Zytonic Experimental Demonstration Research Design
Winter 2019-20 Demonstration Project: Broccoli

Objective: Demonstrate the difference soil characteristics between soil treated with Zytonic or compared to untreated soil using weights of harvested broccoli to quantify benefits.

Summary of Results: Because the Zytonic was applied at randomly assigned blocks, we can utilize a basic linear regression model to get at the casual effect of the Zytonic applications. When we test a basic linear regression model, we find that Zytonic application had a positive effect of 94.44 grams and a p-value of 0.0288. This means the effect of the Zytonic application is statistically significant and is causally increasing the weights of the broccoli by 94.4 grams when it is present in the soil. This means, Zytonic has a positive effects on weights.

Project: 1 acre plot of broccoli to demonstrate use of Zytonic to students and growers.

Zydex Agrees to:

- Provide product MSDS, application or technical manual, and technical support.
- Provide $xxxx for miscellaneous monitoring equipment (e.g., plot-scale flow meters)
- Provide a $xxxx stipend for student scholarships for completion of the project.
- Provide $xxxx research grant to cover the cost of 1 acre of Farm of the Future Demonstration plot (costs of water, irrigation, installation of monitoring equipment, and farm management).
- Provide $xxxx in salary support for project oversight by ag faculty who will also incorporate corresponding soils concepts into curriculum.

West Hills Agrees to:

- Provide students to manage the project and complete associated tasks
- Provide a 1 acre plot designated for controlled growth of a crop (broccoli for Fall/Winter demonstration)
- Provide the following tasks associated with the crop
  - Application of Zytonic in a randomized strip or block arrangement
  - Georeferencing of blocks/plots/strips with GPS and the entry into a GIS project.
  - Planting of broccoli plants
  - Setup of irrigation system
  - Scouting and daily assessment of crop
  - Data collection including
    - Irrigation water metering on both control and treated areas
    - Images of broccoli, with ruler to show length and quality
    - All factors to be the same except for Zytonic application and possibly water applied (water applications will be based on crop water stress measurements, thus, e.g., the amount of chemical and fertilizer applied to be the same on both treated and untreated plots).
    - Average amount of product at harvest in each treatment as well as estimate of standard error in the average value.
    - Images of control and treated crops before harvest
- Provide regular monthly informal updates on progress of this ongoing study and biannual formal reports on results.
- Provide a session on Zytonic at the November 14th Open Farm at West Hills Farm of the Future

Detailed Description:

The broccoli will be planted on one (1) acre at the Farm of the Future on a 132ft x 330 ft plot in Field #2/3. The rows of the broccoli will be hand planted by students to run east to west with 6" between plants and 14" between rows. There will be 65 rows planted on 40" beds, double rows on each bed. The Farm of the Future Farm Manager and student workers will prepare the bed. Irrigation will be accomplished with three sprinkler lines watering the 65 rows for complete and even

coverage. We will be able to measure how much water is being applied to the plot using small plot flowmeters. In addition, six soil moisture sensors with dataloggers will be used within each treatment in the field to determine soil moisture conditions. These will be randomly assigned to three control and three treatment blocks to monitor temporal changes in soil moisture.

Six blocks will be created by dividing the plot into 55' wide blocks (numbered 1 – 6 west to east, as illustrated in the Diagram A). Each block will be divided into north and south sections that are 66' long. Each section will be approximately 3500 sq ft.

Treatment (Zytonic application at recommended rates) and Control (no application) will be randomly assigned to one section of each of the six blocks for a total of six treatment and six control blocks for the plot. The design methodology is shown in the diagram below; this design provides random assignment while maintaining paired control and treatment sections. (NOTE: this is not yet the actual random assignment, but is provided as an example).

Due to the variety in growth rates for the broccoli, we will wait to begin harvesting until the field is 70-80% ready to harvest. We will harvest and weigh 60 broccoli heads from each control and treatment section within in a block (120 broccoli from Block1, Block2, etc) for a total section weight. We will also weigh individual broccoli heads from each section of the blocks to calculate average head weight to determine how the individual broccoli vary compared to the aggregate of the blocks. We will weigh 30 individual broccoli from each of the sections for a statistically robust sample within the blocks. We will ensure that the break of where each section starts/stops is marked using GPS and visually using stakes and flags for the research team to ensure we know which block, section, and the treatment type we are working in.

