants. The amount of glycerin produced from biodiesel production is about 10% of the amount of biodiesel. Thus, it's a big issue and a growing issue as the biodiesel industry is growing. In 2008, about 530 million pounds of raw glycerin was produced in the US (Urbanchuk, 2008). Some composting facilities do accept waste glycerin and the expansion of composting of glycerin would be environmentally preferable to incineration. The University of Arkansas currently composts waste glycerin. Glycerin, which is a humectant (moisture retaining substance), has been suggested as a dust suppressant and has been used for dust control on the personal property of individual biodiesel producers. However, it has not as yet had wide application as a dust suppressant.

Currently, most waste glycerin is incinerated, releasing the carbon to the atmosphere. In some cases the glycerin replaces other fuels, but in general incineration is contrary to the desire to use biodiesel to be more carbon-neutral. There are a few other uses for waste glycerin – an additive to animal feeds, for soap, an additive to pharmaceutical and personal care products, and a possible source of industrial chemicals. The glycerin demand for these uses, however, is small compared to the large volume of glycerin being produced. Some composting facilities do accept waste glycerin and the expansion of composting of glycerin would be environmentally preferable to incineration.

Using glycerin as a soil amendment is a promising disposal alternative that has not received much study. The use of this byproduct as a soil amendment has implications for carbon sequestration and greenhouse gas emission and may be a beneficial use (Cayuela et al., 2010). Glycerin could actually have positive impacts on the soil because it would raise the organic content of the soil. However, as Biodiesel Magazine says, the toxicity of glycerin is unknown. Because it is derived from natural crop or animal sources, it is generally assumed to be nontoxic, but studies of its effects on microbial, biological, and plant systems are lacking.

In an initial study, Schoenau et al. (2009) found that glycerol was effective in increasing soil

organic content, but required supplemental fertilizer to account for nitrogen and phosphorus tie-up by microorganisms during decomposition in the soils. The immobilization of phosphorus might be a benefit to soils with excess phosphorus due to poultry litter application.

The proposed research will examine the effects of glycerin application on microbial, earthworm, and plant growth and survival in soil. A microbial respiration test will be used to evaluate toxicity to microorganisms. An earthworm test will be used to evaluate effects on biological systems. And a plant test will be used to evaluate glycerin's phyto-toxicity.

If glycerin from biodiesel waste is shown to be nontoxic when used as a soil amendment, it can be used by as a beneficial resource in dust control, replanting, and landscaping applications rather than incinerated as a waste product. In addition to disposing of the glycerin in a more environmentally friendly and more carbon neutral manner, it may also show beneficial effects on soils and reduce the use of other soil amendments. If the glycerin is shown to be benign or beneficial, guidelines for application rates will be produced based on the results of this research.

PROJECT OBJECTIVES

The objectives were to evaluate the toxicity and growth effects of methanol-stripped glycerol from biodiesel waste on microbial, biological, and plant systems in soils. Three tests were used: 1. Respirometry; 2. Earthworm toxicity; and 3. Plant toxicity.

The specific objectives were:

1. Measure microbial respiration in soil samples with and without glycerin and determine if the addition of glycerin at several concentrations inhibits or enhances microbial respiration.
2. Evaluate the survival of earthworms in soils with and without glycerin and determine if glycerin affects earthwork survival and if survival is related to glycerin concentration.
3. Observe and measure emergence and growth of plants in soils with and without added glycerin and determine whether the presence or concentration of glycerin affects emergence and growth.

RELATED WORK

Schoenau et al. (2009) studied several biofuel, crop, and animal processing by-products as soil amendments. They found that glycerin was effective in increasing soil organic content, but required supplemental fertilizer to account for nutrient tie-up by microorganisms during decomposition in the soils. Neither glycerin nor the other amendments had any biologically significant effects. Glycerin at the study application rates did not affect soil chemical parameters measured including soluble metals, pH, or salinity. The authors noted that "glycerin addition may be of greatest benefit in increasing soil organic carbon content and carbon sequestration, compared to the alternative of incinerating the glycerin." They found that application rates as high as 10,000 kg glycerin per hectare (which converts to 1% by weight if a soil depth of 0.1 m is assumed) were beneficial for microbial and plant growth but that such a high rate would require unrealistically high rates of fertilizer application. They suggested that a realistic field application rate would be ~1000 kg per hectare. The authors note that because the glycerin immobilizes nutrients, additional fertilizers are needed. On the other hand, we might speculate from this that glycerin addition could be beneficial by immobilizing phosphorus in soils with excess phosphorus. The effects of glycerin on nutrient transport from soil could be a interesting study, but is not within the scope of this proposed research. The Schoenau research group will have a paper in *Journal of Plant Nutrition* 2011 by Qian et al. on thin tillage and glycerin. Another paper on effects of biofuel byproduct amendments on microbial enzyme activity will appear in 2011 in the journal *Applied Soil Ecology* by Alotaibi et al.

Cayuela et al., (2010) added ten different bioenergy byproducts to soil and investigated the soil carbon and nitrogen cycling. They found that biofuel byproducts contain high amounts of easily decomposable C leading to short-term N immobilization in soil, limiting their potential as short-term fertilizers. They also suggested that byproducts should be used in a way that allows them to degrade to an extent to maintain biological activity and nutrient cycling but still show some persistence in soil.

Hall (2010) examined soy-based foam insulation as a soil amendment. He examined toxicity in activated sludge systems, earthworm systems, and plant systems. He found it very difficult to maintain stirred soil cultures or activated sludge cultures for respirometry and that is why the proposed study uses a different respirometry method to measure microbial toxicity. The earthworm and plant studies proposed in the present research follow closely to what Hall did, but the glycerin studies will be performed with a liquid rather than with chunks of solid foam. Hall's study did not observe any toxicity effects of the foam. The controls in the study had a high precision, but the samples using foam had high variability in all three tests. This is likely due to physical effects of the foam chunks. This will not be an issue with glycerin, which can be mixed uniformly with the soil.

Dror et at. (2000) evaluated the effects of soil amendments, including sewage sludge, on the dynamics of kerosene attenuation on field plots. The plots were then leached using mini-sprinkler irrigation. The test was run for 100 days. They found that soil amendments may enhance the rate of kerosene degradation and reduce the residual amount left compared to the untreated soil.

In a report by W.C. Chung et. al. (2005) glycerin was used as an ingredient for a granulate biofungicide to control *Rhizctonia solani* colonization in soil to prevent damping-off of Chinese cabbage. The cabbage germination was not affected by the glycerin as well (Chung, Huangb, & Huang, 2005). Due to fungicide resistance and the need for more sustainable disease practices in the agricultural sector, Siddiqui and Shaukat (2002) tested the effects of zinc and glycerin alone and in combination to improve biocontrol activity of indigenous and nonnative bacteria namely *Pseudomonas aeruginosa* and *Ps. fluorescens*. They concluded that zinc and glycerin alone or in combination increased biocontrol efficacy against root-knot nematode as well as improved tomato plant growth and bacterial rhizosphere colonization. Glucose alone was found to inhibit the nematicidal activity of the bacteria (Siddiqui & Shaukat, 2002).

Rod Rodriguez-Kabana of Auburn University has developed and patented a glycerin-based product that is injected into soil to control weeds and crop-destroying nematodes (AAES, 2008). Dr. Kabana suggests that his product could be used in organic farm production and expects his product to be on the market within five years.

In a study of glycerin in animal feed, Kerr et al. (2007) concluded that crude glycerin from biodiesel production was an important energy source for livestock, namely swine, broiler chickens, and laying hens since glycerin has between 3,200-3,800 kcal/kg for these animals. The glycerin fed to these animals had little to no effect on the animal performance, composition, or meat quality which is valuable information for companies which could benefit from disposing of glycerin from biodiesel producers by selling it as feed to farmers.

The toxicity of glycerin once separated from the biodiesel is based on the concentration of methanol in the glycerin. Methanol will evaporate out of the glycerin when left open to the atmosphere for around a week. Heating the glycerin will also increase the evaporation rate of the methanol. Afterwards, the glycerin is considered non-toxic and biodegradable (Tickell, 2003). According to the Material Safety Data Sheet on glycerin, it is considered a skin and eye irritant with no carcinogenic effects on animals or humans as well as non-hazardous if ingested (EMD Chemicals Inc., 2004). In a study of glycerin in animal feed, Kerr et al. (2007) concluded that crude glycerin from biodiesel production was an important energy source for livestock, namely swine, broiler chickens, and laying hens since glycerin has between 3,200-3,800 kcal/kg for these animals. The glycerin fed to these animals had little to no effect on the animal performance, composition, or meat quality which is valuable information for companies which could benefit from disposing of glycerin from biodiesel producers by selling it as feed to farmers.

MATERIALS AND METHODS

Glycerin

Tests used glycerin obtained from the University of Arkansas Facilities management. The glycerin was then stored in an open container in a fume hood to allow methanol to evaporate. The glycerin is an oily liquid and has a pH of 10.1. Pure glycerol is fairly neutral in pH but sodium hydroxide or potassium hydroxide is used as a catalyst in biodiesel production and the resulting glycerin waste has a high pH.

Plant Test.

The plant tests were performed using OECD method 208 "Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test". Bermuda grass seed was applied to test cells. The cells were 3x3x3 inches. 60 grams of commercial potting soil and 20 mL of water were initially added to each cell. Seed was applied at a rate of 0.16 grams per cell, which is 5 lb/1000 sq ft. In the first scan, glycerin amounts between 8.4 g and 42 g (16.8% to 79% of the 60 g soil mass) were added to the cells. Each treatment was done in triplicate.

In the first scan, only the control showed germination and growth. We hypothesized that the lack of germination and growth in the glycerin cells was due to pH effects. In the second scan, the pH of the soil mixtures was adjusted using acetic acid to a pH of between 6.81 and 6.96. Again the higher concentration treatments did not germinate.

The third scan used much lower glycerin rates of 0.01% to 1%. The cells were given three sprays of water each day. The seeds germinated and numbers of sprouts were monitored. At the end of 14 days, the seedlings were weighed and measured. A fourth scan evaluated concentrations between 3% and 15%.

A fifth scan used rye grass and allowed the grass to grow for two months. Cells were photographed regularly during the test and the mass of grass in each cell was measured at the end of the test. The photo below shows this last scan:

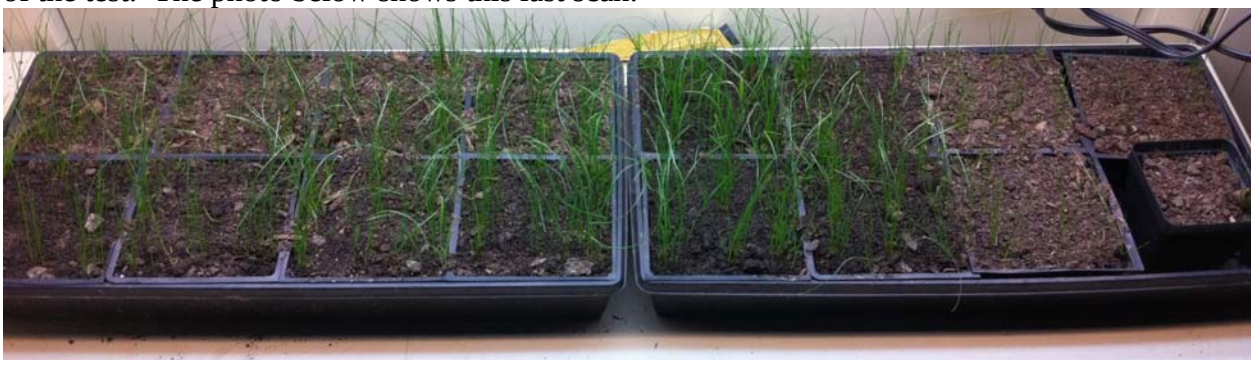


Figure 1. Plant Test

Microbial Toxicity Test.

Microbial respiration inhibition tests were performed using an eight position respirometer with eight solid reactor columns. The AER-200 Respirometer system was provided by Challenge Technology of Springdale, Arkansas. The reactor cells were packed with a test soil that was a mixture of commercial top soil and compost obtained from Nitron Industries, Johnson, Arkansas. Each cell held approximately 65 grams of compost and various amounts of glycerin. The tests measured oxygen uptake by a microbial community in a solid matrix with and without glycerin. Tests were performed with several concentrations of glycerin. Tests were run from one to four weeks. A total of 15 tests were run. The figure below shows the experimental setup.

