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SOYBEAN CYST NEMATODE POPULATIONS AS INFLUENCED BY A NON-TREATED SOYBEAN CROP, A FLUOPYRAM-TREATED SOYBEAN CROP AND A NON-HOST CORN CROP

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SOYBEAN CYST NEMATODE POPULATIONS AS INFLUENCED BY A NON-TREATED SOYBEAN CROP, A FLUOPYRAM-TREATED SOYBEAN CROP AND A NON-HOST CORN CROP

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"Knowledge is the kindling of a flame, not the filling of a vessel." – Socrates

"Choose my instruction rather than silver, and knowledge rather than pure gold. For wisdom is far more valuable than rubies. Nothing you desire can compare with it." Proverbs 8:10-11 NLT

Throughout the entirety of my young adult years, I have been on the receiving end of a multitude of support from the people that have been the most wonderful Christian examples, and parental figures I have ever known. Dennis and Lee Ann; thank you from the bottom of my heart for pushing me to pursue my dreams.

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ABSTRACT

Soybean cyst nematodes are the most significant plant pathogen to soybeans in all soybean producing areas in the United States. Rotating to non-host crops, and using resistant varieties have been the main ways of managing this plant pathogen throughout the history of soybeans in this country. Fluopyram is a seed treatment that is labelled for the control of early season plant parasitic nematodes in soybean. Up until this point, there has been little to no published research conducted on the effectiveness of fluopyram against soybean cyst nematode. For this study, the objective was to conduct research to determine whether fluopyram would be a suitable management strategy for soybean cyst nematode. The questions that guided the study were: (1) Will fluopyram significantly reduce soybean cyst nematode populations and increase yields when compared to untreated soybeans? (2) Will treated seed yield more than untreated seed? and (3) Will fluopyram show population decreases comparable to a non-host cropping treatment? The three treatments used to address these questions were: (1) Soybean seed treated with fluopyram, (2) untreated soybeans, and (3) a non-host crop, in this case white corn. This was the first year this study was conducted. The plots were planted on May 31, 2016 on the Murray State University Hutson Farm. There were eight replications of the three treatments, resulting in 24 total plots. The soybean cyst nematode populations were monitored through egg count samples. These samples were taken once before the plot location was decided, in order to provide population levels to determine which location should be used, then once early in season (initial sample), and once after harvest (final sample). The egg counts revealed that all populations for all treatments were extremely variable for both sampling periods. The yield data between soybean treatments, and the percent reduction for all treatments was collected and analyzed through SAS. The average yield for soybeans treated with fluopyram was 64.99 bu/ac, while the average yield for untreated soybeans was 63.42. Therefore, there was no significant difference found between the two soybean treatments for yield. The average percent soybean cyst nematode population reduction for all treatments is as follows: soybeans treated with fluopyram (treatment 1) was 64.26%, non-treated soybeans (treatment 2) was 61.89%, and a non-host white corn crop (treatment 3) was 67.78%. There was no significant difference found between soybeans treated with fluopyram and untreated soybeans. There was also no significant difference between soybeans treated with fluopyram and the non-host crop treatment. Due to this being the first year of the study, care should be taken when generalizing the results of this work.

CHAPTER 1

Introduction

Soybean cyst nematode (*Heterodera glycines*) is one of the major soybean pests affecting soybean production in the United States today. Soybean cyst nematodes are microscopic roundworms that attack the roots of soybean plants (Tylka, 1994). Soybean cyst nematode populations must be above a certain density for symptoms to appear above ground in plants or for a yield inhibition to occur. According to the University of Minnesota, 300 eggs/100 cm^3 of soil is a healthy population level at which minimal damage to the soybean plants will be inflicted (Chen, Kurle, Malvick, Potter, & Orf, 2011). However, populations that are above this healthy level can be extremely hard to manage even with methods that have been found to be successful.

Since soybean cyst nematode is such a prevalent pathogen, there have been many studies on effective management strategies varying from the influence of winter annual weed removal timing on population density (Mock, Creech, Ferris, Hallett, & Johnson, 2010) to the evolution and selection of the RhG1 locus (Lee, Kumar, Diers, & Hudson, 2015). Some of the most common ways in which to manage this pathogen are through the implementation of crop rotation, resistant varieties, and nematicides (Niblack, 2005). The effectiveness of the management strategies is a subject that has debated by many agronomists. Crop rotation has been seen to consistently reduce soybean cyst nematode populations, but most growers already implement a crop rotation strategy of corn and beans, therefore consecutive years of growing non-host crops is often not an option for those growers (Niblack, Lambert, & Tylka, 2006).

Therefore, most growers will rotate crops in conjunction with utilizing resistant varieties. Nematicides have fairly recently made their debut for the management of soybean cyst nematode, but there has been no compelling evidence provided stating that the highly priced chemicals provide consistent results in reducing nematode populations (Niblack, et al., 2006).

Statement of the Problem

There have been many studies researching soybean cyst nematode management, but there have been very few published studies that have researched the use of fluopyram and how it works to change nematode population levels or how it differs from non-host management strategies. In this study, the problems to be evaluated are, (1) Will fluopyram significantly reduce soybean cyst nematode populations and increase yields when compared to untreated soybeans? (2) Will fluopyram-treated seed yield more than untreated seed?, and (3) Will fluopyram show population decreases comparable to a non-host cropping treatment?

Purpose of the Study

The purpose of this study is to determine whether fluopyram is an effective management strategy for high soybean cyst nematode populations. This will be done through examining the populations of soybean cyst nematodes once soon after planting plots of three different treatments, and once after the nematodes have been exposed to a full growing season of the treated plots. Data will be collected concerning the soybean cyst nematode population levels, the HG types of the population, and soybean yield. After analyzing the results of the research performed on the test plots, statistics will be run to determine if the fluopyram seed treatment generated statistically significant results concerning the change in soybean cyst nematode population levels, and the difference between yield in soybeans that are untreated and soybeans treated with the product fluopyram. All soybeans planted were of the variety Caverndale CF 447

RR/STSn which demonstrates resistance to soybean cyst nematodes of groups 3 and 14. This variety was planted due to the high overall level of the soybean cyst nematode population in the field.

Research Questions

The following questions guided the study:

1. Will fluopyram significantly reduce soybean cyst nematode populations and increase yields when compared to untreated soybeans successfully over one growing season?
2. Will the soybeans that are treated with fluopyram yield more than the untreated soybeans?
3. Will fluopyram show population decreases comparable to a non-host cropping treatment?

Hypotheses

H_1^A: Soybeans treated with fluopyram will demonstrate a significant yield increase as compared to untreated soybeans.

H_0^A: Soybeans treated with fluopyram will have no significant yield increase when compared to untreated soybeans.

H_1^B: Soybeans treated with fluopyram will show a significant decrease in soybean cyst nematode populations compared to untreated soybeans.

H_0^B: Soybeans treated with fluopyram will not show a significant decrease in soybean cyst nematode populations compared to untreated soybeans.

H_1^C: Soybeans treated with fluopyram will demonstrate population decrease comparable to treatments of non-host crops (corn).

H_0^C: Soybeans treated with fluopyram will not demonstrate population decrease comparable to treatments of non-host crops (corn).

Assumptions

The following assumptions were made regarding this study:

1. All replications occurred at the same site.
2. Variability was minimized using a randomized complete block design.
3. The same hand-harvesting methods were used for all plots.
4. All data collected was done so in a precise and consistent manner to minimize error.
5. Soil samples were pulled using the same methods both before and after crop.

Definition of Terms

HG Types – HG Types describe the variation in Soybean cyst nematode virulence. This is determined by the number of Soybean cyst nematode females that can develop on one of seven indicator lines that are supposed to have some sort of resistance (Chen et al., 2011).

Inoculum Level – the amount of eggs added to each seedling from a sample of soil in the HG type test (University of Missouri – HG Type Test Results).

Races or Race Type – an indication of the ability of a population of nematodes to reproduce on a specific host (Koenning, 2000).

Soybean cyst nematode – A parasitic nematode that inhibits the yield of soybean plants through the attack of the soybean root system (Tylka, 1994).

Seed Treatment – The act or process of applying a pesticide or other beneficial substance to a seed

Bushels of soybean – 60 pounds of soybean achieving a moisture of 13%

Delimitations

The following are delimitations of the study:

1. The study was limited to the same treatments throughout all replications.
2. The study was limited to one variety of soybean and one hybrid of corn.
3. The soybean treatments in this study were limited to no-tillage soybeans at 30-inch rows.
4. The corn treatments in this study were limited to no-tillage corn at 30-inch rows.

Limitations

The following are limitations of the study:

1. This study is limited to the environmental conditions of the plot in the 2016 crop year.
2. This study may only apply to certain races and HG types of Soybean cyst nematode.
3. This study is limited to the soil type(s) in the plot area.

Significance of Study

The results of this study should provide greater understanding of soybean cyst nematode management. The information provided in the data interpretation should allow greater insight into the effectiveness of crop rotation versus seed treatment in decreasing soybean cyst nematodes. Future researchers and growers should be able to use the results of the research to make better informed decisions regarding soybean cyst nematode management.

Chapter Summary

Soybean cyst nematodes are one of the main yield-reducing pathogens of soybean today. While crop rotation has played a significant role in soybean cyst nematode management up until this point, the opportunities for seed treatments to play a role in soybean cyst nematode management are now becoming an option. Fluopyram is a seed treatment which has been labelled for soybean cyst nematode. There is little published research on the role of fluopyram in soybean cyst nematode management. Through analyzing fluopyram compared to untreated soybeans and a non-host cropping system, results may be attained to provide growers with another management strategy that can be used to withstand high levels of soybean cyst nematode.