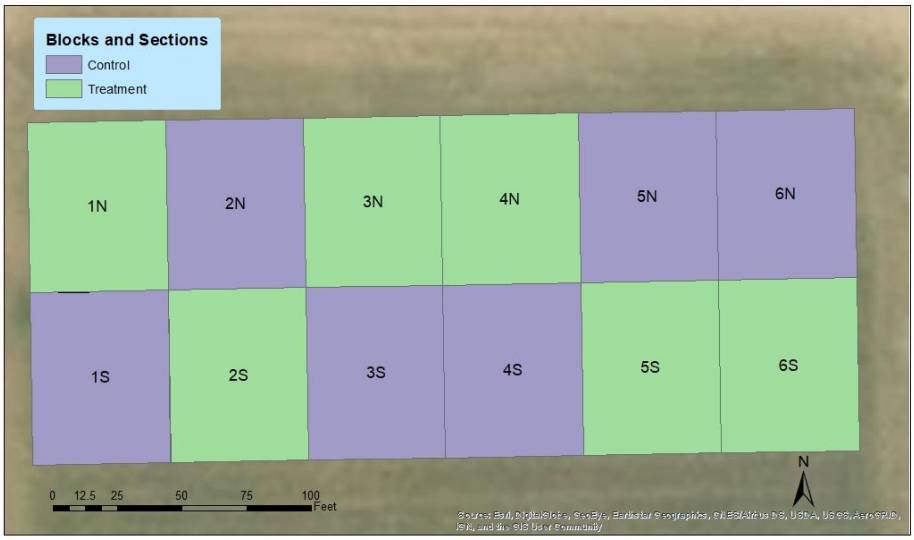
We will also ensure that we do not include broccoli that are in close proximity to treatment/control block 'line' to ensure we are not picking up confounding problems. By this, we mean we will not sample broccoli that are the rows next to where the treatment/control breakoff is. This way, we ensure we are only picking up the treatment effects of the Zytonic application and not confounding the results in anyway.

The following are guidelines used for data collection:

- All samples or collections locations will be randomized and recorded as georeferenced points.
- Students from Introduction to Precision Agriculture will be georeferencing sample locations and entering the spatial data into ArcGIS.
- Trimble Juno 3B units will be used by students for data collection; point averaging over a 2-minute period will be used for sub-foot accuracies.
- All factors to be the same except for Zytonic application on sections randomly selected as treatment.
  - Dependent on approval by Zydex, water applications will be based on crop water stress, transpiration, and soil moisture measurements.

Demonstration Project between Zydex, West Hills College Coalinga Farm of the Future, and West Side Research and Extension Center

A controlled study will be completed on a 132' X 330' one acre plot on the field directly south of the Ag & Ind Science complex. Treatment will consist of a recommended application of Zytonic. Broccoli will be planted and all production practices will be duplicated for all blocks and sections.
Blocks: 1 - 6
Sections: North (N) and South (S) for each block



Detailed Results:
Where the acreage of the project was split up into twelve blocks, six on the north side and six on the south side. Zytonic was applied to the areas where a red minus is present.

For the collection of the broccoli, we also followed the original plan of taking a composite weight of sixty broccoli from each of the twelve blocks. We randomly sampled where to sample in the field, and stayed away from rows which were close to the marked boundaries between blocks. By ensuring random sampling and staying away from boundaries, we could get a clear representative sample from each of the blocks on broccoli weights. After getting the composite weights, we then randomly sampled the sixty broccoli from each of the blocks to weigh thirty pieces of broccoli individually for each of the blocks. We did this to ensure that no bias was introduced when we weighed the individual samples. We recorded the weights of the individual broccoli by placing them on a scale and recording the biomass above ground weight for these broccolis. Here are the descriptive statistics for the aggregate weights:

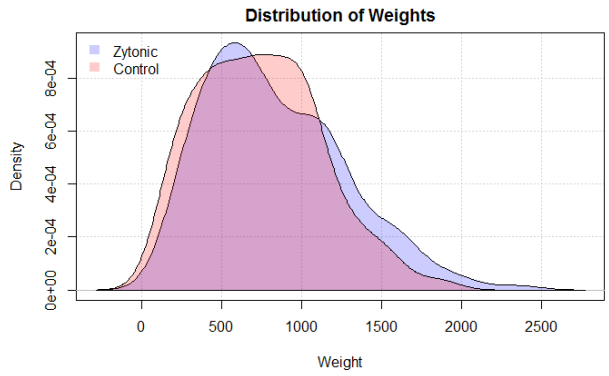
Weights in pounds (Aggregate 60 Broccoli)					
Block 1 N	Block 2 N	Block 3 N	Block 4 N	Block 5 N	Block 6 N
46.6	79	131	113.4	127.6	146.6
Block 1 S	Block 2 S	Block 3 S	Block 4 S	Block 5 S	Block 6 S
60.4	87	77.6	78	151	154.8