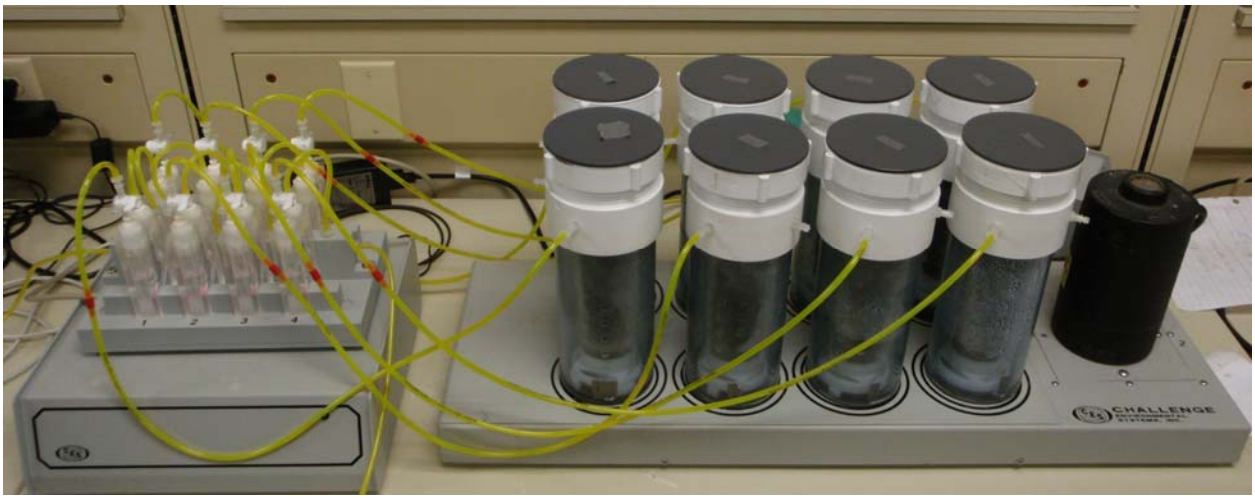


Figure 2. AER-200 Respirometer from Challenge Technology

The respirometer has 8 sealed cells. Each cell has an internal chamber for soil that has holes to allow oxygen in and screens to contain the soil. Inside the cell on the bottom of the cell is a fan that is turned by a magnetic stirrer. The fan allows air to circulate within the cell. Inside the cell on top of the cell is a beaker containing KOH, which captures CO_2 produced in the cell. As oxygen is used up, the lower pressure in the cell draws oxygen in through a tube. A bubble counter between the respirometer and the oxygen supply records the number of bubbles of oxygen taken in by each cell. The counter is calibrated to calculate the mg of oxygen taken in, and this is the data value recorded by the computer.

Respirometer data, mg of oxygen uptake, were recorded every minute. The length of runs varied between a few days and several weeks. The slope of the plot of uptake versus time during the main period of activity is the Oxygen Uptake Rate (OUR), expressed in units of mg/hr.

Earthworm Test.

The earthworm test methods were based on OECD method 207 “Earthworm, Acute Toxicity Tests”. Earthworms were obtained from Parker Farms, Fayetteville, AR. The tests were performed in plastic containers each containing 655 g of soil. Each treatment was done in four replicates. Moisture content and pH were measured for each cell. Worms were rinsed with DI water and dried with paper towels before being applied to the test cells. 10 worms added to each container after weighing. After 7 days, the worms were collected, counted, and weighed. Then they were returned to the test cells for another 7 days. After a total of 14 days, the worms were again collected and weight and the pH and moisture content of the soil was measured again. Various amounts of glycerin were used. The tests were repeated with the test cells adjusted to a pH of 7 using acetic acid.

RESULTS

Plant Test Results

Figure 3 shows the results of grass growth versus glycerol concentration (log-log scale). Up to a concentration of 1% by weight glycerol, growth in the glycerol-treated cells was comparable to or greater than the control (0%). At 1% and above, germination and growth were less than the controls. The soil in the cells at the high concentrations was dry and crunchy, even though those cells received the same amount of water. Glycerol is a humectant, meaning it has hygroscopic (water retaining) properties. The glycerol absorbed the water, making it unavailable to the plants and drying out the soil.

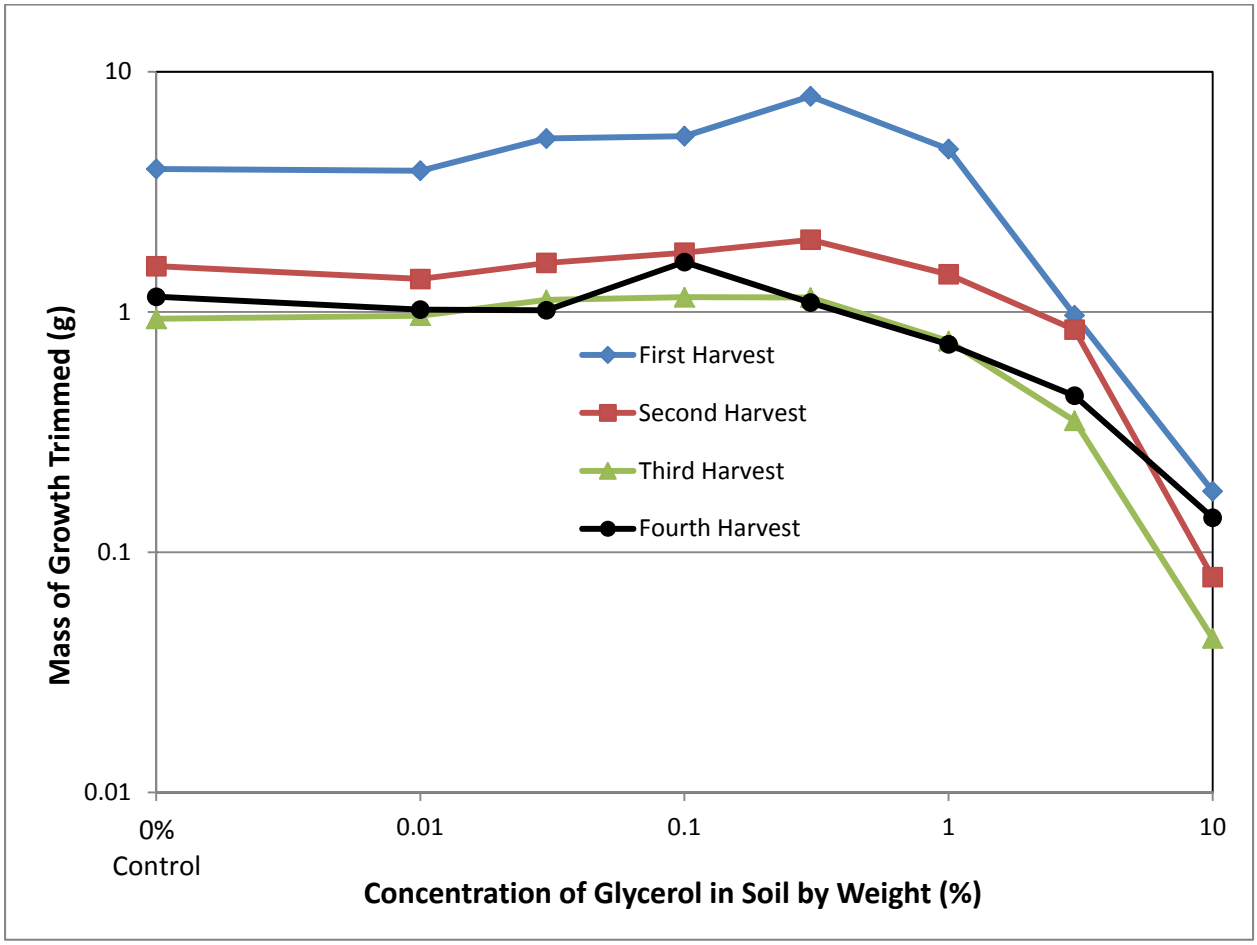


Figure 3. Mass of Grass Clippings versus Glycerol Concentration in Soil (log scale)

The growth data are listed in Table 1. Table 2 expresses the plant mass as a growth rate (g/day) by dividing the harvested mass by the number of days of growth.

Table 1. Mass of plant trimmings at different percentages of glycerol

	Mass of Trimmings (g)			
Conc (%)	Harvest 1	2	3	4
0	3.93	1.55	0.94	1.16
0.01	3.87	1.37	0.96	1.02
0.03	5.27	1.60	1.12	1.02
0.1	5.39	1.76	1.15	1.61
0.3	7.90	2.00	1.15	1.09
1	4.76	1.44	0.76	0.73
3	0.97	0.84	0.35	0.45
10	0.18	0.08	0.04	0.14

Table 2. Plant growth rate: mass of plant trimmings divided by days of growth

	Growth Rate (g/day)				
Conc (%)	Harvest 1	2	3	4	average
0	0.127	0.310	0.134	0.083	0.163
0.01	0.125	0.274	0.138	0.073	0.152
0.03	0.170	0.320	0.160	0.073	0.181
0.1	0.174	0.353	0.164	0.115	0.202
0.3	0.255	0.400	0.164	0.078	0.224
1	0.154	0.287	0.108	0.052	0.150
3	0.031	0.169	0.050	0.032	0.071
10	0.006	0.016	0.006	0.010	0.009

Analysis of Variance (ANOVA) was conducted on the growth rates to determine if there were any differences between concentrations or between harvests. The analysis evidenced significant differences in both concentrations ($p = 9x10^{-6}$) and harvests ($p = 5x10^{-8}$), indicating that there are differences between at least one pair of concentrations and at least one pair of harvests.

To investigate which differences were significant, a series of paired t-tests (two-tail) were performed to identify any significant differences between the growth rate with glycerol and the control. The method compares the growth rate of the glycerol cells at each harvest to the growth rate of the control. These results are listed in Table 3. Three of the treatments showed significant differences ($p<\alpha$) from the control at an $\alpha = 0.05$ level. The 3% and 10% glycerol treatments were significantly less than the control, which could be seen in Figure 3. The 0.1% glycerol treatment showed significantly more growth than the control.

Table 3. Results of Paired t-test between treatment and control

Glycerol %	p value	conclusion
0.01	0.303	
0.03	0.225	
0.1	**0.002**	**> control**
0.3	0.132	
1	0.400	
3	**0.016**	**< control**
10	**0.050**	**< control**

In Figure 4, which shows the growth rate on a linear scale, we can more clearly see that the increase in growth in the range of 0.1 – 0.3 % glycerol.