CHAPTER 2

Review of Relevant Literature

Introduction

This chapter will be used to review the affiliated literature for this study. The review of this literature will investigate the adequacy of the fluopyram seed treatment in lowering soybean cyst nematode populations and identify areas in need of further research. The review of relevant literature will be divided into 9 sections: (1) Introduction; (2) History of soybean cyst nematode; (3) History of soybean cyst nematode management (4) Crop rotation as a management strategy; (5) Resistant varieties as a management strategy; (6) Seed treatments (7) Nematicidal activity of fluopyram; (8) Theoretical framework; and (9) Summary.

Soybean cyst nematode in high populations can significantly impact the life and yield of a soybean plant. Within season, soybean cyst nematode can result in severely chlorotic or even stunted plants. The effect of the soybean cyst nematode is also felt at the season's end, where yield losses up to 20 percent can be seen in the cases with the most severe populations (Davis & Tylka, 2000). This pathogen often goes unnoticed and untreated as the effects of its presence are often credited towards other circumstances such as weather or incorrect chemical application. The lack of treatment results in even further damage as the pathogen is suited to increase in population, and the cycle begins again. In most cases, crop rotation and resistant varieties have been the main means of treatment in the presence of soybean cyst nematode.

History of Soybean cyst nematode

Soybean cyst nematode was first reported in Japan 1915 by “S. Hori”, though it’s effects were known as “Moonlight disease” for as many as 30 years prior. Soybean cyst nematode was then reported in the United States of America in 1954 in Hanover, North Carolina, an area known for importing bulbs from Japan (Riggs & Wrather, 1992; Davis & Tylka, 2000). Since the first report of the nematode in the United States, scientists have disagreed upon whether the nematode was native to the United States or imported. It is very likely that the nematode was imported due to the agricultural practices of early American farmers when growing soybeans. When the soybean was first brought to the United States seed cleaning practices were minimal, if present at all, and sometimes soil was imported to gain nitrogen fixing bacteria for optimum soybean growth (Riggs & Wrather, 1992). However, the difference in tail length between three different forms of the nematode species suggests that a different form of the nematode may have been present in the United States prior to the introduction of the form that was indigenous to Asia (Ferris, Ferris, & Murdock, 1985).

In the first years of soybean production in the United States, soybeans were not grown for grain, and so any yield loss caused by nematodes was of little importance. Once grain production in soybean began; fertilizer, insect pests, and disease preceded all importance over the nematode, which was not well known. Additionally, most soybean cultivars grown in the U.S. during that time were black seeded (Riggs & Wrather, 1992). All known sources of resistance to soybean cyst nematode in early management for the pathogen were black seeded, which may have been why farmers did not notice symptoms caused by soybean cyst nematode or the damage within their crop. Crop rotations of soybean every 3 to 4 years were utilized heavily during the beginning of soybean growth in America, however these methods were abandoned in the peak of the World War 2 era, which began the problematic populations of soybean cyst nematode that can be found in the United States today.

Soybean cyst nematodes go through four juvenile stages and four molts before reaching maturity. The nematode will molt once in its egg prior to hatch, (Riggs & Wrather, 1992) and thus will hatch from its egg as a second stage juvenile. Hatch time can depend on various environmental conditions, for example some eggs may hatch once they reach a certain temperature, others may only hatch once a certain amount time has passed, and still others may require exudates from the soybean plant (Niblack, 2005).

The second stage juvenile will then enter the vascular tissue of the soybean by producing a variety of enzymes and cellulases, and will pierce the cell wall and insert its stylet into the plasma membrane, priming this area of the root for feeding (Niblack, 2005). Once the nematode develops into the third and fourth juvenile stages, feeding sites within the soybean root are completely established and the juvenile becomes sedentary for the next few stages of its life.

The nematode will molt multiple times prior to reaching the adult stage. Once this stage is reached the female nematode will remain sedentary and its body will protrude from the surface of the soybean root. This is what makes the soybean cyst nematode identifiable to the naked eye. In contrast, the mature male nematode will become slender following its final molt, and will exit the root tissue. These males are necessary for reproduction and are often seen in the gelatinous matrices that the female excretes into the soil (Riggs & Wrather, 1992). After the mating process, the female will excrete a very small amount of the produced eggs which will continue the cycle in the same growing season. However, most of the eggs will remain inside the female body after death, at this point the female is referred to as a cyst, which protects the unhatched eggs. These eggs can survive up to nine years before they hatch into the soil. (Chen et al., 2011)

Soybean cyst nematodes are often referred to as races or HG types, however there is a difference in the two systems, while both are a means of dividing the populations of soybean cyst nematode, they have different methods of doing so. The race system was the first means of measuring the ability of a population to reproduce on resistant soybean lines (Niblack et al., 2006). Though it was adopted by many soybean breeders, the race system had many flaws. For example, if seed was labelled as resistant to a certain race, that would imply that all nematodes of the said race would be the same and behave the same way, which is proven to be incorrect. Furthermore, rather than using the race system as a means of population separation, multiple researchers tried to use race synonymously with genotype (Niblack, Arelli, Noel, Opperman, Orf, Schmitt, & Tylka, 2002). The races were created based on phenotypic information without taking into consideration the genotypes that may contribute, making this use of races scientifically incorrect (Niblack et al., 2006).

HG types were delineated in 2002 to fix some of the issues that were apparent with the race system. HG types describe the variability of the soybean cyst nematode virulence, and are adaptable to the creation of new resistant lines and different geographies (Niblack et al., 2006). The HG type test involves the use of seven resistant soybean lines that are referred to as index numbers. If ten percent of the population of soybean cyst nematode can reproduce on the given resistant line, the population would be referred to as positive for that line (Tylka, 2016; Niblack et al., 2006). Each index number corresponds to a specific line of resistance, for example Index number 1 corresponds with PI 548402 (Peking), therefore a soybean cyst nematode population that would be referred to as HG type 1 would have more than 10% reproduction on the line of resistance associated with that soybean (Tylka, 2016). If the HG type was 1. 3. 4. 7, that would mean that ten percent of the nematodes in that population were resistant to lines 1, 3, 4, and 7. In this way we are able to document all resistance of a certain population, rather than to fit it in one category as the race system did. The HG type system is the most recent, and therefore is used more frequently to categorize soybean cyst nematode populations today than is the race system (Tylka, 2016).

Soybean cyst nematode Management

Soybean cyst nematode management strategies are not concrete, nor does one method always work better than another. As stated by Dr. T.L. Niblack, recommending a management strategy has been no small feat due to the fact that no study has pointed to one tactic being superior over another (Niblack, 2005). Historically, rotating to non-host crops and using varieties that are resistant to soybean cyst nematode have been the most effective means of managing this plant pathogen.

Another recommended management strategy has been to use various strategies in conjunction, such as to rotate to non-host crops, use soybean cultivars that are resistant to soybean cyst nematode, and to switch up the resistant cultivars that are used each year. Using crop rotation and resistant varieties as management strategies in conjunction was said to provide further assurance that soybean cyst nematode would not be a problem (Niblack, 2005; Chen et al., 2011).

There have been significant disadvantages that have been demonstrated by the utilization of crop rotation as well as the utilization of resistant varieties to manage soybean cyst nematode. One example of this is that some non-host crops are not as profitable as soybean, or cannot be grown in the infected field as much as necessary, making soybean a more desirable crop for growers to plant. Furthermore, soybean cyst nematode has been observed to be very adaptable to resistant strains of soybean and different environments, making it a particularly persistent soybean pest (Niblack et al., 2006). In addition, there has been evidence in the past that has shown that resistant varieties produce 5-10% less yield than susceptible varieties when grown without the presence of the nematode (Chen et al., 2011). In recent years, using seed treatments to prevent yield loss from soybean cyst nematode has been discussed as a management strategy. Traditionally, seed treatments have not been looked at as a significant solution to soybean cyst nematode management.

Crop Rotation as a Management Strategy

The rotation to non-host crops has proved to be the most effective way to manage areas that are highly or heavily infested with populations of SCN, as it diminishes the food source for the females, and lowers active populations (Niblack, 2005). Over time, research has found that corn yield significantly declines with increased frequency of corn in the crop rotation (Conley, Gaska, Pedersen, & Esker, 2011) making it undesirable to use corn increasingly in a cropping system to control the nematode problem. It can be easy to implement other non-host crops into a rotation due to the fact that soybean cyst nematodes feed on very few crops that are commonly used in agricultural production. Some of these crops include: potato, wheat, oats, red and white clover, sorghum, sugar beets, and rice. The problem that can sometimes arise from not using solely corn in these rotations, is that profitability is reduced in most other non-host crops compared to corn, or these non-host crops do not have a successful market in all areas affected by soybean cyst nematode (Conley, et al., 2011).

If non-host crops are grown, there is no specified amount of time known for effectively reducing the soybean cyst nematode populations (Chen et al., 2011). However even if non-host crops are grown in the rotation, the decline in soybean cyst nematode populations is very unpredictable due to the environmental conditions that may be present during that growing season (Davis & Tylka, 2000). Due to the variability and ineffectiveness of control when using crop rotation as the only form of management, this management practice is not recommended to be used on its own, but in conjunction with resistant varieties, or other management practices (Davis & Tylka, 2000; Chen, et al., 2011; Niblack, 2005; Niblack et al., 2006).

