1-1-2017

Management of Plant-Parasitic Nematodes using Gene Manipulation and Biological Nematicides Biological Nematicides

Weasam Adnan Radhi Aljaafri

Management of plant-parasitic nematodes using gene manipulation and biological nematicides

By

Weasam Adnan Radhi Aljaafri

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Life Science
in the Department of Biochemistry, Molecular Biology, Entomology, and Plant Pathology

Mississippi State, Mississippi

August 2017

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Weasam Adnan Radhi Aljaafri

Approved

Gary Lawrence (Major Professor)	Vincent Klink (Co-Major Professor)

Katheryn Lawrence
(Committee Member)

Shien Lu
(Committee Member)

David Long
(Committee Member)

Kenneth Willeford
(Graduate Coordinator)

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Weasam Adnan Radhi Aljaafri

Date of Degree: August 11, 2017

Institution: Mississippi State University

Major Field: Life Science

Major Professor: Dr. Gary W. Lawrence

Title of Study: Management of plant-parasitic nematodes using gene manipulation and biological nematicides

Pages in Study 161

Candidate for Degree of Doctor of Philosophy

Soybean cyst nematode *(H. glycines*), reniform nematode (*R. reniformis),* and Root-Knot nematode *(M. incognita)* are three damaging plant-parasitic nematodes on soybean. Syntaxin proteins are involved in the process of membrane fusion. T wo *G. max* syntaxin genes (Gm-SYP22-1, and Gm-SYP22-2) that were similar in amino acid composition have been found to contribute to the ability of *Glycine max* to defend itself from infection by the plant parasitic nematode *Heterodera glycines*. Syntaxin genes SYP22-1 and SYP22-2 were identified to be expressed specifically in syncytia undergoing a resistant reaction to *H. glycines* parasitism. The Gm-SYP22-1 and Gm-SYP22-2 genes were isolated by molecular means and genetically engineered in *G. max* [Williams 82/PI 518671], a genotype typically susceptible to *H. glycines* parasitism. Genetically engineered control plants in *G. max* [Williams 82/PI 518671] that lack the overexpression of Gm-SYP22-1 or Gm-SYP22-2 genes were produced to serve as a comparison. The transgenic Gm-SYP22-1 or Gm-SYP22-2 overexpression lines with their pRAP15 control have then been infected with *H. glycines*. In another study, tests include three separate tests in 2015 and one test in 2016 that evaluated different biological products, application rates and product

combinations as seed treatments on soybeans. Results collected from soybean plants that were infested with either *H. glycines*, *M. incognita* or *R. reniformis* indicated that many of these biological products significantly reduced the nematode reproduction compared to control. The number of cyst, juveniles, and eggs recovered were significantly reduced compared with the non-treated control. Other findings identified *Burkholderia renojensis* variant 2 (BioST Nematicide) as being a more consistent nematicide candidate when referencing data from all nematodes and rate ranges. Combinations of *B. renojensis* variant 2 with selected SAR (systemic acquired resistant) products numerically improved the efficacy and consistency of the biological nematicide. Another study focused about investigated of biological seed treatments on *H. glycines*, *and F. virguliforme* indicated that many of these biological products significantly reduced the nematode reproduction over the fungicide only check. Foliar disease severity happened more in the treatments that infested with *H. glycines + F. virguliforme combination* than *F. virguliforme alone.*

DEDICATION

This dissertation dedicated to my father (Adnan Radhi Hamzah Aljaafri) and my mother (Lahida Jassim Hamzah Aljaafri) for their support, love and encouragement to continue my education. Also, I would like to share my wife (Ghaidaa Munean Jawad) and my kids (Ameer, Mortadh, Mohammed, and Youssif). In addition, I would like to include my brothers and sisters.

ACKNOWLEDGEMENTS

I would like to give my deep thankfulness to my major advisor, Dr. Gary Lawrence, for his careful guidance counselor and constant encouragement to be a freethinking researcher with a perspective toward hard research efforts. I thank him sincerely for giving awareness of nematology and truly encouraging me to develop into a good researcher. I would like to clear my truthful gratefulness to my co-advisor Dr. Vincent Klink, for graciously giving his valuable time and guidance to implement this study my graduate profession. Also, I would like to give him my deep thankful for give me a great chance to do a lot of works in the molecular techniques in the biology Sciences. I see myself indebted to my consultative committee member Dr. Kathy. S. Lawrence for her help specially to identified the isolation of my fungus. I would like to swift my deep respect and thankfulness to my committee member Dr. Shien Lu for presenting constructive advices and encouragement me at all the time during my work that supported in the successful completion of my research. I would like to clear my truthful gratefulness to my committee member Dr. David H Long, the "Seed Treatment Technical Manager, Albaugh, LLC" for graciously provide with his valuable time and orientation to implement this study my graduate profession. I would like to give him my very deep thankful for his support in the all the multiple facets of scientific research. I am especially appreciative to Dr. Jan DuBien for her help in the design and analysis the experiments, Dr. Michael A. Caprio, Dr. Jeffrey F.D. Dean, and Dr. Sead Sabanadzovic,

for being extremely useful to encourage me to complete my research. I am especially appreciative Mrs. Karin Lee the director of the international institute office for being immensely helpful to carry out this study. I am especially grateful Dr. Daniel Fleming, Dr. J. Santos Portugal for being highly helpful to carry out this study. I am especially grateful Mrs. Leslie Hunter Woolington for being highly helpful to support me in this study. I would like to admit my previous and present lab group members Dr. Shanker Pant, Dr. Aparna Krishnavajhala, Brant McNeece, Bisho Ram Lawaju, Prakash M. Niruala, and Keshav Sharma, Ismail for their supporting me to achieve my academic and research targets. I am also would like to say thank you to my lab classmate Msr. Yi Xiu Pinnix (Jan). I would like to give my especially thankful to undergraduate student workers (Paul Vaughan, Nathan Gillis Gaudin, Cody Roman) for their invaluable assistance. I want to give my deep thankful to my parents and family for their support me to finish my high degree in the United States. Also, I would like to thank my deep loving Ghaidaa Jawad (my wife) for her support during all the time of my study. My truthful thank will be to the Department of Biochemistry, Molecular Biology, Entomology, & Plant Pathology, Agriculture and life science. Mississippi State University. I would like to show my deep respect and thankfulness to my sponsor (The Higher Committee for Education Development in Iraq) to give me this scholarship for PhD. Degree in the United States. Also, I would like to thank all my Iraqi friends at Mississippi State University.

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CHAPTER I

SOYBEAN (*GLYCINE MAX*)

Introduction

Soybean (*Glycine max*) is a legume crop (Barrett 2006), is grown worldwide and is processed into soybean meal or oil for human consumption (Ali 2010), animal feed, or processed into biofuel. Soybean seeds are approximately 38% protein and 18% oil. Approximately 95% of the oil is consumed by humans with the remainder used for cosmetics and hygiene products or plastics (Liu 2008). Approximately 98% of the soybean meal is used for aquaculture and livestock feed. The remainder is processed into protein and soy flour for human consumption. A small percentage of soybean production is grown as a fresh market vegetable, in Japan as edamame, the United States as a green vegetable, and China as "mao dou", (Shanmugasundaram and Yan 2010).

Soybean production around the world varies by continent. North America & the Caribbean produced 83.9 million tons (38.6%), Asia produced 27.4 million tons (12.6%) and South America produced 101.8 million tons (46.8% of the world total). The United States is the top soybean producer in the world producing 37.0% (80.6 million tons). This is followed by Brazil with 53.9 million tons (24.8%), Argentina 41.4 million tons (19.0%), China 15.8 million tons (7.3%), and India producing 8.9 million tons (4.1%). Together, these countries accounted for more than 90 percent (92.2%) of the world total of soybean production and other counties with 10%. (Barrett 2006; Dwevedi 2011).

Soybean is affected by many diseases. These include foliar fungal diseases (aerial web blight, bacterial blight, Septoria leaf blight, Cercospora blight, downy mildew, frogeye leaf spot, soybean rust, and target spot) soil borne diseases (charcoal root rot, Phytophthora root rot, red crown rot, southern blight, stem canker, sudden death syndrome), and viruses (bean pod mottle virus, soybean vein necrosis-associated virus, and soybean mosaic virus). In addition to the microbial pathogens, plant-parasitic nematodes are a major, ubiquitous, dominant and persistent problem for soybean cultivation worldwide. Soybean are hosts to over 100 species of nematodes (Sinclair and Backman, 1989). The major soybean pest species of nematodes include *Meloidogyne incognita, Rotylenchulus reniformis, Heterodera glycines, Pratylenchus spp.,* and *Belonolaimus longicaudatus.* Among these*, H. glycines,* the soybean cyst nematode (SCN) causes more damage than the rest of the diseases and nematodes combined (Wrather and Koenning, 2006). SCN causes approximately 1 billion dollars in damage to soybeans producers each year, which was about a 7-10% production loss (Khan et al, 2004).

Plant-Parasitic nematodes

Soybean cyst nematode (*Heterodera glycines*)

The first scientific characterization of the soybean cyst nematode was by Ichinole (1952). The combination of increasing production of soybean, lack of agricultural practices to prevent the spread of plant-parasitic nematodes, and the biology of *H. glycines* set the stage for its rapid dispersal. *H. glycines* was first identified in the U.S. in 1954 in North Carolina (Winstead et al. 1955) and by 1957 the soybean cyst nematode (*H. glycines*) had been identified as far west as Mississippi (Riggs, 2004). Soybean cyst

nematodes have spread to 31 states in the United States. In the U.S., *H. glycines* infection has caused higher soybean yield losses than all other pathogens combined (Wrather et al. 2006), a value of approximately one billion dollars. *H. glycines* caused an estimated loss around 23 million metric tons of production loss on the soybean (Bradley and Koenning, 2014). This demonstrates the importance of *H. glycines* on soybean production, however oftentimes the losses resulting from this nematode are undervalued. Losses attributed to this nematode vary from year to year and are affected by the variety of the soybean, soil biotic and abiotic factors, and climat condition. Losses may reach as higher 30% or greater when *H. glycines* is widespread in a field. The highest losses occur in sandy soils which adds additional stress to soybeans in drought years.

H. glycines may be present in a field without causing noticeable symptoms. When symptoms do develop on plants, the first indication of soybean cyst nematode (*H. glycines*) are circular or elliptical shaped areas of the field in which plants are often stunted, less vigorous, and may be chlorotic in color. The size of the infested areas will depend on the length of time a field has been infested. Often, there is a sharp separation between the interface of apparently healthy and stunted plants. Plants growing in infested soils may remain stunted for the entire plant production season. Infected plants are slow to have canopy closure, thereby resulting in more weed growth. Below-ground symptoms are not easy to associate to *H. glycines* and may appear like symptoms of other root pathogens or resemble nutrient deficiencies. *H. glycines* reduces root growth and which results in a decrease in nitrogen fixating nodules on the roots. Nematode infections can also make the roots more susceptible to other root pathogens. This type of symptom

is difficult to observe and the causal agent can only be discerned when compared to plants with less or no infection.

The major diagnostic sign of *H. glycines* infection, is the presence of the female nematode in varying stages of development and of mature cysts attached on the soybean roots. Young females are small, white and partly buried in the root with only part of the nematode protruding on the surface. Older females are larger almost completely on the surface of the roots and appear yellowish or brown depending on maturity. Dead brown cysts may also be present on the roots of soybean plants (Agrios, 2005).

H. glycines life cycle

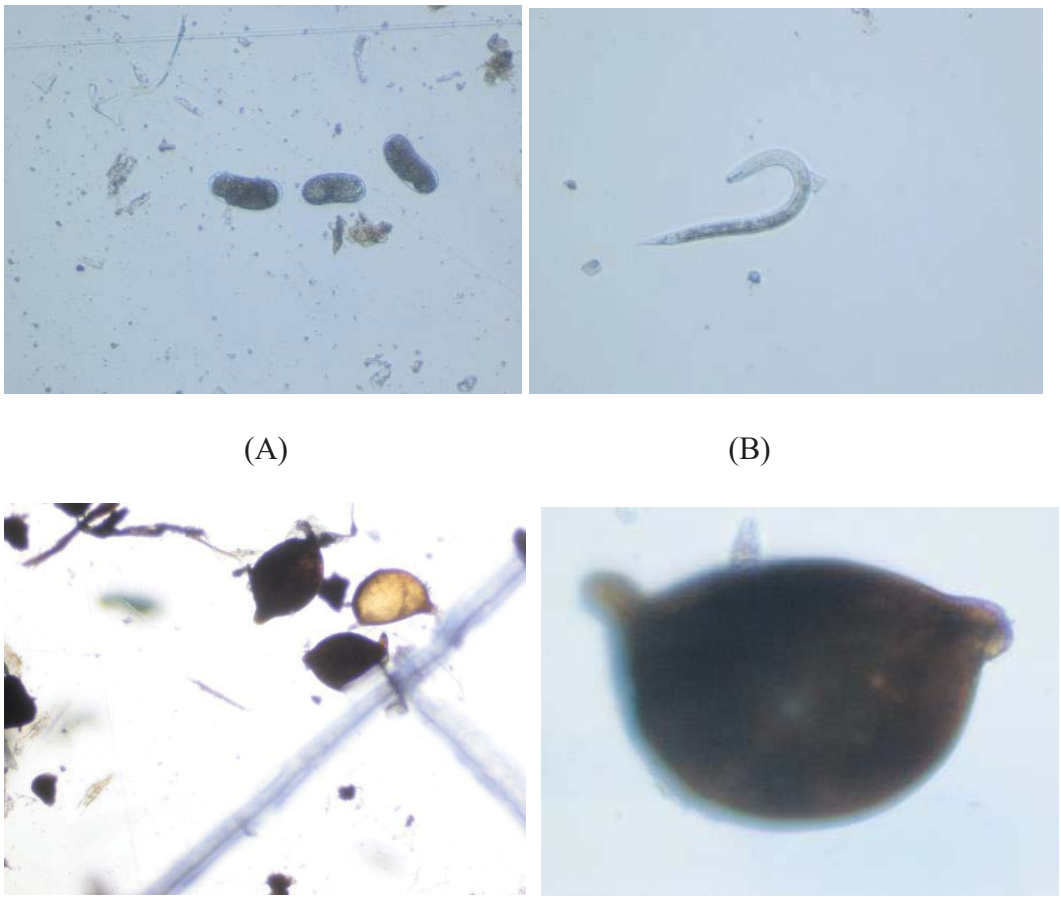
The soybean cyst nematode life cycle consists of six developmental stages. Eggs are encased within the cyst. The eggs will stay dormant in the cyst until appropriate environmental conditions are available. These environmental conditions include adequate moisture, temperature, and specific exudates produced by the host plant. Following egg hatch, there are four stages of juvenile development (Figure 1A) (Klink et al, 2009; Davis 2005). The first-stage juvenile molts in the egg to form of the second-stage juvenile (J2) or the pre-infective second stage juvenile. The second stage juvenile emerges from the cyst, migrates through water between soil particles toward a host root, and burrows into root tissue of the host. The pre-infective second stage juvenile is attracted by root exudates to actively growing roots and upon finding and establishing itself in a suitable root, becomes an infective J2 (i-J2) and will penetrate the host close to the root tip (Figure 1B). When the nematode reaches the pericycle, the stylet is injected into the host cell. At this point, the nematode becomes parasitic (p-J2). The p-J2 then injects substances (proteins) into the plant cell. The parasitized pericycle cell wall will fuse with

neighboring cell walls and forms the complex feeding site known as a syncytium (Davis 2005; Opperman 1998). During this process, proteins are synthesized in the soybean cyst nematode esophageal and/or sub ventral gland cells. The process starts via breakdown of cell wall material close to the plasmodesmata. The cell wall will increase in size, permitting the free flow of cytoplasm, and nuclei in and out of former cellular boundaries and organelles. The repeated cell fusion events produce a syncytium. The syncytium, may include approximately 200 cells sharing a general cytoplasm (Jones and Northcote 1972; Jones. 1981). The infective second stage (i-j2) starts to enlarge, becomes sausage-shaped, and molts three times becoming an adult.

The p-J2 nematodes that develop into males feed for several days. Males will be sedentary during feeding and the feeding process continues until the end of their J3 life stage. The males will stop feeding and subsequently molt remaining in the second stage and third stage cuticles. The adult male molts a final time to become a slender, vermiform motile individual and which burrows out of the cuticle and root for mating. In contrast, the pre-infective stage juveniles that ultimately will develop into females stay sedentary after the establishment of its nurse cell. Through feeding, the adult female will increase in size (Figure 1C). The process is followed third and fourth stage juvenile molts. Through growth, the posterior of the female will erupt out of the root boundary and through the root epidermis. Juveniles that develop into females are sedentary and only able to move their head to feed on the syncytial cells (Davis 2005; Opperman 1998). The posterior of the female erupting beyond the root boundary gives access to the male to mate. After copulation, adult females will produce their eggs in the gelatinous matrix outside of their bodies. The life cycle of the female ends when the cuticle color changes to a creamy

white or yellow-tan that is an indicating signal for mortality of the cyst females. Females will continue to lay the eggs, some oe eggs will be inside her body and other outside in gelatinous matrix. Egg may remain viable up nine years (Inagaki and Tsutsumi, 1971).

