ALTERNATIVE SOLUTIONS TO ASIAN SOYBEAN RUST:

FROM AN ENVIRONMENTAL STANDPOINT

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Acknowledgement

I want to thank Amélio Dall 'Agnol and his family for kindly hosting me during my stay in Londrina, Brazil. They gave me the best accommodations and treated as one of their own family members. This internship would not have been possible without them.

I also want to thank Embrapa Soybean and its fellow staff for agreeing to take on an amateur like me. Embrapa gave me a glimpse of what research is like and provided the opportunity to pursue my interest.

Of course I must thank Lisa Fleming and all of World Food Prize for making this entire trip possible. Thank you for giving the chance of a life time to go to Brazil and intern at a world renowned agricultural research center. This paper is dedicated to you.

Getting Involved

Coming from China to America at a young age has helped me increase my global awareness. Although I came from a middle-class family, I often witnessed the disastrous consequences of poverty and food insecurity. The sight of famished women and children begging for food, or sometimes too weak to even beg, has propelled me to commit to the worldwide effort of ending hunger in our lifetime. Through the guidance of helpful teachers and enthusiastic students who participated in World Food Prize, I found an organization that is dedicated to this goal and has already realized part of this dream.

Not only am I committed to ending world hunger, I am also equally passionate about environmental activism. Knowing that in the next ten years, millions of people will be displaced and robbed of a living due to climate change, a man-made phenomenon, reaffirms my belief and my dedication. I want to use my abilities to try to solve this imminent catastrophe and also galvanize others to join in this movement. Although I cannot reverse climate change by myself, I can, however, contribute my efforts in stopping the alarming progression.

After expressing my enthusiasm about the environment to World Food Prize, the foundation kindly assigned me to Embrapa Soybean in Brazil. I then was designated the role of working with organic fungicide to treat the virulent Asian soybean rust. Because soybean rust is a threat to food security in South America, and more importantly the plant pathogen has become resistant to current fungicide treatments, organic fungicides are an ideal solution to ending world hunger and saving the environment at the same time. As I would soon find out, not only did I work with environmental aspects of agriculture, I also made a direct impact on decreasing food insecurity in the world.

Smooth Transition

Embrapa Soybean is situated in the country outskirts of Londrina, Parana in Brazil. As a leader in cultivation of soybeans in tropics by genetically engineering plants to be resistant to certain stresses and major diseases, Embrapa strives now to incorporate biological control for soy pests and other pathogens, which contributes to reducing the use of pesticides and fungicides. Because it is a relatively new scientific field in Embrapa, the department is slowly expanding and gaining attention and anticipation from worldwide farming industries. The 73 researchers at Embrapa Soybean are dedicated in finding innovative solutions for soybean production stability.

I was fortunate to be a part of the Embrapa Soybean team and participate in the current research with Asian soybean rust control. My supervisors, Claudine Dinali Santos Seixas and Clara Beatriz Hoffmann-Campo introduced me to this field and provided me with so much guidance. Claudine is a plant pathologist who also happens to be passionate about environmental protection. She integrates environmental friendly methods into her treatments of pests and diseases. Since Asian soybean rust is an undeniable menace to tropical cultivars of soybeans, she devotes most of her time to finding a solution to this invasive fungus. She often works in collaboration with Clara Beatriz, who formally specialized in entomology and has recently been assigned to pathology. Because of Beatriz's extensive understanding of entomology, she has provided valuable research materials for me that broadened my knowledge base of the intricate relationship between biological control and pests. Both of my supervisors provided an abundance of opportunities for me to explore organic agriculture and also gave me the freedom and independence to conduct the research. Whenever I was in need of help, they often came to my rescue. The success of my research was a direct product of their teaching and guidance.

I am so thankful for my supervisors, but I am equally thankful for the warmhearted college interns in the Embrapa laboratories and all the employees. They made my transition from America to Brazil so much easier. Thanks to their kindness and hospitality, I did not feel any cultural shock. I was cordially invited to all office parties and small gatherings. Even though I tried to learn Portuguese, they also worked hard to learn English. But when hand gestures and miming failed, Google translator became a convenient aid (although at times the translator did create some misunderstandings). So without too much effort, I made some unforgettable friends and despite the language barrier I became a part of the Embrapa family.

Responsibilities

The research on organic fungicide is a continual process at Embrapa. I had the opportunity to initiate the experiment with Asian soybean rust. I tested the effectiveness of three organic treatments against the virulent pathogen. However, two months is not enough time to do satisfactory research, so while I started the experiment, other Embrapa scientists will finish the project.

With Claudine and Clara Beatriz's help, I set up the experiment and then used high performance liquid chromotography (HPLC) to analyze the specified compounds produced by the soybean plants. The lab technicians trained me in the identification process, and with Clara Beatriz's suggestions, I learned the basic mechanism of HPLC. Although Claudine and Clara Beatriz gave me guidelines in the research process, they expected that I carry out the experiment independently. I completed the collection of samples, preparation for HPLC, and the identification process autonomously, after my supervisors trained me. Of course, if I had any doubts and any errors, Claudine and Clara Beatriz would help me and give me recommendations. After I completed my two months study, I provided them with the data I collected and analyzed. As it will be delineated later, four repetitions were supposed to be carried out, but due to the time constraints, I only completed one full repetition. The researchers and interns will use my data to finish the analyzation of the three fungicides.

Impact

As the research will be presented later proves, of the three products tested only one of which showed promising results. However, it did not stop the progression of the rust in the soybean plants. The treatment only alleviated the symptoms. Although the research was not entirely successful, it reaffirms the need to augment our efforts on the research of organic agriculture. The current research will not prevent the dire consequences caused by pests and other diseases, which could worsen with the diseases' expansion into new territories. However, this research also shows promising results. Although it cannot substitute traditional fungicides entirely, it can be used alongside the available treatments. This research can temporarily relieve food insecurity problems in certain regions of the world; it cannot, however, solve the dilemma entirely. Researchers around the world should focus more attention to organic agriculture and discover innovative solutions that can reduce the stress on the environment and provide an abundant supply of food.

I might not have had a huge impact on food security, but this research experience definitely influenced my beliefs. My environmental fervor did not end; it only grew stronger after going to Brazil. I understood the need for people to pioneer this new field, and the world needs individuals like me who have the motivation and the determination to answer these conundrums. After all, if I do not do anything, who else would? The change starts with me. I am so thankful that World Food Prize has given this opportunity of a lifetime to me. Because of the research at Embrapa, I am committed to reverse environmental degradation and end food insecurity.

Abstract

Three products were tested for effectiveness in preventing Asian soybean rust (Phakopsora pachyrhizi) and promoting the production of phytoalexins in soybean culture BR 154. The three products are Sodium Silicate, Fish Fertil (composed of fish, sugar cane molasses, phosphoric acid, and crust, and crustaceous) and Calda Viçosa (a mixture of salts and calcium). All three products are composed of organic elements or that of nontoxic compounds, which are biodegradable and therefore less environmentally detrimental than the conventional fungicide.