Resistant Varieties as a Management Strategy

While resistant soybean varieties are still hosts of soybean cyst nematode, they are poor hosts, which will result in the decline of population density over time (Riggs & Wrather, 1992). It has been found that the initial infection of soybean plants after planting by soybean cyst nematode is the most important or damaging infection compared to later infections. Therefore, varieties that have the capability to prevent or lessen the severity of the initial infection should prove effective in preventing yield loss (Riggs & Wrather, 1992). Resistant varieties are not one-hundred percent effective at removing nematodes from the field (Tylka, 2008). However, planting these resistant varieties can cause the reduction in populations of the nematode by keeping most of the juveniles from feeding, thus ending their life cycle (Davis & Tylka, 2000).

When soybean cyst nematode was first discovered, it was difficult to find varieties resistant to the nematode. As we have gained further knowledge and understanding of what genotypes are necessary for soybean cyst nematode resistance, more and more varieties have become available. The number of varieties available to growers today has increased substantially from the 90's, and there are now hundreds of varieties available in all different maturity groups (Tylka, 2008; Tylka & Mullaney, 2016; Niblack et al., 2006).

There are some significant downfalls of resistant varieties when not used correctly. The HG type of the soybean cyst nematode population must be known before choosing a resistant variety, as the HG types will reveal which indicator lines the specific nematode populations can reproduce on (Tylka, 2016; Niblack et al., 2006). Furthermore, if the same source of resistance is used each year, the nematode population can develop a resistance to this indicator line as well (Koenning, 2000; Davis & Tylka, 2000; Tylka, 2008; Hershman, Heinz, & Kennedy, 2008; Niblack et al., 2006). It is also recommended not only to utilize rotation of resistant varieties, but to rotate to non-host crops as well (Davis & Tylka, 2000; Chen, et al., 2011; Niblack, 2005; Niblack et al., 2006).

Seed Treatments

A relatively innovative approach to soybean cyst nematode management is using seed treatments to manage the population density. As stated earlier, studies show that disturbing the initial infection of the soybean by nematodes is more beneficial to yield than disturbing later infections (Riggs & Wrather, 1992). Using seed treatments may allow for that initial infection by soybean cyst nematode to be interrupted.

Seed treatments have appeared throughout history in some shape or form from the mid-1800s to current times, though primitive forms of a "seed treatment" involved non-chemical agents (Buttress & Dennis 1947). In the 1700s, a regular treatment of cereals occurred after the discovery that wheat sunken in seawater and then sowed had good germination and was not infected by smut, this caused farmers to begin to treat their wheat with a very strong brine and quicklime in order to prevent infection (Tull, 1733). There are many seed treatments today that are available as both fungicides and nematicides for soybean. Seed treatments used as nematicides will not kill the entire population of soybean cyst nematode, but provide a promising management solution and can be used in conjunction with other management practices (Davis & Tylka, 2000). Traditionally, corn seed goes through seed treatment more commonly than soybean. This is because soybean seed is not as expensive as corn and often is not carried over from year to year (for planting) since soybean does not store well (Hoeft, Aldrich, Nafziger, & Johnson, 2000). However, in an instance which nematodes are a known problem, and a seed treatment is a known solution, growers will either buy pre-treated seed from a facility, or treat seed themselves prior to planting.

Nematicidal Activity of fluopyram

Fluopyram is a group 7 fungicide and succinate dehydrogenase inhibitor (Bayer CropScience, 2016). Succinate dehydrogenase inhibitors prevent fungal respiration resulting in organism death (Zaworski, 2014). Fungicides come in two categories, contact and systemic fungicides. Systemic fungicides move through the plant and can stop disease and/or infection for a period, whereas contact fungicides do not move through the plant and only act as an initial barricade to infection (Hoeft, et al., 2000). Fluopyram is labelled as a systemic seed treatment for soybean to protect against early season nematodes (Bayer CropScience, 2016). Fluopyram works by being absorbed into the germinating seed and moves systemically throughout the entire plant and roots (Bayer CropScience, 2015). This is the initial feeding site for soybean cyst nematode, thus the seed treatment should inhibit the reproduction cycle of the nematode, resulting in decrease in population density. Fluopyram does cause a “Halo Effect” on cotyledons due to the metabolization of the seed treatment through the plant, but current companies producing the chemical state that it does not impact the growth rate of the plant, nor is it seen past the cotyledon stage (Bayer CropScience, 2015). There are not many published studies on the effects of fluopyram on soybean cyst nematode, though it is an effective inhibitor of soybean sudden death syndrome (SDS), another viable threat to soybean yield. However, early testing of the product by Bayer CropScience revealed that the product has some effect on soybean cyst nematode populations. If this is the case, the product could provide further management options to those who depend on soybean growth in infected fields.

Theoretical Framework

There is little to no published research on the effectiveness of the fluopyram technology on soybean cyst nematode. Fluopyram is registered by Bayer Crop Sciences as having activity on both sudden death syndrome and soybean cyst nematode. The high population of soybean cyst nematode in the plots researched in this manuscript should provide an ideal research environment when comparing non-host crop plots, and the fluopyram seed treated plots, with untreated soybeans. Based on the research done on the effects of non-host cropping systems on soybean cyst nematodes, and the lack of research in the fluopyram seed treatment, more research is needed to determine the overall effectiveness of fluopyram as a management strategy for soybean cyst nematode. The variability of soybean cyst nematode populations throughout the entire plot should provide some insights into the usage of both systems in different environments with different populations of the nematode.

Summary

Soybean cyst nematode is one of the most problematic pathogens affecting soybean production today. Traditional management methods such as crop rotation or resistant varieties may not always work well into a grower's management plan. Therefore, the need for other management strategies is necessary in order to add greater diversity to management practices, and to allow farmers growing soybeans in infected fields to be able to maximize the yields necessary to make a living. Seed treatments such as fluopyram could offer a solution for some farmers to diversify and intensify their management strategies if it provides control similar or greater than that provided by crop rotation.

CHAPTER 3

Material and Methods

Methodology

This chapter consists of the methods used to conduct this study. These methods will provide the construct for the analysis of the relationship between fluopyram and the management of soybean cyst nematode. This chapter is divided into the following sections: (1) research design; (2) site selection; (3) instrumentation; (4) data collection; and (5) data analysis.

Research Design

This study used a randomized complete block experimental research design. The randomized complete block (RCBD) design is widely used throughout agricultural research and works well with experiments that do not have large treatments. The signature feature of the RCBD method are the use of blocks of equal size containing all treatments (Gomez & Gomez, 1984). The blocks in the study reduce experimental error by grouping experimental units to minimize variability. The groups in this study consisted of 24 different plots separated into 3 treatments in an area with high soybean cyst nematode populations. The treatments administered consisted of a) non-host crop (in this case, corn) b) soybeans treated with the fluopyram seed treatment, and c) untreated soybeans. The soybean variety planted, Caverndale CF 447 RR/STSn, did have a known resistance to soybean cyst nematode groups 3 and 14. This variety was chosen due to the high population density of nematodes throughout the research area. It is also extremely difficult to find soybeans with glyphosate resistant traits that did not have some line of soybean cyst nematode resistance.

Variables

The variables in this study consist of the soybean cyst nematode populations in each plot and the different treatments. The soybean cyst nematode populations were different for each plot, and were monitored at the beginning and at the end of the study. The nematode populations are a dependent variable in this study. The treatments were randomized within each replication using random assignment. The relationship between the variables will be examined to determine the effectiveness of each treatment in managing soybean cyst nematode populations over the course of a season, making the treatments an independent variable.

Natural population fluctuations will occur in this study and cannot be controlled. These fluctuations will be considered as a moderating variable.

Data Source/ Site Selection

Plot Procedure

To have a population substantial enough for proper research, a field with known soybean cyst nematode populations was separated into four quadrants. The layout of these quadrants can be seen in Appendix I. Soil samples were collected from each quadrant and sent in to the University of Missouri Extension Plant Nematology Laboratory, which specializes in soybean cyst nematode testing, to see which area of the field had the highest population. The quadrant with the highest population was chosen for the study. The chosen quadrant was then broken down into 24 smaller plots measuring 30 ft x 20 ft. Soil samples were taken to provide the initial and final soybean cyst nematode populations for each individual plot following the planting and harvest of the plots. The treatment locations for each replication were chosen through simple random sampling methods.

Plot Location and History

The quadrant that was chosen for the plot via the methods described above was located on the Murray State University Hutson Farm in Murray, Kentucky near Highway 80E. (36.65208 N, -88.35549 W) The Hutson Farm has been in the possession of Murray State University since 2013. Since this area has been under the management of Murray State University, it has been under a no-till crop plan, which we continued this year. The location of each individual treatment was chosen using simple random assignment techniques, which is standard for the randomized complete block experimental design. Both the plot assignments (Figure 1) and an aerial image of the plot location (Figure 2, (Land.db, 2017)) can be found in the figures below.