The adult female will be lemon-shaped and commonly is visible on the root system of susceptible plants without high magnification (Figure 1D). A pheromone is released by the female to attract males for mating. The total life cycle requires 30-40 days to complete; however, this is influenced by the environment (mainly adequate temperature and moisture). The optimum soil temperature is 75 °F to initiate egg hatch, 82 °F for root penetration, and 82-89 °F for juvenile. da Rocha et al. (2008). Therefore, many generations of soybean cyst nematode can be completed in a typical soybean growing season. Figure (1.1).



(A) (B)

(C) (D)

Figure 1.1 Life cycle of soybean cyst nematode (*Heterodera glycines*).

(A) *H. glycines* eggs under the microscope (20X); (B) *H. glycines juvenile* second stage (infective stage, J2) under the microscope (20X); (C) *H. glycines* white adult female under the microscope (20X), (D) *H. glycines* cyst nematode undermicroscope (20X). (Photos by Weasam Aljaafri).

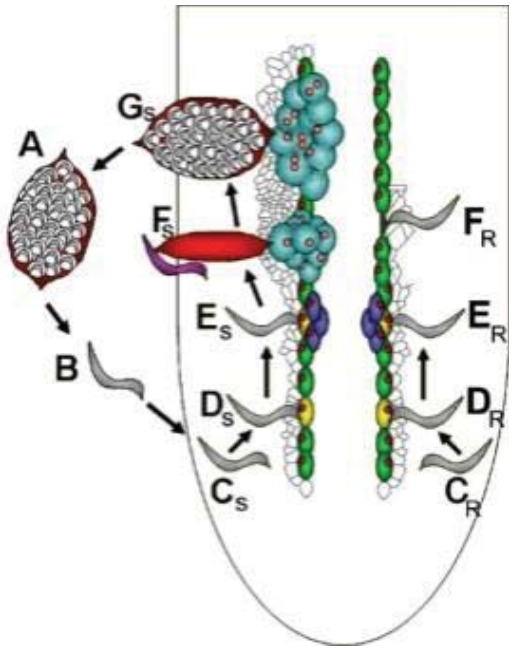


Figure 1.2 Life cycle of soybean cyst nematode (*H. glycines*).

A, Cysts of nematode. B, pi-J2 (gray color) hatch and migrate across the root of host. CS, CR i-J2 nematodes burrow within the root and migrate across the pericycle (green color). DS, DR, i-J2 select a cell (yellow color) for feeding site establishment. ES, i-J2 soybean cyst nematode has molted into third stage. ER, I- J2 nematodes will not increase in the size. FS, the third stage of juvenile undergo the subsequent molt into the fourth stage of juvenile nematodes. Meantime, the female keeps growing circumferentially as nematode feeds. The male discontinues feeding at the end of their third stage of juvenile. Male and female in the fourth stage of juvenile nematodes that be adults. The vermiform male (blue color) burrows outside the root and subsequently copulates with the female. FR, the syncytium collapses and the nematodes do not grow. G, after ~30 days, the female with eggs is clearly visible and emerging from the root. Figure adapted from Klink et al. (2009a).

Reniform nematode (*Rotylenchulus reniformis*)

The reniform nematode (*Rotylenchulus reniformis*) occurs primarily in the tropical and subtropical regions worldwide. Reniform nematodes were first identified in 1931 by Hagan and Yap from cowpea in rotation with pineapple on the Island of Ohau, Hawaii (Linford, M.B., and F. Yap. 1940; Linford, M. B., and J. M. Oliveira. 1940). The first confirmation of the genus and species *Rotylenchulus reniformis* was made in 1940 by Linford and Olivera (Linford, M.B. and J.M. Oliveira. 1940). Currently *R. reniformis*

has been recorded in most of the Gulf Coast States and including Tennessee, Arkansas, Georgia, Missouri, North Carolina, South Carolina, and Hawaii. (Bird, G. W, et al., 1973; Linford, M. B., F. Yap. 1940; Heald, C.M., and A. F. Robinson.1990; Linford, M. B., J.M. Oliveira. 1940; Fassuliotis, G., R. V. et al., 1968). *R. reniformis* was identified in the Gold Coast of West Africa in 1956 as a parasite for soybean (Peacock, F.C. 1956). *R. reniformis* was first discovered in Mississippi in 1968 on centipede grass (Patel, M. V. 1990). In Mississippi, *R. reniformis* has been observed in the 51 counties, and yield losses have been estimated at an average of 29% of the total field (Lawrence and McLean, 2002). This nematode has been identified in 55%, 30%, and 32% of the cotton acreage of Louisiana, Alabama, and Mississippi, respectively (Lawrence and McLean, 1999). *R. reniformis* has increased from a proportional uncommon nematode to the major pathogens the United States (Lawrence et al., 2005).

There are many species of plants that may serve as hosts for *R. reniformis*. (Caswell, E.P. et al.1991; Birchfield, W. and L.R. Brister.1962; Peacock, F.C. 1956; Linford, M.B. and F. Yap. 1940,). The major crop hosts for *R. reniformis* are cotton, tobacco, soybean, sweet potato, and many vegetables. (Scumbiato, G.L. and D.L Turnage. 1992). Recently, *R. reniformis* has replaced the root-knot nematode as the most common parasitic species on cotton in the southeastern Cotton Belt (McLean and Lawrence, 2000).

Annual cotton yield losses due to *R. reniformis* is estimated around $100 million yearly (Blasingame and Patel 2013). The losses by *R. reniformis* is estimated around 12% in the United States according to the Cotton Disease Loss Estimate Committee (Lawrence

et al., 2014). The yield losses, Mississippi's due to *R. reniformis* averages 8.6% annually (Lawrence et al., 2014).

The symptoms of *R. reniformis* on soybeans include stunting, empty pods, chlorosis, and root decay (Sinclair, J. B. and P. A. Backman. 1989). Also, nematode is known to parasitize the rhizobium on the roots system and reduced the yield around 33.1%. (Meredith, J.A., et al. 1983; Rebois, R. V. et al., 1968).

Life cycle of *Rotylenchulus reniformis*

Rotylenchulus reniformis is considered a semi-endoparasite due to the way the nematode penetrates and parasitizes the host. The adult females oviposit eggs in a gelatinous matrix (Figure 1.3 A). The first stage of juvenile nematode molts within the egg producing second stage of juvenile which emerges from the eggs (Figure 1.3 B, C). After emerging, three additional molts will occur in the soil within nine or ten days. Vermiform present males and females, the vermiform adult female will penetrate and parasitize the host plant by infecting new roots of the plant or re-infecting the roots currently parasitized by other females (Figure 1.3 D). The vermiform adult female penetrates the roots with the anterior part of its body until the head region is in the phloem and cortex of the host roots. The posterior part of the adult female which nematode outside that will start to swell after 24 hours and within four to five days they assume the characteristic reniform shape. The adult females oviposit their eggs within eight to nine days after insemination in a gelatinous matrix. Each female may lay 60 to 200 eggs. The males remain vermiform and have not been observed to feed Males are often observed coiled around the adult female in the gelatinous matrix. The total life

cycle of *R. reniform* nematode on the host takes around 16 to 23 days when the soil temperature around 29 C (Riggs, R.D. 1982).

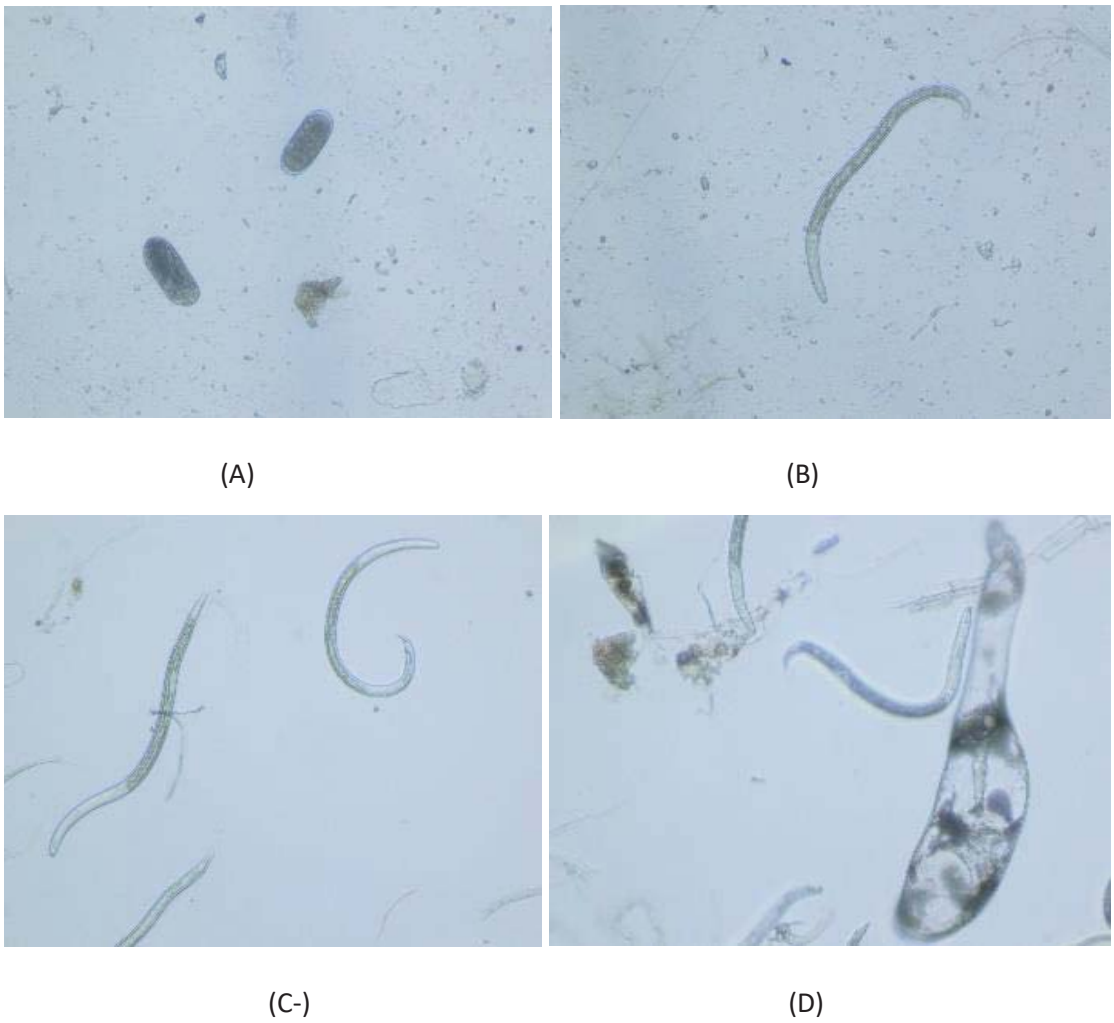


Figure 1.3 Life cycle of *Rotylenchulus reniformis*.

(A) *R. reniformis* eggs under the microscope (20X); (B) *R. reniformis* second stage (J2) female under microscope (20X); (C) *R. reniformis* second stage male (J2) under microscope (20X); (D) *R. reniformis* adult female- infective stage under microscope (20X). (Photos by Weasam Aljaafri).

Root-knot nematode (*Meloidogyne incognita*)

The southern root-knot nematode (*M. incognita, (Kofoid and White) Chitwood* has been considered an economically significant pest on many crops that are grown in the southern and western regions of the United States. *M. incognita* causes estimated crop losses around of 5 to 10% for several major crops including vegetables, field crops, ornamentals, and fruits (Haseeb et al., 1984; Stokes, 1977; Bird and Hogger, 1973; Hogger and Bird, 1976; Schroder et al., 1993; Walker et al., 1994). *M. incognita* is an obligate, sedentary endoparasite. The host range of the *M. incognita* is very broad. The parasite modifies the cells to supply the female with a sufficient source of the nutrients to complete the life cycle (Eisenback and Triantaphyllou, 1991). The infection of the host via *M. incognita* is both intracellular and intercellular and results in the damage of epidermal and sub-epidermal cells (Wyss, V. 1975). The mechanical acts of the stylet (Linford, 1942) and enzymes excreted by the sub-ventral esophageal gland (Bird et al, 1975) allow for intracellular penetration. *M. incognita* causes significant yield loss on the soybean crop (Weaver, D.B., et al. 1988) by as much as 90% to susceptible soybean varieties (Kinloch, R.A., 1974.). Yearly soybean production losses in the United States by *M. incognita* nematode exceed 99,000 metric tons (Wrather, J.A., et al. 2003).

M. incognita cause physiological alterations and dramatic morphological changes in the cells of plants. The symptoms and signs that are associated with root-knot nematodes infection include root galls and root rots, stunted growth, shoot chlorosis, and other symptoms and signs that are commonly associated with nutritional deficiencies, including chlorosis (Bala and Hosein, 1996; Bird, 1974; Misra et al., 2002; Zarina and Abid, 1995), and common decline (Nigh, 1972), including poor yield, and wilting

(Rajendran et al., 1975). The root-knot nematode is easily recognized by the characteristic knots or galls that are produced on the roots where the nematode feeds and develops (Caillaud et al., 2008).

Life cycle of Root-knot nematode (*Meloidogyne incognita*)

M. incognita is a sedentary endoparasitic nematode getting food from inside the roots. The first stage of the life cycle of *M. incognita* is the egg (Figure 1.4 A). The first stage juvenile stage is found and indroducing the first molt inside the egg, forming second stage juvenile (J2) prior hatching. (Abad et al. 2009). The second juvenile hatches from the egg and once find host plant will penetrate near the root tip (Figure 1.4 B) (Abad et al. 2003). The second stage juvenile (J2) migrates intercellular and intracellularly to meristematic region of the root (Abad et al. 2009). J2 will move intercellulary during the cortex and intracellilary through the vascular tissue. Then, second stage (J2) will establish the feeding site on vascular tissue. After migration, the second stage reaches the improving vascular root tissue. In order to get nutrients and sustain their subsequent sedentary parasitic stages, each second stage encourages the differentiation of 5 to 7 parenchymatic root cells inside a multinucleate and hypertrophied feeding cells often referred to the giant cells. Giant cells will grow very large in the size. The giant cells get high metabolic activity when modified via secretions of *M. incognita* and these cells will be hypertrophy under this reaction will produce root galls on the roots. Root cells in the neighboring of the giant-cells also will be enlarge and divide quickly and outcoming in gall formation presumably as a results of plant growth regulator diffusion. *M. incognita* second stage will feed from the giant cells and molt three times to reach the reproductive mature adult phase. Males will molt back to the vermiform shape and then migrate out of

the root to meet with females (Figure 1.4 C). Females will be pear-shaped (Figure 1.4 D) and produce 200-1000 eggs, and release the eggs on the root surface in the protective gelatinous matrix. The eggs mass will produce outside of her body for *M. incognita*. The life cycle might be completed in 20 days in the optimum temperature of 25 - 30 °C. (Abad et al. 2009).

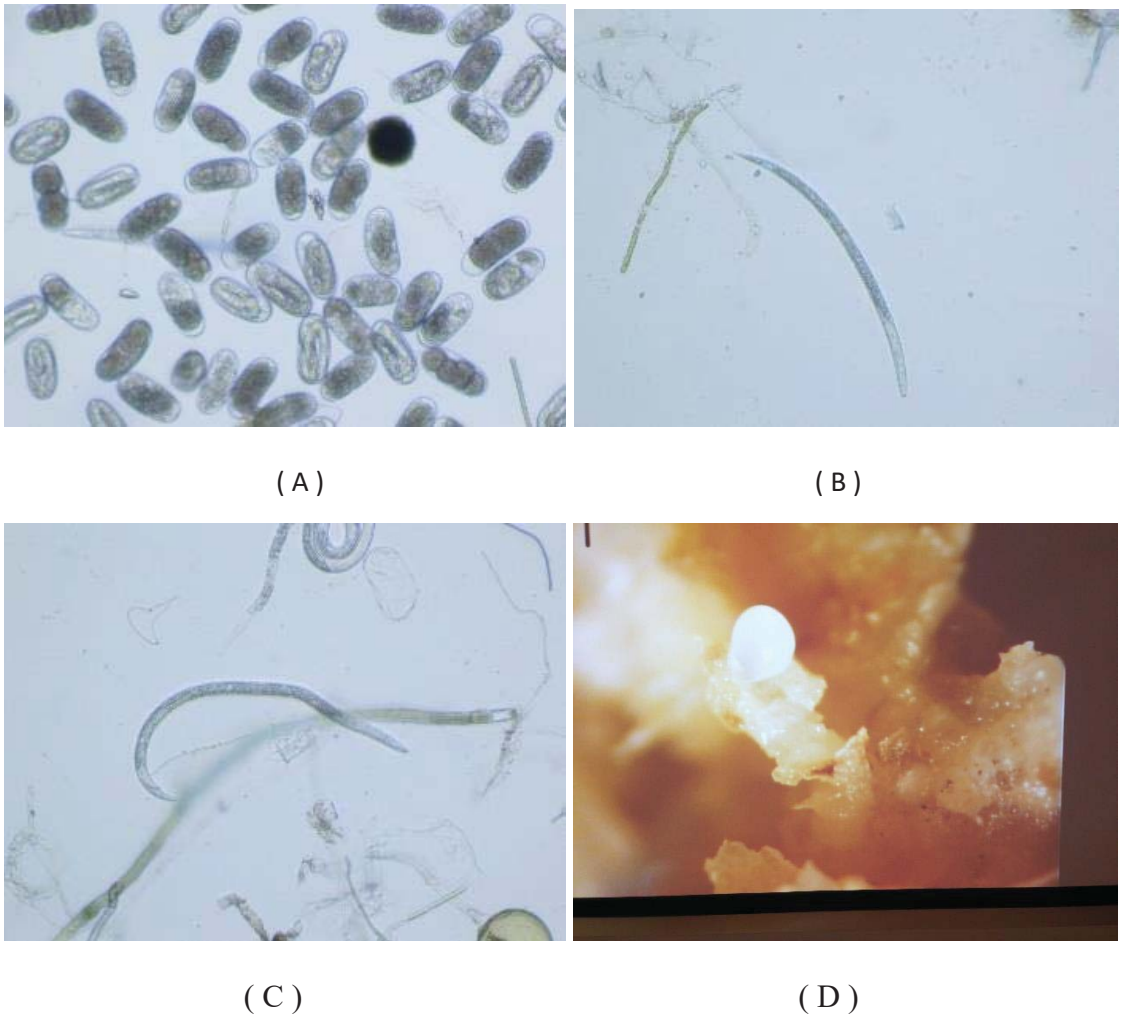


Figure 1.4 Life cycle of *Meloidogyne incognita*.