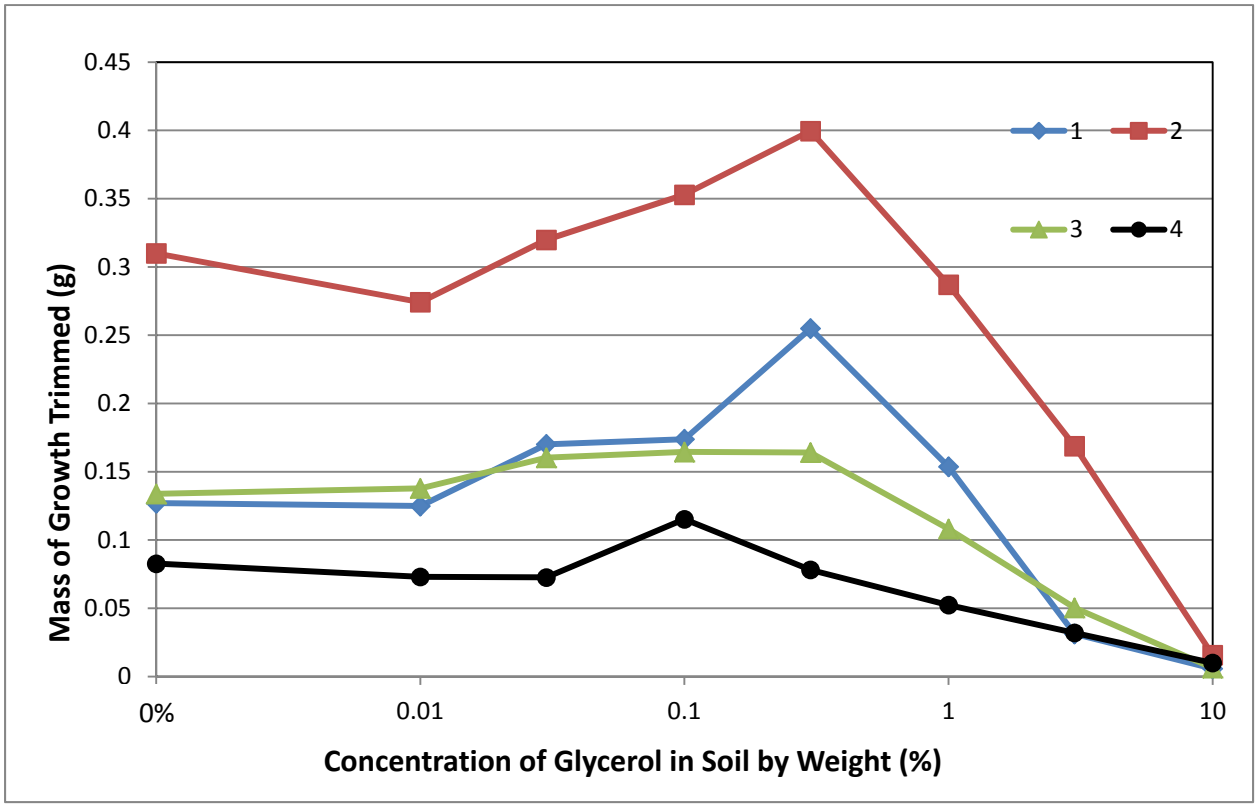


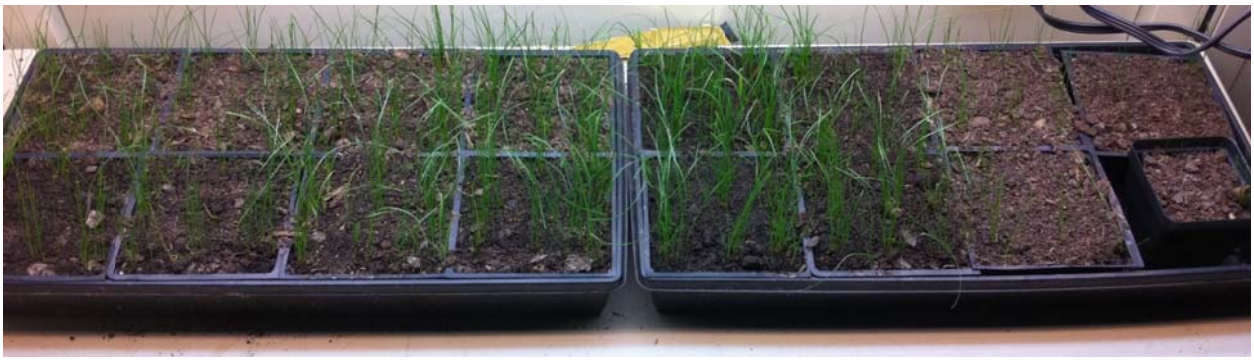
Figure 4. Growth rate of grass at difference concentrations of glycerol.

Figures 5 and 6 show photos of the test plots through the experiment.

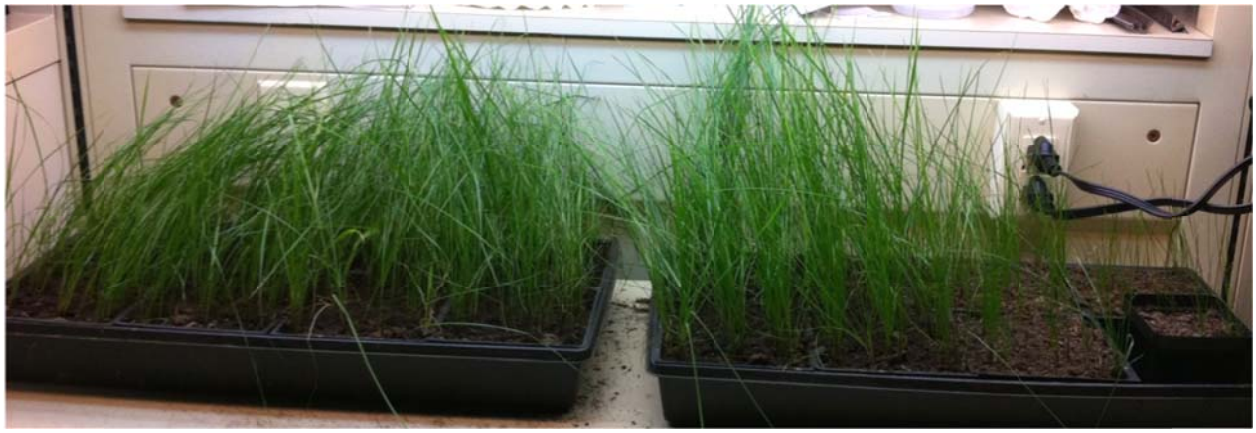
Glycerol concentration (%)

0 0.01 0.03 0.1 0.3 1.0 3.0 10

a. Germination at 6 days



b. Growth at 9 days



0 0.01 0.03 0.1 0.3 1.0 3.0 10

Glycerol concentration (%)

c. Before first harvest: 37 days

Figure 5. Grass growth over time at different glycerol concentrations. a. Germination at 6 days; b. Growth at 9 days; Before first harvest at 37 days. Glycerol concentrations (L-R): 0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10

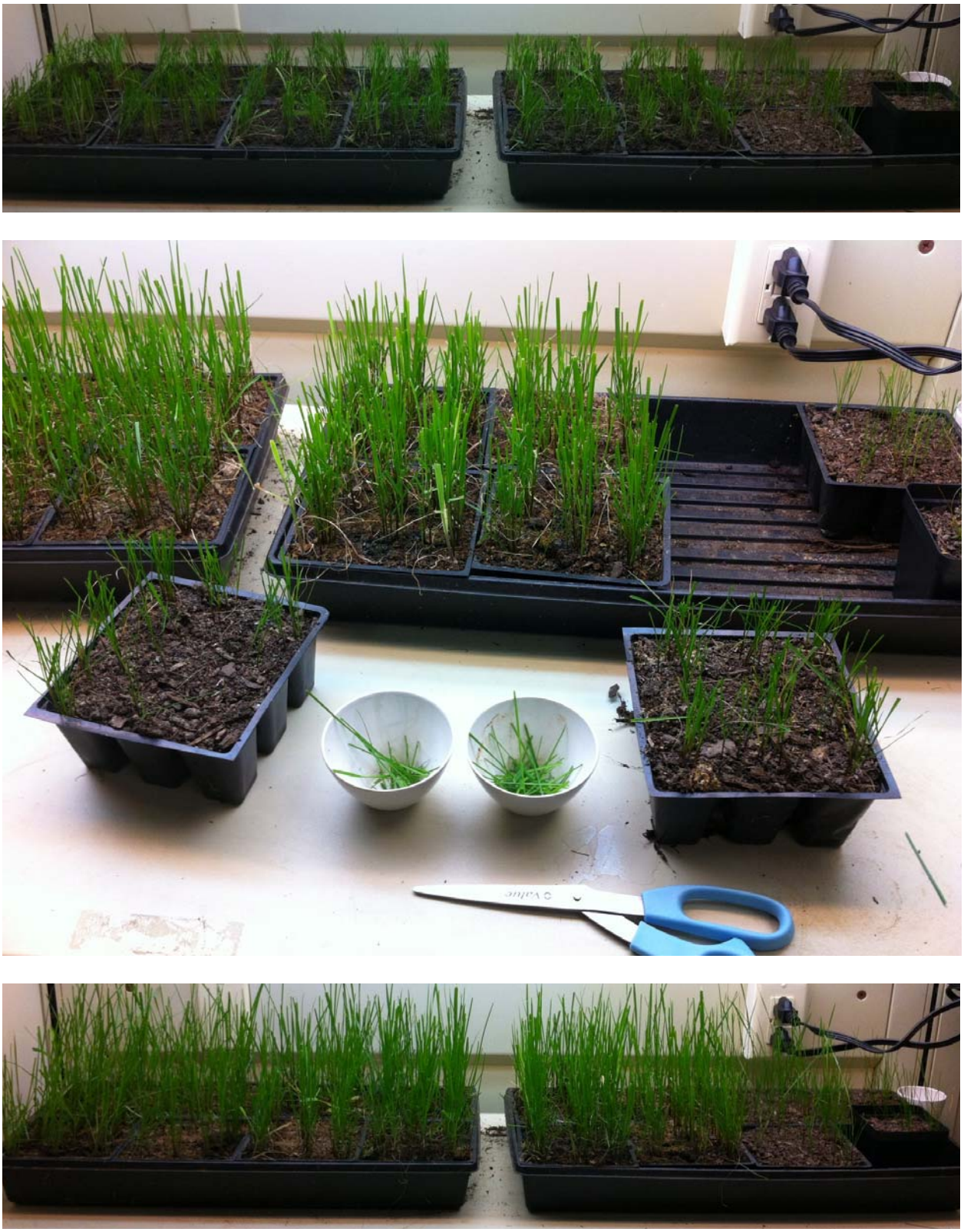


Figure 6. Test grass harvesting

In germination tests, initially glycerol amounts between 16.8% and 79% were used. Only the control showed germination and growth. We hypothesized that the lack of germination and growth in the glycerin cells was due to pH effects. In the second scan, the pH of the soil mixtures was adjusted using acetic acid to a pH of between 6.81 and 6.96. Again the higher concentration treatments did not germinate.

The third scan used much lower glycerin rates of 0.01% to 1%. The results of these tests are shown in Table 4. Data for the tests are also provided in Appendix A.

Table 4. Grass germination and growth results: 0.01 to 1%

Concentration (%)	Number sprouts			Total Number sprouts	Plant length (cm): avg +/- stand dev	
	Pot 1	Pot 2	Pot 3		Root	Stem
0 control	15	110	115	240	6.5 +/- 0.9	2.3 +/- 0.7
0.01	0	6	28	34	7.7 +/- 1.5	1.5 +/- 0.6
0.13	1	3	29	33	7.2 +/- 1.1	1.7 +/- 0.6
0.25	0	13	53	66	7.5 +/- 1.1	1.8 +/- 0.6
0.50	15	19	103	137	6.8 +/- 0.8	1.5 +/- 0.8
1	70	107	105	282	7.0 +/- 0.7	1.1 +/- 0.3

The results of these experiments are inconsistent. It is not clear why there were large differences between the three pots for a given treatment. It would appear that concentrations between 0.01 and 0.25% exhibit less germination, but germination numbers at 0.5 and 1% are similar to the control and there are no significant differences between the plant lengths as their confidence intervals overlap.

A fourth scan evaluated concentrations between 3% and 15%; these results are shown in Table 5.

Table 5. Grass germination and growth results: 3 to 14%

Concentration (%)	Number sprouts	Plant length (cm): avg +/- stand dev	
		Root	Stem
0 control	77	6.9 +/- 1.2	1.5 +/- 0.6
3	13	6.6 +/- 1.3	1.3 +/- 0.5
5	9	5.7 +/- 0.7	1.0 +/- 0.5
9	7	5.0 +/- 0.9	0.9 +/- 0.4
14	0	-	-

These experiments show a decrease in germination with glycerol concentrations of 3% and above. Of the plants that germinated, there is an apparent decrease in plant length with increasing glycerol. However, this decrease is not statistically significant as the confidence intervals overlap.

Due to the inconsistency in the data, it is difficult to make conclusive inferences from the germination experiments. It is clear, however, that plants could germinate and grow in low concentrations of glycerol below 10% but would not germinate in concentrations above 10%. It is also clear that the failure of germination at glycerol concentrations above 16% is not solely due to the high pH of the glycerol.

Microbial Test Results

Figure 7 shows a representative respirometer run. The respirometer had 8 cells. In the run shown in Figure 7, two cells had no glycerol (control), four cells had 0.03% glycerol, and two cells had 10% glycerol. Two of the 0.03% replicates tracked very closely together. A third 0.03% replicate started with the other two but then hit a constant, that is, there was no further uptake. A fourth 0.03% replicate had no uptake at all during the experiment. These errors were likely due to a failure in the cell or tubing that didn't allow more oxygen in or a different failure of the system for that particular cell.

Similar errors were observed in a number of the respirometer runs. Another commonly seen error was a nearly vertical rise in the uptake curve due to air leaks in the respirometer cells or tubing. In the lower of the two 10% runs in this experiment, there was an initial jump in the apparent oxygen uptake to 974 mg within the first 30 minutes and then no additional uptake for 3 hours, which is similar to the lag period in the other cells. When the initial jump was subtracted off, the uptake curve was similar to that of the other 10% cell. The adjusted data are what is shown in Figure 7. Note that this adjustment does not affect the oxygen uptake rate (OUR), which is the slope of the uptake versus time plot during the growth period (after the lag).