The experimental plants were classified into three categories and two subcategories, and one control (total of seven categories). These were the following categories: Sodium Silicate (with inoculation/without inoculation), Fish Fertil (with inoculation/without inoculation), Calda Viçosa (with inoculation/without inoculation), no products and no inoculation

The plants were then assigned an identification number and then randomly placed in a treatment. Seven plants (each with their own treatment) were then placed on a table in a greenhouse. Because the conditions of a greenhouse are not always uniform, seventy plants were used and placed on ten different tables. Each table represented the variety of environmental situations in the greenhouse. Samples (leaflet of each individual plant) were collected before inoculation, three days, four days, and five days after inoculation, which made for a total of four collections. There should be four repetitions, each with seventy plants. However, due to the time constraints, only one repetition was completed.

The samples were then analyzed using HPLC (high performance liquid chromatography) to identify specific compounds produced by the soybean plants. Because soybeans produce abundant isoflavonoids and phytoalexins that have shown to have anti-fungi mechanisms, measuring the levels of these compounds in each treatment and then comparing the results would test the effectiveness of the products. The treatment that promotes the most isoflavonoids and phytoalexins productions would be the most effective against the pathogen.

In this experiment, Fish Fertil tested to promote the most isoflavonoids and phytoalexins production, while the other two promoted about the same level of productions. This showed a direct correlation between the effectiveness of the product and the level of production of the compounds. Fish Fertil was the most effective against the fungus, followed by Calda Viçosa.

Introduction

Asian rust (*Phakopsora pachyrhizi*) has caused huge devastation to soybean growers around the world. It has been reported that yield losses could be as high as 40 percent in Japan, 10-50 percent in China, and 23-90 percent in Taiwan. In the Eastern Hemisphere, the causal fungus has infected 64 leguminous hosts. Unfortunately, the fungus has spread to the West. It was first reported in 1976 in Puerto Rico during an experimental planting and then later in Brazil and Colombia (Sinclair, Backman). According to J. T. Yorinori, the rust has been found on an estimated 60% of soybean acreages, and it is particularly destructive in the south of Brazil. In the southern states of Brazil, Goias and Mato Grosso, yield losses has been as high as 30-75 percent in 2002, which is the equivalent to monetary losses of US $125.5 million, at $220.5 per ton.

The disease usually begins with small, water-soaked lesions, which then increase in size, turning from gray to tan or brown. They usually assume a polygonal shape and can reach a size of 2-5 mm2. Lesions generally appear on leaves, but can also surface on petioles, pods, and stems. These lesions are associated with leaf chlorosis, and premature defoliation and early maturity (Sinclair, 1989).

The causal organism best survive in environments where the mean daily temperature is less than 28 degrees C, with precipitation or long periods of humidity. These conditions are quite common in Brazil during its winter months. The development of rust is inhibited by dry environments, excessive precipitation, or daily mean temperatures greater than 30 degrees or less than 15 degrees.

Because the rust is especially virulent, much of soybean farmers' time and money has been devoted to controlling the rust. In 2002, 80 percent of acreages in Brazil had an average of two fungicides sprays. At the cost of $40.00/ha, a total $592 million was spent on control (Yorinori, 2002). But due to the frequent sprays, the rust has become resistant to some of the common fungicides used. The effectiveness of the products decreases as the year goes on, and so new measures must be introduced to control and prevent the rust from spreading. Also many of these products have detrimental effects on the environment. If some of the toxic compounds seep into ground water and pollute the rivers, it can contaminate the food supply and more importantly disrupt the balance of the ecosystem. To avoid these potential man-made calamities, new methods must also incorporate environmental safe measures. All of these requirements highlight organic products as a potential answer to rust control.

However, despite the numerous benefits that organic fungicide may offer, it can only prevent and build the plants' immune response. It cannot, however, completely kill the rust. So in soybeans they must be able to promote phytoalexin productions. Phytoalexins are toxic antimicrobial substances produced in plants only after stimulation by injury caused by pathogenic microorganisms, chemical or mechanical. Isoflavonoids are the most commonly produced phytoalexins in the legume family (Agrios, 105). These inducible pterocarpan phytoalexins have been shown to have fungitoxic effects. The most common soybean isoflavonoids are conjugates of daidzin and genistin. Although daidzin and genistin are primarily constitutive, the hydrolyzed aglycone daidzein is the immediate precursor of glyceollins. Glyceollin, another phytoalexin in soybeans that has been extensively studied, is strongly induced in response to stresses of all types, and infections of pathogenic fungi (Parniske, 1991). To test the effectiveness of the products against the Asian soybean rust, it is essential to measure the amount of phytoalexins, isoflavones and glyceollins in the plants before and after infection.

Materials and Methods

Three products were tested: sodium silicate, Fish Fertil, and Calda Viçosa. 70 plants of the BRS 154 variety were used. The BRS 154, developed by Embrapa Wheat and Embrapa Soybean, originated from a crossing between Embrapa 1 x Braxton. The cultivar was designed to be cropped in southern Brazil. This variety has a high yield potential and good adaptation under no-tillage system. It has resistance to certain pathogenic fungus, like stem canker caused by *Diaporthe phaseolorum*, but it has no resistance against soybean rust (Bertagnolli, 2002).

Sodium Silicate solution was prepared mixing 20 mL of the prepared compound with 2000 mL of distilled water.

Fish Fertil composed of fish, sugar cane molasses, phosphoric acid, crustaceous was also prepared in a solution. 50 mL of the product was drawn with a syringe and mixed with 2000 mL of distilled water in a 2 liter plastic bottle.

Calda Viçosa is composed of 2 kg of $CuSO_4$, 4 kg of $ZnSO_4$, 1.2 kg of $MgSO_4$, 0.4 kg of H_3BO_3, 0.64 kg of Ca. The product was modified because the original formula contained ammonium, which is an unnecessary source of nitrogen for soybeans. After all the individual components were weighed, they were dissolved in 2000 mL of distilled water in a 2 liter plastic bottle.

The 70 plants selected were all grown in greenhouse conditions in individual pots. Unfortunately, all the plants were infected by another pathogenic fungus, powdery mildew (*Microsphaera diffusa*). The pathogen grows best in dry environments with a mean temperature between 18 and 24 degrees C. If left untreated, the fungus could cause stunting and distortion of leaves, buds, growing tips, and fruit. It also can reduce growth and vigor of the plant. Because the plants were grown in conditions best for combating Asian rust, which requires high humidity, the powdery mildew had an advantageous environment to grow. To correct for this unexpected situation, milk was used as a fungicide to control the mildew. Approximately 1000 mL of milk mixed with 2000 mL of water was sprayed on the leaves of all plants. Although milk has proven successful in treating mildew, the exact reason behind this phenomenon is unknown at this time.