(A)*Meloidogyne incognita* eggs under the microscope (20X); (B) *M. incognita* second stage juvenile (J2), infective stage female under the microscope (20X); (C) *Meloidogyne. Incognita* second stage male under the microscope (20X); (D) *M. incognita* female attach the roots. (Photos by Weasam Aljaafri).

Management of plant-parasitic nematodes

Historically, plant-parasitic nematodes have been managed using a combination of chemical control, biological control, crop rotation, and resistant germplasm.

There are two basic types of chemicals for nematodes management. These are fumigants and non-fumigants (Schneider et al. 2003; Rosskopf et al. 2005). These are need for pre- and post-plant nematode management tactics. Due to the loss of many of these major management tactics for the soybean cyst nematode (*Heterodera glycines*) (Rosskopf et al. 2005), other strategies needed to be identified for nematode management. A nematicide that can safely be applied to growing plants and translocate to the roots in sufficient quantities to kill both ecto- or endoparasites nematodes has not been developed. However, many recertly biological products have been studied and shown nematicide properties (Lawrence et al. 2016; Xiang et al. 2013). The newest of these to be made commercially available is imidacloprid+ fluopyram (known as Velum Total™Bayer Crop Science, Raleigh, NC). In 2016, Lawrence, et al. (unpublished) studied Velum Total™ as in-furrow spray in cotton with seed treatments such as Aeris (Imidacloprid+ Thiodicarb, 0.75 mg ai/seed, Bayer Crop Science, Raleigh, NC). The results of this tests have been shown the Velum Total plus Aeris had activity against nematodes. (Lawrence et al., 2016).

Seed treatment nematicides were introduced in 2005 and crop production and management practices started to change. Management practices have changed from the standard granular in-furrow applications to seed treatments. Seed treatments can be used to manage the pathogen as a contact or systemic nematicides (Mueller et al., 2013). The systemic products are maintainal for a larger permid of tree and inside the plant tissue

and continue during growning stage that potential foliar butter management for soil-borne diseases (Mueller et al., 2013).

There are number of products that have been registered as seed treatments for the management of plant- parasitic nematodes. These seed biological nematicides have shown activity on a number of plant-parasitic nematodes and used for several crops.

These include Avicta® (abamectin, Syngenta) was effctive (*M. incoginta* on tomato, tobacco, and cotton (Qiao et al., 2012; Muzhandu et al., 2014 Faske and Starr, 2007). VOTiVO® (*Bacillus firmus*, Bayer CropScience) votivo has shown activity for *Rotylenchulus reniformis*, *Herodera glycines,* and *M. incoginta* (Castillo et al., 2013; Schrimsher et al., 2011; Mendoza et al., 2008). Another, and Clariva® (*Pasteuria nishizawae*, Syngenta). Clariva has shown activity against *H. glycines* management.

Crop rotation is one of these practices that useful for nematodes management. The goal in crop rotation is to reduce populations of plant-parasitic nematodes below harmful levels. (Francl and Dropkin, 1986; Sasser and Uzzell, 1991; Koenning et al. 1993). Rotations usually involves planting a non-host crop for two to three years to reduce economically significant levels of plant-parasitic nematodes in the field (Ross, 1962; Francl and Dropkin. 1986; Chellemi, 2002). Management of nematodes with crop rotation is difficult with species such as the *Meloidogyne incognita* nematode which has a wide host range.

Host plant resistance is the primary means of nematodes management. (Niblack and Chen, 2004). Planting resistant cultivars is the most efficient means of managing plant-parasitic nematodes. Plant breeders have examined host resistance in soybean and cotton for this response to several plant-parasitic nematodes including, *H. glycines* and

M. incognita. To opened the development of method resistant varties technologies were used to identified sequences. Gutiérrez found that simple sequence repeats (SSRs) were closely associated with genes for resistance to *M. incognita* on chromosomes 11 and 14 of upland cotton (Gutiérrez et al. 2010). Jenkins et al developed markers to detect SSR to help identify *M. incognita* resistant plants (Jenkins et al. 2012). This work assists breeders to quickly develop *M. incognita* resistant cultivars. Kadam et al. (2016) analyzed the phylogenetic variety of the Rhg1 and Rhg4 loci in soybean and developed SNP signs to detect resistance for *H. glycines* by using resistant genes and QTL. Shi et al. (2015) identified SNPs and evolved marker examination for high-rise throughput to choose soybean varieties with resistance to soybean cyst nematode *H. glycines*. Carter et al. (2011) developed and released the 'N7003CN' soybean line with high yield and resistance to *H. glycines* race 2. Genetic engineering has become a possible means to generate nematode resistance (McLean et al. 2007; Matsye et al. 2012; Klink et al. 2009).

Sudden Death Syndrome

Sudden death syndrome (SDS) was first time observed in 1971 in Arkansas by H.J. Walters on plants exhibiting interveinal chlorotic lesions (Roy et al. 1997). In 2010 the losses attenuated to SDS were estimated 4.7 million metric tons. (Bradley and Koenning, 2014). The fungus that causes this disease is *Fusarium virguliforme*. (Aoki et al., 2005). The SDS disease cycle starts with the infection stage of the roots for soybean via germinating chlamydospores, which are the overwintering structures for the fungus and can survive across a high range of temperatures and soil types. The chlamydospores produce the mycelium which infect the roots of plants (McLean and Lawrence, 1995). After infection, symptoms develop as discoloration of the roots and blue spore masses

which may be seen on the taproot. (Luo et al., 1999; Roy et al., 1997). Foliar symptoms of SDS consist of interveinal chlorotic lesions, which may eventually become necrotic.

Recently, a few products have been developed for SDS management. ILeVO® fluopyram, Bayer CropScience Co.) applied as seed treatment has been shown to be effective to control SDS (Avenot and Michailides, 2010; Avenot et al. 2012).

Primaries all attempts at SDS management have been by host resistance and cultural practices. However, Leandro et al. (2013) dtermined that SDS can develop in any cultivar during times of suboptimal environmental conditions. Other management strategies include delayed planting, tillage, and rotation with non-host plants. (Wrather et al., 1995; De Bruin and Pederson, 2008). SDS cause significant damage by itself is also interacts with (*H. glycines*). (McLean and Lawrence, 1995; Xing and Westphal, 2006; Xing and Westphal, 2009). The presence of *H. glycines* in a field will lead to a greater severity of SDS and higher yield losses (McLean and Lawrence, 1995. Lawrence, et al 1988).

Management of nematodes (*H. glycines, M. incognita, R. reniformis)* will continue to play a mager role in soybean production. While the introduction of molecular technology and goal of developing safer and environmentally friendly nematicides, the objective of this study are:

Objectives of Study

1-Evaluate the impact of specific selected genes for resistance to nematode.

2- Evaluate the effect of seeds treated with different biological nematicides on nematode management and their effects on plant growth.

3- Evaluated of biological seed treatments nematicides on SCN and effect on SDS disease on soybean.

References

Abad, P., Castagnone-Sereno, P., Rosso, M. N., Engler, J. D. A., and Favery, B. 2009. Invasion, feeding and development. P. 163 - 176. In Root-knot nematode. Eds. Perry, R. N., Moens, M., and Starr, J. L. CAB International. Wallingford, OX, UK.

Abad, P., Favery, B., Rosso, M. N., and Castagnone-Sereno, P. 2003. Root-knot nematode parasitism and host response: molecular basis of a sophisticated interaction. Molecular Plant Pathology. 4:217 - 224.

Agrios, G. N., 2005. Plant Pathology. Academic Press. Fifth edition.

Ali, N. 2010. Soybean processing and utilization. In G. Singh (Ed.), the soybean (pp. 345–374). CABI.

Aoki, T., O'Donnell, K., and Scandiani, M. M. 2005. Sudden death syndrome of soybean in South America is caused by four species *of Fusarium: Fusarium brasiliense sp. nov., F. cuneirostrum sp. nov., F. tucumaniae, and F. virguliforme*. Mycoscience 46:162-183.

Avenot, H. F., Biggelaar, H. v. d., Morgan, D. P. and Michailides, T. J. 2012. Fungicidal activity of fluopyram for suppression of Alternaria species pathogenic on California pistachio. Resistant Pest Management Newsletter 22:10-14.

Avenot, H. F. and Michailides, T. J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Protection 29:643-651.

Bala, G., and F. Hosein. 1996. Plant parasitic nematodes associated with anthuriums and other tropical ornamentals. Nematropica 26:9-14.

Barrett, J. 2006. The Science of Soy: What do we really know? Environmental Health Perspective 114(6): A352-A358

Birchfield, W., and L.R. Braister.1962. New host and non-host of renifor nematode. Plant Disease Reporter 46: 683-685.

Bird, A. F. 1974. Plant response to root-knot nematode. Annual Review of Phytopathology 12:69- 85.

Bird, A. F., W. J. F. Downtown, and J. S. Hawker. 1975. Cellulose secretion by second stage larvae of the root-knot nematode (*Meloidogyne javanica*). Marcellia 38:165-169.

Bird, G. W., and Hogger, C. H. 1973. Nutsedges as hosts of plantparasitic nematodes in Georgia cotton fields. Plant Disease Reporter 57:402.

Bird, G. W., J.L. Crawford, and N. E. McGlohn. 1973. Distribution, frequency of occurrence, and population dynamics of *Rotylenchulus reniformis* in Georgia. Plant Disease Reporter 57: 399-401.

Blasingame, D., Patel, M. V .2013. Cotton disease loss estimate committee report. In: Proceedings of the 2013 Beltwide cotton conferences, San Antonio, pp 1242–1246. 7–10.

Bradley, C. A., and Koenning, S. R. 2014. Soybean disease loss estimates graph- 2009-2011. http://unitedsoybean.org/wp-content/uploads/Soybean-Disease-Loss-Estimates-Graph-2009-2011.pptx

Caillaud, M.C., Abad, P., Favery, B., 2008. Cytoskeleton reorganization, a key process in root-knot nematode induced giant cell ontogenesis. Plant Signaling and Behavior 3 (10), 816–818.

Carter, T. E., Koenning, S. R., Burton, J. W., Rzewnicki, P. E., Villagarcia, M. R., Bowman, D. T., and Arelli, P. R. 2011. Registration of 'N7003CN' maturity-group-VII soybean with high yield and resistance to race 2 (HG Type 1.2.5.7-) soybean cyst nematode. Journal of Plant Registrations. 5:309.

Caswell, E.P., J. Defank, W. J. Apt, and C.S. Tong. 1991. Influence of nonhost plants on population decline of *Rotylenchulus reniformis*. Journal of Nematology 23: 91-98.

Castillo, J. D., Lawrence, K. S., and Kloepper, J. W. 2013. Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 on cotton. Plant Disease. 97:967 - 76.

Chellemi, D. O. 2002. Nonchemical management of soilborne pests in fresh market vegetable production systems. Phytopathology 92:1367–1272.

Davis, E., Tylka G. 2005. Soybean Cyst Nematode Disease. The Plant Health Instructor.

da Rocha, M. R., Anderson, T. R. and Welacky, T. W. 2008. Effect of inoculation temperature and soybean genotype on root penetration and establishment of *Heterodera glycines*. Journal of Nematology 40:281-285.

De Bruin, J. L. and Pedersen, P. 2008. Soybean seed yield response to planting date and seeding rate in the upper Midwest. Agronomy Journal 100:696-703.

Dwevedi, A., Kayastha. 2011. A. Soybean: A multifaceted legume with enormous economic capabilities. Dwevedi A., Kayastha A. Soybean: A multifaceted legume with enormous economic capabilities. Soybean-Biochemistry, Chemistry, and Physiology

Einsenback, J. D., and H.H. Triantaphyllou. 1991. Root-knot nematodes: Meloidogyne species and races. Pp. 191-274. In: Nickle, W. R., ed M annual of Agricultural Nematology. Marcel Dekkler Inc., New Yourk. NY.

Fassuliotis, G., and G.J. Raw. 1967. The reniform nematode in South Carolina. Plant Disease Reporter 51: 557.

Faske, T. R. and Starr, J. L. 2007. Cotton root protection from plant-parasitic nematodes by abamectin-treated seed. Journal of Nematology 39:27-30.

Francl, L.J., and Dropkin, V. H. 1986. *Heterodera glycines* population dynamics and relation of initial population density tp soybean yield. Plant Disease: 70: 791-795

Gutiérrez, O. A., Jenkins, J. N., McCarty, J. C., Wubben, M. J., Hayes, R. W., and Callahan, F. E. 2010. SSR markers closely associated with genes for resistance to root-knot nematode on chromosomes 11 and 14 of Upland cotton. Theoretical and Applied Genetics. 121:1323 - 1337.

Hartman, K. M. and J. N. Sasser. 1985. Identification of *Meloidogye* species on the basis of differential host test and perineal-pattern morphology. Pp. 69-76 *in* K. R Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne*, vol. 2. Methodology.

Haseeb, A., R. Pandey, and A. Husain. 1984. Some new host records of the root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949. Current Science 53:1151-1152.

Heald, C.M., and A.F. Robinson. 1990. Survey of current distribution of *Rotylenchulus reniformis* in the United States. Supplement to Journal of Nematology 22 (4s): 695-699.

Hogger, C. H., and Bird, G. W. 1976. Weed and indicator hosts of plant-parasitic nematodes in Georgia cotton and soybean fields. Plant Disease Reporter 60:223–226.

Ichinohe, M. 1952. On the soybean nematode, *Heterodera glycines* n. sp., from Japan. Magazine of Applied Zoology 17: 1-4

Inagaki, H., and Tsutsumi M. (1971) Survival of the soybean cyst nematode, *Heterodera glycines* Ichinohe (*Tylenchida: Heteroderidae*) under certain storage conditions. Appl Entomol Zool (Jpn) 8: 53–63

Jenkins, J. N., McCarty, J. C., Wubben, M. J., and Hayes, R. 2012. SSR markers for marker assisted selection of root-knot nematode (*Meloidogyne incognita*) resistant plants in cotton (*Gossypium hirsutum* L). Euphytica. 183:49 - 54.

Jones, M. G. K. 1981. the development and function of plant cells modified by endoparasitic nematodes. Pages 255-279 in: Plant Parasitic Nematodes, Vol. III. B. M. Zuckerman and R. A. Rohde, eds. Academic Press, New York, U.S.A.

Jones, M. G. K., Northcote D.H. 1972. Nematode-induced syncytium-a multinucleate transfer cell. J Cell Sci 10: 789–809

Kadam, S., Vuong, T. D., Qiu, D., Meinhardt, C. G., Song, L., Deshmukh, R., Patil, G., Wan, J. R., Valliyodan, B., Scaboo, A. M., Shannon, J. G., and Nyuyen, H. T. 2016. Genomic-assisted phylogenetic analysis and marker development for next generation soybean cyst nematode resistance breeding. Plant Science. 242:342 - 350.

Khan, R., Alkharouf N., Beard H., MacDonald M., Chouikha I., Meyer S., Grefenstette J., Knap H., and Matthews B. 2004. Microarray Analysis of Gene Expression in Soybean Roots Susceptible to the Soybean Cyst Nematode Two Days Post Invasion. Journal of Nematology 36(3):241-248

Kinloch, R. A. 1974. Response of soybean cultivars to nematicidal treatments of soil infested with Meloidogyne incognita. J. Nematol., 6: 7-11.

Klink, V.P., Kim K-H., Martins V.E., MacDonald M.H., Beard H.S., Alkharouf N.W., Lee S-K., Park S-C., and Matthews B.F. 2009. A correlation between host-mediated expression of parasite genes as tandem inverted repeats and abrogation of the formation of female *Heterodera glycines* cysts during infection of *Glycine max*. Planta 230:53-71

Koenning, S. R, Schmitt DP, Barker KR. 1993. Effects of cropping systems on population density of *Heterodera glycines* and soybean yield. Plant Disease 77: 780-786

Lawrence, G. W., and K. S. McLean. 1999. Managing the reniform nematode with nematicides. Pp. 100-101 *in* C. P. Dugger and D. A. Richter, eds. Proceedings of the Beltwide Cotton Conferences, Jan. 3-7, Orlando, FL: National Cotton Council of America. www.cotton.org./beltwide/proceedings/1999/ abstracts/43.cfm

Lawrence, G. W., K. W. Roy, and K. S. Mclean. 1988. Soybean cyst nematode associations with sudden death syndrome of soybeans. Phytopathology 78: 1514. (Abstract).