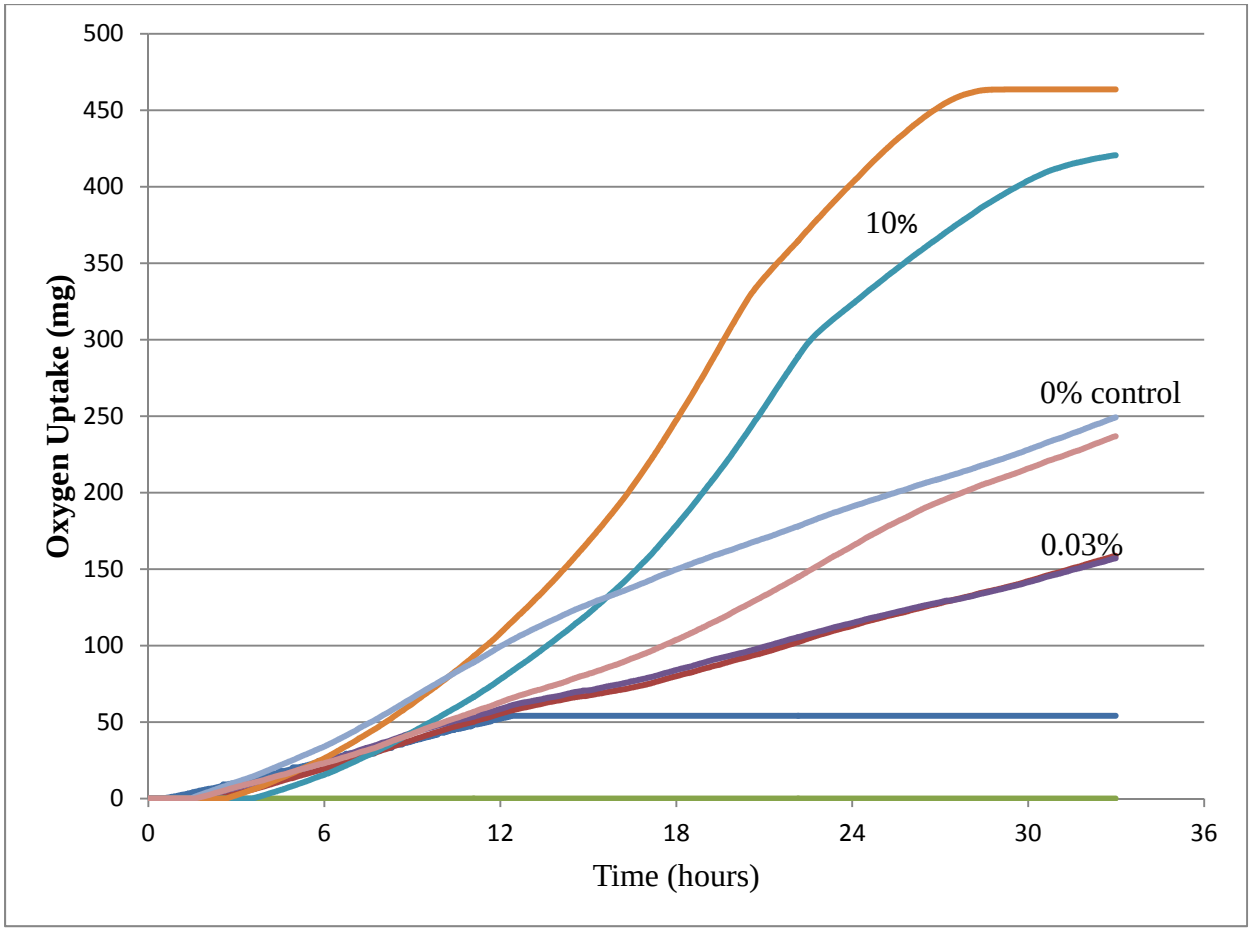


Figure 7. Oxygen Uptake during respirometer run

From this run, the OUR was calculated as the slope from 8 to 25 hours, which is the major period of microbial activity. The 10% curves plateaued after this point. For the 0.03% cell that stopped at 54 mg uptake at 12.4 hours, the rate was the slope from 8 to 12 hours. The uptake rates are shown in Table 6.

Table 6 Oxygen Uptake Rate (OUR) and Glycerol Content ([1]rate 8-12 hours; [2]no uptake data).

Glycerol %	OUR (mg/hr)
0	8.095
0	8.068
0.03	[1]4.872
0.03	4.787
0.03	------[2]
0.03	4.697
10	19.327
10	23.963

There is remarkable agreement between replicates for the same treatment (with the exception of the failed cells). However, it is not clear why the 0.03% glycerol would be less than the control and the 10% more. Each treatment was prepared in one batch and then separated into replicates. Perhaps there is a physical factor that is consistent among the replicates but is different between the preparations. From this run, although we cannot conclude whether the glycerol increases or decreases microbiological activity, we can conclude that there is microbial activity in the presence of glycerol and that glycerol does not severely inhibit microbial activity.

Figure 8 shows a respirometer run with all 8 cells run without glycerol. We can see that two of the cells had an apparent uptake at the beginning of the test and two of the cells had a longer lag than the other cells. In spite of these anomalies, we can use the slope of the curve to calculate an OUR. One of the cells had zero uptake and cannot be used. There is good precision among the values and a tight confidence interval can be calculated. Minor wiggles in the curve that are seen in all curves at the same time are likely due to temperature changes in the lab. Table 7 is a summary of the OUR values for this test.

Table 7. OUR values of cells without glycerol

OUR values (mg/hr)	4.006	3.593	3.405	3.473	4.177	4.113	4.536
mean	3.900						
95% confidence interval	+/-	0.389	=	(3.512, 4.289)			

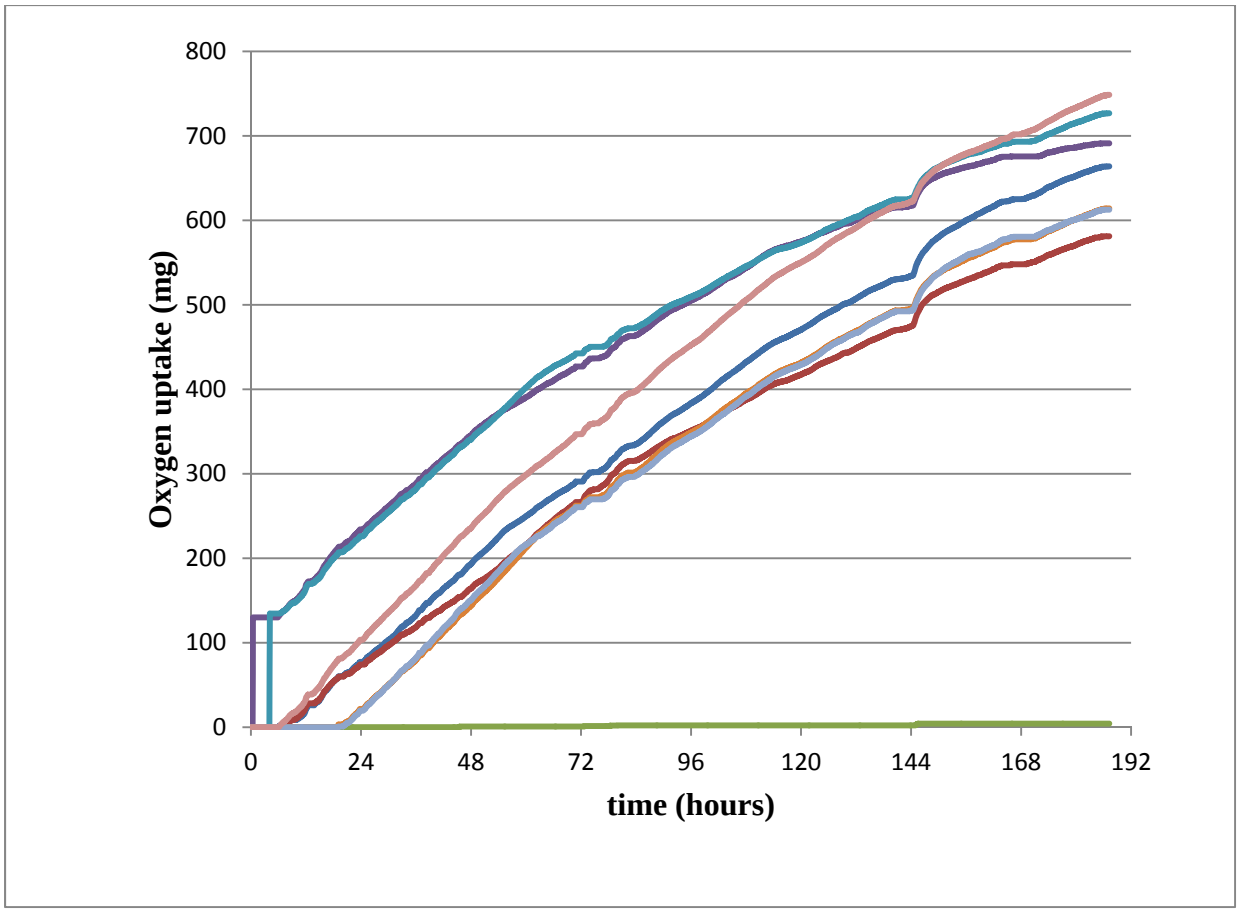


Figure 8. Respirometer run with 8 replicates with no glycerol

Figure 9 shows a run with four replicates each of two glycerol concentrations – 0.1% and 3%. We observed that the 3% cells had higher oxygen uptake than the 0.1%. One of the 0.1% cells failed. Table 8 is a summary of the data:

Table 8. Results of respirometer run with 0.10% and 3% glycerol

0.10%				
OUR values	6.720	6.647	5.827	
mean	6.398			
95% confidence interval	+/-	1.232	= (5.166, 7.631)	
3%				
OUR values	23.187	23.663	25.112	23.349
mean	23.828			
95% confidence interval	+/-	1.399	= (22.429, 25.226)	

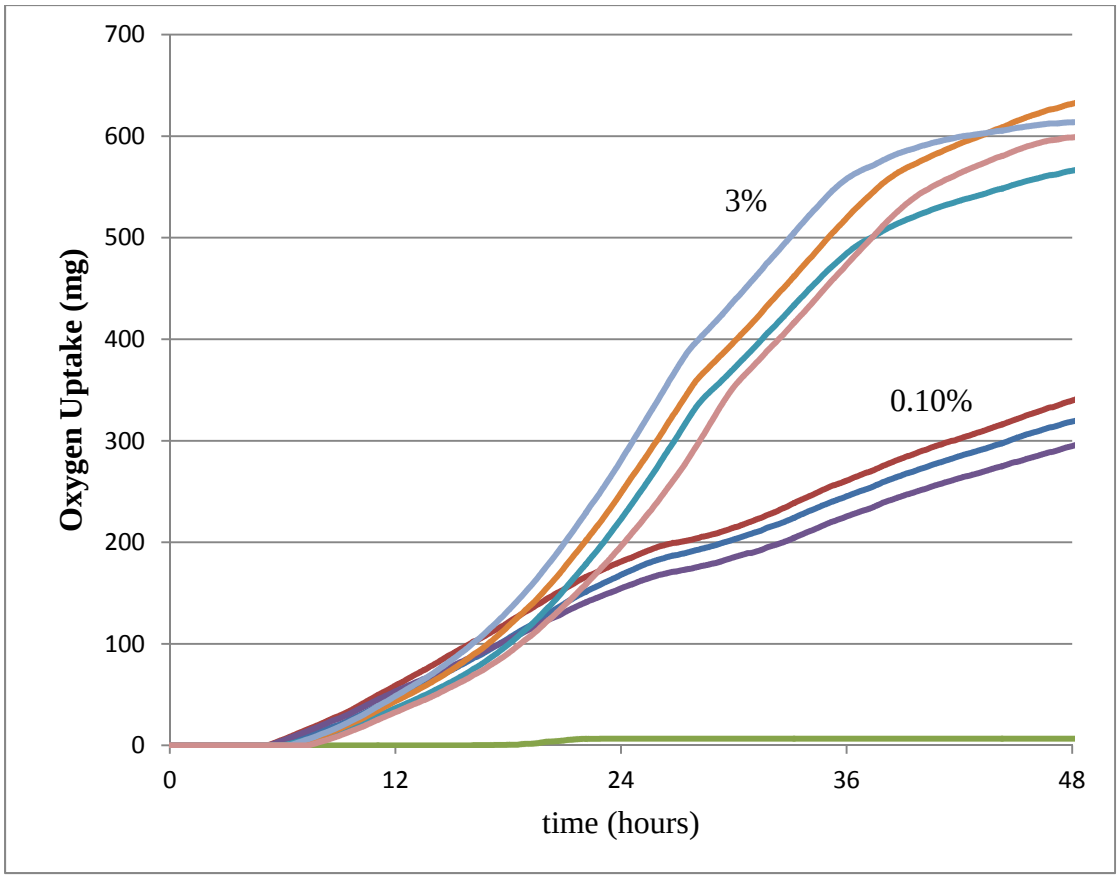


Figure 9. Respirometer run with 0.10% and 3% glycerol

From these results it is clear that the oxygen uptake for 3% is significantly more than that for 0.10%. The oxygen uptake rates for the 3% glycerol cells are similar to that for the 10% cells in the other run. It appears that glycerol as an organic substrate is easily utilized by soil microbes. Again, it is clear that glycerol does not prevent microbial activity. Table 9 summarizes the data for the three respirometer runs summarized.