Figure 1 Plot Assignment

701	Rep 7 702	703		801	Rep 8 802	803
501	Rep 5 502	503		601	Rep 6 602	603
301	Rep 3 302	303		401	Rep 4 402	403
101	Rep 1 102	103		201	Rep 2 202	203
Hwy 80						

Treatment 1 Soybean + fluopyram
Treatment 2 Soybean Control
Treatment 3 Corn

The aerial imagery was annotated using the Land.db software. (Land.db, 2017) The soil type of the entire plot location was Grenada silt loam. An illustration of the soil map can be found in

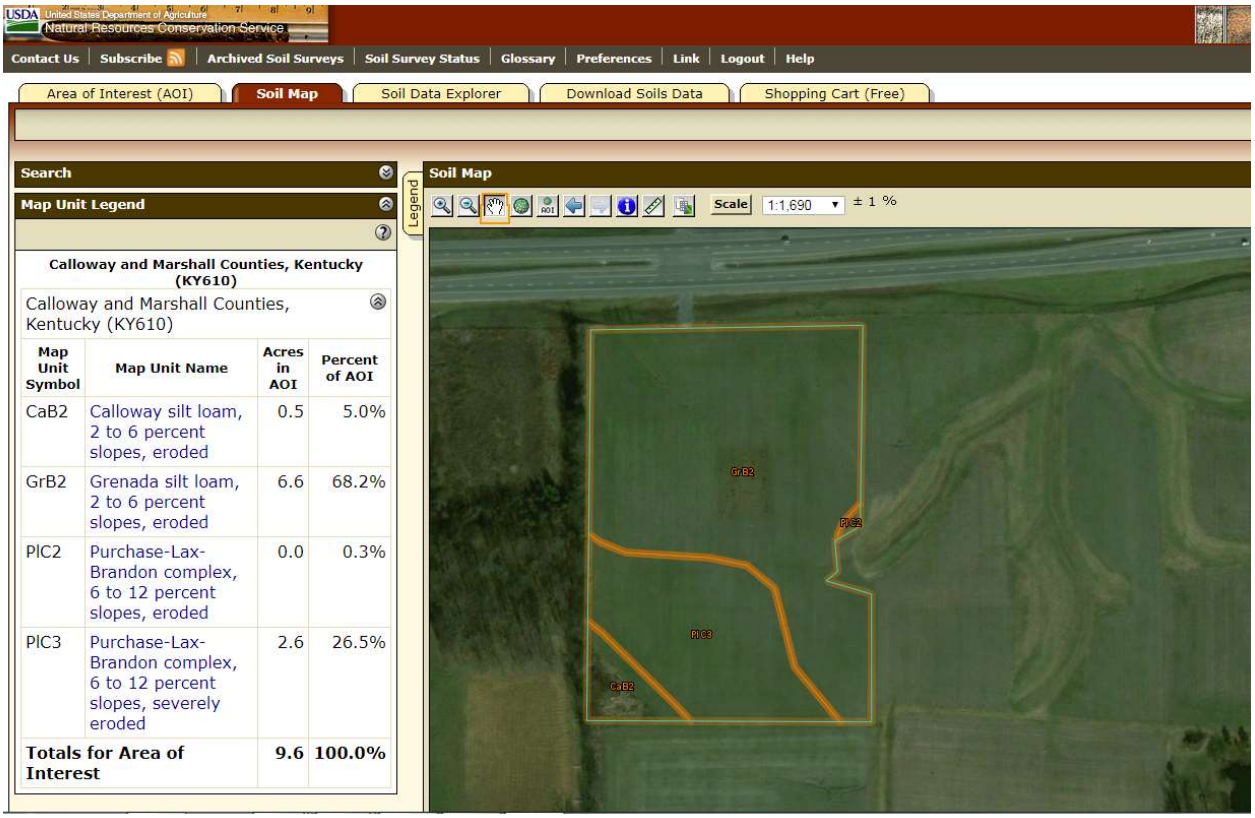
Figure 3 below. An illustration of soybean cyst nematode variability for each plot can be found in Appendix II.

Figure 2 Aerial Imagery of SCN Plot Location (Land.db, 2017)

Seed Variety and Planting

The seed variety that was chosen for the soybean plots of both treatments was Caverndale Farms CF447/RRSTSN, which was a glyphosate and sulfonylurea tolerant variety in the 4.4 maturity group. Both treatments of soybean were planted at a population rate of 139,000 seeds per acre. The soybean cyst nematode rating for this soybean variety was 3,14. The corn hybrid that was chosen was P1477WHR which is a 115-day glyphosate resistant white corn. Both the corn and the soybeans were planted on May 31, 2016.

Figure 3 Web Soil Survey for Hutson Farm (USDA NRCS Web Soil Survey, 2017)



This was late for corn, but due to the high rainfalls in the area during the season, the plots fell right in line with the planting of other growers in the county. All plots were planted in 30 inch rows so that the corn and soybean plots would be consistent.

Fertilizer and Herbicide Applications

Both the corn and soybean plots were treated with herbicides for weed control. The corn plots also had nitrogen fertilizer applied. On June 3, 2016, the corn and soybean plots were treated with a pre-emergent herbicide mix. This mix consisted of 0.84375 pounds of acid equivalent glyphosate per acre, 2.55 ounces of pyroxasulfone active ingredient per acre (ai/ac), and 0.25% volume per volume (v/v) of nonionic surfactant. The corn plots were also fertilized with 180 units of nitrogen per acre using ammonium nitrate fertilizer on June 30, 2016. The corn and soybean plots were treated with a post-emergent herbicide mix of 0.84275 pounds of acid equivalent glyphosate and 0.25% v/v of nonionic surfactant. This post-emergent herbicide mix was applied on June 21, 2016.

Fluopyram Rate

The treated soybeans were treated at a rate of 0.25 mg of fluopyram/seed. This measures out to be 58 ml of ILeVO product/140,000 soybeans seeds, or 58 ml per bag of seed. Using the Cimbria Heid CentriCoater seed treater, only 4.4 lbs of soybean seed were able to be treated at one time. To accommodate this, an equation was used to figure how many ml of active ingredient would need to be mixed with the 4.4 lb of seed. There were 2082 seeds/lb, which meant for 4.4 lb there was approximately 9161 seed. A 15 ml mixture was made of 5.68 mL ILeVO, 0.75 ml of colorant, and 8.57 ml of distilled water. Ten ml of this mixture was directly applied to the 4.4 lb of seed.

Instrumentation

Instrument Selection

The instrument used to sample the soil was a standard JMC 36 inch soil probe. This soil probe was used each time soil samples were taken for consistency. Soybean cyst nematode samples were stored in sandwich bags to retain moisture for the nematodes. Soybean seed was treated with fluopyram using a Cimbria Heid CentriCoater. When harvesting, we used hand harvesting methods and collected the ears (for corn), or the whole plant (for soybeans) in feed sacks. The soybean seed was then put through a portable threshing machine owned by the university. To clean soybean grain after harvest and to collect weights we used grain dockage sieves one was a 20/64" round sieve, and the other was an 18/64" round sieve. This allowed most of the soybeans to fall through to a smaller sieve, whereas the bottom pan collected the trash. We measured both the corn and the soybean weights with and Ohaus Adventurer digital scale. For soybean moisture, an M-3G Dickey-John Portable Moisture Tester was used. To calculate corn moisture after harvest a forced air oven was used to remove the moisture from the corn ears after the initial weight was taken. The same scale used to measure soybean weights was used to measure initial and final weights after drying in corn.

Data Collection Methods

Soybean cyst nematode Sampling Procedure

When sampling for the location of the research plots, 25 soil samples were taken and put into gallon bags. These samples were taken sporadically around each quadrant, with care to make sure that the samples were not too close to the edge of the quadrant. The quadrant with the highest soybean cyst nematode population was chosen for the research plot.

After the chosen quadrant was measured out into 30 foot by 20 foot plots, each plot was sampled for its individual soybean cyst nematode population. 6 to 8 samples were collected within each plot sporadically. Each plot was sampled by the same person, within the same day, using the same sampling technique.

Plot harvest

When crops reached physiological maturity, and dried down, they were hand harvested to later collect yield data. Both the corn and soybean plots had one inner row harvested as a representative from the plot. The corn plots were harvested by collecting all ears off each plant in the row, and storing them in grain bags. To determine which inner row was to be harvested, both rows were walked down to determine weed or disease presence, the row which had the least of these was the row that was chosen for harvest. The soybean plots were harvested using the same row choice method, but rather than pulling all pods off the plant, hand shears were used to remove the entire plant from the ground. Once the plants were removed they were also stored in grain bags.

The corn did not require any further harvest techniques. The soybeans however, had to be shelled. A portable bean threshing machine was used to remove the beans from the pods. The beans collected from each plot were run through the thresher multiple times to ensure cleanliness that resembled combine harvesting. The beans were further cleaned in the lab prior to weighing for yield.

Weight and moisture

Once the plots were harvested, the corn and beans from each plot were weighed and tested for moisture. Corn was weighed on the ear in grams. To determine corn moisture, three ears that looked uniform in damage and had all kernels were selected and placed in paper bags. These ears were weighed initially, and then stored in the forced air oven at a temperature of 60 degrees Celsius for two weeks. Once they were removed they were weighed for their final weight. Soybeans from each plot were weighed in pounds and were tested for moisture using a Dickey – John moisture sensor. The moisture was measured three times out of different samples for each plot for consistency.

Data Analysis

After data points were collected, all data was run through the SAS software (SAS Institute, 2003) The ANOVA procedure was run for soybean yield data. Descriptive statistics were run using Microsoft Excel 2016.

Chapter Summary

The May 31st planting date was a little late for the non-host crop, but was ideal for the planting of the soybean treatments. The plots chosen were ideal for the study due to high levels of soybean cyst nematode populations in fields that were commonly planted to soybean. The harvest methods used promoted good yield collection and removed the possibility of combine error. Once all plots were hand harvested, data collection occurred in the crops lab.