Lawrence, K. S., Lawrence, G. W., and van Santan, E. 2005. Effect of controlled cold storage on recovery of *Rotylenchulus reniformis* from naturally infested soil. Journal of Nematology 37:272–275.

Lawrence, G. W., and K. S. McLean. 2002. The reniform nematode. Pp. 145-146 *in* J. McRae and D. A. Ritcher, eds. Proceedings of the Beltwide Cotton Conferences, Jan. 8-12, Atlanta, GA: National Cotton Council of America. www. cotton.org/beltwide/proceedings/2002/abstracts/ B2003.cfm.

Lawrence, K. S., M. Olsen, T. Faske, R. Hutmacher, J. Muller, J. Mario, B. Kemerait, C. Overstreet, G. Sciumbato, G. W. Lawrence, S. Atwell, S. Thomas, S. Koenning, R. Boman, H. Young, J. Woodward, and H. L. Mehl. 2014. Cotton disease loss estimate committee report. Pp. 247-248 *in* S. Boyd, M. Huffman, and B. Robinson, eds. Proceedings of the Beltwide Cotton Conferences, Jan. 6-8, New Orleans, LA: National Cotton Council of America. www.cotton.org/beltwide/ proceedings/2005-2014/data/conferences/2010/ S1842.HTM

Lawrence, K. S., Lawrence, G. W., Faske, T., Kemerait, B., Overstreet, C., Wheeler, T., Young, H., and Mehl, H. 2016. Beltwide nematode research and education committee 2015 nematode research report cotton varietal and nematicide responses in nematode soils. 1:737 – 740.

Leandro, L. F. S., Robertson, A. E., Mueller, D. S. and Yang, X.B. 2013. Climatic and environmental trends observed during epidemic and non-epidemic years of soybean sudden death syndrome in Iowa. Online. Plant Health Progress doi: 10.1094/PHP-2013- 0529-01-RS.

Linford, M. B. 1942. The transient feeding of root-knot nematode larvae. Phytopathology 32: 5880-589.

Linford, M.B., and F. Yay. 1940. Some host plants of the reniform nematode in Hawaii. Proceedings of the Helminthological Society of Washington 7: 42-44.

Linford, M.B., and J.M. Olivera. 1940. *Rotylenchulus reniformis*, nov. gen., n. sp., a nematode parasite of roots. Proceedings of the Helminthological Society of Washington 7: 34-42.

Liu, K., 2008. Food use of whole soybeans. In L. Johnson, P. J. White, & R. Galloway (Eds.), Soybeans: chemistry, production, processing, and utilization (pp. 441–482). Urbana, IL: AOCS Press.

Luo, Y., Myers, O., Lightfoot, D. A. and Schmidt, M. E. 1999. Root colonization of soybean cultivars in the field by *Fusarium solani* f. sp. *glycines*. Plant Disease 83:1155- 1159.

Matsye, P. D., Lawrence G.W., Youssef R.M., Kim K-H., Matthews B.F., Lawrence K.S., and Klink V.P. 2012. The expression of a naturally occurring, truncated allele of an □-SNAP gene suppresses plant parasitic nematode infection. Plant Molecular Biology 80:131-155

McLean, M. D., Hoover G.J., Bancroft B., Makhmoudova A., Clark S.M., Welacky T., Simmonds D.H., and Shelp B.J. 2007. Identification of the full-length Hs1pro-1 coding sequence and preliminary evaluation of soybean cyst nematode resistance in soybean transformed with Hs1pro-1 cDNA. Can J Botany 85: 437-441

McLean, K. S. and Lawrence, G. W. 1995. Development of *Heterodera glycines* as affected by *Fusarium solani*, the causal agent of sudden death syndrome of soybean. Journal of Nematology 27:70-77.

McLean, K. S., and G. W. Lawrence. 2000. A survey of plant-parasitic nematodes associated with cotton in northeastern Louisiana. Supplement to the Journal of Nematology 32:508-512.

Meredith, M.B., R.N. Inserra, and D. Monzon de Fernandez. 1983. Parasitism of Rotylenchulus reniformis on soybean root rhizobium nodules in Venezuela. Journal of Nematology 15: 211-214.

Misra, R. L., S. D. Mishra, and S. Misra. 2002. Root-knot nematode infestation in *Kalanchoe fedtschenkoi* Hamet & Pirr. Journal of Ornamental Horticulture, New Series 5:61.

Mueller, D. S., Wise, K. A., Dufault, N. S., Bradley, C. A. and Chilvers, M. I. 2013 Fungicides for field crops. The American Phytopathological Society. Pages 4-9.

Muzhandu, R. T., Chinheya, C. C., Dimbi, S. and Manjeru, P. 2014. Efficacy of abamectin for the control of root knot nematodes in tobacco seedling production in Zimbabwe. African Journal of Agricultural Research 9:144-147.

Mendoza, A. R., Kiewnick, S., and Sikora, R. A. 2008. In vitro activity of *Bacillus firmus* against the burrowing nematode Radopholus similis, the root-knot nematode *Meloidogyne incognita* and the stem nematode *Ditylenchus dipsaci*. Biocontrol Science and Technology. 18:377 - 389.

Niblack, T. L, and Chen SY. 2004. Cropping systems and crop management practices. Breeding for resistance and tolerance. Pp. 181-206.

Nigh, E. L. Jr. 1972. Susceptibility of Arizona-grown ornamentals to attack by several nematode species. Plant Disease Reporter 56:914-918.

Opperman, C., and Bird D. 1998. The soybean cyst nematode, *Heterodera glycines*: a genetic odel system for the study of plant-parasitic nematodes. Current Opinion in Plant Biology 1:342-346

Patel, M.V., Plant pathology lab report .1990. Mississippi Cooperative Extension Service, Mississippi State, MS.

Peacock, F.C. 1956. The reniform nematode in the Gold Coast. Nematologica 1: 307-310.

Qiao, K., Liu, X., Wang, H. Y., Xia, X. M., Ji, X. X. and Wang, K. Y. 2012. Effect of abamectin on root-knot nematodes and tomato yield. Pest Management Science 68:853-857.

Rajendran, G., P. Veluswamy, T. S. Muthukrishnan, and R. Sivagami. 1975. Susceptibility of *Crossandra* and related genera to the root-knot nematode, *Meloidogyne incognita* (Kofoid, 1889) Chitwood, 1949. South Indian Horticulture 23:147-148.

Rebois, R. V., W.C. Johnson, and E.J. Cairns. 1968. Resistance to soybean, Glycine max L. Merr., to the reniform nematode. Crop Science 8: 394-395.

Riggs, R. D. 2004. History and distribution. In D. P. Schmitt, J. A. Wrather, & R. D. Riggs (Eds.), Biology and management of soybean cyst nematode (2nd ed., pp. 41–56). Marceline: Schmitt & Associates.

Riggs, R. D. 1982. Nematology in the Southern United States. Southern Cooperative Bulletins. 205pp.

Ross, J. P. 1962. Crop rotation effects on the soybean cyst nematode population and soybean yields. Phytopathology 52: 815-818

Rosskopf, E. N., Chellemi, D. O., Kokalis-Burelle, N., and Church, G. T. 2005. Alternatives to Methyl Bromide: A Florida Perspective. American Phytopathological Society. APSnet feature, http://www.apsnet.org/publications/apsnetfeatures/Documents/2005/MethylBromideAlternatives.

Roy, K. W., Rupe, J. C., Hershman, D. E., and Abney, T. S. 1997. Sudden death syndrome of soybean. Plant Dis. 81:1100-1111.

Sasser, J. N., Uzzell, G, Jr. 1991. Control of the soybean cyst nematode by crop rotation in combination with nematicide. Nematology Journal 23: 344-347

Schneider, S. M., Rosskopf, E. N., Leesch, J. G, Chellemi, D. O, Bull, C.T., and Mazzola, M. 2003. United States Department of Agriculture-Agricultural Research Service research on alternatives to methyl bromide: pre-plant and post-harvest. Pest Manag Sci. 59: 814-826

Schroeder, J., Thomas, S. H., and Murray, L. W. 1993. Yellow and purple nutsedge and chile peppers host southern root-knot nematode. Weed Science 41:150–156.

Schrimsher, D. W., Lawrence, K. S., Castillo, J., Moore, S. R. and Kloepper, J. W. 2011. Effects of *Bacillus firmus* GB-126 on the soybean cyst nematode mobility in vitro. Phytopathology 101: S161.

Scumbiato, G.L., and D.L. Turnage. 1992. Southern United States soybean disease loss estimate for 1991. Proceedings of the Southern Soybean Disease Workers 19: 18-22.

Shanmugasundaram, S., & Yan, M.-R. ,2010. Vegetable soybean. In G. Singh (Ed.), the soybean (pp. 427–460). CABI.

Shi, Z., Liu, S. M., Noe, J., Arelli, P., Meksem, K., and Li, Z. L. 2015. SNP identification and marker assay development for high-throughput selection of soybean cyst nematode resistance. BMC genomics. 16:1.

Sinclair, J.B., and P.A. Backman. 1989. Compendium of Soybean Diseases. APS Press St. Paul, Minnesota. 106pp.

Stokes, D. E. 1977. Effects of root-knot nematodes on ornamental plants. Nematology Circular 24. Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville.

Walker, J. T., J. B. Melin, and J. Davis. 1994. Sensitivity of bedding plants to southern root-knot nematode, *Meloidogyne incognita* race 3. Journal of Nematology 26:778-781.

Weaver, D.B., R. Rodriguez-Kabana and E.L. Carden, 1988. Multlple-species nematode resistance in soybean: Effect of genotype and fumigation on yield and nematode numbers. Crop Sci., 28: 293-298.

Winstead, N. N., Skotland, C. B., and Sasser, J. N. 1955. Soybean cyst nematode in North Carolina. Plant Disease Reporter. 39:9 - 11.

Wrather, J. A, and Koenning, S. R. 2006. Estimates of disease effects on soybean yields in the United States 2003-2005. J Nematology 38: 173-180

Wrather, J. A., Kendig, S. R., Anand, S. C., Niblack, T. L. and Smith, G. S. 1995. Effects of tillage, cultivar, and planting date on percentage of soybean leaves with symptoms of sudden death syndrome. Plant Disease 79:560-562.

Wrather, J.A., S.R. Koenning and T.R. Anderson, 2003. Effects of diseases on soybean yield in the United States and Ontario (1999 to 2002). Plant Health Progress www.plantmanagementnetwork.org/ pub/ review/2003/soybean/.

Wyss, V. 1975. A routine method for investigations on the histology of feeding sites of plant-parasitic nematodes. Nematologica 21:110-111.

Xiang, N., Lawrence, K. S., Schrimsher, D., and Nightengale, S. 2013. Evaluation of Poncho Votivo, Aeris, Temik, and UFS0 738 on cotton for root knot management in Alabama, 2013. Plant Disease Management Report.7: N005. Doi: 10.1094/PDMR07.

Xing, L. J. and Westphal, A. 2006. Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in sudden death syndrome of soybean. Phytopathology 96:763-70.

Xing, L. J. and Westphal, A. 2009. Effects of crop rotation of soybean with corn on severity of sudden death syndrome and population densities of *Heterodera glycines* in naturally infested soil. Field Crops Research 112:107-117.

Zarina, B., and M. Abid. 1995. New host records of root-knot nematodes (*Meloidogyne* species) in Pakistan. Pakistan Journal of Nematology 13:49-50.

CHAPTER II

ANALYSIS OF THE *GLYCINE MAX* ROLE OF SYNTAXIN 22 (SYP22) IN RESISTANCE TO *HETERODERA GLYCINES*

Abstract

Syntaxin proteins are involved in the process of membrane fusion. Two *G. max* syntaxin genes (Gm-SYP22-1, and Gm-SYP22-2) that were similar in amino acid composition have been found to contribute to the ability of *Glycine max* to defend itself from infection by the plant- parasitic nematode *Heterodera glycines*. The Gm-SYP22-1 and Gm-SYP22-2 genes were expressed in root cells (syncytia) undergoing a resistant reaction while not being expressed in control cells. The experiments have identified SYP22-1 and SYP22-2 to be expressed specifically in syncytia undergoing a resistant reaction to *H. glycines* parasitism. The Gm-SYP22-1 and Gm-SYP22-2 genes have been isolated from genetically engineered in *G. max* [Williams 82/PI 518671], a genotype typically susceptible to *H. glycines* parasitism. Genetically engineered plants in *G. max* [Williams 82/PI 518671] that lack the overexpression of Gm-SYP22-1 or Gm-SYP22-2 genes have also been produced to serve as a control. The transgenic Gm-SYP22-1 or Gm-SYP22-2 overexpression lines with their pRAP15 control have then been infected with *H. glycines*. Infection was allowed to proceed for 30 days. At the end of the 30-day life span, *H. glycines* cysts were extracted from the soil, enumerated and compared to control plants. Plants overexpressing Gm-SYP22-1 or Gm-SYP22-2 had suppressed *H. glycines*

parasitism. In contrast, the gene expression levels of Gm-SYP22-1 and Gm-SYP22-2 were reduced in transgenic lines engineered for their RNA interference (RNAi) in *G. max* [Peking/PI 548402], a genotype normally resistant to *H. glycines*. In comparison to genetically engineered control *G. max* [Peking/PI 548402] lines, RNAi of Gm-SYP22-1 or Gm-SYP22-2 resulted in an increase in parasitism in the normally *H. glycines* resistant *G. max* [Peking/PI 548402]. The role *G. max* SYP22 has in defense was explained by the vacuole serving as a site of storage for enzymes and conjugated glucosides, becoming activated during pathogen invasion and agreed with a defense role found for SYP22 in *Arabidopsis thaliana*.

Introduction

The lipid bilayer membrane is a unifying component of all cells. In eukaryotes membranes are capable of merging, undergoing a fusion process. This fusion event is reliant on the engagement of different types of proteins to accomplish the task. Genes whose protein products function in membrane fusion are found in all eukaryotes, originally identified genetically in *Saccharomyces cerevisiae* as *Secretion* (*Sec*) genes (Novick et al. 1980, 1981). Notably, a number of additional important genes functioning in membrane fusion not identified in the original genetic screen have been identified in subsequent genetic studies (reviewed in Jahn and Fasshauer 2012) (Figure 1). There are three proteins that form the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) (reviewed in Jahn and Fasshauer 2012). These proteins include the suppressors of sec one/syntaxin 121 (SYP121/Sso1p), synaptobrevin/vesicle associated membrane protein (SYB/VAMP/Sec22p) and synaptosomal-associated protein 25 (SNAP-25/Sec9p) (reviewed in Jahn and Fasshauer 2012). Another protein recruited

to this SNARE complex is mammalian uncoordinated-18 (Munc18/Sec1p) (reviewed in Jahn and Fasshauer 2012). Associated with these proteins is a calcium sensor called synaptotagmin/Tricalbin-3 (SYT/Tcb3p) (reviewed in Jahn and Fasshauer 2012). Once a stable SNARE complex is assembled, membrane fusion is mediated by two cytoplasmic proteins including N-ethylmaleimide-sensitive fusion protein (NSF/Sec18p) and alpha soluble NSF attachment protein (alpha-SNAP/Sec17p) (reviewed in Jahn and Fasshauer 2012) (Figure 2.1); therefore, during membrane fusion, vesicle and target membrane proteins bind while other cytoplasmic proteins provide the energy for membrane fusion to occur (reviewed in Jahn and Fasshauer 2012). This entire unit is called the 20 S particle due to its observed sedimentation properties occurring during its biochemical isolation (Sollner et al. 1993a, b). The central function these proteins have in homeostasis makes it understandable that perturbing them has drastic and sometimes lethal consequences.

The normal *PENETRATION* gene has been shown to be related to a family of proteins known as syntaxins (Mayer et al. 1991; Collins et al. 2003). The analysis has shown that this particular syntaxin is syntaxin121 (SYP121) (Sanderfoot et al. 2000; Collins et al. 2003). In plants. syntaxins have been originally identified genetically in *A. thaliana* by Mayer et al. (1991). In those studies, the *A. thaliana* syntaxin known as *KNOLLE* localizes to the cell plate while functioning in cytokinesis (Mayer et al. 1991; Lukowitz et al. 1996; Waizenegger et al. 2000). However, the phenotype of the *knolle* mutant in this case is embryo lethal. Subsequent studies have shown that KNOLLE protein binds the Sec1 homolog KEULE during cytokinesis, however, the localization pattern of KNOLLE protein suggests it has roles throughout development in all somatic tissues (Assad et al. 2001).

The demonstrated importance of SYP121 during plant defense to pathogen infection and the conserved nature of the protein throughout eukaryotes indicates that homologs existing in other plants may perform important defense roles. This prediction is important from an applied standpoint since the identification of defense roles for syntaxins, especially in agricultural crops, may lead to improvements in food production. Recent work performed in *Glycine max* has led to the identification of components of the 20 S particle, including syntaxins, that perform important roles in resistance to the plant-parasitic nematode *Heterodera glycines* (Matsye et al. 2011, 2012; Pant et al. 2014; Sharma et al. 2016). These studies indicate that other syntaxins may also be important to the process of defense (Klink et al. 2017).