Table 9. Summary of respirometer runs

Glycerol concentration	OUR: Mean +/- 95% CI
0%	8.095, 8.068 (two values)
0%	3.900 +/- 0.389
0.03%	4.785 +/- 0.217
0.10%	6.398 +/- 1.232
3%	23.828 +/- 1.399
10%	19.327, 23.963 (two values)

Worm Test Results

Figures 10 and 11 and Tables 10 and 11 summarize the worm growth and survival tests. Glycerol concentrations of 9% and above were nearly instantaneously fatal to the worms. The worms vigorously tried to get away from the glycerol amended soil. The worms appeared deflated after dying from contact with the glycerol, suggesting that the hygroscopic properties of the glycerol dehydrated and deflated the worms.

At concentrations of 2% and below, there was no mortality of the worms. Figure 11 shows the growth of the worms. The growth is the percent change in total mass of the surviving worms. At 3% glycerol and above there was a reduction in growth beyond that due to the mortality. So, beside the mortality, the glycerol in concentrations of 3% and above affects the growth of the worms.

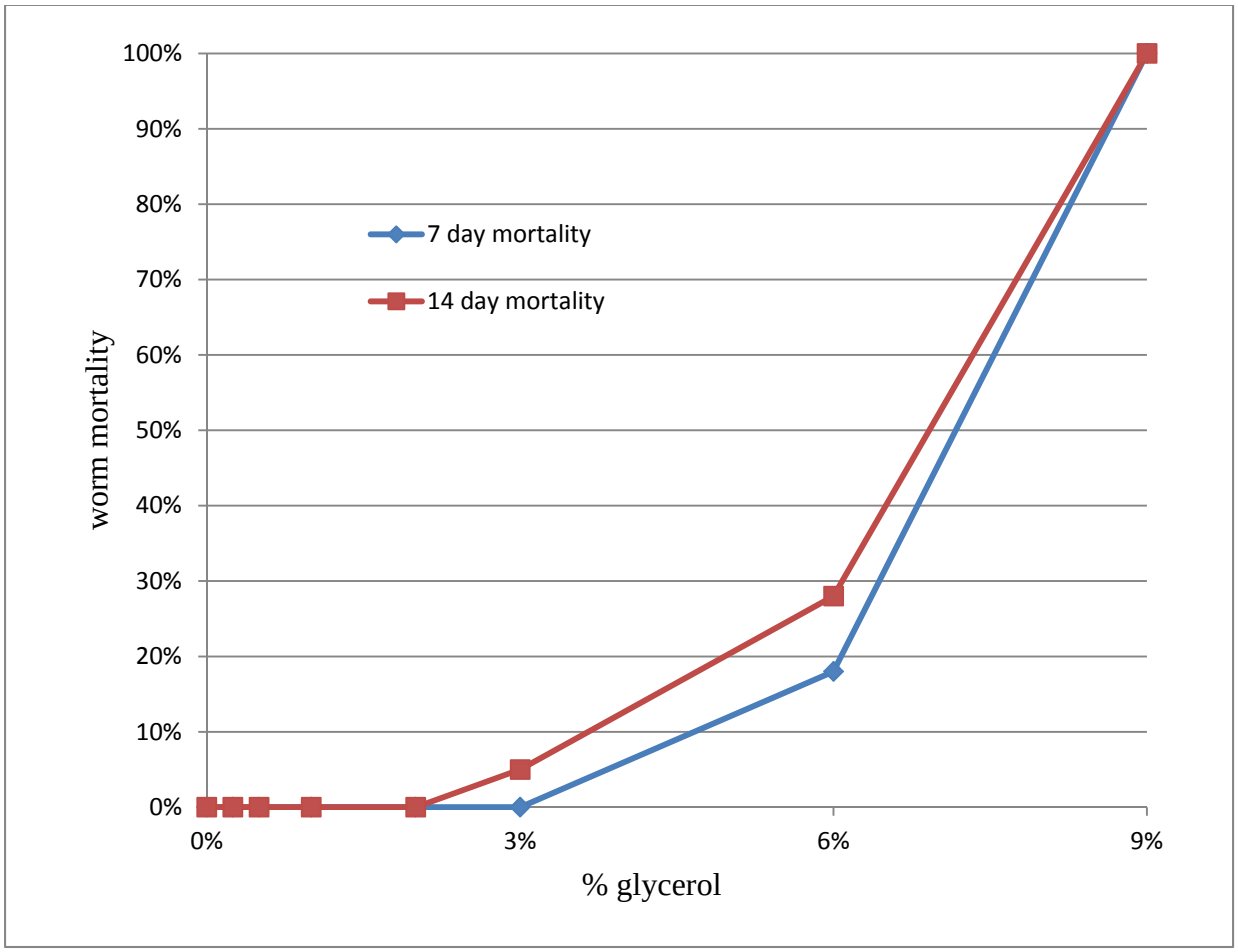


Figure 10. Worm mortality versus % glycerol

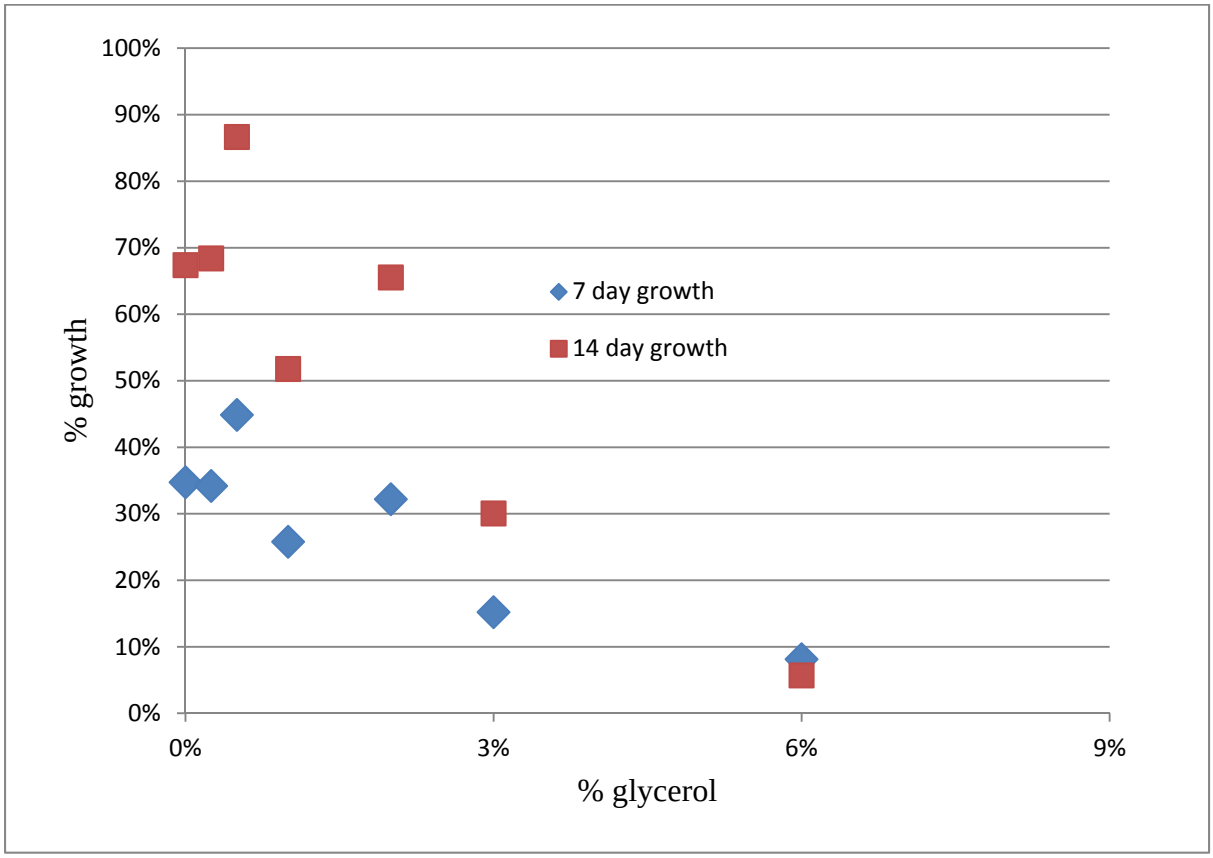


Figure 11 Growth of worms versus % glycerol. The growth is the change in total mass of the surviving worms.

Table 10. Worm mortality

Percent glycerol	7 day mortality	14 day mortality
0%	0%	0%
0.25%	0%	0%
0.50%	0%	0%
1%	0%	0%
2%	0%	0%
3%	0%	5%
6%	18%	28%
9%	100%	100%

Table 11. Worm growth

	7 day growth	14 day growth
0%	0.347351	0.673675
0.25%	0.341909	0.683817
0.50%	0.448517	0.866492
1%	0.257959	0.517551
2%	0.321869	0.655203
3%	0.151943	0.300353
6%	0.081353	0.056875

Glycerol in high concentrations is harmful or fatal to worms, but the worms can survive and grow in concentrations of 2% and below. The data for the worm studies are given in Appendix B.

CONCLUSIONS AND RECOMMENDATIONS

This study assessed the toxicity of biodiesel waste glycerol to plant, microbiological, and biological systems. Plant tests, respirometer tests, and worm studies were used to make the assessments.

Plant tests indicated that glycerol concentrations greater than 1% by weight are detrimental to grass germination and growth. This inhibition was observed even when the soil pH was adjusted to overcome the high pH of the glycerol, indicating that inhibition is not solely due to pH effects. Concentrations less than 1% do not inhibit growth and may by beneficial to growth.

Microbial tests showed that glycerol is an organic substrate that is easily utilized by soil microbial communities. Concentrations up to 10% exhibited microbial activity as evidenced by oxygen uptake. Uptake rates increased at high glycerol concentrations compared to low concentrations and controls.

Worm tests showed that glycerol in concentrations above 2% were fatal to worms. Observations indicated that the lethality of the glycerol is due to the hygroscopic nature of glycerol, which dehydrates the worms. Glycerol concentrations above 2% also retarded growth in worms that survived. At concentrations of 2% and below, there was no observed impact on the survival and growth of worms.

Based on the results of this study, it is recommended that application rates of glycerol to soil be under about 10,000 kg/hectare (10,000 lb/acre), which converts to approximately 1% if the glycerol is distributed in a 0.1 m depth. The rate of 1000 kg/hectare suggested by Schoenau (2010) is an appropriate rate and should not result in toxicity to plant, microbiological, or biological systems.

REFERENCES

Alabama Agricultural Experiment Station (AAES) (2008), "Biofuel By-Product Shows Promise on Crops", *AG Illustrated*, Vol. 6, Issue 1, Fall 2008, College of Agriculture, Auburn University, Auburn, AL.

Cayuela, M.L., Oenema, O., Kuikman, P.J., Bakker, R.R., (2010), "Bioenergy by-products as soil amendments? Implications for carbon sequestration and greenhouse gas emissions", *GCB Bioenergy*, Vol. 2, pp. 201-213.

Chung, W., Huangb, J., & Huang, H. (2005). "Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (Brassica chinensis) caused by Rhizoctonia solani." *Biological Control*, 32(2), 287-294.

Dietrich, Janet, (2007) "Potential Impacts of Biofuel Production on Soils", *Capturing Feed Grain & Forage Opportunities 2007 Proceedings - "Farming for Feed, Forage and Fuel"*, December 11-12, 2007, Government of Alberta, Canada.

Dror, I., Gerstl, Z., Braester, C., Rubin, H., and Yaron, B., (2000) "In situ effect of soil amendments on the dynamics of kerosene attenuation", *Land Contamination& Reclamation*, Vol. 8, No. 4.

EMD Chemicals Inc. (2004, 12 6). Material Safety Data Sheet for Glycerin. Retrieved April 13, 2010, from https://www.vwrsp.com/msds/10/VW5/VW5712-7.pdf

Hall, Luke (2010) *Biobased Foam as a Soil Additive*, M.S. Thesis, Civil Engineering, University of Arkansas, in progress.

Kerr, B.J., Dozier III, W.A., Bregendahl, K. 2007. "Nutritional Value of Glycerin for Nonruminants." In: *Proceedings of Minnesota Nutrition Conference, September 18, 2007, Minneapolis, MN*. p. 220-234. Retrieved April 14, 2010, from http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=214725

Schoenau, Jeff, P. Qian, K. Alotaibi, and R. Urton, 2009, *Potential For Using Biofuel, Crop And Animal Processing By-Products As Soil Amendments To Increase Fertility: Effect of Soil Amendment with Dehydrated Alfalfa, Dry and Wet Distillers Grains, Thin Stillage and Glycerol on Growth of Canola, Soil Properties, and Microbial Activity Final Report,* Department of Soil Science, College of Agriculture, University of Saskatchewan, Saskatoon, SK, Canada.