CHAPTER 4

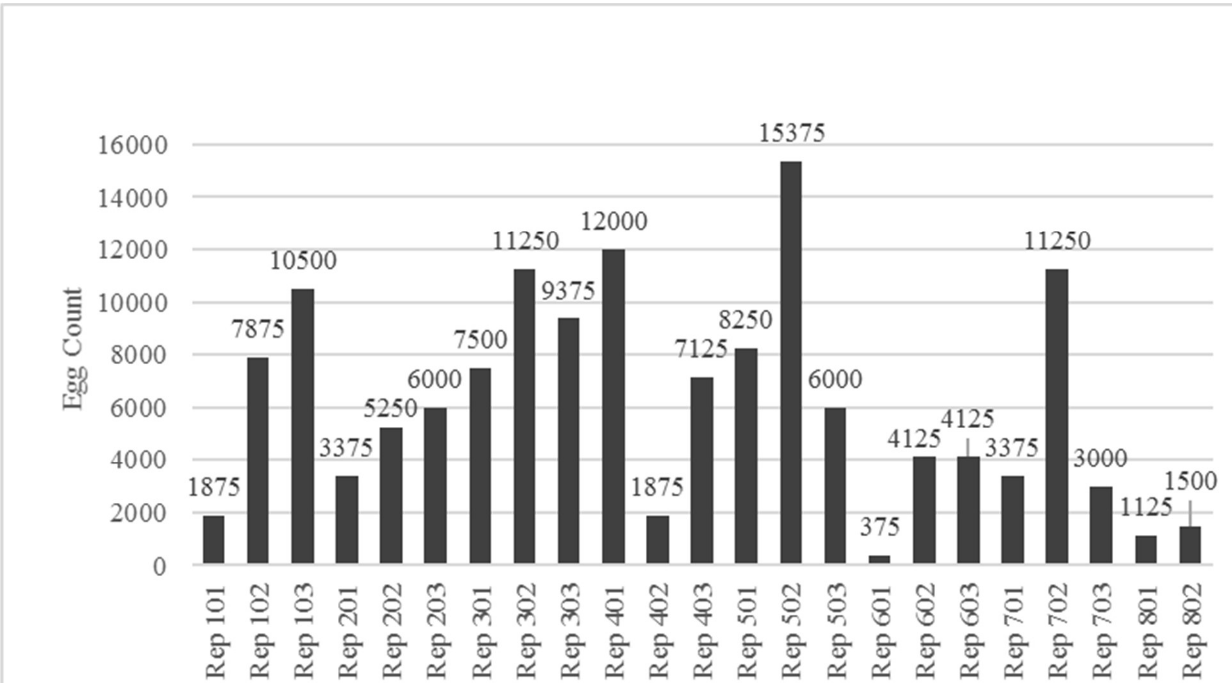
Results

This chapter provides the results of the study and data analysis. This data was interpreted by the SAS program for yield comparisons between the two soybean varieties, and percent reduction in soybean cyst nematode populations for all treatments. Microsoft Excel 2016 was used for all other descriptive statistics. All data will be summarized in a variety of tables.

The field was sampled for soybean cyst nematodes in 4 different quadrants labelled Quadrant 1 – Quadrant 4. The quadrant was chosen based on the highest population, or area with the highest egg count. The quadrant that was chosen in had an egg count of 19,500 eggs per cup or 230 cm^3 of soil. Once the quadrant was chosen, it was measured into plots and soil tests, and initial soybean cyst nematode egg tests for each individual plot were collected. HG type tests were collected over the entire plot site as well. Soil test results from Waters Agricultural Laboratory are in Appendix III. Initial soybean cyst nematode egg counts can be found in Appendix IV. The soybean cyst nematode egg counts were measured per cup or 250 cm^3 of soil sent in. There are three levels of soybean cyst nematode levels. 1 to 500 eggs is considered a low egg count, 500 to 10,000 is a moderate egg count, and 10,000 and greater is a high egg count. Figure 4 is a graph illustrating the initial population counts for each treatment area. The egg counts in all treatment areas were deemed moderate to high counts. The HG Type test (Appendix V) was conducted using an inoculum level of 1,000 eggs. These results were recorded in Female

Index (FI) and percent. Low percentages of FI mean that populations were not able to develop well on the line, whereas High FI percentage indicates that the nematode population was resistant to that line. In the percent column, lines that are indicated with a (+) sign, are lines in which there was

Figure 4 Initial Soybean Cyst Nematode Egg Count



an FI greater than 10%, meaning that the nematode was resistant to that line. The official results for the HG Type Test conducted at the University of Missouri Extension Nematology Laboratory show that the HG Type from this location was 2.7 and can be found in Appendix V.

Soybean cyst nematode populations were extremely variable for both the initial and final samples. This was to be expected as the study was not in a controlled environment, but in a naturally occurring ecosystem. The variability in populations contributed to many of the results that were seen in the study, and will be taken into account when conclusions and recommendations are made.

Hypothesis One

Hypothesis one considered the possible yield increase with seed treated with fluopyram compared to untreated seed. Yield data was collected for soybeans at the end of the growing season. Yield data was analyzed to compare the yield of soybeans treated with fluopyram and the untreated soybeans. Table 1 shows that, for soybeans treated with fluopyram (Treatment 1), the average yield was 64.987 bushels per acre. The average yield for untreated soybeans (Treatment 2) was 63.419 bushels per acre. Therefore, no significant difference in was found between the yield of soybeans treated with fluopyram and untreated soybeans. Table 1 shows the descriptive statistics for both soybean treatments. Yield data for all plots can be found in Appendix VI.

Table 1
Yield Data for Soybean Treatments

Seed Treatment	Average Yield
Fluopyram Treated	64.99
Non-Treated	63.42
Pr > F	0.3958
% CV	5.398
LSD (P = 0.10)	NS

Note: NS = Not significant

Hypothesis Two

Hypothesis two examined the soybean cyst nematode population change in plots planted with soybeans treated with fluopyram and untreated soybeans to determine whether soybeans treated with fluopyram demonstrated a significant population decrease as compared to untreated beans. It is important to note that replication 6 was completely removed from this portion of the study, due to the population levels being much greater than two times the standard deviation. As displayed in Table 2, the average percent reduction in soybean cyst nematode population for soybeans treated with fluopyram (Treatment 1) was 64.26%, while the percent reduction average for untreated soybeans (Treatment 2) was 61.89%. Therefore, there was no significant difference found between the populations of the soybeans treated with fluopyram and the untreated soybeans. Table 2 illustrates the percent reduction for the two soybean treatments. The very large %CV demonstrate the large amount of variability in soybean cyst nematode populations even within a small area. Population levels for all treatments can be found in Appendix IV.

Table 2
Percent Reduction of Soybean Cyst Nematode Populations for All Treatments

Treatment	Avg Initial Pop.	Avg Final Pop.	% Reduction Average
Fluopyram Treated	7553.57	2387.57	64.260
Non-Treated	6026.86	2209.00	61.886
Non-host	5571.43	1366.29	67.784
Pr > F			0.8346
% CV			28.343
LSD (0.10)			NS

Note: NS = Not Significant

Hypothesis Three

Hypothesis three observed the population change in plots planted with soybeans treated with fluopyram and plots planted in a non-host crop to determine whether soybeans treated with fluopyram demonstrated a significant population decrease as compared to a non-host crop. The soybean cyst nematode egg counts were compared between the treatments of soybeans treated with fluopyram and the non-host crop. The sixth replication was excluded from this comparison as well due to soybean cyst nematode populations that far exceeded two times the standard deviation. Table 2 exhibits that percent reduction of soybeans treated with fluopyram (Treatment 1) with a percent reduction average of 64.26%, while the percent reduction of the non-host crop was 67.78%. These results revealed that there was no significant difference found between the population level of the non-host crop and the population levels of the soybeans treated with fluopyram. Table 2 illustrates the percent reduction for the non-host treatment and the soybeans treated with fluopyram. Egg counts indicated population levels for all treatments can be found in Appendix IV.

Chapter Summary

Soybean cyst nematode levels were moderate to high in all plots across all treatments. This promoted the optimum test environment for this study. However, the population levels of soybean cyst nematode were extremely variable throughout the season. The yield data between soybean treatments revealed no statistically significant difference between the soybeans treated with fluopyram and the untreated soybeans. Likewise, the population levels in the soybeans treated with fluopyram and the untreated soybeans were not found to be significantly different, either. However, while the soybean cyst nematode populations in the soybeans treated with fluopyram and the non-host crop were not statistically different, they were comparable. The data from replication 6 had to be removed from the soybean cyst nematode population portion of the study due to populations that exceeded 2 times the standard deviation.

CHAPTER 5

Conclusions and Recommendations

Soybean cyst nematodes pose a significant threat to soybean yields throughout all soybean producing states in the continental US. Crop rotation and the use of resistant varieties have been the consistent management strategy for soybean cyst nematode up until this point in history, in which soybean seed treatments have become more readily available and less costly. Fluopyram is a group 7 fungicide that is labelled for the seed treatment in soybean in order to protect against Sudden Death Syndrome and “damage caused by early season plant pathogenic nematodes” (Bayer CropScience, 2016). There has previously been little to no published research showing soybean cyst nematode population decrease through the use of fluopyram. This study was conducted in order to obtain results about the use of fluopyram as a control for soybean cyst nematode populations, as a method of achieving yield increase in fields with high levels of soybean cyst nematode, and as a comparable control strategy to the implementation of a non-host cropping system.

Conclusions for Hypothesis One

It was hypothesized that soybeans treated with fluopyram would demonstrate a significant yield increase as compared to untreated soybeans. Yield data was shown in Table 1 in chapter IV. It can be seen that there was no significant yield difference between the soybeans treated with fluopyram which yielded an average of 64.99 bushels per acre (bu/ac), and the untreated soybeans which yielded 63.42 bu/ac. The maximum yield achieved by the soybeans treated with fluopyram was 67.88 bu/ac in plot 403, while the untreated soybeans had a yield of 69.80 bu/ac. Due to the lack of significant yield difference between the two soybean treatments, the null hypothesis will be accepted for this section of the study. H_0^A: Soybeans treated with fluopyram had no significant yield increase when compared to untreated soybeans.