The *A. thaliana* genome encodes 24 genes that are related to syntaxin (Sanderfoot et al. 2000). The cellular localization pattern of a number of these syntaxin proteins has been determined (Sanderfoot et al. 2000). These syntaxins have a number of different functions that relate to their cellular localization pattern, but have a common role in membrane fusion. The plant cell has a number of membrane-containing compartments that function in various cellular processes. These components include, but are not limited to the endoplasmic reticulum (ER), exocyst, *trans*-Golgi network/early endosome (TGN/EE), Golgi-associated retrograde protein (GARP) complex, homotypic fusion and protein sorting (HOPS) complex, conserved oligomeric Golgi (COG) complex, class C core vacuole/endosome tethering (CORVET), trafficking protein particle (TRAPP) I–III complexes, depends on SLY1-20 (Dsl1) complex, endosome-associated retrograde protein (EARP) complex, and plasma membrane (PM) (Vukašinovi´ and Žárský 2016; Klink et al. 2017). Different macromolecular protein complexes are used to facilitate their

interactions and fusion events, but involve different types of syntaxin proteins that localize to these different compartments. One of these specialized syntaxins is syntaxin 22 (SYP22).

SYP22 is a component of the endosome or prevacuolar compartment (PVC) (Sanderfoot et al. 2000). The endosome is a membrane delimited structure that forms from materials that are endocytized from the PM. Materials captured in the endosome may then become targeted for degradation or become targeted back to the *trans*-Golgi network. In *A. thaliana*, SYP22 has been first identified in a mutagenic screen and called AtVAM3 because it is a vacuolar associated membrane protein. SYP22 is closely related in primary amino acid sequence composition to another syntaxin called SYP23. SYP23 has been first identified in a mutant screen and called AtPLP. (Sanderfoot et al. 2000).

While the genome of *A. thaliana* has 24 syntaxins, in comparison, *G. max* has 54 syntaxins (Pant et al. 2014). Using a phylogenetic approach, Klink (unpublished data) has performed a comparative analysis of all syntaxin proteins found in the genomes from *A. thaliana* (dicot), *G. max* (dicot), *Gossypium hirsutum* (dicot), *Zea mays* (monocot), *Oryza sativa* (monocot), *Selaginella moellendorffii* (lycophyte), *Physcomitrella patens* (bryophyte) and *Chlamydomonas reinhardtii* (green algae). In that, the amino acid sequences of the syntaxins have been aligned by taxonomic group, assembled together using the ClustalW Multiple Alignment feature in Bio Edit 7.0 (Hall, 2007). The sequences then have been manually edited. Phylogenetic trees have been constructed using BLAST 1.7.4 (Drummond and Rambaut 2012). The analyses assumed a related clock and a strict clock model with WAG+I+G substitution model and a Yule Process Prior Distribution for 10,000,000 generations sampling every 1,000 trees (Whelan and

Goldman 2001; Gernhard, 2008). In those studies, Bayes Factors (1,000 replicates) have been calculated between the strict and relaxed clock models using Tracer v1.5.0 (Rambaut and Drummond 2007). A maximum clade credibility tree (from 10,001 trees) had been generated using Tree Annotator 1.7.4, visualized using FigTree v1.3.1 (Rambaut, 2009, 2012). One outcome of the analysis was the grouping of the 54 different *G. max* syntaxins with the 24 known syntaxins of *A. thaliana*. A notable observation made from these studies was the identification of 4 *G. max* genes that are closely related to *A. thaliana* SYP22 (Klink et al. unpublished). SYP22 is a protein having important roles in development, salt tolerance, vacuolar assembly and auxin transport (Sato et al. 1997; Sanderfoot et al. 1999; Ohtomo et al. 2005; Ueda et al. 2006; Hamaji et al. 2009; Shirakawa et al. 2010; Uemura et al. 2010; Ebine et al. 2012). The central role that *A. thaliana* SYP22 performs in basic aspects of plant biology indicated it could perform an important role in defense.

Rationale for proposed work

The role of the *G. max* SYP31 in defense has been made because prior studies show it to be expressed to relatively high levels specifically in the syncytium cells undergoing the process of defense to *H. glycines* (Pant et al. 2014). The effective nature of the overexpression of these genes in defense opened questions as to whether other SYP genes also functioned in defense. This prediction has been realized in studies showing that *G. max* SYP6, SYP8, SYP71 and SYP131 also function in defense (Klink et al. 2017). As will be shown, in the analysis presented here, the expression of SYP22 during the resistant reaction that *G. max* has in syncytia during parasitism by *H. glycines* made it a reasonable candidate for molecular analyses in examining the process of resistance. In

the analysis presented here, *G. max* homologs of SYP22 have been identified to be expressed in root cells undergoing the process of resistance (i.e. syncytia). This observation indicates the *G. max* SYP22 performs a role in defense to *H. glycines*.

Objective of study

To determine of induced expression of membrane fusion components homologous to those comprising the prevacuolar compartment (PVC) soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) (i.e. SYP22) indicates a function in defense during *Glycine max* resistance to *Heterodera glycines*.

Materials and Methods

Selection of candidate genes

The selection of candidate genes was by mining data from published gene expression experiments (Klink et al. 2007, 2009a, 2010a, b, 2011; Matsye et al. 2011). This procedure is an effective means to identify genes that function in *G. max* defense to *H. glycines* parasitism, proven further in independently-performed genetic mutational analyses (Matsye et al. 2012; Liu et al. 2012; Matthews et al. 2013, 2014; Pant et al. 2014, 2015). To summarize those published experimental procedures used to identify the candidate resistance genes employed here, *G. max* [Peking/PI 548402] and *G. max* [PI 88788] were infected with *H. glycines* [NL1-Rhg/HG-type 7/race 3], resulting in a resistant reaction proven histologically in unengineered roots which is the natural resistance response found in these *G. max* genotypes (Ross 1958; Endo 1965, 1991; Klink et al. 2007, 2009a, 2010a, b, 2011). Roots were then being processed for histology and laser microdissection (LM), a procedure that has been used to collect syncytia undergoing the defense response (Klink

et al. 2005, 2007, 2009a, 2010a, b, 2011). The mRNA was isolated from the syncytia and converted to probe for hybridization onto the Affymetrix® Soybean GeneChip® (Klink et al. 2007, 2009a, 2010a, b, 2011; Matsye et al. 2011). The hybridizations was run in triplicate (arrays 1-3) using probe derived from RNA isolated from LM-collected syncytia obtained from 3 independent replicate experiments each run independently in the two different *H. glycines*-resistant genotypes (Klink et al. 2007, 2009a, 2010a, b, 2011). For the gene to be considered expressed at a given time point (3 or 6 days' post infection [dpi]), probe signal was measurable above threshold on all three arrays for both *G. max* $_{[Peking/PI\ 548402]}$ and *G. max* $_{[PI\ 88788]}$ (6 total arrays), $p < 0.05$ (Klink et al. 2007, 2009a, 2010a, b, 2011). The original analysis procedure was performed as follows; the measurement for a particular probe set (gene) transcript on a single array was determined using the Bioconductor implementation of the standard Affymetrix® detection call methodology (DCM) (Klink et al. 2007, 2009a, 2010a, b, 2011). DCM consists of four steps, including (1) removal of saturated probes, (2) calculation of discrimination scores, (3) p-value calculation using the Wilcoxon's rank test, and (4) making the detection call (present [P]/marginal [M]/absent [A]). Ultimately, the algorithm determines if the presence of a gene transcript is provably different from zero (P), uncertain or marginal (M), or not provably different from zero or absent (A) (Klink et al. 2007, 2009a, 2010a, b, 2011; Matsye et al. 2011). The mined data used in the analysis is presented (Table 2.1). From these data, genes used in the analysis were selected for functional experiments and/or qPCR. The analysis resulted in the identification of gene expression pattern for 4 *G. max* genes that were homologous to *A. thaliana* SYP22 (P93654). The *G. max* SYP22

genes are GmSYP22-1 (Glyma01g01960), GmSYP22-2 (Glyma09g33950), GmSYP22-3 (Glyma16g08200) and GmSYP22-4 (Glyma16g13410).

Gene cloning

G. max root mRNA was isolated according to Matsye et al. (2012) using the UltraClean® Plant RNA Isolation Kit according to the manufacturer's instructions (Mo Bio Laboratories®, Inc.; Carlsbad, CA). Genomic DNA was removed from the mRNA with DNase I according to the manufacturer's instructions (Invitrogen®, Carlsbad, California.). The cDNA was synthesized from mRNA using the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen®) with oligo $d(T)_{20}$ as the primer (Invitrogen®) according to the manufacturer's instructions. The accession numbers and DNA primer sequences for the genes examined in the study presented in Table 2.1. Genomic DNA contamination was assessed by PCR by using beta-conglycinin primer pair that amplifies DNA across an intron, thus yielding different sized products based on the presence or absence of that intron (Klink et al. 2009b) Table 2.1.

Table 2.1 PCR and qPCR primers used to study the genes examined in the analysis of genes under study.

Gene name	Accession	Primer type	Primer 5'-->3'
GmSYP22-1	Glyma01g01960	PCR-F-OE	CACCATGAGCTTTCAGGACATCGAGC
		PCR-R-OE	CTAAGCAGCAAGAACAATGATGACG
		qPCR-F	CACAACGTTGAAGTTAATGCAAGTAAG
		qPCR-R	AAGAAGTGCTTGCGGAACAAA
		qPCR probe	CACAGCGTCTTTCAGCGGAGAGG
GmSYP22-2	Glyma09g33950	PCR-F-OE	CACCATGAGCTTTCAGGACATCGAGG
		PCR-R-OE	CTAAGCAGCAAGAACAATGATGACG
		qPCR-F	CACAATGTTGAAGTTAATACAAGTAAG
		qPCR-R	AAGAAGTGCTTGCGGAACAAA
		qPCR probe	CTCAGCGTCTTTCAGCTGAGAGG
ribosomal S21	expressed sequence tag	qPCR-F	ATGCAGAACGAGGAAGGACAG
		qPCR-R	GAAGCATGGTCCTTAGCG
		qPCR probe	CCTAGGAAGTGCTCTGCCACAAAC
Egfp		PCR	GAATTTGTTTCGTGAACTATTAGTTGCGG
		PCR	GCATGCCTGCAGGTCACTGGATTTTG
Ar-VirG		PCR	ATGCGCCATCTTATTACCGAGTATTTAAC
		PCR	TCAGGCCGCCATCAGACC
β-conglycinin		PCR	CCATGCTGACGCTGATTACCTC
		PCR	CTACCAGGCTTGTTAACGGGTATGG

Genetic transformation of *G. max*

The pRAP plant transformation system used here was designed and tested specifically for studying the interaction between *G. max* and *H. glycines* (Klink et al. 2008, 2009b; Matsye et al. 2012; Matthews et al. 2013, 2014). The pRAP plant transformation system was proven to obtain the same outcomes (resistance to *H. glycines* parasitism) as genetic mutational analyses and virus induced gene silencing (VIGS) (Liu

et al. 2012; Matthews et al. 2013). The pRAP vector system that was proven to function in *G. max* is based off of the published Gateway® cloning vector platform that was developed and proven to work in other plant systems (Curtis and Grossniklaus, 2003; Matsye et al. 2012; Matthews et al. 2013). The published pRAP vector platform used an enhanced green fluorescent protein (eGFP) transgenic reporter system. The pRAP vector platform, depending on the integrated cassette, was used to activate or suppress the transcription of a targeted gene (Jefferson et al. 1987; Fire et al. 1998; Collier et al. 2005; Klink et al. 2009b; Matsye et al. 2012; Matthews et al. 2013, 2014; Pant et al. 2014, 2015). The expression of the gene cassettes was driven by the figwort mosaic virus subgenomic transcript promoter (FMV-sgt) promoter (Bhattacharyya et al. 2002). The FMV-sgt promoter had been proven to drive gene expression in transgenic *G. max* roots throughout the life cycle of *H. glycines* (Klink et al. 2008). The activation of transcription of a targeted gene was accomplished using the pRAP15 vector which was designed to result in an increase in the relative transcript levels of the gene of interest (GOI) (Matsye et al. 2012; Youssef et al. 2013; Matthews et al. 2013, 2014; Pant et al. 2014, 2015). The pRAP17 vector had been designed to result in a decrease in the relative transcript levels of the GOI (Klink et al. 2009b; Pant et al. 2014, 2015). Between the left and right border of the pRAP15 and pRAP17 vectors exists the attR homologous recombination sites of the Gateway® system (Invitrogen®) where the GOI integrates (Klink et al. 2009b; Matsye et al. 2012; Pant et al. 2015). Thus, roots exhibiting the expression of the eGFP visual reporter possessed the GOI, each with their own promoter and terminator sequences (Collier et al. 2005; Klink et al. 2009b; Matsye et al. 2012; Matthews et al. 2013; Pant et al. 2015).

The amplicons representing the GOI were cloned from *G. max* [Williams 82/PI 518671] and ligated into the directional pENTR/D-TOPO® Gateway®-compatible vector (Invitrogen®) according to the manufacturer's instructions. The reaction contents then were transformed into chemically competent *E. coli* strain One Shot TOP10® and selected on kanamycin (50 ug/ml) according to the manufacturer's instructions (Invitrogen®). Gene sequences were confirmed by matching them to the *G. max* [Williams 82/PI 518671] genome accession (Schmutz et al. 2010). Amplicons representing full length genes have been cloned into the pRAP15 overexpression vector (Matsye et al. 2012; Pant et al. 2015). Alternatively, full length genes or subcloned portions of genes were engineered into the pRAP17 RNAi vector (Klink et al. 2009b). This approach was proven effective for RNAi studies in plants (Klink and Wolniak, 2001). In the overexpression studies, the amplicons were ligated into the pRAP15 destination vector using LR Clonase® (Invitrogen®) according to the manufacturer's instructions (Matsye et al. 2012). The pRAP15-*ccd*B control and engineered pRAP15 vector containing the GOI were used to transform chemically competent *Agrobacterium rhizogenes* K599 (K599) (Hofgen and Willmitzer 1988; Haas et al. 1995; Collier et al. 2005). The transformation mix then was plated on LB-agar, selecting with tetracycline (5 ug/ml) (Matsye et al. 2012). A PCR reaction using pRAP15 primers that amplify the 717 bp eGFP gene and the 690 bp *A. rhizogenes* root inducing (Ri) plasmid (EU186381) *Vir*G gene (VirG) confirmed that the K599 contains both plasmids prior to transformation (Table 2.1). The pRAP15 vector containing the GOI was confirmed by PCR using primers for the respective genes and DNA sequencing. Genetic transformation experiments resulting in gene overexpression in *G. max* roots were performed according to Matsye et al. (2012) in

H. glycines-susceptible genetic background of *G. max* [Williams 82/PI 518671] (Concibido et al. 2004; Schmutz et al. 2010). Genetic transformation experiments designed to decrease the level of target gene mRNA were then performed. (Klink et al. 2009b). This procedure used the pRAP17 RNAi vector in the functionally *H. glycines*-resistant genetic background of *G. max* [Peking/PI 548402] (Concibido et al. 2004). The procedure for making genetically engineered plants used in overexpression or RNAi experiments involves the co-cultivation of 7-9-day old *G. max* [Williams 82/PI 518671] (overexpression experiments) or *G. max* [Peking/PI 548402] (RNAi experiments) with the K599 engineered to harbor the appropriate genetic construct. The roots of these plants were excised while the cut plants were immersed in Murashige and Skoog (MS) media containing the K599 harboring the engineered pRAP15-*ccd*B or pRAP17-*ccd*B controls while at the same time different plants were cut and transformed with K599 harboring the engineered pRAP15-GOI or pRAP17-GOI experimental constructs (Murashige and Skoog 1962; Klink et al. 2009b; Matsye et al. 2012; Pant et al. 2014). Due to the way K599 transfers the DNA cassettes situated between the left and right borders of the plasmid into the root cell chromosomal DNA, the subsequent growth and development of the stably transformed genetically engineered cell into a result in the production of a plant that was a genetic mosaic called a composite plant (Collier et al. 2005). These composite, genetically mosaic plants were the entire shoot being non-transgenic and the entire root being transgenic (Haas et al. 1995; Collier et al. 2005; Klink et al. 2008, 2009b; Matsye et al. 2012; Matthews et al. 2013; Pant et al. 2014). In these studies, therefore, each individual transgenic root system functions as an independent transformant line (Tepfer, 1984; Matsye et al. 2012; Matthews et al. 2013; Pant et al. 2014, 2015). Quantitative PCR (qPCR) was used to

confirm the relative levels of transcript abundance in the pRAP15-GOI engineered overexpressing lines or the pRAP17-GOI-engineered RNAi lines.

Quantitative PCR

The DNA sequences for the qPCR primers used in quantitative gene expression experiments are provided in (Table 2.1). The experiments involving *G. max* have used the ribosomal protein gene S21 (S21) as a control (Klink et al. 2005). The Gm-S21 gene was used as a control in prior studies (Klink et al. 2005; Matsye et al. 2012; Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). S21 was a highly-conserved gene proven to be transcribed into mRNA and translated into protein (Morita-Yamamuro et al. 2004). With regard to assessing the relative abundance in transcript levels in qPCR experiments, prior qPCR analyses were shown that the Gm-S21 control performs in the same manner as elongation initiation factor protein 3 (Matsye et al. 2012). Therefore, Gm-S21 was selected to serve as the control for the qPCR experiments presented here.