Siddiqui, S., & Shaukat, I. (2002). "Zinc and glycerol enhance the production of nematicidal compounds in vitro and improve the biocontrol of Meloidogyne javanica in tomato by fluorescent pseudomonads." *Letters in Applied Microbiology*, Vol. 35 , 212–217.

Smith, Sarah (2008), “Pollution Violations May Test Public Support for Biodiesel”, *Biodiesel Magazine*, June 2008.
Tickell, J. (2003). *From the Fryer to the Fuel Tank: The Complete Guide to Using Vegetable Oil as an Alternative Fuel*. New Orleans: Joshua Tickell Publications.

Urbanchuk, John M (2008), *Economic Contribution of the Biodiesel Industry*, National Biodiesel Board.

Appendix A Soil Data and Statistics

Growth Tests – Plant mass

	Raw	Weight	Raw	Weight	Harvest 1	Raw	Weight	Raw	Weight	Harvest 2
10	45.9915	0.0272	46.7222	0.1795	0.1795	45.9815	0.0195	46.6177	0.0787	0.0787
3	46.7351	0.7708	47.708	1.1653	0.96805	47.0945	1.1325	47.0945	0.5555	0.844
1	51.0976	5.1333	50.9356	4.3929	4.7631	47.4022	1.4402	47.9688	1.4298	1.435
0.3	53.116	7.1517	55.1913	8.6486	7.90015	47.9169	1.9549	48.5796	2.0406	1.99775
0.1	50.2064	4.2421	53.075	6.5323	5.3872	47.6514	1.6894	48.3786	1.8396	1.7645
0.03	50.9164	4.9521	52.1324	5.5897	5.2709	47.3826	1.4206	48.3169	1.7779	1.59925
0.01	49.3863	3.422	50.8594	4.3167	3.86935	47.2415	1.2795	48.0014	1.4624	1.37095
0	50.1111	4.1468	50.2654	3.7227	3.93475	47.443	1.481	48.1574	1.6184	1.5497
	45.9643		46.5427			45.962		46.539		

Raw	Weight	Raw	Weight	Harvest 3	Raw	Weight	Raw	Weight	Harvest 4
45.9935	0.032	46.5743	0.0438	0.0438	46.02	0.0548	46.6816	0.1392	0.1392
46.2553	0.2938	46.941	0.4105	0.35215	46.3917	0.4265	47.0117	0.4693	0.4479
46.7143	0.7528	47.2906	0.7601	0.75645	46.7568	0.7916	47.2165	0.6741	0.73285
47.0628	1.1013	47.7254	1.1949	1.1481	47.045	1.0798	47.6503	1.1079	1.09385
47.081	1.1195	47.713	1.1825	1.151	47.9532	1.988	47.7808	1.2384	1.6132
46.9636	1.0021	47.7714	1.2409	1.1215	46.9335	0.9683	47.6055	1.0631	1.0157
46.7751	0.8136	47.6456	1.1151	0.96435	46.925	0.9598	47.6265	1.0841	1.02195
46.8933	0.9318	47.4708	0.9403	0.93605	47.1493	1.1841	47.6719	1.1295	1.1568
45.9615		46.5305			45.9652		46.5424		

9/6/2011	10/13/2011	10/18/2011	10/25/2011	11/8/2011
Concentr	1	2	3	4
10	0.1795	0.0787	0.0438	0.1392
3	0.96805	0.844	0.35215	0.4479
1	4.7631	1.435	0.75645	0.73285
0.3	7.90015	1.99775	1.1481	1.09385
0.1	5.3872	1.7645	1.151	1.6132
0.03	5.2709	1.59925	1.1215	1.0157
0.01	3.86935	1.37095	0.96435	1.02195
0.001	3.93475	1.5497	0.93605	1.1568

ANOVA on plant mass

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
10	4	0.4412	0.1103	0.003682
3	4	2.6121	0.653025	0.089438
1	4	7.6874	1.92185	3.693868
0.3	4	12.13985	3.034963	10.69134
0.1	4	9.9159	2.478975	3.82711
0.03	4	9.00735	2.251838	4.115436
0.01	4	7.2266	1.80665	1.923263
0.001	4	7.5773	1.894325	1.914778
1	8	32.273	4.034125	6.161993
2	8	10.63985	1.329981	0.367156
3	8	6.4734	0.809175	0.166698
4	8	7.22145	0.902681	0.207361

ANOVA

ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	25.50812	7	3.644017	3.354222	0.014657	2.487578
Columns	55.96241	3	18.65414	17.17064	7.25E-06	3.072467
Error	22.81434	21	1.086397			
Total	104.2849	31				

Growth Rates

g/day					
Growth Rates					
	31	5	7	15	days
	1	2	3	4	average
0	0.127	0.310	0.134	0.083	0.163
0.01	0.125	0.274	0.138	0.073	0.152
0.03	0.170	0.320	0.160	0.073	0.181
0.1	0.174	0.353	0.164	0.115	0.202
0.3	0.255	0.400	0.164	0.078	0.224
1	0.154	0.287	0.108	0.052	0.150
3	0.031	0.169	0.050	0.032	0.071
10	0.006	0.016	0.006	0.010	0.009

ANOVA on growth rates						
Anova: Two-Factor Without Replication						
SUMMARY	*Count*	*Sum*	*Average*	*Variance*		
0	4	0.653217	0.163304	0.01007		
0.01	4	0.609768	0.152442	0.007371		
0.03	4	0.722643	0.180661	0.010531		
0.1	4	0.806338	0.201584	0.010836		
0.3	4	0.89654	0.224135	0.018882		
1	4	0.601059	0.150265	0.010026		
3	4	0.282327	0.070582	0.004365		
10	4	0.03773	0.009433	2.11E-05		
1	8	1.041065	0.130133	0.006412		
2	8	2.12797	0.265996	0.014686		
3	8	0.924771	0.115596	0.003402		
4	8	0.515818	0.064477	0.001058		
ANOVA						
ce of Varic	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.140253	7	0.020036	10.88515	9.37E-06	2.487578
Columns	0.177648	3	0.059216	32.17052	4.86E-08	3.072467
Error	0.038655	21	0.001841			
Total	0.356556	31				

Example of paired t-test

t-Test: Paired Two Sample for Means		
	10	*0*
Mean	0.009433	0.163304
Variance	2.11E-05	0.01007
Observations	4	4
Pearson Correlation	0.802222	
Hypothesized Mean Difference	0	
df	3	
t Stat	-3.18249	
P(T<=t) one-tail	0.024999	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.049998	
t Critical two-tail	3.182446	

Germination and growth tests

Scan 1.

application rate = 5lb/1000sqft					length (in	width (in)	
plant test				1pot=	3.1875	3.1875	
common bermuda grass seed				18	pots total		
app rate lb/ft^2=		0.005		1.27002	total area ft^2		
app rate g/ft^2=		2.27		2.882944	total weight of seed, g		
				0.317505	total volume of soil, ft^3		
	depth, in	area in^2	area ft^2	volume, ft^3		mass, g per pot	
	3	10.16016	0.070557	0.017639		0.16	

	60	g fafard soil germination mix	=	1	3 inch size pot amount of soil with 0.25 inch available space at top	
	3/29/2011					
				per pot	29-Mar-11	
					% glycerin	
	soil, g=	60			g, glycerin	
	water, mL=	20			g, soil+water+glycerin per batch	
					pH of soil+water+glycerin per batch	
					pot #s	
					g, soil+water+glycerin per pot	
Instructions						
1. Weight all soil for 3 pots in tared plastic container						
2. add all water for 3 pots and glycerin toegether in tared glass container and mix thoroughly						
3. mix soil into water/glycerin glass container						
4. Put small sample from total mixture into test tube for pH and moisture content tests						
5. Weigh remaining mixture in glass container and record						
6. divide weighed total mixture into 3 pots roughly equal						
7. weigh seeds for each pot on tared paper						
8. Per pot, remove small amount of surface soil, spread seeds, and cover with about 0.25 inch of removed soil						
9. After all pots are prepared, squirt water on soil surface until well moistened						

- used same water volume in each pot and varied te glycerin mass concentration.
- counted water sprays per pot
to moisten soil surface.
- added 30 mL of DI water to 65g of soil, but not all the soil could fit into the pot.
- tried 50 g of soil plus 24 mL DI water, all fit into pot.
-next tried 60 g soil + 24 mL DI water +70% by weight glycerin, all fit into pot. So used 60g soil + 20mL DI water per pot as baseline.

pH glycerin=10.27 pH soil=8.01

no change in pH of glycerin or soil

mass, g of glycerin per pot																	
0%			14%			28%			42%			56%			70%		
0			8.4			16.8			25.2			33.6			42		
253.6			277.9			302.7			328.7			354			382.4		
7.13			8.69			9.11			9.58			9.94			10.09		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
84.42	84.25	84.17	92.26	92.13	92.09	100.53	100.45	100.37	109.23	109.15	109.07	117.47	117.28	117.52	127.02	126.93	126.89

% glycerin	0	14	28	42	56	70

bermuda grass only grew in control with 0% glycerin

did not bother with measuring root and stem lenghts since only control grew

Moisture content								
M1=	mass empty container							
M2=	mass wet soil +container				in oven 24 hr at temp=100.3-100.9F			
M3=mass dry soil+container								
mass moisture in soil=M2-M3								
Mass dry soil=M3-M1								
March 29, no pH adjustment								
March 29, 2011 plants beginning	control	14%	28%	42%	56%	70%		
M1, g	1.48	1.48	1.47	1.48	1.49	1.46		
M2, g	2.02	2.05	2.11	2.26	2.17	2.08		
M3, g	1.79	1.77	1.78	1.86	1.81	1.75		
MC,g=	0.23	0.28	0.33	0.4	0.36	0.33		
MC%=	74.19355	96.55172	106.4516	105.2632	112.5	113.7931		
pH	7.13	8.69	9.11	9.58	9.94	10.09		
april 11, 2011 plants end	control	14%	28%	42%	56%	70%		
M1, g	1.48	1.49	1.48	1.48	1.47	1.47		
M2, g	1.95	1.99	2.04	2.15	2.07	2.01		
M3, g	1.8	1.82	1.75	1.77	1.8	1.71		
MC,g=	0.15	0.17	0.29	0.38	0.27	0.3		
MC%=	46.875	51.51515	107.4074	131.0345	81.81818	125		
pH	7.11	8.7	9.14	9.55	9.97	10.07		

lab 1147 temp approx 75F, humidity 30-60%

lights off 3am-11am		water sprays from bottle									
date	**time**	**control**	**14%**	**28%**		**42%**	**56%**	**70%**	**humidity**	**temp range, F**	
3/29/2011	20:00	7	4	4		4	4	4	21-25	78-81	
3/30/2011	6:30	2	2	2		2	2	2	30-45	77-80	
3/30/2011	22:10	6	4	4		4	4	4	30-65	65-73	
3/31/2011	15:30	6	6	6		4	4	4	29-67	68-74	
4/1/2011	6:00	8	5	4		4	4	4	33-61	71-75	
4/2/2011	10:20	5	4	4		4	4	4	35-59	73-77	
4/3/2011	7:45	6	4	4		4	4	4	29-69	75-77	
4/4/2011	15:00	6	4	4		4	3	3	45-52	63-71	
4/4/2011	22:45	2	3	3		3	3	3	38-76	65-71	
4/5/2011	13:00	3	3	3		3	3	3	38-73	66-72	
4/5/2011	21:30	2	2	2		2	2	2	33-40	72-73	**
4/6/2011	12:00	1	1	1		1	1	1	33-81	65-72	
4/7/2011	11:00	2	2	2		2	2	2	45-82	61-71	
4/7/2011	21:10	2	2	2		2	2	2	45-83	66-70	
4/8/2011	11:30	3	3	3		3	3	3	68-99	65-70	
4/8/2011	18:15	1	2	2		2	2	1	66-99	68-70	
4/11/2011	11:30	ended							63-99	66-69	

**added humidifier to grow box after readings this day due to low humidity

Sprouts			**# sprouts, total number**			
			Control			
date	**time**	Pots	1	2		3
4/4/2011	15:00		2	1		
4/4/2011	22:45		5	2		
4/5/2011	13:00		12	3		
4/5/2011	21:30		13	4		3
4/6/2011	11:00			5		14
4/7/2011	11:00					17
4/7/2011	21:10					22
4/8/2011	10:35					24
4/11/2011	11:30					
		total	13	5		24