The experiment was divided into six treatments and one control. The treatments were as followed:

1. Plants treated with sodium silicate with inoculation of Asian soybean rust
2. Plants treated with sodium silicate without inoculation of rust
3. Treatment of Fish Fertil with inoculation of rust
4. Treatment of Fish Fertil without inoculation of rust
5. Treatment of Calda Viçosa with inoculation of rust
6. Treatment of Calda Viçosa without inoculation of rust

The control was no treatment and no inoculation. Beside the treatments, the experiment also included ten conditions. Since the environment within a greenhouse cannot be guaranteed to always remain uniform, the effectiveness of the products can also vary depending on the amount of sunlight exposure and humidity. Ten tables were set up in the greenhouse, where each table represented a different micro-environment. Seven plants were assigned to a table, and each of those seven plants represented one of the seven treatments. Because it is an experimental setup, the plants were randomly assigned a treatment. Below is a table of the assignment plan.

Treatment:

1. Sodium Silicate with inoculation
2. Sodium Silicate without inoculation
3. Fish Fertil with inoculation
4. Fish Fertil without inoculation
5. Calda Viçosa with inoculation
6. Calda Viçosa without inoculation
7. Without products and inoculation

ID		**ID**		**ID**		**ID**		**ID**	
101	4	201	5	301	2	401	5	501	3
102	2	202	1	302	3	402	6	502	2
103	5	203	4	303	6	403	1	503	5

104	3	204	3	304	4	404	3	504	6
105	1	205	6	305	1	405	7	505	1
106	6	206	7	306	7	406	2	506	7
107	7	207	2	307	5	407	4	507	4
ID		**ID**		**ID**		**ID**		**ID**	
601	1	701	3	801	1	901	5	1001	5
602	4	702	7	802	3	902	6	1002	3
603	5	703	1	803	4	903	1	1003	6
603	2	704	4	804	5	904	4	1004	2
605	3	705	2	805	7	905	7	1005	4
606	7	706	6	806	6	906	2	1006	7
607	6	707	5	807	2	907	3	1007	1

There were four collections, before inoculation, 72 hours after inoculation, 96 hours after, and 120 hours after. All collections followed the same procedures. The samples were collected from each plant by cutting the 3 leaflet on top of the plant and then wrapped in aluminum foil and labeled with the appropriate ID number. Then to preserve the samples before analyzing them back in the laboratory, all samples were placed in a styrofoam box filled with liquid nitrogen.

After preparing the formula for each product according to the above instructions, they were placed in three separate spray cans. All plants marked for treatments were taken outside of the greenhouse to be sprayed. Each of the treatment was sprayed on the leaves of plants and then the plants were left outside for about 10 minutes for the spray to condensate.

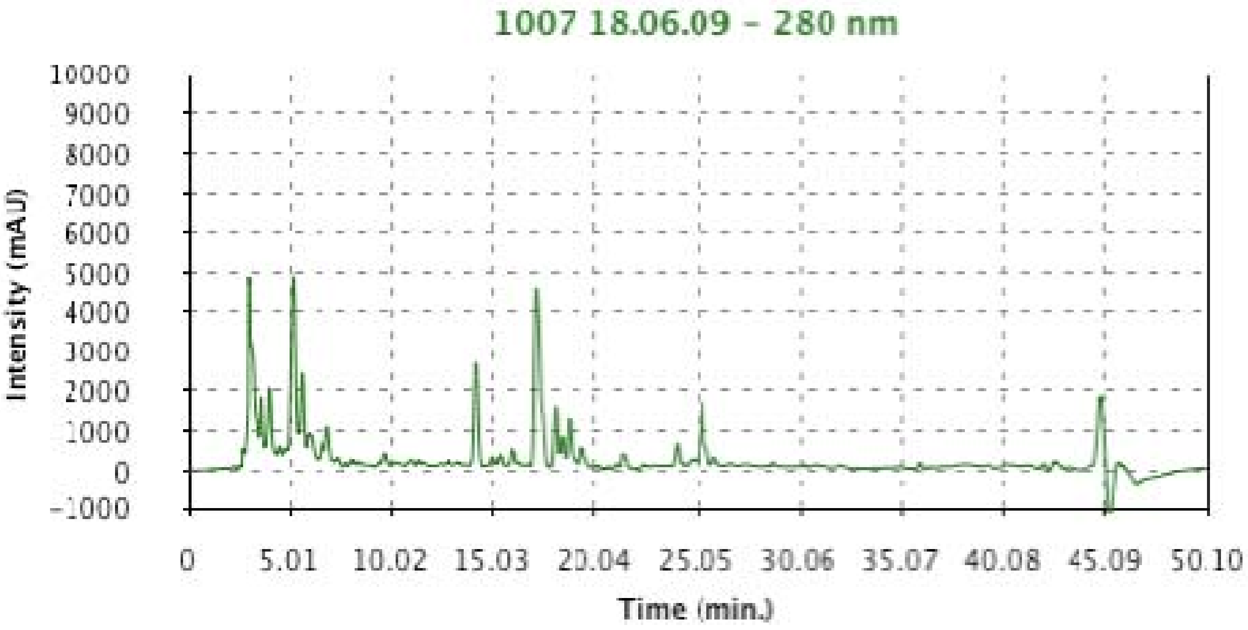
To prepare the inoculum, spores from the rust were collected from plants in a different greenhouse by gently tapping the infected plants' leaves. Then spore concentration was measured using the Neubauer Square method. Below is the process.

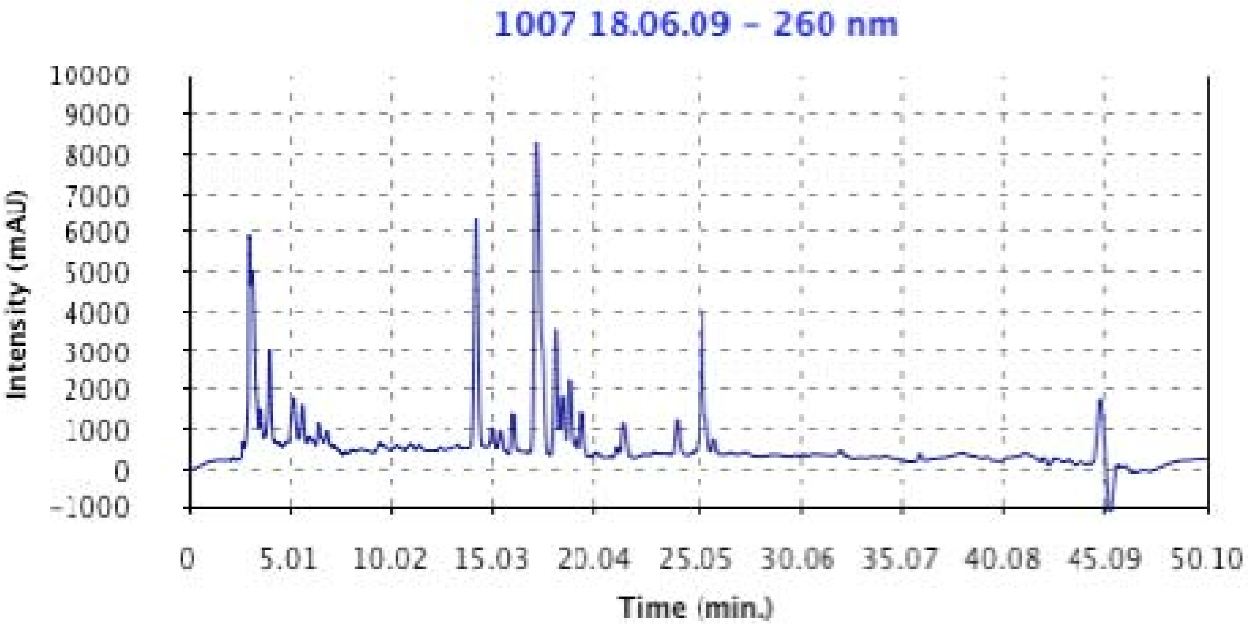
	First Square	**Second Square**	**(1st + 2nd)/2**
1st Repetition	33	21	27
2nd Repetition	26	24	25
3rd Repetition	19	22	20.5
Total Average	24.166		
Spore Volume	241666.7/mL		