Conclusions for Hypothesis Two

It was also hypothesized that the soybeans treated with fluopyram would demonstrate significant decrease in soybean cyst nematode populations compared to the soybeans that were untreated. Table 2 in chapter IV shows the initial and final average egg counts for each treatment, as well as the average percent reduction for each treatment. The difference in percent reduction for the soybeans treated with fluopyram (64.26%) and the untreated soybeans (61.89%) was a mere 2.37%. Therefore, there were no significant results found in favor of the hypothesis. The null hypothesis will be accepted for this section of the study. H_0^B: Soybeans treated with fluopyram did not show a significant decrease in soybean cyst nematode populations compared to untreated soybeans.

Conclusions for Hypothesis Three

The final hypothesis addressed the comparability of the use of fluopyram to control soybean cyst nematode populations versus a non-host crop. Table 2 in Chapter IV shows the average initial and final egg counts for soybeans treated with fluopyram and the non-host crop (white corn), along with the average percent reduction for each. The difference in percent reductions for soybeans treated with fluopyram (64.26%) and the non-host crop (67.78%)

indicate that there was no significant difference between the two treatments. However, the averages in percent reduction were comparable. The statistics that were run did not indicate any difference, whether higher or lower, between the fluopyram treatment and the non-host treatment. Therefore, the hypothesis for this segment of the study will be accepted. H_1^C: Soybeans treated with fluopyram will demonstrate population decrease comparable to treatments of non-host crops (corn).

Recommendations for Future Research

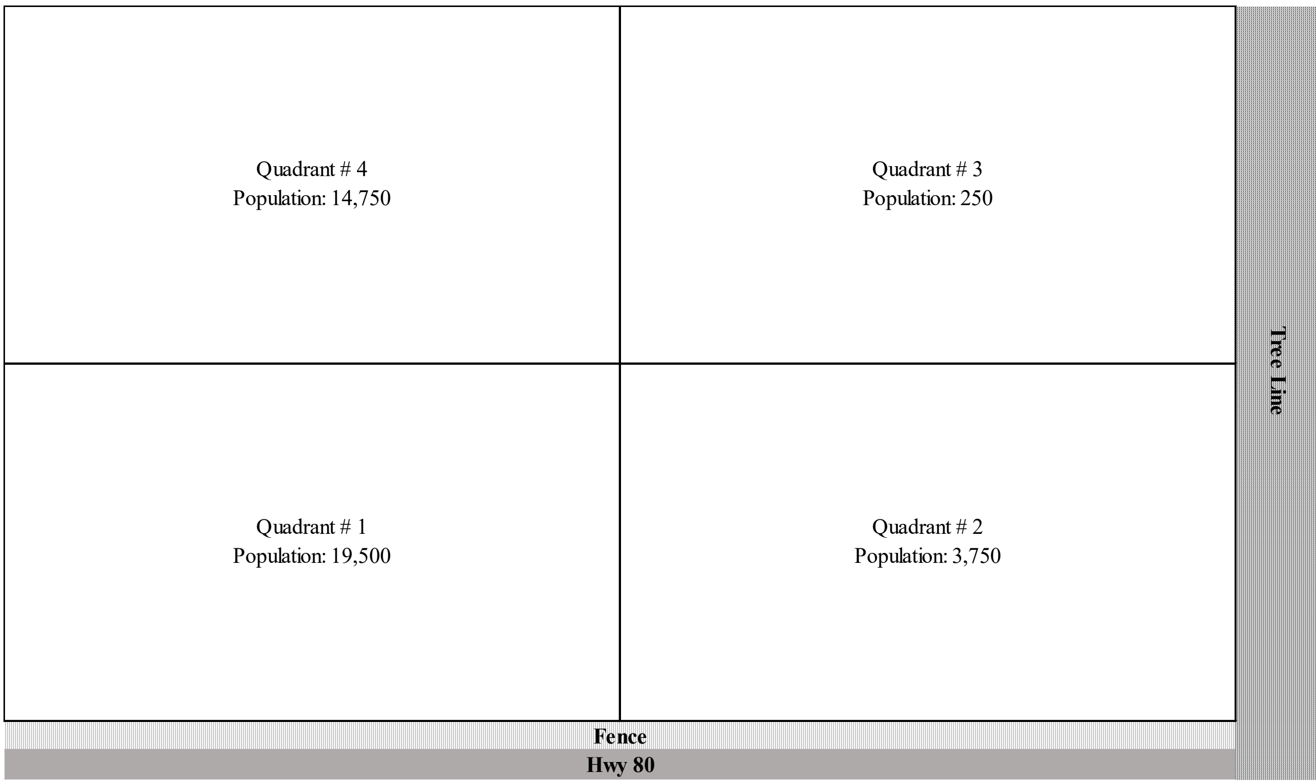
The results provided evidence that fluopyram may not be a seed treatment that causes much difference in soybean yield and soybean cyst nematode population levels when used in conjunction with resistant varieties. It is very possible that the resistant variety that was chosen for this study, CF447/RRSTSN, which was resistant to groups 3, 14, exhibited more control for soybean cyst nematodes than was previously thought. In the future, it would be recommended to include treatments of resistant varieties, along with treatments of susceptible varieties treated with fluopyram in order to see the true effects of both treatments independently. It is not known whether the use of the resistant variety, or the use of fluopyram contributed to the comparable results of the non-host crop and the treated soybeans. For future studies on this topic it would be advised to utilize six different treatments, (1) SCN susceptible soybeans with seed treatment, (2) SCN susceptible soybeans without seed treatment, (3) SCN resistant soybean varieties with seed treatment, (4) SCN resistant soybean varieties without seed treatment, (5) non-host crop, and (6) fallow treatment. The utilization of these treatments would require the implementation of conventional herbicides as nearly all soybeans that are glyphosate tolerant are soybean cyst nematode resistant as well.

Conclusions

Conclusions at this time are limited. Due to variability in soybean cyst nematode populations, along with this being the first year of this study, a continuation of this project will be necessary in order to develop sound conclusions. There are many ways in which this research project could be enhanced in the future. A treatment containing resistant varieties for the HG type in the field being studied will be needed in order to determine the effectiveness of resistant varieties as compared to fluopyram. Data for resistant varieties within the plots would help to establish whether or not the values in this study were due to the combination of management strategies. Furthermore, changing the fluopyram treatment to be applied on susceptible varieties would allow further insight into the control provided by this seed treatment. Finally, if more locations were added, there would be more understanding as to how the different treatments work across different soil types, and microbial ecosystems. The extreme variability of the soybean cyst nematode populations throughout all treatments makes it necessary to revise and conduct this research project further over many more years.

Appendix I

Quadrant Layout



Appendix II

Plot Variability

701 Initial Pop: 4,125 Final Pop: 2,750 % Reduction: 33.333	Rep 7 702 Initial Pop: 3,375 Final Pop: 750 % Reduction: 77.777	703 Initial Pop: 11,250 Final Pop: 3,300 % Reduction: 70.666		801 Initial Pop: 3,000 Final Pop: 1,925 % Reduction: 35.833	Rep 8 802 Initial Pop: 1,125 Final Pop: 963 % Reduction: 14.4	803 Initial Pop: 1,500 Final Pop: 688 % Reduction: 54.133
501 Initial Pop: 7,125 Final Pop: 3,300 % Reduction: 53.684	Rep 5 502 Initial Pop: 8,250 Final Pop: 2,000 % Reduction: 75.757	503 Initial Pop:15,735 Final Pop: 6,500 % Reduction: 57.723		601 Initial Pop: 6,000 Final Pop: 500 % Reduction: 91.666	Rep 6 602 Initial Pop: 375 Final Pop: 3,500 % Reduction: -833.33	603 Initial Pop: 4,125 Final Pop: 5,250 % Reduction: -27.272
301 Initial Pop: 6,000 Final Pop: 3,000 % Reduction: 50	Rep 3 302 Initial Pop: 7,500 Final Pop: 5,000 % Reduction: 33.333	303 Initial Pop: 11,250 Final Pop: 2,250 % Reduction: 80		401 Initial Pop: 9,375 Final Pop: 1,250 % Reduction: 86.666	Rep 4 402 Initial Pop: 12,000 Final Pop: 188 % Reduction: 98.433	403 Initial Pop: 1,875 Final Pop: 500 % Reduction: 73.333
101 Initial Pop: 2,063 Final Pop: 375 % Reduction: 81.822	Rep 1 102 Initial Pop: 1,875 Final Pop: 563 % Reduction: 69.973	103 Initial Pop: 7,875 Final Pop: 3,750 % Reduction: 52.380		201 Initial Pop: 10,500 Final Pop: 1,688 % Reduction: 83.923	Rep 2 202 Initial Pop: 3,375 Final Pop: 625 % Reduction: 81.481	203 Initial Pop: 5,250 Final Pop: 375 % Reduction: 92.857
			Hwy 80			

Treatment 1 Soybean + Ilevo	
Treatment 2 Soybean Control	
Treatment 3 Corn	

Appendix III

Soil Analysis

Waters Agricultural Laboratories, Inc.
2101 Calhoun Rd. Hwy 81 Owensboro, KY 42301
(270) 685-4039 FAX (270) 685-3989

"Improving Growth... With Science"