The qPCR experiments used Taqman® 6-carboxyfluorescein (6-FAM) probes and Black Hole Quencher (BHQ1) (MWG Operon; Birmingham, AL). The qPCR differential expression tests were performed using mRNA samples isolated from three independent replicates. The qPCR reaction conditions included a 20 µl Taqman Gene Expression Master Mix (Applied Biosystems; Foster City, CA), 0.9 µl of 100 µM forward primer, 0.9 µl of 100 µM reverse primer, 2 µl of 2.5 µM 6-FAM (MWG Operon®) probe and 9.0 µl of template DNA. The qPCR reactions were performed on an ABI 7300 (Applied Biosystems®). The qPCR conditions were included a preincubation of 50° C for 2 min, followed by 95° C for 10 min. This step was followed by alternating 95° C for 15 sec

followed by 60° C for 1 min for 40 cycles. The accepted universal standard for qPCR statistical analysis, using $2^{-\Delta\Delta C}{}_{T}$ to calculate fold change, was followed according to the derived formula presented in Livak and Schmittgen (2001) (Klink et al. 2005; Matsye et al. 2012; Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017).

The infection of *G. max* by *H.* glycines

H. glycines [NL1-Rhg/HG-type 7/race 3] was proven to generate a susceptible reaction in unengineered and pRAP15-*ccd*B control-engineered *G. max* [Williams 82/PI 518671] (Klink et al. 2007, 2009a, 2010a, b; 2011; Matsye et al. 2011, 2012; Youssef et al. 2013; Matthews et al. 2013, 2014). In contrast, *H. glycines* [NL1-Rhg/HG-type 7/race 3] had been proven to generate a resistant reaction in unengineered and pRAP17-*ccd*B control-engineered *G. max* [Peking/PI 548402] (Klink et al. 2007, 2009a, 2010b, 2011; Matsye et al. 2011; Pant et al. 2014, 2015). Female *H. glycines* [NL1-Rhg/HG-type 7/race 3] used in the analysis were purified by sucrose flotation (Jenkins, 1964; Matthews et al. 2003; Klink et al. 2007, 2009b, 2011; Matsye et al. 2012; Pant et al. 2014, 2015). Each root was inoculated with one ml of *H. glycines* at a concentration of 2,000 second stage juveniles (J2s)/ml per root system and infected for 30 days according to Matsye et al. (2012). At the end of the experiment, the cystslife stages was collected over nested 20 and 100-mesh sieves (Matsye et al. 2012; Pant et al. 2014, 2015). Furthermore, the soil has been washed several times and the rinse water sieved to assure collection of all cysts (Matsye et al. 2012; Matthews et al. 2013; Pant et al. 2014, 2015).

Calculation of the effect the expression of the transgene has on *H. glycines* parasitism

The overexpression and RNAi experiments have 3 independent biological replicates. In every experiment, each biological replicate had multiple experimental replicates represented by 5-20 individual plants. The community-accepted assay used to determine if an experimental condition exerts an influence on *H. glycines* development (parasitism) was calculated and presented as the female index (FI) (Golden et al. 1970). The FI was calculated as FI = (Nx/Ns) X 100, where Nx was the average number of females on the test cultivar and Ns was the average number of females on the standard susceptible cultivar (Golden et al. 1970; Riggs and Schmitt, 1988, 1991; Niblack et al. 2002; Klink et al. 2009; Matthews et al. 2013). In the experiments of Golden et al. (1970), Riggs and Schmidtt (1988, 1991), Kim et al. (1998) and Niblack et al. (2002), the labs that originally developed and modified the FI, the FI is calculated from a total of 3-10 experimental and 3-10 control plants. In those studies, each individual plant serves as a replicate and biological replicate might or might not be performed (Golden et al. 1970; Riggs and Schmidtt 1988, 1991; Kim et al. 1998; Niblack et al. 2002). All of the experiments presented here at least meet and in most cases, exceed these published standards (Golden et al. 1970; Riggs and Schmidtt 1988, 1991; Kim et al. 1998; Niblack et al. 2002). The FI assay was also the community-accepted standard analysis method used in experiments in other labs employing genetically engineered constructs in *G. max*, including those using K599, to examine *H. glycines* biology (Steeves et al. 2007; McLean et al. 2007; Mazarei et al. 2007; Li et al. 2010; Melito et al. 2011; Liu et al. 2011, 2012; Matthews et al. 2013, 2014). Following the published methods employed in those studies, Nx is the pRAP15-GOI or pRAP17-GOI-transformed line and Ns was the pRAP15-*ccd*B

or pRAP17-*ccd*B control. Because the pRAP15 or pRAP17 control had the *ccd*B gene located in the position where, otherwise, the GOI was inserted during the LR clonase reaction, those control vectors also control for non-specific effects caused by gene overexpression or RNAi (Klink et al. 2009b; Matsye et al. 2012; Matthews et al. 2013; Pant et al. 2014, 2015; Sharma et al. 2016; Klink et al. 2017). Therefore, by definition, the pRAP15-*ccd*B or pRAP17-*ccd*B transformed plants serve as a control. The FI was calculated and presented as a function of the cysts per mass of the whole root (wr) and also cysts per gram (pg) of root. The cyst per gram analysis was done to account for any possible root growth effect that may result by the overexpression or RNAi of a GOI. The experiments were analyzed statistically using the Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test, $p < 0.05$ cutoff (Matsye et al. 2012; Pant et al. 2014). Following community-accepted, standard published methods, error bars were not calculated when using the FI analysis (Golden et al. 1970; Riggs and Schmidtt 1988, 1991; Kim et al. 1998; Niblack et al. 2002). The effect that the overexpressed gene exerts on root growth was taken from a representative experiment and determined as a function of root mass tested statistically using the Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test, $p < 0.05$ cutoff (Mann and Whitney, 1947; Matsye et al. 2012; Pant et al. 2014).

Results

Selection of candidate genes for genetic analyses

Prior analyses identified four SYP22-related genes in the *G. max* genome (Pant et al. 2014). The identified candidate *G. max* SYP22 genes were being studied to determine if they perform a role in defense to *H. glycines* parasitism**.** Data derived from prior published reports on *G. max* resistance to *H. glycines* parasitism was examined (Klink et

al. 2010a, b). The *G. max* SYP22 gene was considered expressed in syncytia undergoing defense if the probe set representing the gene measures probe in all 6 examined arrays (3 arrays for *G. max* [Peking/PI 548402] and *G. max* [PI 88788]) at a statistically significant level above background ($p < 0.05$) for a given time point (3 or 6 dpi) while not being expressed in control cells (Table 2.2) (Klink et al. 2010a, b). SYP22-1 and SYP22-2 were expressed specifically in syncytia undergoing a resistant reaction to *H. glycines* parasitism (Table 2.2). In contrast, GmSYP22-3, lacked specificity by also being expressed in control cells. The expression of Gm-SYP22-4 could not be measured by the experimental methods used because the Affymetrix® Gene Chip® lacked a probe set on the array. These results show that these genes are expressed at some point during the resistant reaction while not being expressed in control cells (Table 2.2). Consequently, these results led to the determination of focusing in on SYP22-1 and SYP22-2 since they fit the criteria of being expressed specifically in the cells undergoing a resistant reaction.

Table 2.2 Summary of *G. max* SYP22 candidate gene expression.

	Time point (dpi)		
Gene	0 (control)	3	6
GmSYP22-1	N/M	M	M
GmSYP22-2	N/M	N/M	M
GmSYP22-3	M	M	M
GmSYP22-4	n/a	n/a	n/a

Footnote: Blue denotes replicates where gene expression is not statistically significant. Red denotes replicates where gene expression is statistically significant ($P < 0.05$). Yellow, genes employed in the functional genetic studies. M, measured. N/M, not measured. n/a, not applicable because no Affymetrix® probe set existed on the microarray used to measure gene expression of that gene. Details are provided in Supplemental Table 2.1.

Functional analysis of the GmSYP22 genes during *H. glycines* parasitism

The objective of using the complimentary approaches of gene overexpression and RNAi in studying a developmental process was that the combined opposite outcomes, respectfully, were hallmarks of the involvement of the gene in the process (Zhou et al. 2005; Baena-González et al. 2007; Sun et al. 2014; Pant et al. 2014; Doczi et al. 2015). These opposite outcomes were engineered resistance in the normally *H. glycines*-susceptible *G. max* [Williams 82/PI 518671] and engineered impairment of resistance in the normally *H. glycines*-resistant *G. max* [Peking/PI 548402] (Pant et al. 2014, 2015; Sharma et al. 2016). Experiments were performed in *G. max* leading to the experimentally induced or suppressed expression of *G. max* SYP22-1 and SYP22-1 (Figure 2.1).

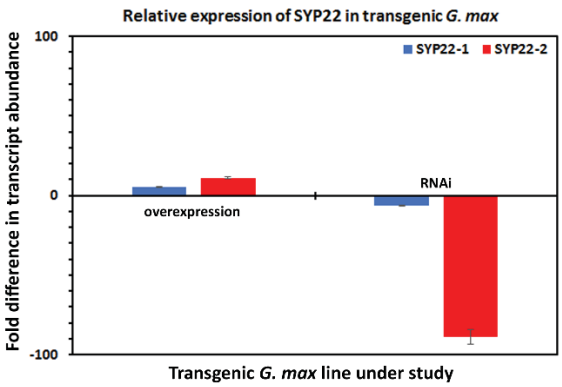


Figure 2.1 The relative levels of transcript abundance have been measured by qPCR in transgenic *G. max* overexpression and RNAi lines.

Overexpression and RNAi lines of SYP22-1 (blue) and SYP22-2 (red), revealing experimentally induced or suppressed mRNA levels. Error bar represents standard deviation.

The transgenic SYP22-1 and SYP22-2 overexpression lines with their pRAP15 control were then been infected with *H. glycines*. Infection was allowed to proceed for 30 days. At the end of the 30-day life span, *H. glycines* cysts were extracted from the soil,

enumerated and compared to control plants. The experiments show that plants overexpressing SYP22-1 or SYP22-2 have impaired *H. glycines* parasitism (Figure 2.2).

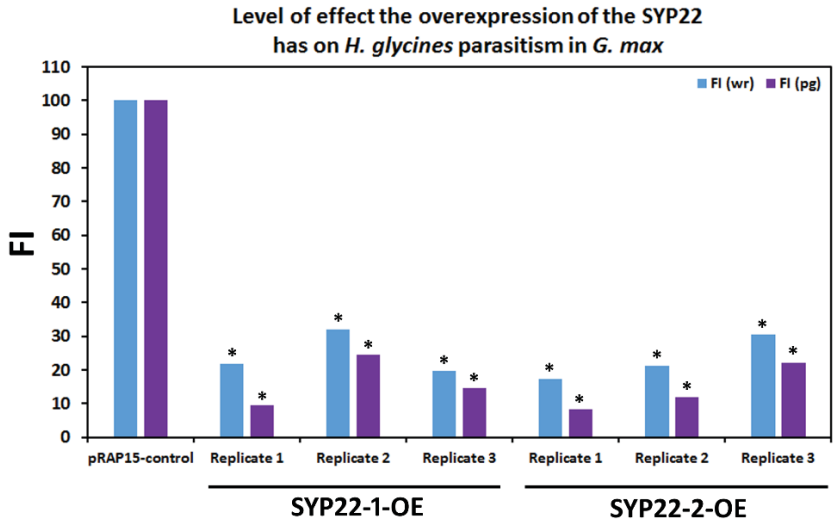


Figure 2.2 Level of effect the overexpression of *G. max* SYP22 has on *H. glycines* parasitism in *G. max* as indicated by its FI.

*, Statistically significant using Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test, $P < 0.05$ (Mann and Whitney 1947).

The transgenic SYP22-1 and SYP22-2 RNAi lines with their pRAP17 control were then infected with *H. glycines*. Infection was allowed to proceed for 30 days. At the end of the 30-day life span, *H. glycines* cysts were extracted from the soil, enumerated and compared to control plants. The experiments show that the SYP22-1 or SYP22-2 RNAi lines had impaired resistance to *H. glycines* parasitism (Figure 2.3).

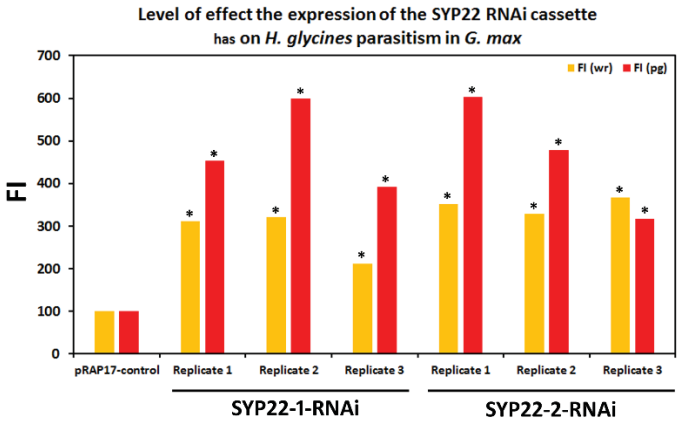


Figure 2.3 Level of effect the RNAi of *G. max* SYP22 has on *H. glycines* parasitism in *G. max* as indicated by its FI.

*, $P < 0.05$. *, statistically significant using Mann–Whitney–Wilcoxon (MWW) Rank-Sum Test, $P < 0.05$ (Mann and Whitney 1947).

The data obtained from these complimentary approaches of gene overexpression and RNAi in studying SYP22 resulted in combined opposite outcomes, respectfully. The opposite outcomes were impaired susceptibility to *H. glycines* parasitism in the SYP22 overexpression lines and impaired resistance to *H. glycines* in the SYP22 RNAi lines. These opposite outcomes were hallmarks of the involvement of the gene in the process of resistance (Pant et al. 2014; Sharma et al. 2016).

Discussion

A number of recent studies have pointed to the importance of components of the membrane fusion apparatus having a role during *G. max* resistance to *H. glycines* (Matsye et al. 2012; Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). Included in these analyses are observations of defense function of different members of the syntaxin gene family (Klink et al. 2017). The results presented here continue with the characterization of the *G. max* syntaxin gene family by functionally examining the

syntaxin of plants two family members, SYP22. The result, along with a series of subsequent analyses have revealed the importance of vesicle transport, mediated by SYP121, to plant defense because the vesicles are responsible for the delivery of antimicrobials, enzymes and structural elements to the site of defense (Collins et al. 2003; Lipka et al. 2005; Stein et al. 2006; Humphry et al. 2010; Johansson et al. 2014).

The *G. max* genome has multiple copies of SYP22

The *G. max* genome has 4 genes having amino acid sequence relatedness to *A. thaliana* SYP22 (Klink, unpublished data). In *A. thaliana*, SYP22 has been shown to have a number of biological functions. For example, it has been described in *A. thaliana* as Suppressor of yeast vacuolar morphology 3 (vam3) mutant (VAM3) (Sato et al. 1997). SYP22 has also been described as shoot gravitropism 3 (sgr3) (Yano et al. 2003). Other studies identified *A. thaliana* SYP22 as the short stem and midrib (SSM) gene (Ohtomo et al. 2005). However, any potential defense role for *A. thaliana* SYP22 was not clear in these studies because such a role had not been tested. In contrast, a defense function for *A. thaliana* SYP22 could be extrapolated from work done in other genetic studies. For example, *A. thaliana syp22* mutants have been observed to have an altered distribution of myrosin cells (Ueda et al., 2006). These myrosin cells are idioblasts and are present along leaf veins. Myrosin cells accumulate the defense molecule thioglucoside glucohydrolase myrosin, encoded by thioglucoside glucohydrolase1 (TGG1) and thioglucoside glucohydrolase1 (TGG2) (Ueda et al. 2006). From these studies, it is clear that the *G. max* SYP22 gene could have a defense role, especially if it is actively expressed in syncytia undergoing the process of resistance. Subsequent studies have shown that these membrane fusion components also have roles in defense to pathogen attack. Studies in

the plant genetic model *Arabidopsis thaliana* have identified a mutant that facilitates the ability of the plant pathogen *Blumeria graminis* f. sp. *hordei* to successfully penetrate leaf tissue (Collins et al. 2003). The identified mutant (*penetration1* [*pen1*]) resulted in successful penetration of the hyphae into the leaf cell (Collins et al. 2003).

G. max SYP22 is expressed specifically within syncytia undergoing a resistant reaction

Prior studies had shown that the expression pattern of SYP genes presages their involvement in resistance (Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). Analyses presented here have resulted in the identification of four *G. max* genes that are related to the *A. thaliana* SYP22 (Klink, unpublished data). An examination of the gene expression characteristics of those *G. max* SYP22 genes were done using previously published microarray data (Klink et al. 2010a, b). The analyses resulted in the identification or both SYP22-1 and SYP22-2 being expressed specifically in syncytia undergoing the process of resistance in *G. max* [Peking/PI 548402] and *G. max* [PI 88788]. Important in the design of those studies is that each genotype can undergo a resistant reaction to *H. glycines*. Prior studies performed in *G. max* that have tested gene function through genetic analyses demonstrate that genes expressed in the cells specifically undergoing the process of resistance have functional roles in resistance (Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). In contrast, SYP22-3 was shown to be expressed in both control cell types and cells undergoing the process of resistance. Furthermore, Gm-SYP22-4 does not exhibit measurable expression. Genes not showing measurable expression in the tested cells have been shown to not have a role in resistance (Sharma et al. 2016; Klink et al. 2017). These observations indicate SYP22-1 and SYP22-2 had the

highest probability of functioning during defense. These genes then were examined in functional studies including their experimentally induced overexpression and RNAi.