After determining that the spore volume was sufficient to ensure effective infection, the spores were mixed with 5 mL of 0.001% Tween 20 solution (Tween separates the spores). 2 L of water was added to the mixture, and the solution was then placed in a spray can. Before spraying, all the pots marked for inoculation (Treatment 1, 3, 5) were taken to a separate room of the greenhouse to isolate them and prevent any transfer of inoculum to the other plants. The inoculum was sprayed on all the plants' leaves, and 1 L of it was sufficient to cover all the plants. After the plants were sprayed, they were placed back in the original positions. The plants on each table were rearranged to accommodate the plants not inoculated and to ensure that the rust did not spread to nearby plants. However, due to space limits, the spores of the rust could transfer. Once the plants were placed in their designated spots, nebulization of the greenhouse was turned on and operated once every hour. Because rust depends heavily on humidity and wind, nebulization assured that the spores would germinate and infect the soybean plants.

Once the samples from each plants were collected before inoculation, 72 hours after, 96 hours after, and 120 hours after, the samples were then prepared for High Performance Liquid Chromatography (HPLC). Soybean leaves from each collected sample were grounded with pistil and mortar. Liquid nitrogen was added to the sample to make the grounding process more effortless. After grounding the foliage into a fine powder, 500 mg of sample were placed in a test tube. Then 300 mL of an 80 percent of methanol solution, measured with a pipette, were placed in the test tube. All test tubes were then subjected to sonication in an ultrasound machine for 20 minutes. The purpose of sonication is to mix the foliage with the methanol completely. Extracts were then filtered to remove unnecessary plant debris and membranes and placed in a vacuum to be dried. After the samples were completely dried, they were re-dissolved in 100 mL of 80 percent of methanol and then filtered through Acrodiscs using a filter-syringe. Approximately 20 µL of samples were ready for HPLC injection.

The HPLC identified and quantified isoflavones and glyceollins. Compounds were eluted in a linear gradient system, composed of solvents: (a) 2 percent of acetic acid, (b) solution of methanol, acetic acid, water (18:1:1). The initial gradient were 75 percent of (a) and 25 percent of (b), changing to 25 percent of (a) and 75 percent of (b) in 40 minutes, and then returning to initial condition at 45 minutes. The HPLC run flux was 1.0 mL/min and the absorption was measured at 260 and 280 nanometers. Finally, retention times on the column spectra of observed compounds were compared with those obtained from standards. Below are two examples of the graphs (Plant 1007) observed at 260 nm and 280 nm.

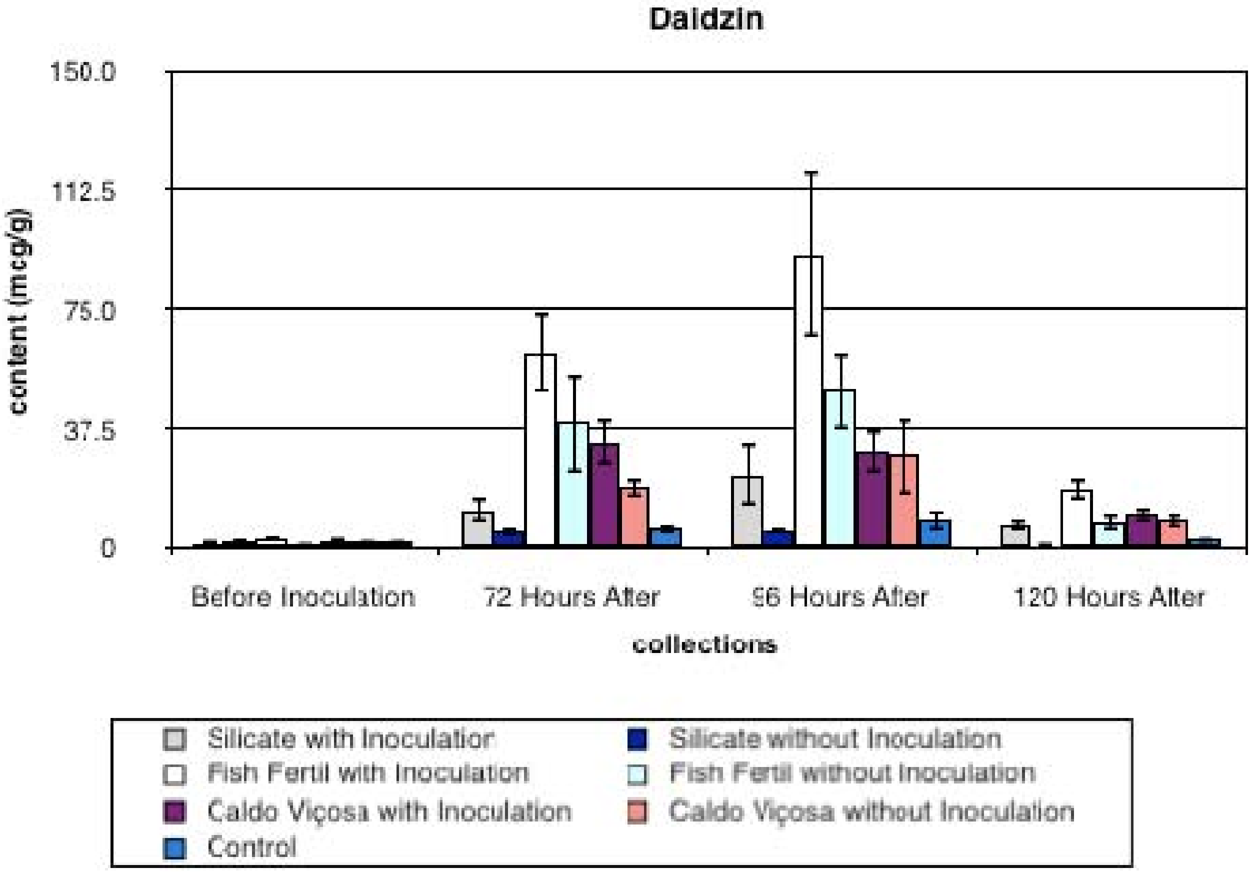

1007 18.06.09 - 280 nm
Intensity (mAU)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
0
5.01
10.02
15.03
20.04
25.05
30.06
35.07
40.08
45.09
50.10
Time (min.)