Soil Analysis

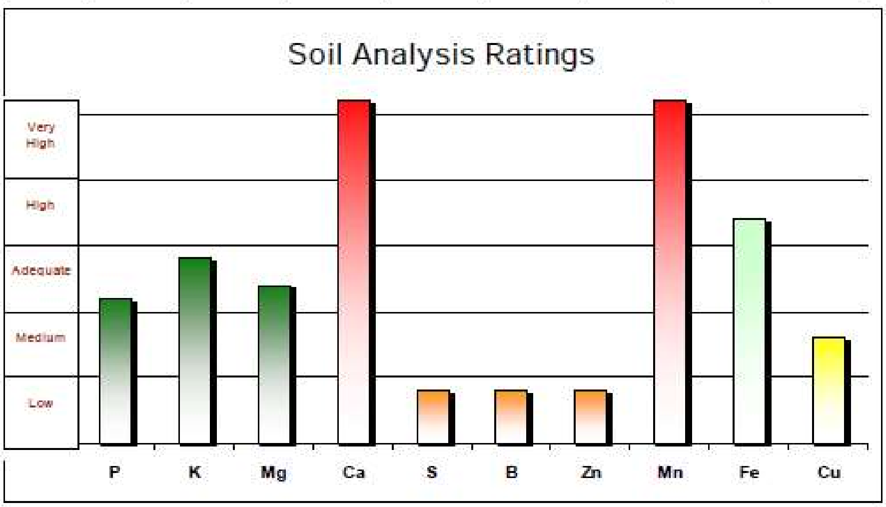
MURRAY STATE UNIVERSITY	Grower: DAVID FERGUSON	Received: 03/22/2016
DAVID FERGUSON	Farm ID: QUADRANT	Processed: 03/24/2016
213 S. OAKLEY APPLIED SCI	Sample ID: 1	Account #: 60187
MURRAY, KY 42071		

Lab Results
lbs. per Acre

Lab Number: 112437XO

Target pH: 6.5
Test Method: Mehlich III

P Phosphorus	K Potassium	Mg Magnesium	Ca Calcium	Soil pH	Buffer pH	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
102 A	301 A	174 A	3203 VH	6.3	7.65	17 L	0.6 L	2.9 L	667 VH	261 H	2.3 M
Aluminum	Sodium	Nitrate N	Soluble Salts	Organic Matter	ENR	Molybdenum	NH4	Nickel	BiCarbs		
			mmhos/cm	1.95 %	39	ppm		ppm	meq/l		

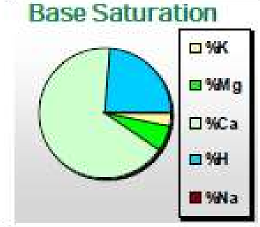


Cation Exchange Capacity: 11.9 meq/100g

Base Saturation

K: 3.2 %
Mg: 6.1 %
Ca: 67.2 %
H: 23.5 %
Na: %

Base Saturation



Fertility Recommendations
lbs. per Acre

Crop: NO CROP

Yield:

Lime Tons/Acre	Gypsum Tons/Acre	N Nitrogen	P2O5 Phosphate	K2O Potash	Mg Magnesium	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
0.5			*	*							

Comments:

* = Maintenance Recommendation

Waters Agricultural Laboratories, Inc.
2101 Calhoun Rd. Hwy 81 Owensboro, KY 42301
(270) 685-4039 FAX (270) 685-3989

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Soil Analysis

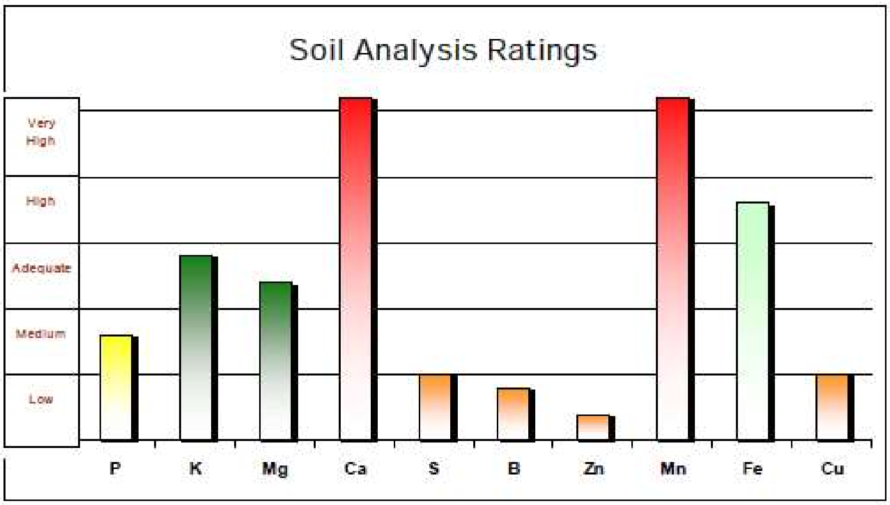
MURRAY STATE UNIVERSITY	Grower: DAVID FERGUSON	Received: 03/22/2016
DAVID FERGUSON	Farm ID: QUADRANT	Processed: 03/24/2016
213 S. OAKLEY APPLIED SCI	Sample ID: 2	Account #: 60187
MURRAY, KY 42071		

Lab Results
lbs. per Acre

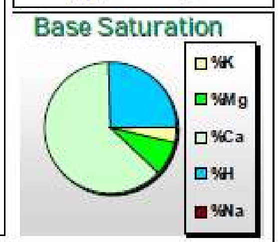
Lab Number: 112438XO

Target pH: 6.5
Test Method: Mehlich III

P Phosphorus	K Potassium	Mg Magnesium	Ca Calcium	Soil pH	Buffer pH	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
72 M	295 A	179 A	2389 VH	5.6	7.70	22 L	0.7 L	1.4 L	562 VH	312 H	1.5 L
Aluminum	Sodium	Nitrate N	Soluble Salts mmhos/cm	Organic Matter 1.77 %	ENR 35.4	Molybdenum ppm	NH4	Nickel ppm	BiCarbs meq/l		



Cation Exchange Capacity	9.5 meq/100g
Base Saturation	
K:	4.0 %
Mg:	7.9 %
Ca:	62.9 %
H:	25.3 %
Na:	%



Fertility Recommendations
lbs. per Acre

Crop: NO CROP

Yield:

Lime Tons/Acre	Gypsum Tons/Acre	N Nitrogen	P2O5 Phosphate	K2O Potash	Mg Magnesium	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
2.0			*	*							

* = Maintenance Recommendation

Comments:

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Soil Analysis

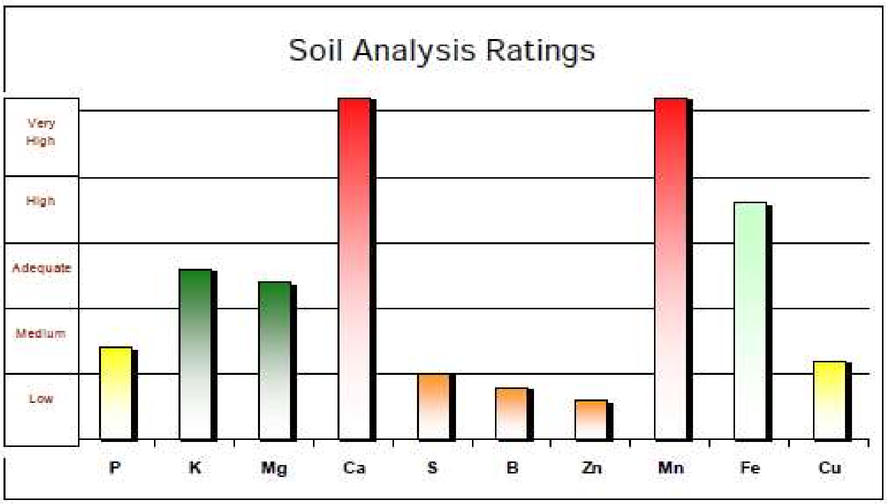
MURRAY STATE UNIVERSITY	Grower: DAVID FERGUSON	Received:	03/22/2016
DAVID FERGUSON	Farm ID: QUADRANT	Processed:	03/24/2016
213 S. OAKLEY APPLIED SCI	Sample ID: 3	Account #:	60187
MURRAY, KY 42071			

Lab Results
lbs. per Acre

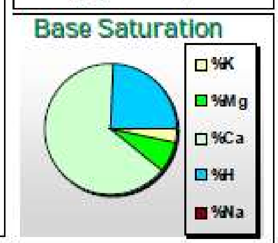
Lab Number: 112439XO

Target pH: 6.5
Test Method: Mehlich III

P Phosphorus	K Potassium	Mg Magnesium	Ca Calcium	Soil pH	Buffer pH	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
53 M	274 A	172 A	2607 VH	5.7	7.70	22 L	0.7 L	1.8 L	716 VH	304 H	1.8 M
Aluminum	Sodium	Nitrate N	Soluble Salts	Organic Matter	ENR	Molybdenum	NH4	Nickel	BiCarbs		
			mmhos/cm	1.58 %	31.6	ppm		ppm	meq/l		



Cation Exchange Capacity	10.0 meq/100g
Base Saturation	
K:	3.5 %
Mg:	7.2 %
Ca:	65.3 %
H:	24.0 %
Na:	%



Fertility Recommendations
lbs. per Acre

Crop: NO CROP

Yield:

Lime Tons/Acre	Gypsum Tons/Acre	N Nitrogen	P2O5 Phosphate	K2O Potash	Mg Magnesium	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
2.0			*	*							

* = Maintenance Recommendation

Comments:

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2101 Calhoun Rd. Hwy 81 Owensboro, KY 42301
(270) 685-4039 FAX (270) 685-3989