Transgenic *G. max* plants made to genetically induce the expression of Gm-SYP22-1

Prior studies in *G. max* have demonstrated that it was possible to isolate SYP genes from cDNA synthesized from isolated root mRNA and examine them functionally for any potential role in resistance (Pant et al. 2014; Sharma et al. 2016; Klink et al. 2017). In the analysis presented here, SYP22-1 and SYP22-2 have built on those studies. Each gene was engineered into plasmid vectors that could experimentally induce their expression through overexpression (Matsye et al. 2012). In contrast, SYP22-1 and SYP22-2 were engineered into plasmid vectors that could experimentally reduce their expression through RNAi (Klink et al. 2009b). The results of these experiments confirm transgenic roots of *G. max* containing SYP22-1 and SYP22-2 overexpression cassettes did exhibit higher relative transcript levels of each gene. In contrast, transgenic roots containing SYP22-1 and SYP22-2 RNAi cassettes exhibited lower relative transcript levels of each gene. These results demonstrate that the transgenic roots are behaving as they would be expected to function, based on the genetic cassette with which they have been engineered. With the transgenic roots made, each gene could be examined experimentally. These experiments allow the determination if they have a function during the process of defense that *G. max* has toward *H. glycines*.

Gm-SYP22 role in *G. max* defense to *H. glycines*

Using the complimentary approaches of gene overexpression and RNAi in studying a developmental process is that the combined opposite outcomes, respectfully,

are hallmarks of the involvement of the gene in the process (Zhou et al. 2005; Baena-González et al. 2007; Sun et al. 2014; Pant et al. 2014; Doczi et al. 2015). This procedure has been used to study the role of candidate *H. glycines* resistance genes in *G. max* (Pant et al. 2014, 2015; Sharma et al. 2016). In *G. max*, plants engineered to experimentally induce SYP22-1 or SYP22-2 gene expression result in a significant decrease in the FI. These results indicate they perform important roles in defense. In contrast, *G. max* plants engineered to experimentally reduce the relative transcript abundance of SYP22-1 or SYP22-2 result in a statistically significant increase in the FI. It is clear from the analyses that SYP22 performs and important role during *G. max* defense to *H. glycines*.

Conclusion

The observation that *G. max* SYP22 functions in defense fills an important gap in our current understanding of resistance to *H. glycines* and, perhaps, root pathogens in general. The results explain how materials can be delivered to the vacuole, a structure that is central to cellular homeostasis while also having important roles in defense. The role *G. max* SYP22 has in defense is explained by the vacuole serving as a site of storage for enzymes and conjugate glucosides that can become activated during pathogen invasion. The results presented here were in agreement with observations made in *A. thaliana* of SYP22 performing an important role in defense. Future studies examining *G. max* myrosinase genes will help clarify the involvement of SYP22 and likely explain the process of defense in more detail.

Table 2.3 Supplemental. Table Gene expression data used in the analysis.

Gene	Time point (dpi)	*G. max*: Genotype 1 p-value			*G. max*: Genotype 2 p-value			
		Peking/PI 548402			PI 88788			
		array 1	array 2	array 3	array 1	array 2	array 3	Outcome
SYP22-1	0	0.0376841	0.106612	0.016427	0.003823	0.00382	0.00292	N/M
SYP22-2		0.0081843	0.186972	0.186972	0.001673	0.00222	0.01643	N/M
SYP22-3		0.9623159	0.813028	0.97477	0.238453	0.12387	0.67168	N/M
SYP22-4		n/a	n/a	n/a	n/a	n/a	n/a	n/a
SYP22-1	3	0.0016729	0.001673	0.02043	0.001673	0.00292	0.00167	M
SYP22-2		0.0131156	0.02523	0.143002	0.00222	0.01312	0.00222	N/M
SYP22-3		0.4645763	0.328321	0.535424	0.123873	0.81303	0.07743	N/M
SYP22-4		n/a	n/a	n/a	n/a	n/a	n/a	n/a
SYP22-1	6	0.0029236	0.003823	0.016384	0.00222	0.00292	0.00167	M
SYP22-2		0.0038229	0.004963	0.001673	0.006396	0.00167	0.00167	M
SYP22-3		0.2668473	0.856998	0.97477	0.761547	0.53542	0.46458	N/M
SYP22-4		n/a	n/a	n/a	n/a	n/a	n/a	n/a

Footnote. Klink et al. (2010a, b) presented gene expression analyses of RNA isolated from syncytia undergoing the process of resistance in two different *H. glycines*-resistant genotypes, *G. max* $_{[Peking/PI\ 548402]}$ and *G. max* $_{[PI\ 88788]}$. Three independently replicated studies performed independently in two different *G. max* genotypes utilized Affymetrix® microarrays to measure the presence or absence of transcript at 0 (control) 3 and 6 days' post infection. For genes represented by probe sets, those not measuring probe provably above background ($p \geq 0.05$) in at least one of the three analyzed microarrays in any genotype (blue) were not considered for further examination in transgenic studies. The p values were calculated according to the Wilcoxon's rank test (Mann and Whitney 1947). Probe sets measuring probe provably above background ($p < 0.05$) (red) (i.e. SYP22-1, SYP22-2) were considered selected for examination in transgenic studies.

Table 2.4 Supplemental. Table Transgenic plants used in the analysis.

Overexpression							
Gene	Accession	# of control plants	# of OE plants	FI (wr)	p-value (wr)	FI (pg)	p-value (pg)
GmSYP22-1	Glyma01g01960	22	27	21.79	0	9.63	0
		24	29	32.00	0	24.65	0
		27	26	19.74	0	14.55	0
GmSYP22-2	Glyma09g33950	22	28	17.36	0	8.30	0
		24	27	21.33	0	11.93	0
		27	27	30.41	0	22.24	0
RNAi							
Gene	Accession	# of control plants	# of RNAi plants	FI (wr)	p-value (wr)	FI (pg)	p-value (pg)
GmSYP22-1	Glyma01g01960	21	20	311.58	0.0019	454.09	0.0002
		21	22	320.53	0.0016	598.60	0.0002
		21	18	212.39	0.0128	392.69	0.0135
GmSYP22-2	Glyma09g33950	21	21	351.34	0.0012	603.11	0.0001
		21	22	327.86	0.0252	477.83	0.0152
		21	18	367.94	0.0027	316.98	0.0135

Footnotes. Accession is the genome accession of the gene. OE, overexpression, wr, cysts per whole root analysis. pg, cysts per gram analysis. p-values calculated by the Wilcoxon's rank test (Mann and Whitney 1947).

References

Assaad, F.F., Qiu, J.L., Youngs, H., Ehrhardt. D., Zimmerli. L., Kalde. M., Wanner, G., Peck. S.C., Edwards. H., Ramonell, K., Somerville, CR., Thordal-Christensen H. 2004. The PEN1 syntaxin defines a novel cellular compartment upon fungal attack and is required for the timely assembly of papillae. Mol Biol Cell 15: 5118–5129.

Baena-González, E., Rolland, F., Thevelein, J.M. and Sheen, J. 2007. A central integrator of transcription networks in plant stress and energy signalling. Nature. 448: 938-942.

Bhattacharyya, S., Dey, N. and Maiti, I.B. 2002. Analysis of cis-sequence of from the Figwort mosaic virus and comparison of promoter activity with the cauliflower mosaic virus promoters in monocot and dicot cells. Virus Res. 90: 47–62.

Collier, R., Fuchs, B., Walter, N., Lutke, K.W. and Taylor, C.G. 2005. Ex vitro composite plants: an inexpensive, rapid method for root biology. Plant J. 43: 449–457.

Collins, N.C., Thordal-Christensen H, Lipka, V., Bau, S., Kombrink, E., Qiu, J.L., Hückelhoven, R., Stein, M., Freialdenhoven, A., Somerville, S.C., Schulze-Lefert P. 2003. SNARE-protein mediated disease resistance at the plant cell wall. Nature 425**:** 973-977.

Concibido, V.C., Diers, B.W., and Arelli, P.R. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci. 44: 1121–1131.

Curtis, M. D., and Grossniklaus, U. 2003. A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol. 133: 462-469.

Dhonukshe, P., Bargmann, B.O., and Gadella, T.W. Jr. 2006. Arabidopsis tubulin folding cofactor B interacts with alpha-tubulin in vivo. Plant Cell Physiol. 47: 1406-1411.

Doczi, R., Brader, G., Pettko´-Szandtner, A., Rajh, I., Djamei, A., Pitzschke, A., Teige M, Hirt H. 2015. The Arabidopsis mitogen-activated protein kinase kinase MKK3 is upstream of Group C mitogen-activated protein kinases and participates in pathogen signaling. Plant Cell, Vol. 19: 3266–3279.

Drummond, A.J., Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214

Ebine, K., Uemura, T., Nakano, A., Ueda, T. 2012. Flowering time modulation by a vacuolar SNARE via FLOWERING LOCUS C in Arabidopsis thaliana. PLoS One. 2012;7(7): e42239. doi: 10.1371/journal.pone.0042239.

Endo, B.Y. 1965. Histological responses of resistant and susceptible soybean varieties, and backcross progeny to entry development of *Heterodera glycines*. Phytopathology. 55: 375–381.

Endo, B.Y. 1991. Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. Revue Nématol. 14: 73–84.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. 1998. Potent and specific genetic interference by double stranded RNA in Caenohrabditis elegans. Nature. 391: 806–811.

Gernhard, T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* 253: 769-778

Golden, A.M., Epps, J.M., Riggs, R.D., Duclos, L.A., Fox, J.A., and Bernard, R.L. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode *(Heterodera glycines).* Plant Disease Reporter. 54: 544-546.

Haas, J.H., Moore, L.W., Ream, W., and Manulis, S. 1995. Universal PCR primers for detection of phytopathogenic Agrobacterium strains. Appl. Environ. Microbiol. 61: 2879-2884.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98

Hamaji, K., Nagira, M., Yoshida, K., Ohnishi, M., Oda, Y., Uemura, T., Goh, T., Sato, M.H., Morita, M.T., Tasaka, M., Hasezawa, S., Nakano, A., Hara-Nishimura, I., Maeshima, M., Fukaki, H., Mimura, T. 2009. Dynamic aspects of ion accumulation by vesicle traffic under salt stress in Arabidopsis. Plant Cell Physiol 50:2023-2033.

Hofgen, R., and Willmitzer, L. 1988. Storage of competent cells for Agrobacterium transformation. Nucleic Acids Res. 16: 9877.

Humphry, M., Bednarek, P., Kemmerling, B., Koh, S., Stein, M., Göbel, U., Stüber, K., Pislewska-Bednarek, M., Loraine, A., Schulze-Lefert, P., Somerville, S., Panstruga, R. 2010. A regulon conserved in monocot and dicot plants defines a functional module in antifungal plant immunity. Proc Natl Acad Sci USA 107: 21896-21901.

Jahn, R., and Fasshauer, D. 2012. Molecular machines governing exocytosis of synaptic vesicles. Nature. 490: 201-207.

Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. 1987. GUS fusions: □-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 6: 3901–3907.

Jenkins, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Dis. Rep. 48: 692.

Johansson, O.N., Fantozzi, E., Fahlberg, P., Nilsson, A.K., Buhot, N., Tör, M., Andersson, M.X. 2014. Role of the penetration-resistance genes PEN1, PEN2 and PEN3 in the hypersensitive response and race-specific resistance in Arabidopsis thaliana. Plant J 79: 466-476.

Kim, D.G., Riggs, R.D., and Mauromoustakos, A. 1998. Variation in Resistance of Soybean Lines to Races of *Heterodera glycines*. J. Nematol. 30: 184-191.

Klink, V.P., and Wolniak, S.M. 2001. Centrin is necessary for the formation of the motile apparatus in spermatids of Marsilea. Mol. Biol. Cell. 12: 761-776.

Klink, V.P., Alkharouf, N., MacDonald. M., and Matthews, B.F. 2005. Laser capture microdissection (LCM) and analysis of Glycine max (soybean) syncytial cells formed by the soybean cyst nematode Heterodera glycines. Plant Mol. Biol. 59: 969-983.

Klink, V.P., Overall, C.C., Alkharouf, N., MacDonald, M.H., and Matthews, B.F. 2007. Laser capture microdissection (LCM) and comparative microarray expression analysis of syncytial cells isolated from incompatible and compatible soybean roots infected by soybean cyst nematode (Heterodera glycines). Planta. 226: 1389-1409.

Klink, V.P., MacDonald, M.H., Martins, V.E., Park, S.C., Kim, K.H., Baek, S.H., and Matthews, B.F. 2008. MiniMax, a new diminutive Glycine max variety, with a rapid life cycle, embryogenic potential and transformation capabilities. Plant Cell, Tissue and Organ Culture 92: 183-195.

Klink, V.P., Hosseini, P., Matsye, P.D., Alkharouf, N.W., and Matthews, B.F. 2009a. A gene expression analysis of syncytia laser microdissected from the roots of the *Glycine max* (soybean) genotype PI 548402 (Peking) undergoing a resistant reaction after infection by *Heterodera glycines* (soybean cyst nematode). Plant Mol. Biol. 71: 525-567.

Klink, V.P., Kim, K.H., Martins, V.E., MacDonald, M.H., Beard, H.S., Alkharouf, N.W., Lee, S.K., Park, S.C., and Matthews, B.F. 2009b. A correlation between host-mediated expression of parasite genes as tandem inverted repeats and abrogation of the formation of female Heterodera glycines cysts during infection of *Glycine max.* Planta. 230: 53-71.

Klink, V.P., Hosseini, P., Matsye. P.D., Alkharouf. N.W., and Matthews. B.F. 2010a. Syncytium gene expression in *Glycine max* [PI 88788] roots undergoing a resistant reaction to the parasitic nematode *Heterodera glycines*. Plant Physiology and Biochemistry. 48: 176-193.

Klink, V.P., Overall, C.C., Alkharouf, N.W., MacDonald, M.H., and Matthews, B.F. 2010b. Microarray detection calls as a means to compare transcripts expressed within syncytial cells isolated from incompatible and compatible soybean (Glycine max) roots infected by the soybean cyst nematode (Heterodera glycines). Journal of Biomedicine and Biotechnology. 1-30.

Klink, V.P., Hosseini, P., Matsye, P.D., Alkharouf, N.W., and Matthews. B.F. 2011. Differences in gene expression amplitude overlie a conserved transcriptomic program occurring between the rapid and potent localized resistant reaction at the syncytium of the *Glycine max* genotype Peking (PI 548402) as compared to the prolonged and potent resistant reaction of PI 88788. Plant Mol. Biol. 75: 141-165.

Klink, VP, Sharma, K., Pant, S.R., McNeece, B., Niraula, P., and Lawrence, G.W., 2017. Components of the SNARE-containing regulon are co-regulated in root cells undergoing defense. Plant Signaling & Behavior e1274481 (in press)

Li, J., Todd, T.C., Oakley, T.R., Lee, J., and Trick, H.N. 2010. Host derived suppression of nematode reproductive and fitness genes decreases fecundity of *Heterodera glycines*. Planta. 232: 775-785.

Lipka, L., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S., and Schulze-Lefert, P. 2005. Pre- and Postinvasion Defenses Both Contribute to Nonhost Resistance in Arabidopsis. Science 310: 1180-1183.

Liu, X., Liu, S., Jamai, A., Bendahmane, A., Lightfoot, D.A., Mitchum, M.G., and Meksem, K. 2011. Soybean cyst nematode resistance in soybean is independent of the Rhg4 locus LRR-RLK gene Funct Integr Genomics. DOI 10.1007/s10142-011-0225-4.

Liu, S., Kandoth, P.K., Warren, S.D., Yeckel, G., Heinz, R., Alden, J., Yang, C., Jamai, A., El-Mellouki, T., Juvale, P.S., Hill, J., Baum, T.J., Cianzio, S., Whitham, S.A., Korkin, D., Mitchum, M.G., and Meksem, K. 2012. A soybean cyst nematode resistance gene points to a new mechanism of plant resistance to pathogens. Nature 492: 256-260.

Livak, K.J., and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408.

Lukowitz, W., Mayer, U., and Jürgens, G. 1996. Cytokinesis in the Arabidopsis embryo involves the syntaxin-related KNOLLE gene product. Cell 84: 61-71.

Mann, H.B., and Whitney DR. 1947. On a test of whether one of two random variables is stochastically larger than the other. Annals of Mathematical Statistics 18:50–60.

Matsye, P.D., Kumar, R., Hosseini, P., Jones, C.M., Tremblay, A., Alkharouf, N.W., Matthews, B.F., and Klink, V.P. 2011. Mapping cell fate decisions that occur during soybean defense responses. Plant Mol. Biol. 77: 513-528.

Matsye, P.D., Lawrence, G.W., Youssef, R.M., Kim, K.H., Matthews, B.F., Lawrence, K.S., and Klink, V. P. 2012. The expression of a naturally occurring, truncated allele of an □-SNAP gene suppresses plant parasitic nematode infection. Plant Mol. Biol. 80: 131-155.

Matthews, B., MacDonald, M.H., Thai, V.K., and Tucker, M.L. 2003. Molecular characterization of argenine kinase in the soybean cyst nematode (*Heterodera glycines*). J. Nematol. 35: 252-258.