1007 18.06.09 - 260 nm
Intensity (mAU)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
0
5.01
10.02
15.03
20.04
25.05
30.06
35.07
40.08
45.09
50.10
Time (min.)

Results

Once the isoflavones and glyceollins were identified and quantified, the data was reorganized to find the average of each compound found in each treatment. The data was then compared to find a correlation. The following compounds were found in every treatment: daidzein, glycitein, genistein, and their respective conjugates (acetyl, malonyl), and glyceollins.

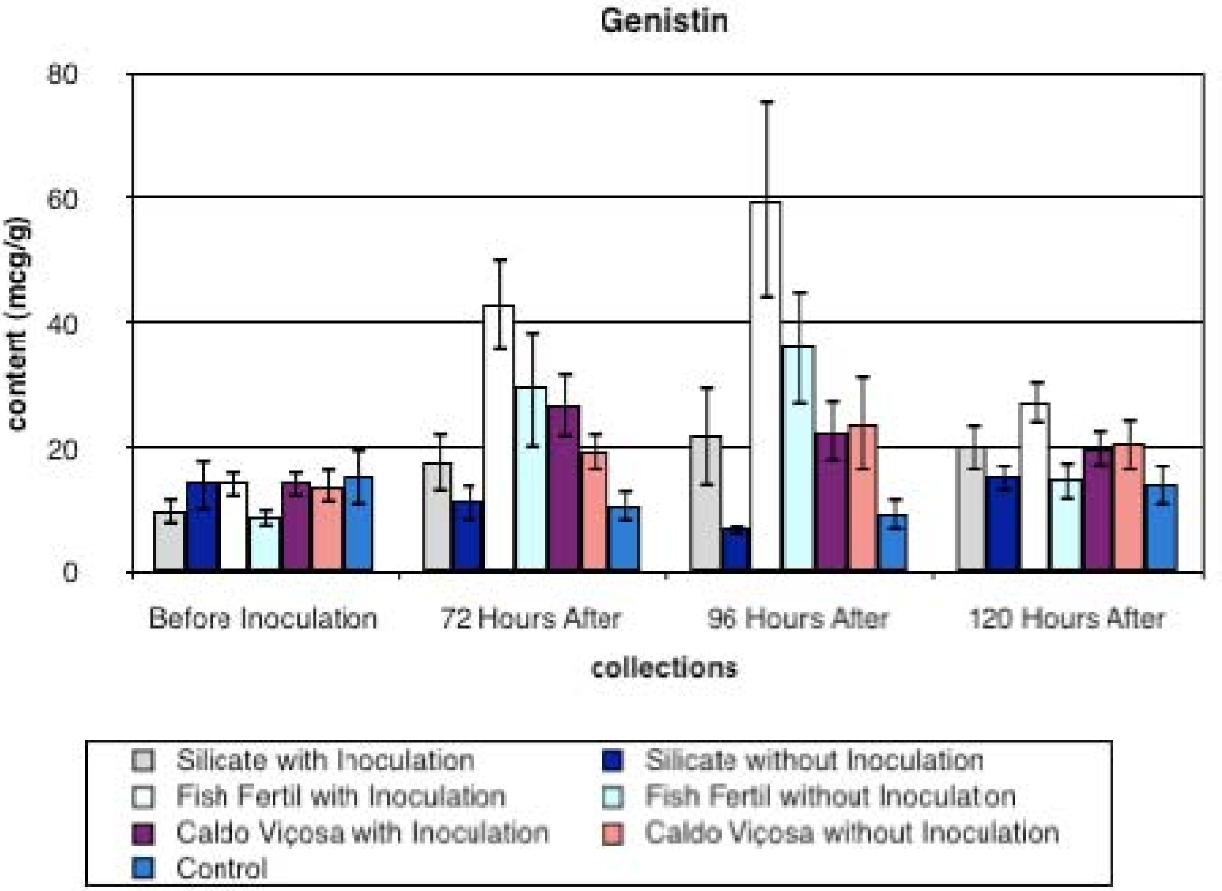
Daidzin, although found in every treatment in every period, followed a similar pattern in each case. Before inoculation, only trace amounts, around 1.0 to 2.0 µg/g, was found in all treatments. But by 72 hours and 96 hours, the amount of daidzin increased significantly. However, by 120 hours, the concentration dropped back to that of before inoculation. (See below) Compared with the control concentration, which remained less than 10.0 µg/g, all other treatments' concentrations increased. However, only plants treated with Fish Fertil reached about 50 µg/g and higher. Calda Viçosa followed in a distant second, and Silicate did not seem to promote daidzin production when compared with the control.