Looking at the descriptive statistics for all the collected individual zytonic and control broccoli weights (30 from each block, for a total of 360 individual weights, 180 for zytonic and 180 for control weights). It looks like the following:

Descriptive Statistics - Control Weights in Grams					
Minimum	1st Quantile	Median	Mean	3rd Quantile	Max
75.0	448.2	728.5	742.3	1009.0	1886.0

Descriptive Statistics - Zytonic Weights in Grams					
Minimum	1st Quantile	Median	Mean	3rd Quantile	Max
140.9	499.0	743.5	836.7	1118.0	2358.0

As you can see here, the Zytonic broccoli are higher weights in all categories reported above. I'd like to show this graphically as shown below for the density plots of these weights:



As you can see in the above image, Zytonic weights are reported in blue and the controls are red. This is a density histogram plot, so it is showing us where most of the weights lie for the control and zytonic plots. We see that zytonic has an overlap with the control weights but has a lot of weights

over the controls on the heavier end. This is why we can see more blue sticking out at about 1000 grams and it keeps going out to the 2500s. We can see that in the descriptive stats table above, but this graphically shows us the Zytonic weights are typically above the control weights. Now we need to see if these two groups are statisticaly identically, or if they are statistically different from each other.

Statistical Testing:

Graphics are great for us to see the overlap and differences between groups, but it is not the official way for us to test if the two groups are different. To test this difference, we utilize the Welch two sample t-test to test whether or not the two groups are statistically different from each other. The test itself is looking to see whether the true differences in the means of the two groups is equal to zero, written in statistical notion

H0 (Null hypothesis) = True difference in means equals zero
H1 (Alternative hypothesis) = True difference in means is not equal to zero

If we reject the null hypothesis, then we are saying we have picked up a statistically significant difference in the two groups.

To make this decision for rejection of the null hypothesis, we will base the decision on the p-value from the Welch test. If the p-value is above the significance level, then we will fail to reject the null hypothesis. If the p-value is below the significance level, then we will reject the null hypothesis listed above. The significance level, determined at the start of this project, is a 95% significance level and an associated .05 alpha level. The alpha level, inferred from the significance level, will be the comparison made here. What we are doing then, is comparing the p-value of the Welch test to the 0.05 alpha level. Anything, below 0.05 will mean there is a difference in the groups and a p-value above 0.05 will mean the groups are similar.

Detailed Summary:
Running a Welch t-test, I find a p-value of 0.028 which is below the 0.05 so I reject the null hypothesis. This means there is a statistically significant difference between the groups. This means the difference that you can see in the descriptive stats above for the groups is statistically significant and that the Zytonic broccoli weigh more.

Then we tested the locations of the blocks to see if any difference was present there as well. Using the same alpha level from before, we tested to see if there was a difference between the North and South blocks (comparing all the North blocks to the South blocks). There, I get a Welch t-test p-value of .23 which means there is no statistically significant difference between the North and South weights. This means, there isn't an effect from the North or south position being picked up in the Zytonic broccoli weights. When we do the same testing looking at East vs West weights (Block 1, Block 2, Block 3 vs Block 4, Block 5 and Block 6)I get a Welch t-test p-value of 0.00007 meaning that there is an effect present from comparing West and East blocks. The East side blocks (Block 4 – Block 6) are statistically significantly heavier broccolis than the West side. This may be due to some field effects, but you can see the difference in weights when you look at the original weights in pounds above. I won't speculate to what could have caused this, but I am sure Dr. Ellsworth has some theories as to why this may occur.

Because the Zytonic was applied at randomly assigned blocks, we can utilize a basic linear regression model to get at the casual effect of the Zytonic applications. That is, what is the estimated effect of the Zytonic application on the broccoli weights? When we test a basic linear regression model, we find that Zytonic application had a positive effect of 94.44 grams and a p-value of 0.0288. This means the effect of the Zytonic application is statistically significant and is causally increasing the weights of the broccoli by 94.4 grams when it is present in the soil. This means, Zytonic has a positive effects on weights.