Scan 2.
Instructions

1. Weightall soil for 3 pots in tared plastic container
2. add glycerin to mixing container for all three pots and add vinegar amount specified in table about volume of vinegar to add based on glycerin %
3. measure pH, and add more vinegar until pH is approx 7
4. subtract total vinegar volume from the amount of DI water suppose to be added to determine the remaining amount of DI water needed, then add it to mixing container
5. add all water for 3 pots and glycerin toegether in tared glass container and mix thoroughly
6. mix soil into water/glycerin glass container
7. Put small sample from total mixture into test tube for pH and moisture content tests
8. Weigh remaining mixture in glass container and record
9. divide weighed total mixture into 3 pots roughly equal
10. weigh seeds for each pot on tared paper
11. Per pot, remove small amount of surface soil, spread seeds, and cover with about 0.25 inch of removed soil
12. After all pots are prepared, squirt water on soil surface until well moistened

	pH adjustment with distilled white vinegar, diluted by manufacturer to 5% acidity				
		vinegar pH=	2.44		
	8.4 g glycerin in 40 mL of DI water, pH=		10.08		
		mL of vinegar	pH of glycerin, DI water, vinegar mix		
		0	10.08		
soil, g=	60	1	8.92		
water, mL	20	2	8.54		
		3	8.21		
		4	7.99		
		5	7.67		
% glycerin by weight of 60g soil per plant		6	7.22		
		7	6.65		
	now just add vinegar to glycerin % to be tested and check pH				
	glycerin % by weight of soil	glycerin, g per pot	vinegar added, mL	finished pH of mix	
	0	0	0	10.1	
	0.14	8.4	5	6.96	
	0.28	16.8	10	6.81	
	0.42	25.2	16	6.86	
	0.56	33.6	21	6.82	
	0.7	42	27	6.87	

	pH adjustment of glycerin using vinegar								
11-Apr-11	mass, g of glycerin per pot								
% glycerin	0%			14%			28%		
g, glycerin	0			8.4			16.8		
g, soil+water+vinegar+glycerin per batch	240.52			265.33			290.58		
pH of soil+water+glycerin per batch	7.15			6.96			6.81		
pot #s	1	2	3	4	5	6	7	8	9
g, soil+water+glycerin per pot	80.10	79.89	79.73	88.20	87.84	87.76	96.55	96.13	96.24
	42%			56%			70%		
	25.2			33.6			42		
	316.1			343.45			387.84		
	6.86			6.82			6.87		
	10	11	12	13	14	15	16	17	18
	105.07	104.86	104.72	114.15	113.97	113.81	129.12	128.59	128.44

lights off 3am-11am		**water sprays from bottle**								
date	**time**	**control**	**14%**	**28%**	**42%**	**56%**	**70%**	**humidity range %**	**temp range, F**	
4/11/2011	19:30	4	4	4	4	4	3	31-35	67-71	1*
4/12/2011	11:00	3	3	3	2	2	2	41-50	67-70	
4/12/2011	17:15	3	3	3	3	3	2	38-41	69-71	2*
4/13/2011	11:00	3	3	2	2	1	1	38-75	66-72	
4/13/2011	19:00	2						52-70	66-69	
4/14/2011	11:00	1	2	2	2	1	1	56-99	64-69	
4/14/2011	17:00	1	2	2	2	2	1	58-73	67-69	
4/15/2011	11:00	2	2	2	2	1	1	57-99	64-70	
4/16/2011	11:00	3	3	2				30-60	67-75	
4/17/2011	7:00	3	3	3	3	3	3	32-61	68-73	
4/17/2011	20:30	3	3	3	3	1	1	39-99	65-71	
4/18/2011	12:00	ended						65-99	64-70	
			1* humidifier turned off at beginning for experimentation purposes							
			2* humidifier turned back on after this reading							

Sprouts			# sprouts, total number in pot per day			only control grew				
			Control			did not measure shoots or stems for that reason				
date	**time**	**Pots**	**1**	**2**	**3**					
4/17/2011	20:30			3	8					
4/18/2011	12:00				18					
		total		3	18					

April 11, 2011, Plants beginning with pH adjustment to approximately 7 using vinegar, acetic acid 5% solution

April 11, 2011 plants beginning	control	14%	28%	42%	56%	70%
M1, g	1.47	1.49	1.48	1.48	1.49	1.48
M2, g	2.74	2.53	2.48	2.19	2.65	2.81
M3, g	2.23	1.99	1.91	1.79	1.97	2.01
MC,g=	0.51	0.54	0.57	0.4	0.68	0.8
MC%=	67.10526	108	132.5581	129.03	141.6667	150.943396
pH	7.15	6.96	6.81	6.86	6.82	6.87
April 18, 2011 plants end	control	14%	28%	42%	56%	70%
M1, g	1.48	1.49	1.49	1.47	1.48	1.48
M2, g	2.03	2.1	2.24	1.98	1.91	2.05
M3, g	1.88	1.89	1.82	1.68	1.65	1.69
MC,g=	0.15	0.21	0.42	0.3	0.26	0.36
MC%=	37.5	52.5	127.2727	142.86	152.9412	171.428571
pH	7.14	6.99	6.77	6.84	6.79	6.88

Scan 3.

	mass, g of glycerin per pot								
% glycerin	0%			0.01%			0.13%		
g, glycerin	0			0.01			0.08		
g, soil+water+glycerin per batch	240.22			240.26			240.48		
pH of soil+water+glycerin per batch	7.12			7.19			7.26		
pot #s	1	2	3	4	5	6	7	8	9
g, soil+water+glycerin per pot	80.05	79.84	79.46	80.02	79.73	79.59	80.10	79.82	79.53
	0.25%			0.50%			1.0%		
	0.15			0.30			0.60		
	240.69			241.12			242.06		
	7.32			7.51			7.76		
	10	11	12	13	14	15	16	17	18
	80.13	79.86	79.62	80.28	80.01	79.89	80.61	80.13	80.18

lights off 3am-11am		**water sprays from bottle**							
date	time	control	0.01%	0.13%	0.25%	0.5%	1.0%	humidity range %	temp range, F
4/19/2011	21:00	3	3	3	3	3	3	69-88	68-71
4/20/2011	13:00	3	3	3	3	3	3	49-88	64-70
4/21/2011	13:30	3	4	3	3	3	3	42-72	66-71
4/22/2011	12:30	3	3	3	3	3	3	45-81	66-71
4/24/2011	17:00	3	3	3	3	3	3	64-99	66-70
4/25/2011	22:00	3	3	3	3	3	3	65-99	66-70
4/26/2011	14:45	3	3	3	3	3	3	58-99	66-71
4/27/2011	15:30	3	3	3	3	3	3	51-78	67-72
4/28/2011	13:30	3	3	3	3	3	3	37-60	68-74
4/29/2011	18:15	3	3	3	3	3	3	43-58	67-71
5/2/2011	10:00	ended						37-68	67-73
			10 sprays	15	mL				
13			so, 1 spra	1.5	mL, approximately				
			soil, g=	60					
			water, ml	20					

	Sprouts		Plant Pots																
			control			0.01%			0.13%			0.25%			0.50%			1%		
	date	time	pot #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			# sprouts, total																	
	4/22/2011	12:30	numb	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4/24/2011	9:45		0	45	40	0	0	10	0	0	20	0	11	20	0	10	25	10	15	21
	5/2/2011	14:00	**total**	**15**	**110**	**115**	**0**	**6**	**28**	**1**	**3**	**29**	**0**	**13**	**53**	**15**	**19**	**103**	**70**	**107**	**105**

	Control						0.01%						0.13%						0.25%				0.50%				1.00%					
	Pot 1, lengths in cm		Pot 2		Pot 3		Pot 4		Pot 5		Pot 6		Pot 7		Pot 8		Pot 9		Pot 10		Pot 11		Pot 12		Pot 13		Pot 14		Pot 15		Pot 16		Pot 17		Pot 18	
	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stems	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem
average									8.7	1.4	7.4	1.5	8	2	7.3	1.3	7.2	1.9	0	0	8.2	1.4	7.0	2.1	6.7	1.1	7.1	1.2	7.2	2.3			7.1	1.5	6.5	1.5
average																																		
average per 15	6.9	1.2																																
average per 15			7.2	2.4	7.3	2.3	0	0															7.1	2			6.5	1.2						
average per 25			7.9	2.4	6.5	1.5																	6.5	2.1					6.4	1.5	7.9	0.6	6.9	1.1	6.6	1.1
average per 25			5.6	3.1	6.2	3																							7.5	2.1	7.6	0.9	7.4	1.6	7.3	1.2
average per 25			4.7	2.7	5.9	1.3																							6.1	1.2			7.6	1.4	5.7	1.6
average per 25					6.7	2.2																							6.9	2.6			7.1	0.9	5.9	1.4
average per 20			6.1	2.8													5.8	1.7													7.5	0.7				
average per 20																																				
total	6.9	1.2	6.3	2.7	6.5	2.1	0.0	0.0	8.7	1.4	7.4	1.5	8.0	2.0	7.3	1.3	6.5	1.8	0.0	0.0	8.2	1.4	6.9	2.1	6.7	1.1	6.8	1.2	6.8	1.9	7.7	0.7	7.2	1.3	6.4	1.4
	1.6	0.33381			0.89	0.664					7.7	1.5					7.2	1.7					7.5	1.8					6.8	1.5					7.045	1.136
					6.5	2.3					1.5	0.6					1.1	0.6					1.1	0.6					0.8	0.8					0.713	0.341
	thickest clumps of roots for 1%																																			
	thinnest clumps for control																																			

April 19, plants beginning no pH adjustment						
april 19, 2011 plants	control	0.01%	0.13%	0.25%	0.50%	1.00%
M1, g	1.49	1.48	1.48	1.48	1.48	1.47
M2, g	1.88	1.88	1.91	1.86	1.88	1.86
M3, g	1.72	1.71	1.72	1.68	1.72	1.7
MC,g=	0.16	0.17	0.19	0.18	0.16	0.16
MC%=	69.57	73.91	79.17	90.00	66.67	69.57
pH	7.12	7.19	7.26	7.32	7.51	7.76
May 2, 2011 plants end	control	0.01%	0.13%	0.25%	0.50%	1.00%
M1, g	1.49	1.48	1.46	1.49	1.47	1.48
M2, g	1.92	1.87	2.03	2.02	2.03	1.9
M3, g	1.81	1.7	1.78	1.77	1.81	1.72
MC,g=	0.11	0.17	0.25	0.25	0.22	0.18
MC%=	34.38	77.27	78.12	89.29	64.71	75
pH	7.15	7.21	7.31	7.28	7.59	7.83

Scan 4

Plants	soil and glycerin added without pH adjustment
60	g soil
20	mL, DI water per pot
0.16	g, seeds per pot

Instructions

1. Weight all soil for 3 pots in tared plastic container
2. add all water for 3 pots and glycerin toegether in tared glass container and mix thoroughly
3. mix soil into water/glycerin glass container
4. Put small sample from total mixture into test tube for pH and moisture content tests
5. Weigh remaining mixture in glass container and record
6. divide weighed total mixture into 3 pots roughly equal
7. weigh seeds for each pot on tared paper
8. Per pot, remove small amount of surface soil, spread seeds, and cover with about 0.25 inch of removed soil
9. After all pots are prepared, squirt water on soil surface until well moistened

	mass, g of glycerin per pot								
% glycerin	0%			3%			5%		
g, glycerin	0			1.8			3		
g, soil+water+glycerin per batch	240.4			277.9			302.7		
pH of soil+water+glycerin per batch	7.09			7.98			8.01		
pot #s	1	2	3	4	5	6	7	8	9
g, soil+water+glycerin per pot	80.01	79.85	79.74	92.11	92.25	92.17	100.58	100.14	100.27

	9%			12%			15%		
	5.4			7.2			9		
	328.7			354			382.4		
	8.23			8.47			8.76		
	10	11	12	13	14	15	16	17	18
	109.21	109.25	109.13	117.59	117.24	117.08	126.98	127.16	127.02

lights off 3am-11am		**water sprays from bottle**							
date	time	control	3%	5%	9%	12%	15%	humidity range %	temp rang
5/3/2011	18:10	4	4	4	4	4	4	35	71
5/4/2011	13:00	3	3	3	3	3	3	34-37	71-74
5/5/2011	10:00	2	2	2	2	2	2	37-68	67-73
5/6/2011	17:30	2	2	2	2	2	2	43-59	67-71
5/7/2011	13:00	3	3	3	3	3	3	47-99	67-73
5/8/2011	13:00	3	3	3	3	3	3	51-88	68-73
5/9/2011	20:00	3	3	3	3	3	3	54-88	67-72
5/10/2011	11:15	2	2	2	2	2	2	66-99	63-70
5/11/2011	14:30	2	2	2	2	2	2	63-89	66-72
5/12/2011	13:00	2	2	2	2	2	2	62-86	65-70
5/13/2011	14:45	2	2	2	2	2	2	61-86	64-69
5/14/2011	15:00	3	3	3	3	3	3	58-99	66-70
5/15/2011	21:00	3	3	3	3	3	3	63-81	64-71
5/16/2011	12:00	3	3	3	3	3	3	71-83	66-71
5/17/2011	15:00	3	3	3	3	3	3	35-62	66-72
5/18/2011	14:30	3	3	3	3	3	3	44-72	65-71
5/19/2011	14:15	4	4	4	4	4	4	44-86	66-70
5/20/2011	13:00	ended						61-99	66-70