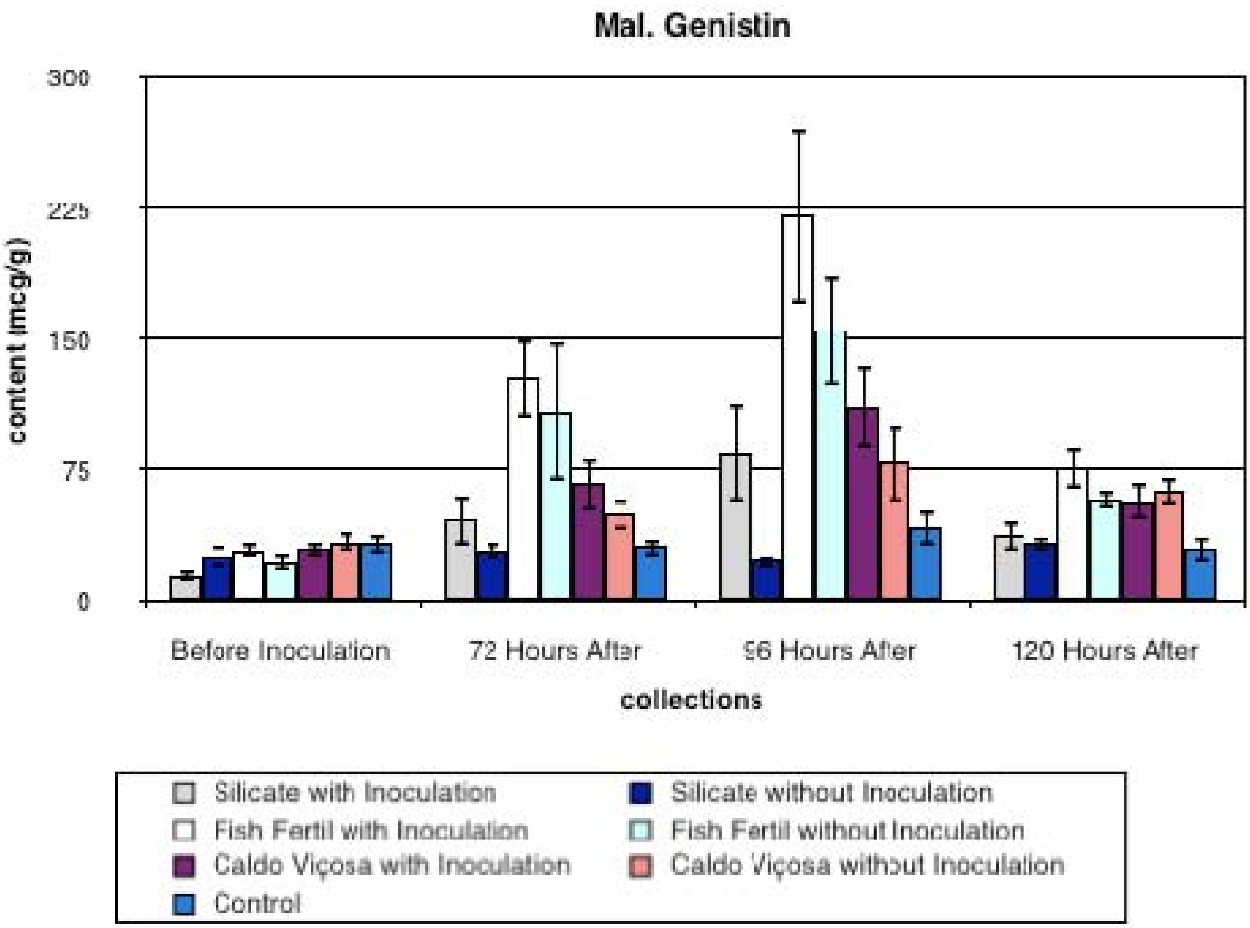


Glycitin, another isoflavone, was only found in some treatments but not in others. Even when it was discovered, only trace amounts existed. The exception was treatment 3 (Fish Fertil with inoculation), which seemed to induce the production of glycitin in 72 hours and 96 hours after, high above the control concentration.

Genistin, on the other hand, was generously produced in every treatment and in every time frame. But it followed a similar pattern as that of daidzin. The control concentration remained between 2.59 µg/g and 4.75 µg/g. Treatment 3 and treatment 4's concentration, again, increased dramatically by 72 hours and 96 hours after. Treatment 4 and 5 also promoted genistin production. However, the difference between the two latter treatments' concentrations is not as significant as that of treatment 3 and 4. Modest amount of genistin was found in treatment 1 and 2, but again not as dramatic as the other treatments. Treatment 2's concentration are more in line with that of the control and does not seem to induce much production of isoflavones. (See below)

Malonyl daidzin was barely detected by HPLC before inoculation, but greatly increased in concentration by 72 hours after and 96 hours after in majority of the treatments. Again, in treatment 3 and 4, the production of this phytoalexin increased tremendously. The concentration of malonyl daidzin in treatment 3 at 96 hours after inoculation was about 8 times that of the control in the same time frame. (See below) Even the concentration of treatment 4 was 5 times that of the control. Treatment 5 and treatment 6 also produced abundant amount of this isoflavone, but not nearly as much as the previous two treatments. Treatment 1 did not induce much production of malonyl daidzin, and treatment 2's concentration was less than the control at certain time frames.