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Soil Analysis

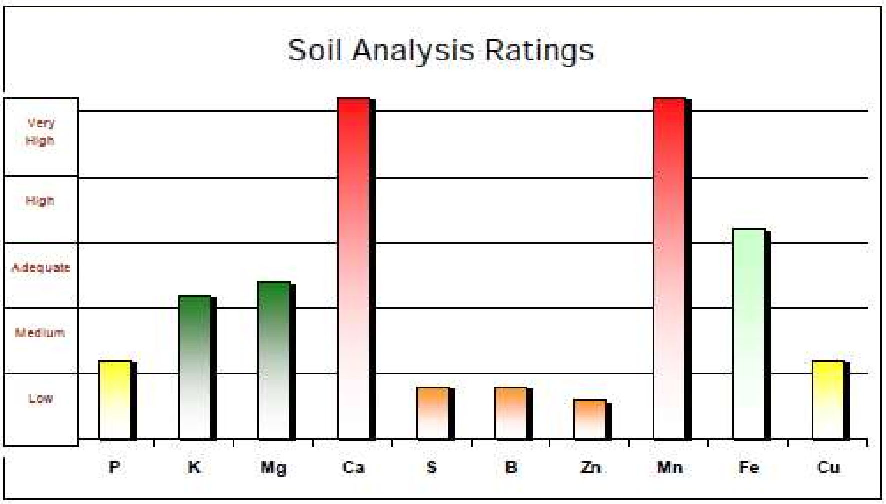
MURRAY STATE UNIVERSITY	Grower: DAVID FERGUSON	Received: 03/22/2016
DAVID FERGUSON	Farm ID: QUADRANT	Processed: 03/24/2016
213 S. OAKLEY APPLIED SCI	Sample ID: 4	Account #: 60187
MURRAY, KY 42071		

Lab Results
lbs. per Acre

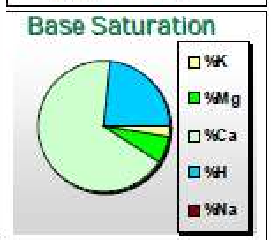
Lab Number: 112440XO

Target pH: 6.5
Test Method: Mehlich III

P Phosphorus	K Potassium	Mg Magnesium	Ca Calcium	Soil pH	Buffer pH	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
45 M	237 A	190 A	3268 VH	6.3	7.65	16 L	0.7 L	1.6 L	642 VH	232 H	1.7 M
Aluminum	Sodium	Nitrate N	Soluble Salts	Organic Matter	ENR	Molybdenum	NH4	Nickel	BiCarbs		
			mmhos/cm	1.74 %	34.8	ppm		ppm	meq/l		



Cation Exchange Capacity	12.1 meq/100g
Base Saturation	
K:	2.5 %
Mg:	6.6 %
Ca:	67.7 %
H:	23.2 %
Na:	%



Fertility Recommendations
lbs. per Acre

Crop: NO CROP

Yield:

Lime Tons/Acre	Gypsum Tons/Acre	N Nitrogen	P2O5 Phosphate	K2O Potash	Mg Magnesium	S Sulfur	B Boron	Zn Zinc	Mn Manganese	Fe Iron	Cu Copper
0.5			*	*							

* = Maintenance Recommendation

Comments

Appendix IV

Soybean Cyst Nematode Egg Counts

A. Initial Tests

Soybean Cyst Nematode Egg Count Report

Extension Plant Nematology Lab
23 Mumford Hall
University of Missouri
Columbia, MO 65211

8/1/2016

David Ferguson — David Ferguson, Murray State University
Rm 213 Applied Science Bldg, South
KY — Murray KY 42071

Lab ID	Grower	Sample ID	Soybean cyst nematode eggs/cup of soil (250 cm3)
160781	David Ferguson	Plot 101	2,063
160782	David Ferguson	Plot 102	1,875
160783	David Ferguson	Plot 103	7,875
160784	David Ferguson	Plot 201	10,500
160785	David Ferguson	Plot 202	3,375
160786	David Ferguson	Plot 203	5,250
160787	David Ferguson	Plot 301	6,000
160788	David Ferguson	Plot 302	7,500
160789	David Ferguson	Plot 303	11,250
160790	David Ferguson	Plot 401	9,375
160791	David Ferguson	Plot 402	12,000
160792	David Ferguson	Plot 403	1,875
160793	David Ferguson	Plot 501	7,125
160794	David Ferguson	Plot 502	8,250
160795	David Ferguson	Plot 503	15,375
160796	David Ferguson	Plot 601	6,000
160797	David Ferguson	Plot 602	375
160798	David Ferguson	Plot 603	4,125
160799	David Ferguson	Plot 701	4,125
160800	David Ferguson	Plot 702	3,375
160801	David Ferguson	Plot 703	11,250

Monday, August 01, 2016

8/1/2016

David Ferguson — David Ferguson

KY — KY

Lab ID	Grower	Sample ID	Soybean cyst nematode eggs/cup of soil (250 cm3)
160802	David Ferguson	Plot 801	3,000
160803	David Ferguson	Plot 802	1,125
160804	David Ferguson	Plot 803	1,500

An egg count of less than 500 is considered low. Soybeans without SCN-resistance can be planted. If no eggs are detected, sample fields every 2 to 3 years at harvest. This increases the probability of finding the nematode if it is present in the field. Monitor areas of the field where SCN is likely to be introduced, such as field entrances, areas that flood, fence rows or places where waterfowl congregate. If fewer than 500 eggs are detected, sample after a susceptible variety is grown.

An egg count of 500 to 10,000 is considered moderate. Plant SCN-resistant soybeans. Rotate sources of SCN resistance whenever possible. If varieties with different sources of resistance to SCN are not available, then grow a different SCN-resistant variety every year soybeans are planted. Resistant varieties increase selection pressure on the nematodes. This can reduce the long-term effectiveness of the resistance.

An egg count greater than 10,000 is considered high. Plant a non-host crop. These include alfalfa, barley, canola, clover, corn, cotton, forage grasses, oats, sorghum, tobacco, rye and wheat. Rotate non-host crops with SCN-resistant varieties. An HG type (race) test may be appropriate if resistant varieties are being used and the egg count continues to increase. An HG type test will tell you which sources of SCN resistance would be good to plant in your field and which would be poor.

B. Final Populations

SCN DIAGNOSTICS
Plant and Nematode Screening Services
1721 East Campus Loop
University of Missouri
Columbia, MO 65211-5315

Soybean Cyst Nematode Egg Count Report

1/17/2017

David Ferguson | **David Ferguson, Murray State University**
Rm 213 Applied Science Bldg, South
KY | Murray KY 42071

Lab ID	Grower	Sample ID	Soybean cyst nematode eggs/cup of soil (250 cm3)
170018	David Ferguson	101	375
170019	David Ferguson	102	563
170020	David Ferguson	103	3,750
170021	David Ferguson	201	1,688
170022	David Ferguson	202	625
170023	David Ferguson	203	375
170024	David Ferguson	301	3,000
170025	David Ferguson	302	5,000
170026	David Ferguson	303	2,250
170027	David Ferguson	401	1,250
170028	David Ferguson	402	188
170029	David Ferguson	403	500
170030	David Ferguson	501	3,300
170031	David Ferguson	502	2,000
170032	David Ferguson	503	6,500
170033	David Ferguson	601	500
170034	David Ferguson	602	3,500
170035	David Ferguson	603	5,250
170036	David Ferguson	701	2,750
170037	David Ferguson	702	750
170038	David Ferguson	703	3,300
170039	David Ferguson	801	1,925
170040	David Ferguson	802	963
170041	David Ferguson	803	688

Appendix V

HG Type Test

OUTREACH & EXTENSION
UNIVERSITY OF MISSOURI
COLUMBIA

Lab Code: 160370

HG Type Test

Extension Nematology Laboratory
23 Mumford Hall
Columbia, Missouri 65211
573-884-9118

David Ferguson, Murray State University	Phone: (270) 293-5681
Rm 213 Applied Science Bldg, South	Email: dferguson@murraystate.edu
Murray, KY 42071	Date: 7/5/2016

Date Received: May 2016 Condition of sample: Fine

Dates of HG Type Test: In 6/7/16 Out 7/5/16 Inoculum level: 1,000 eggs

Sample: Quadrant #1

Number of females on Lee 74: 91

Indicator Line:	Female Index (FI)	≥10%
1) PI 548402 (Peking)	8%	
2) PI 88788	25%	+
3) PI 90763	0%	
4) PI 437654	0%	
5) PI 209322	9%	
6) PI 89772	0%	
7) PI 548316 (Cloud)	56%	+
Pickett	52%	+

HG Type: 2.7	
Race: 5	

Comments: The Peking, PI 90763, PI 437654, PI 209322, and PI 89772 sources of resistance are resistant to your SCN population. PI 88788 is moderately resistant to this population of SCN, and Cloud is moderately susceptible.

Amanda Howland, Research Specialist

Appendix VI

Yield Data for All Plots

Yield Data

Plot	Treatment	Rep #	Crop	Final Yield(bu/A)
101	2	1	Soybean	61.758
102	3	1	Corn	118.539
103	1	1	Soybean	67.503
201	1	2	Soybean	63.018
202	2	2	Soybean	64.871
203	3	2	Corn	120.327
301	3	3	Corn	124.854
302	2	3	Soybean	58.401
303	1	3	Soybean	63.215
401	2	4	Soybean	64.655
402	3	4	Corn	130.534
403	1	4	Soybean	67.876
501	1	5	Soybean	64.921
502	3	5	Corn	123.190
503	2	5	Soybean	60.358
601	3	6	Corn	125.225
602	1	6	Soybean	61.091
603	2	6	Soybean	69.800
701	3	7	Corn	120.529
702	2	7	Soybean	62.563
703	1	7	Soybean	67.165
801	1	8	Soybean	65.105
802	2	8	Soybean	64.949
803	3	8	Corn	112.353

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