Sprouts			Plant Pots																
			control			3%			5%			9%			12%			15%		
date	time		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		# sprouts, total number in pot																		
5/8/2011	8:00	pot	1	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5/9/2011	11:15		2		30															
5/10/2011	14:30		5		33															
5/13/2011	15:00		8		40			7	4			3			1					
5/20/2011	13:00	total	**19**		**58**			**13**	**9**			**7**			**1**					

			Control				3%				5%						9%						12%						15%			
	ot 1, lengths in c		Pot 2		Pot 3		Pot 4		Pot 5		Pot 6		Pot 7		Pot 8		Pot 9		Pot 10		Pot 11		Pot 12		Pot 13		Pot 14		Pot 15		Pot 16		Pot 17		Pot 18	
	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stems	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem	roots	stem
average	6.7	1.3	0	0	7.1	1.8	0	0	0	0	6.6	1.3	5.7	1	0	0	0	0	5.0	0.9	0	0	0	0	3.8	0.4	0	0	0	0	0	0	0	0	0	0
average																																				
average per 15	6.8	1.3																																		
average per 15																																				
average per 25					7.1	2.3																														
average per 25					6.9	2.1																														
average per 25																																				
average per 25																																				
average per 20																																				
average per 20																																				
total	6.8	1.3	0.0	0.0	7.0	2.1	0.0	0.0	0.0	0.0	6.6	1.3	5.7	1.0	0.0	0.0	0.0	0.0	5.0	0.9	0.0	0.0	0.0	0.0	3.8	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
avg	6.9	1.5									6.6	1.3	5.7	1					5.0	0.9																
stdev	1.2	0.6									1.3	0.5	0.7	0.5196					0.9	0.4041																

May 3, plants beginning, no pH adjustment						
May 3, 2011 plants before	control	3%	5%	9%	12%	15%
M1, g	1.48	1.47	1.48	1.47	1.48	1.48
M2, g	2.07	2.03	2.2	2.12	1.95	1.97
M3, g	1.78	1.72	1.79	1.77	1.69	1.7
MC,g=	0.29	0.31	0.41	0.35	0.26	0.27
MC%=	96.66667	124	132.2581	116.6667	123.8095	122.7273
pH	7.09	7.89	8.01	8.23	8.47	8.76
May 20, 2011 plants after	control	3%	5%	9%	12%	15%
M1, g	1.47	1.48	1.48	1.49	1.47	1.48
M2, g	2.15	2.11	2.14	2.19	2.21	2.09
M3, g	1.93	1.78	1.69	1.81	1.92	1.78
MC,g=	0.22	0.33	0.45	0.38	0.29	0.31
MC%=	47.82609	110	214.2857	118.75	64.44444	103.3333
pH	7.12	7.93	8.04	8.18	8.52	8.7

Appendix B. Worm Data

	Instructions for worms
1	tare plastic container and add 655g soil
2	add correct % of glycerin by weight to soil in container, mix well, remove about 5g of mixed soil for moisture content and pH test
3	record total weight and repeat for all 4 containers for specific glycerin
4	rinse worms with DI water to remove soil, pat dry with paper towel
5	weigh 10 worms and determine the average weight, record
6	add worms to correct container
7	after 7 days, sift through soil to find worms
8	collect all worms from one container and rinse with DI water to remove soil, dry with paper towel
9	count collected worms to determine if new worms were birthed or other died
10	return worms to soil and repeat steps 7-9 after 14 days approximately
11	after the 14 day weighing, reserve small amount of soil for pH and moisture content testing

measure average weight of 10 worms before and after test

check moisture content before and after test

Check pH before and after test

approximately 765 g soil per container with a minimum of three containers per sample type was suggested from the earthworm toxicity test OECD method. I used 4 containers per glycerin % test.

glycerin		glycerin neutralizing test with vinegar	
8.4	g	pH	mL vinegar
		10.07	0
		8.92	1
		8.54	2
		8.07	3
		7.61	4
		6.96	5
		1.7	g glycerin/mL vinegar
		to get near neutral glycerin+vinegar mix	

	April 18, worms beginning, no pH adjustment				
April 18, 2011, worms beginning	control	0.25%	0.50%	1.00%	2.00%
M1, g	1.47	1.46	1.47	1.47	1.49
M2, g	2.54	2.55	2.53	2.49	2.7
M3, g	2.14	2.11	2.12	2.11	2.28
MC,g=	0.4	0.44	0.41	0.38	0.42
MC%=	59.70149	67.69231	63.07692	59.375	53.16456
pH	7.28	7.39	7.55	7.68	7.83
May 4, 2011, worms end	control	0.25%	0.50%	1.00%	2.00%
M1, g	1.48	1.48	1.49	1.48	1.47
M2, g	2.99	2.67	2.59	2.63	2.38
M3, g	2.49	2.25	2.19	2.2	2.04
MC,g=	0.5	0.42	0.4	0.43	0.34
MC%=	49.50495	54.54545	57.14286	59.72222	59.64912
pH	7.3	7.36	7.59	7.71	7.88

May 5,worms beginning no pH adjustment				
May 5, 2011, worms before	cotrol	3.0%	6.0%	9.0%
M1, g	1.48	1.46	1.48	1.47
M2, g	3.41	3.13	3.36	4.19
M3, g	2.52	2.4	2.51	2.98
MC,g=	0.89	0.73	0.85	1.21
MC%=	85.57692	77.65957	82.52427	80.13245
pH	7.25	7.94	8.09	8.34
May 19, 2011, worms end	cotrol	3.0%	6.0%	9.0%
M1, g	1.48	1.48	1.46	1.47
M2, g	3.86	3.27	3.41	4.25
M3, g	3.02	2.63	2.69	3.21
MC,g=	0.84	0.64	0.72	1.04
MC%=	54.54545	55.65217	58.53659	59.77011
pH	7.27	7.97	8.05	8.37

April 14, 2011 worms beginning with			
from previous tests of neutralizing glycerin with vinegar, I found that 1.7g gllycerin required			
1 mL of vinegar to bring the pH down to 7 approximately			
so decided to test worms in same concentration of soil as I did the plants on April 11, 2011			
glycerin neutralizing test with vinegar			
pH	mL vinegar		
10.07	0		
8.92	1		
8.54	2		
8.07	3		
7.61	4		
6.96	5		
1.7	g glycerin/mL vinegar		
to get near neutral glycerin+vinegar mix			

765	g soil per container	
765 g soil plus was too much for mixing in plastic containers used for the worm test and barely fit, so I reduced it to 655g for rest of worm tests		
24 total containers for april 14		actual weights

	worms weighed															
14-Apr-11	control				14%				28%				42%			
container label #s	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
10 worms per container, average weight per 10, g	0.354	0.329	0.342727	0.391	0.358	0.362	0.328									
14-Apr	stopped after 3rd container of 14% glycerin because worms were dead in first 2 container of 14% after about 5 minutes of being in soil															
	did not finish with the rest of the glycerin percentages, decided to start over with lower glycerin concentration later															
	did to measure moisture content since I had to start over with new concentrations anyway															

soil prep for worms, april 14, 2011	vinegar											H2SO4
# of batches made due to limit of weight scale	1	1	1	2		2		4				1
batch label	1	1	1	1	2	1	2	1	2	3	4	
% glycerin	control	14%	28%	42%		56%		70%				14%
g, glycerin per worm container	0	107.1	214.2	321.3	321.3	428.4	428.4	535.5	535.5	535.5	535.5	107.1
g, glycerin per mixing batch	0	428.4	856.8	642.6	642.6	856.8	856.8	535.5	535.5	535.5	535.5	428.4
mL vinegar to neutralize glycerin in soil	0	252	504	378	378	504	504	315	315	315	315	4
pH	7.13	6.94	6.99	6.99	6.96	6.97	6.99	7.01	7.01	6.99	7.02	7.01
total weight of glycerin + vinegar	0	690.6	1365.3	1016.5	1029.2	1378.8	1369.8	858.4	866.4	866.1	861.2	434.1
theoretical weight per container, given 4 containers	0	172.65	341.325	508.25	514.6	689.4	684.9	858.4	866.4	866.1	861.2	108.525
container 1	0	172.5	339.4		514.2		679.8	854.1				105.3
container 2	0	176.2	344.5		510.6		680.7		864.3			104.5
container 3	0	168	341.5	508.8		690				863.2		106.2
container 4	0	168.8	333.5	507.7		688.6					857.5	104.1

18-Apr-11	control				0.25%				0.50%				1%				2%			
container label #s	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
weight of glycerin per container, g	0	0	0	0	1.6375	1.6375	1.6375	1.6375	3.275	3.275	3.275	3.28	6.55	6.55	6.55	6.6	13	13	13	13
wieght of soil +glycerin, g	650.02	650.03	650.01	650.02	651.64	651.62	651.66	651.64	653.28	653.28	653.26	653.27	656.57	656.54	656.56	656.54	663.01	663.03	663.04	663.02
10 worms per container, average weight per 10, g	0.294	0.311	0.298	0.286	0.287	0.303	0.306	0.309	0.309	0.291	0.284	0.262	0.288	0.285	0.311	0.341	0.291	0.263	0.278	0.302
deaths or births of worms after 7 days	0	0	0	2+	0	0	0	0	2+	0	0	0	0	0	0	0	0	0	0	0
average weight of remaining worms after 7 days	0.37	0.379	0.408	0.445	0.416	0.398	0.393	0.41	0.473	0.379	0.382	0.426	0.394	0.372	0.388	0.387	0.374	0.351	0.404	0.37
deaths or births of worms after 14 days	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
average weight of remaining worms after 16 days	0.431	0.454	0.516	0.589	0.547	0.486	0.472	0.524	0.622	0.463	0.475	0.579	0.502	0.465	0.451	0.441	0.467	0.479	0.503	0.428
growth	0.076	0.068	0.11	0.159	0.129	0.095	0.087	0.101	0.164	0.088	0.098	0.164	0.106	0.087	0.077	0.046	0.083	0.088	0.126	0.068
				0.10325				0.103				0.1285				0.079				0.09125
			% growth	35%			% growth	34%			% growth	45%			% growth	26%			% growth	32%
growth	0.137	0.143	0.218	0.303	0.26	0.183	0.166	0.215	0.313	0.172	0.191	0.317	0.214	0.18	0.14	0.1	0.176	0.216	0.225	0.126
			avg	0.20025				0.206				0.24825				0.1585				0.18575
			% growth	67%				68%				87%				52%				66%

5-May-11	control				3.00%				6.00%				9%				12%			
container label #s	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
weight of glycerin per container, g	0	0	0	0	19.65	19.65	19.65	19.65	39.3	39.3	39.3	39.3	58.95	58.95	58.95	58.95	78.6	78.6	78.6	78.6
wieght of soil +glycerin, g	650.01	650.03	650	650.02	669.67	669.64	669.68	669.69	689.35	689.27	689.29	689.31	709.03	708.97	708.94	709.06				
10 worms per container, average weight per 10, g	0.324	0.281	0.365	0.331	0.391	0.363	0.324	0.337	0.36	0.346	0.338	0.345	0.377	0.358	0.364	0.405				
deaths or births of worms after 7 days	0	1+	0	0	0	0	0	0	-2	-1	-1	-3	worms died after approx 1 hr in 9% soil							
average weight of remaining worms after 7 days	0.407	0.376	0.472	0.453	0.421	0.409	0.398	0.402	0.385	0.354	0.371	0.392	didn't continue with 12% as previously planned							
deaths or births of worms after 14 days	0	0	0	1+	-1	0	0	-1	-2	-3	-4	-2								
average weight of remaining worms after 14 days	0.496	0.478	0.584	0.563	0.467	0.461	0.457	0.455	0.391	0.337	0.356	0.384								
	0.083	0.095	0.107	0.122	0.03	0.046	0.074	0.065	0.025	0.008	0.033	0.047								
				0.10175				0.05375				0.02825								
			% growth	31%			% growth	15%			% growth	8%								
growth	0.172	0.197	0.219	0.232	0.076	0.098	0.133	0.118	0.031	-0.009	0.018	0.039								
			avg	0.205				0.10625				0.01975								
			% growth	63%				30%				6%								