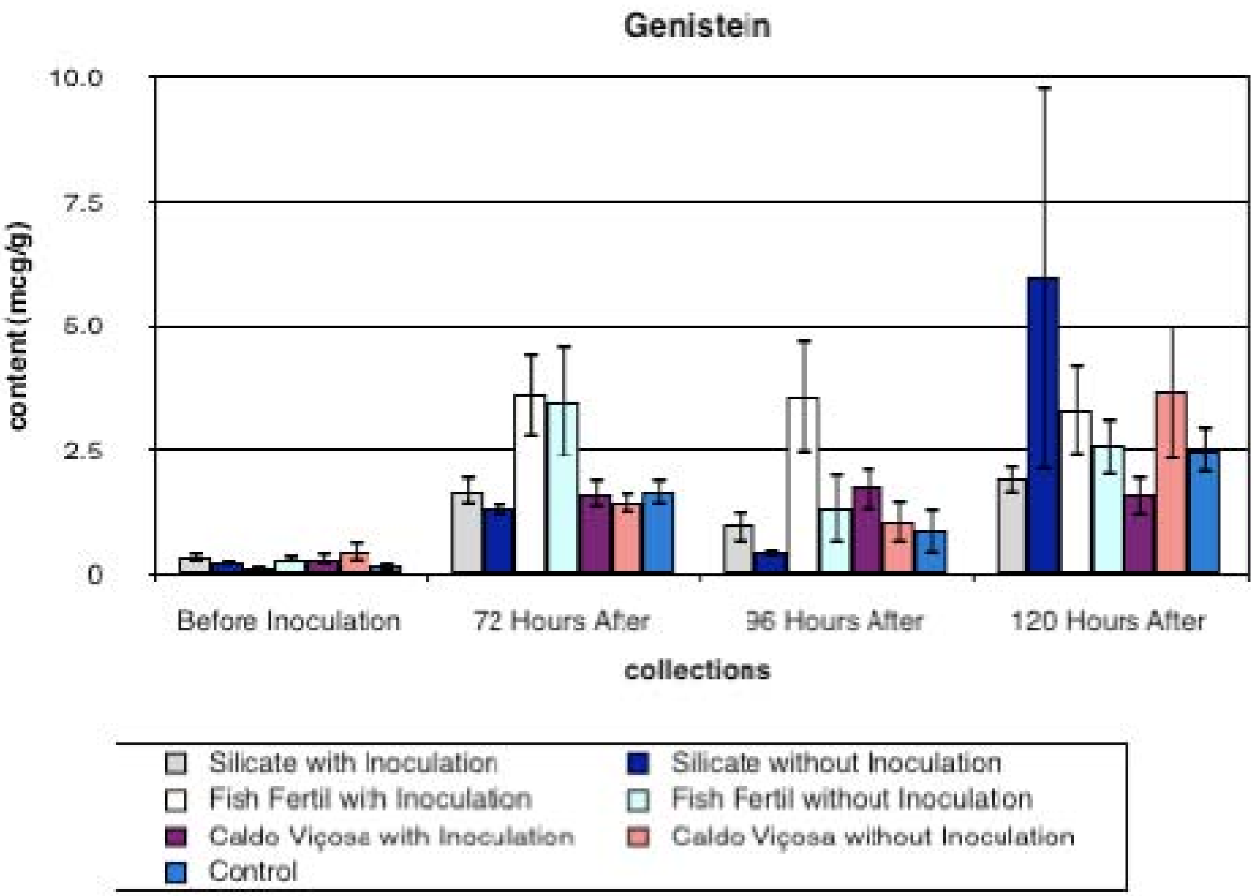


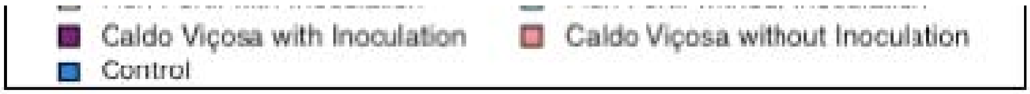


Malonyl glycitin, a conjugate of glycitin, was not found before inoculation in any of the treatments. In the control, HPLC only detected the phytoalexin sporadically. However, in treatments 3, 4, 5, 6, malonyl glycitin presented itself more than in other treatments, especially treatments 3 and 5. Treatment 3 again produced the most in all of the treatments in 72 hours and 96 hours after.

Malonyl genistin, which was produced most abundantly in soybeans, followed the pattern of genistin. In before inoculation, all treatments produced about relatively the same amount. Only treatment 2 and the control induced a steady amount in each time frame. In the other treatments, the isoflavone production increased substantially by 72 hours and 96 hours, before dropping back to before inoculation level in 120 hours after. And like all other compounds previously mentioned, malonyl genistin was abundantly produced in treatment 3, followed closely by treatment 4. Treatments 5 and 6 only produced about half the amount of 3 and 4 respectively. Treatment 1 induced production higher than normal in 96 hours after.

Acetl daidzin and acetl glycitin were also found in some treatments. For acetl glycitin, there appears to be no apparent correlation in what treatments and what time frame seems to generate more production. In both before inoculation and 120 hours after inoculation, no acetl glycitin compounds were identified. The only significant amount detected was in treatment 5 in 96 hours after. Acetl daidzin, on the other hand, was found in majority of the treatments, although in small concentrations. Treatment 3 produced the most in all time frames, followed by





treatment 4. The rest of the treatments' concentrations were fairly close to that of the control. Fish Fertil induced more production compared to the other two fungicides.

Daidzein, the hydrolyzed aglycone of daidzin, has been considered the immediate precursor of glyceollins. It was only generated by some treatments in a slightly higher than control level. Surprisingly, treatment 4 produced the most daidzein in 96 hours after, which reached about 4.5 µg/g. All other treatments produced minute amount. Glycitein, in comparison to daidzein was produced in all treatments, but more so in treatment 3 than any other.

Genistein, the last of the aglycones, was found in all time frames and in all treatments. Surprisingly, however, treatment 2 seemed to secrete more of this isoflavone in 96 hours than in any other treatments, which suggested that some error must have resulted. But even then, the concentration of genistein was minute in all the treatments.

The phytoalexin glyceollin was found in majority of the treatments in every time frame. This was a surprise because glyceollin does not usually appear with such regularity. But in this particular experiment, glyceollin and many derivatives of glyceollins were detected. Because there has yet to be a reliable marker to quantify glyceollins, to measure this phytoalexin can only be done by noting its presence in the specific treatments. There appeared to be no correlation with the data.

Treatment 1

Plant ID #	**105**	**202**	**305**	**403**	**505**	**601**	**703**	**801**	**903**	**1007**
Before Inoculation					🟨	🟨	🟨	🟨		
72 Hours After		Derivative	🟨		🟨	🟨	Derivative	🟨	Derivative	Derivative
96 Hours After	🟨	🟨		🟨	🟨	🟨		🟨	🟨	🟨
120 Hours After	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	Derivative	Derivative

Treatment 2

Plant ID #	**102**	**207**	**301**	**406**	**502**	**604**	**705**	**807**	**906**	**1004**
Before Inoculation	🟨	🟨	🟨	Derivative	🟨		🟨		🟨	🟨
72 Hours After	Derivative		Derivative	🟨			🟨	Derivative	🟨	🟨
96 Hours After	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
120 Hours After	🟨	No data	🟨	🟨	Derivative	🟨	Derivative	🟨	Derivative	Derivative

Treatment 3

Plant ID #	**104**	**204**	**302**	**404**	**501**	**605**	**701**	**802**	**907**	**1002**
Before Inoculation	🟨	🟨	🟨			🟨	🟨		🟨	🟨
72 Hours After	🟨	🟨	🟨	🟨	Derivatve	Derivative	🟨	🟨	🟨	Derivative
96 Hours After	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
120 Hours After	🟨	Derivative	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨

Treatment 4

Plant ID #	**101**	**203**	**304**	**407**	**507**	**602**	**704**	**803**	**904**	**1005**
Before Inoculation	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨	🟨
72 Hours After	🟨	🟨	Derivative	🟨	🟨	🟨	🟨	🟨	🟨	🟨
96 Hours After	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
120 Hours After	Derivative	Derivative	🟨			🟨	🟨	🟨	🟨	🟨

Treatment 5

Plant ID #	**103**	**201**	**307**	**401**	**503**	**603**	**707**	**804**	**901**	**1001**
Before Inoculation	🟨		🟨	🟨	🟨	🟨	🟨	🟨		🟨
72 Hours After	Derivative	Derivative	Derivative	🟨	🟨	🟨	🟨	Derivative	🟨	🟨
96 Hours After	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	Derivative
120 Hours After	🟨	🟨	🟨	🟨	🟨	Derivative	🟨	🟨	🟨	🟨

Treatment 6

Plant ID #	**106**	**205**	**303**	**402**	**504**	**607**	**706**	**806**	**902**	**1003**
Before Inoculation	🟨	🟨	🟨	🟨	🟨	🟨		🟨	🟨	
72 Hours After	🟨	🟨	Derivative	Derivative	🟨	Derivative	🟨	🟨	🟨	🟨
96 Hours After	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	
120 Hours After	🟨	🟨	🟨	Derivative	🟨	🟨	🟨	Derivative	🟨	Derivative

Treatment 7

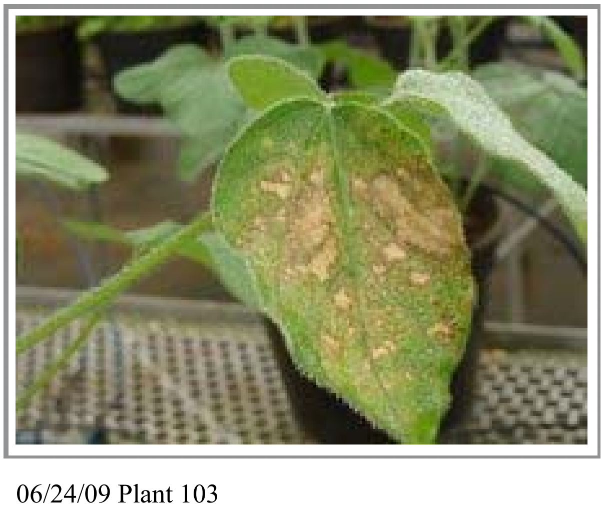
Plant ID #	**107**	**206**	**306**	**405**	**506**	**606**	**702**	**805**	**905**	**1006**
Before Inoculation	🟨	🟨	🟨		🟨	🟨	🟨	🟨	🟨	🟨
72 Hours After	Derivative	Derivative	🟨	Derivative	🟨	🟨	🟨	Derivative	🟨	Derivative
96 Hours After	🟨	🟨	🟨	🟨	🟨	No data	🟨	🟨		🟨
120 Hours After	Derivative	🟨	🟨	Derivative	Derivative	Derivative	🟨	🟨	🟨	🟨

Key	
🟨	Presence of glyceollin
Derivative	Presence of derivative of glyceollin

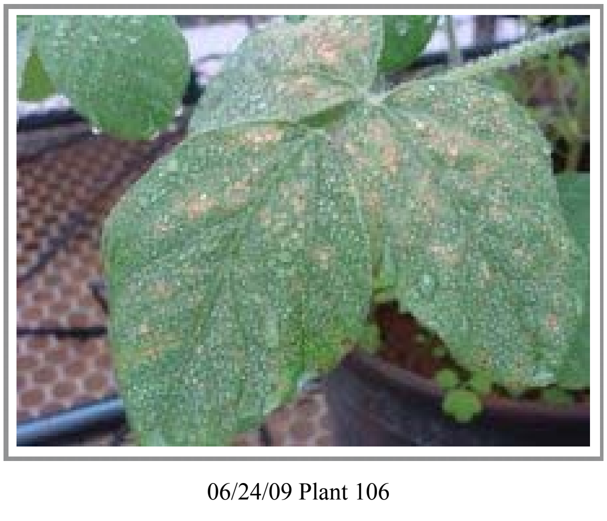
The disease cycle was also carefully noted in all of the plants. Unfortunately, rust developed in many of the plants treated with the products, some more severe than others. Calda Viçosa did not prevent the disease from developing. Plant 103 (picture at bottom) in 120 hours after was observed with the signature mark of brown lesions on many of its leaves. The size of the lesions covered the entire leaves, but the disease was not in its advanced stage. The plant did not appear to reach early maturity or defoliate prematurely. However, surprisingly in plants treated with Calda Viçosa without inoculation, lesions similar to those caused by rust surfaced on the leaves. However, phytotoxicity was observed in some of the plants in treatment 5, 6 before inoculation. Phytotoxicity could cause brown spots and lesions. This often happens when fungicides and herbicides cause unintended spray injury on the crops instead of the pest. (See plant 106).

Plants treated with Fish Fertil, treatment 3 and 4, had less of a serious problem with rust. The plants did not appear to exhibit as many signs of the disease. The lesions of treatment 3, when compared to those of treatment 5, were noticeably smaller and fewer. Only some of the plants in treatment 3 displayed the worst symptoms. Some of the plants showed few brown lesions, around 2 mm2, and not all of the leaves of one plant had these distinct marks. No plants showed defoliation or early maturity. However, Fish Fertil did not prevent the plants from contracting the disease; it only ameliorated the signs and slowed the disease progression. But plants in treatment 4 did not manifest any signs of phytotoxicity, which demonstrated that Fish Fertil was safer for soybeans.

Silicate had a similar effect on the plants as Calda Viçosa had. The plants displayed lesions, some more than those in treatment 5. The lesions were equally big and some of the leaves were ready to defoliate. The plants seemed to have reached maturity early, and chlorosis was observed in two of the seven plants. In treatment 2, the plants did not exhibit any signs of phytotoxicity, so the product did not cause any damage to the plants (See plant 1004).



06/24/09 Plant 103



06/24/09 Plant 106

06/24/09 Plant 1004

Conclusion

Of the three products tested, Fish Fertil was the most effective in treating Asian soybean rust. It induced the plants to mount a fierce immune response against *Phakopsora pachyrhizi*. As shown in treatment 4, even without inoculation, Fish Fertil instigated a response from the plants to produce more isoflavones (daidzin, glycitin, genistin, their conjugates, and their aglycones). The malonyl daidzin, malonyl genistin, and daidzein concentration were the highest in plants treated with Fish Fertil. These compounds were considered precursors of glyceollins, which also helped in the defense response. However, the induction of production of isoflavones and glyceollins did not stop the infection of the rust from spreading. Fish Fertil could not prevent the disease, but it did ameliorate the symptoms and slowed the progression of the pathogen. Interestingly enough, the level of concentration of all the compounds decreased by 120 hours after, at the time when the disease manifested itself. For Fish Fertil to be more effective, it must be sprayed more than once.

Calda Viçosa was the second must effective in treating the rust. However, as mentioned earlier the product does cause phytotoxicity in plants, which farmers must take under consideration when applying the fungicide. The concentration levels of isoflavones and glyceollins in treatment 5 were far from that of treatment 3, and even treatment 4 (with a couple of exceptions). Although glyceollins were detected in treatment 5 and 6, the precursors were not at a relatively high level. Plants without inoculation and treated with Calda Viçosa were observed to produce more isoflavones and glyceollins when compared to the control. Again, the concentrations started to fall at 120 hours after, which indicated that the spray failed to combat the rust. More than one spray could be applied to see results.

Silicate did not treat the disease as nearly as well as the other two products. It did not promote isoflavone and glyceollin production. The concentration of the compounds in treatment 1 did not even reach that of treatment 6. Treatment 2 remained at the same concentration levels as that of the control. Silicate did not induce a strong plant defense response. The plants treated with silicate were the worst affected by the rust. As mentioned earlier, some plants were ready to defoliate, which showed the advanced stage of the disease. Even though the concentration levels started to decrease at 120 hours after, the product did not slow the disease from progressing. More than one spray is unnecessary.

Because of the time constraints of this internship, only one repetition was completed. For more reliable results, at least three more repetitions must be carried out. But with the current results, it is appropriate to draw the conclusion that Fish Fertil would be the most beneficial, organic fungicide to treat soybean rust. It does not cause any phytotoxicity and promotes the plants to mount a strong immune response to the pathogen. However, it does not completely protect the plants from rust infection. It must be applied before inoculation to counter the damaging consequences, as a preemptive measure. Calda Viçosa could also be used, although phytotoxicity is one of the unfortunate side effects. Many factors could influence farmers to utilize one fungicide over the others. But the most effective and environmentally friendly treatment is Fish Fertil.

This research only proves the importance of developing new organic fungicides, ones that can combat the disease and prevent the rust from infecting the soybeans. However, the current research have yet to identify a treatment that embody these characteristics. These three fungicides are only the first step in finding a satisfying treatment.

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