Matthews, B.F., Beard, H., MacDonald, M.H., Kabir, S., Youssef, R.M., Hosseini, P., and Brewer, E. 2013. Engineered resistance and hypersusceptibility through functional metabolic studies of 100 genes in soybean to its major pathogen, the soybean cyst nematode. Planta. 237: 1337-1357.

Matthews, B.F., Beard, H., Brewer, E., Kabir, S., MacDonald, M.H., and Youssef, R.M. 2014. Arabidopsis genes, AtNPR1, AtTGA2 and AtPR-5, confer partial resistance to soybean cyst nematode (Heterodera glycines) when overexpressed in transgenic soybean roots BMC Plant Biology. 14: 96.

Mayer, U., Torres, Ruiz, R.A., Berleth, T., Misera, S., and Jurgens, G. 1991. Mutations affecting body organization in the Arabidopsis embryo. Nature 1991; 353:402-407

Mazarei, M., Elling, A.A., Maier, T.R., Puthoff, D.P., and Baum, T.J. 2007. GmEREBP1 is a transcription factor activating defense genes in soybean and Arabidopsis MPMI. 20: 107-119.

McLean, M.D., Hoover, G.J., Bancroft, B., Makhmoudova, A., Clark, S.M., Welacky, T., Simmonds, D.H., and Shelp, B.J. 2007. Identification of the full-length *Hs1*$^{pro-1}$ coding sequence and preliminary evaluation of soybean cyst nematode resistance in soybean transformed with *Hs1*$^{pro-1}$ cDNA**.** Can. J. Botany**.** 85: 437-441.

Melito, S., Heuberger, A., Cook, D., Diers, B., MacGuidwin, A., and Bent A. 2010. A nematode demographics assay in transgenic roots reveals no significant impacts of the Rhg1 locus LRR-Kinase on soybean cyst nematode resistance. BMC Plant Biol. 10: 104.

Morita-Yamamuro, C., Tsutsui, T., Tanaka, A., and Yamaguchi, J. 2004. Knock-out of the plastid ribosomal protein S21 causes impaired photosynthesis and sugar-response during germination and seedling development in Arabidopsis thaliana. Plant Cell Physiol. 45: 781-788.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plantarum. 15: 473-497.

Niblack, T.L., Arelli, P.R., Noel, G.R., Opperman, C.H., Orf, J.H., Schmitt, D.P., Shannon, J.G., and Tylka, G.L. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. J. Nematol. 34: 279-288.

Novick, P., Field, C., and Schekman, R. 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 21: 205-215.

Novick, P., Ferro, S., and Schekman, R. 1981. Order of events in the yeast secretory pathway. Cell 25: 461-469.

Ohtomo, I., Ueda, H., Shimada, T., Nishiyama, C., Komoto, Y., Hara-Nishimura, I., and Takahashi, T. 2005. Identification of an allele of VAM3/SYP22 that confers a semi-dwarf phenotype in Arabidopsis thaliana. Plant Cell Physiol 46:1358–1365.

Pant, S.R., Matsye, P.D., McNeece, B.T., Sharma, K., Krishnavajhala, A., Lawrence, G.W., and Klink V.P. 2014. Syntaxin 31 functions in *Glycine max* resistance to the plant parasitic nematode *Heterodera glycines* Plant Mol. Biol. 85: 107-121.

Pant, S.R., Krishnavajhala, A., McNeece, B.T., Lawrence, G.W., and Klink, V.P. 2015. The syntaxin 31-induced gene, LESION SIMULATING DISEASE1 (LSD1), functions in *Glycine max* defense to the root parasite *Heterodera glycines*. Plant Signaling and Behaviour. 10: 1, e977737.

Rambaut, A. 2009. FigTree v1.3.1, Available from http://beast.bio.ed.ac.uk/FigTree

Rambaut, A., and Drummond, A.J. 2012. TreeAnnoator v1.7.4, Available from http://beast.bio.ed.ac.uk/TreeAnnotator

Riggs, R.D., and Schmitt, D.P. 1988. Complete characterization of the race scheme for Heterodera glycines. J. Nematol. 20: 392-395.

Riggs, R.D., and Schmitt, D.P. 1991. Optimization of the *Heterodera glycines* race test procedure. J. Nematol. 23: 149-154.

Ross, J.P. 1958. Host-Parasite relationship of the soybean cyst nematode in resistant soybean roots. Phytopathology. 48: 578–579.

Sato, M.H., Nakamura, N., Ohsumi, Y., Kouchi, H., Kondo, M., Hara-Nishimura, I., Nishimura, M., and Wada, Y. 1997. The AtVAM3 encodes a syntaxinrelated molecule implicated in the vacuolar assembly in Arabidopsis thaliana. J Biol Chem 272:24530–24535.

Sanderfoot, A.A., and Raikhel, N.V. 1999. The specificity of vesicle trafficking: coat proteins and SNAREs. Plant Cell 11:629-642.

Sanderfoot, A.A., Farhah, F., Assaad, F.F., and Raikhel, N.V. 2000. The Arabidopsis genome. An abundance of soluble N-ethylmaleimide-sensitive factor adaptor protein receptors. Plant Physiol. 124: 1558-1569.

Sharma, K., Pant, S.R., McNeece, B.T., Lawrence, G.W., and Klink, V.P. 2016. Co-regulation of the Glycine max soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE)-containing regulon occurs during defense to a root pathogen. J Plant Interactions 11:74-93

Schmutz, J., Cannon, S.B., Schlueter, J., Ma, J., Mitros, T., Nelson, W., Hyten, D.L., Song, Q., Thelen, J.J., Cheng, J., Xu, D., Hellsten, U., May, G.D., Yu, Y., Sakurai, T., Umezawa, T., Bhattacharyya, M.K., Sandhu, D., Valliyodan, B., Lindquist, E., Peto, M., Grant, D., Shu, S., Goodstein, D., Barry, K., Futrell-Griggs, M., Abernathy, B., Du, J., Tian, Z., Zhu, L., Gill, N., Joshi, T., Libault, M., Sethuraman, A., Zhang, X.C., Shinozaki, K., Nguyen, H.T., Wing, R.A., Cregan, P., Specht, J., Grimwood, J., Rokhsar, D., Stacey, G., Shoemaker, R.C., and Jackson, S.A. 2010. Genome sequence of the palaeopolyploid soybean. Nature. 463: 178-183.

Shirakawa, M., Ueda, H., Shimada, T., Koumoto, Y., Shimada, T.L., Kondo, M., Takahashi, T., Okuyama, Y., Nishimura, M., and Hara-Nishimura, I. 2010. Arabidopsis Qa-SNARE SYP2 proteins localized to different subcellular regions function redundantly in vacuolar protein sorting and plant development. Plant J 64:924-935.

Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., and Rothman, J.E. 1993a. SNAP receptors implicated in vesicle targeting and fusion. Nature 362:318-324.

Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., and Rothman, J. E. 1993b. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75:409-418.

Steeves, R.M., Todd, T.C., Essig, J.S., and Trick, H.N. 2006. Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress Heterodera glycines reproduction. Funct. Plant. Biol. 33: 991–999.

Stein, M., Dittgen, J., Sánchez-Rodríguez, C., Hou, B.H., Molina, A., Schulze-Lefert, P., Lipka, V., and Somerville, S. 2006. Arabidopsis PEN3/PDR8, an ATP Binding Cassette Transporter, Contributes to Nonhost Resistance to Inappropriate Pathogens That Enter by Direct Penetration. Plant Cell 18: 731-746.

Sun, L., Zhu, L., Xu, L., Yuan, D., Min, L., and Zhang, X. 2014. Cotton cytochrome P450 CYP82D regulates systemic cell death by modulating the octadecanoid pathway. Nat. Commun. 5: 5372.

Tepfer, D. 1984. Transformation of several species of higher plants by Agrobacterium rhizogenes: sexual transmission of the transformed genotype and phenotype. Cell. 37: 959-967.

Ueda, H., Nishiyama, C., Shimada, T., Koumoto, Y., Hayashi, Y., Kondo, M., Takahashi, T., Ohtomo, I., Nishimura, M., and Hara-Nishimura, I. 2006. AtVAM3 is required for normal specification of idioblasts, myrosin cells. Plant Cell Physiol 47:164–175.

Uemura, T., Morita, M.T., Ebine, K., Okatani, Y., Yano, D., Saito, C., Ueda, T., and Nakano, A. 2010.Vacuolar/pre-vacuolar compartment Qa-SNAREs VAM3/SYP22 and PEP12/SYP21 have interchangeable functions in Arabidopsis. Plant J 64:864-873.

Vukašinovi, ´ N., Žárský, V. 2016. Tethering complexes in the Arabidopsis endomembrane system. Front Cell Dev Biol 2016; 4:46 doi: 10.3389/fcell.2016.00046.

Waizenegger, I., Lukowitz, W., Assaad, F., Schwarz, H., Jurgens, G., and Mayer, U. 2000. The Arabidopsis KNOLLE and KEULE genes interact to promote vesicle fusion during cytokinesis. Curr Biol 2000; 2:1371-4; http://dx. doi.org/10.1016/S0960-9822(00)00775-2

Whelan, S., and Goldman, N. 2001. A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Molecular Biology and Evolution 18: 691-699

Yamazaki, S., Harashima, S., Sakaguchi, M., and Mihara, K. 1997. Identification and functional characterization of yeast zeta-COP. J. Biochem. 121: 8-14.

Yano, D., Sato, M., Saito, C., Sato, M.H., Morita, M.T., and Tasaka, M. 2003. A SNARE complex containing SGR3/AtVAM3 and ZIG/VTI11 in gravitysensing cells is important for Arabidopsis shoot gravitropism. Proc Natl Acad Sci USA 100:8589–8594.

Youssef, R.M., MacDonald, M.H., Brewer, E.P., Bauchan, G.R., Kim K.H., and Matthews, B.F. 2013. Ectopic expression of AtPAD4 broadens resistance of soybean to soybean cyst and root-knot nematodes. BMC Plant Biology 13: 67.

Zhou, C., Zhang, L., Duan, J., Miki, B., and Wu, K. 2005. HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. Plant Cell. 17: 1196-1204.

CHAPTER III

EVALUATION OF THE EFFECT OF BIOLOGICAL NEMATICIDE SEED TREATMENTS ON NEMATODE MANAGEMENT AND EFFECTS ON PLANT GROWTH.

Abstract

Soybean cyst nematode *(Heterodera glycines*), reniform nematode (*Rotylenchulus reniformis),* and root-knot nematode *(Meloidogyne incognita)* are three most damaging plant-parasitic nematodes on soybean. One recent strategy for nematode management is the application of biological control products. Biological control is being accepted as an alternative to chemical methods due to less negative effects placed on the environment. Experiments were conducted in the greenhouse at the R.R. Foil Plant Science Research Center at Mississippi State University to determine the efficacy of potential biological control products to manage nematodes on soybean. Experiments include tests to evaluated different selected biological products, application rates and product combinations as seed treatments on soybeans. Treatments were included *Burkholderia renijensis,* bacterial metabolite, SAS-products, and ALB-EXP Bacteria. The study included the effects on plant growth and development and nematode life stage development. Seeds were planted in 500 cm of a steam sterilized sand: soil mix (1:1/ V: V) in 10 cm dia clay pots. Seeds were placed into one 2.54 cm depression in each pot with either the addition of 2500 eggs of *H. glycines, M. incognita*, and 2500 vermiform

life stage of *R. reniformis*. Treatments also included the standard nematicide seed treatments Abamectin and a fungicide alone controls. Treatments were arranged as a randomized complete block design with five replications. Tests ran for 60 days. Results indicated no negative plant effects on the soybean plants from any biological seed treatments. Many of the biological products were statistically similar to the standard nematicide abamectin. These biological products significantly reduced the nematode reproduction of juveniles and eggs recovered compared with the non-treated control. *Burkholderia sp*. variant 2 (BioST^{TM} Nematicide) was a more consistent nematicide candidate. Combinations of *Burkholderia sp* variant 2 with selected SAR (systemic acquired resistant) products numerically improved the efficacy and consistency of the biological nematicide. Stacking biological Technologies that exhibit nematicidal activity appears to be an approach that could improve product performance compared to traditional chemistries used alone.

Introduction

Biological control of nematodes

Biological control is the complete or partial management of pest organisms by other organisms that are common in the environment and leads to suppressed the population of the pathogens and subsequently less damage that are possible. (Agrios, 2005; Eilenger et al. 2001).

Biological management of soil borne diseases with microorganisms has been researched for 65 years (Barker, 1987) and biocontrol of nematodes was first studied by Duddington (1951). The use of biological product is considered an effective alternative for nematode management on vegetables (Van Gundy, 1985; Kerry, 1987; Sikora, 1992).

The three major types of organisms that are antagonistic to nematodes, including (a) predators - organisms which actively seek out nematodes and then consume them; (b) parasites - organisms which grow within their host and obtain their nutrition from the host. and (c) antagonists - which influence nematode abundance through mechanisms other than predation and parasitism (Stirling, 1991). Sikora (1992) has suggested the term "antagonistic potential" for all parasites, predators, pathogens, competitors and other organisms in soil that work together to repel, inhibit, or kill plant-parasitic nematodes. Antagonists most likely to be feasible are: predacious or trapping fungi, endo-parasitic fungi, fungi pathogen/ parasites of females, endo- mycorrhizal and mutualistic fungi, plant-health promoting rhizobacteria and obligate bacterial parasites. Sikora (1992).

There are some biological products that have been marketed for management of plant-parasitic nematrodes. These products include *Bacillus firmus* as (Bio-Nem-WP/BioSafe; Ashdod, Agrogreen, Israel). Keren-Zur et al. (2000), also two strains of *B. amyloliquefaciens* IN937a and *B. subtilis* GB03 (BioYield; Gusrafson LLC, Plano, TX) (Burkett-Cadena et al. 2008). In addition, VOTIVO (*B. firmus*) GB-126 produced by (Bayer CropScience, Raleigh, NC). (Castillo et al. 2013), and Clariva® (*Pasteuria nishizawae)* Syngenta. Askary (2015).

Burkholderia sp.

Burkholderia species are considered to have activity on nematodes and insects and can befound in many types of environments, including inside various organisms, water, and the rhizospheres. (Coenye, T., and Vandamme P. 2003; Parke, J. L., and Gurian-Shermm D. 2001). Some species of *Burkholderia* are known as pathogens for plants; for example, *Burkholderia cepacia* has been discovered as disease on onions.

(Burkholder W.H. 1950). In addition, there are several species of *Burkholderia* have been known as human pathogens which are including some species of *Burkholderia cepacia, B. mallei, B. pseudomallei, B. fungorum* cause melioidosis and glanders. (Parke, J. L., and Gurian-Shermm D. 2001, Cheng, A. C., and Currie B. J. 2005; Nierman, W. C. et al. 2004). However, *Burkholderia spp.* have shown beneficial activities with in the hosts and have the ability to modulate nitrogen in the roots. (Caballero-Mellado, J.; et al. 2007; Chen, W. M., et al. 2007; Caballero-Mellado, J., et al. 2004). Some species of *Burkholderia* have been observed to have activity as biological compounds against foliar disease, disease post harvest, and soil borne disease. Also, *Burkholderia spp* have been used in the bioremediation treatments for contaminated soil and groundwater. (Burkhead, K. D., et al. 1994; Knudsen, G.R., and Spurr. H.W. 1987; Cassida, L., et al. 2004; Zhang, W., and Sulz, M. 1988; Leahy, J. G., et al. 1996; Lessie, T. G., et al. 1996). Additionally, some *Burkholderia spp.* have been release to extracellular enzymes include hemolytic, lipolytic, and proteolytic that have activity as toxins, siderophores, and antibiotics. There are some products produced by *Burkholderia spp.* that have activity as insecticides. (Ennouri, K., et al. 2013). The soil isolated *Burkholderia ambifaria* has antifungal activity used for the biogical control. (Denning, D. W., and Hope, W. W. 2010; Vicente, M. F., et al. 2003). *Burkholderia gladioli* has activity against the *Alternaria alternate.* (Mahamuni, Shrikumar.Vijaykumar., 2015). There are some studies have been shown the activity of *Burkholderia* spp. work as biological products against several of pathogens. *Burkholderia. rinojensis* has the biochemical properties that have it important species biological natural products. (Burkhead, K.D., et al. 1994; Janisiewicz, W.J.; Roitman,

J.1988; He, H., et al. 2014). This bacterial biocontrol agent will be the lead active ingredient for nematode protection in the following studies.

Seed Treatments

Seed treatments have been used widely in United States for more than 30 years. Seed treatments include fungicides, bactericides, nematicides, insecticides, bio-control agents and herbicide antidotes.

Seed treatment nematicides have been available in the market since 2005 and management practices have been changed from the standard granular in-furrow applications to seed treatments, such as Avicta Complete Cotton, (abamectin), Aeris, (thiodicarb), and Votivo, a biological strain of the *Bacillus firmus* strain GB216. Seed treatments have simplified the growing process and reduced producer's exposure to chemicals. There are some examples of bionematicides as seeds treatments including abamectin (Syngenta) has shown activity against soybean cyst nematode (*H. glycines*) and Root-knot nematode (*M. incognita*). Other product is VOTiVO which is *Bacillus firmus* (Bayer Cropsciene). This product has shown activity against *H. glycines*, *Rotylenchulus reniformis*, and *M. incognita.* Clariva (*Pasteuria nishizawae*, Syngenta) that has activity *H. glycines* (Qiao, et al. 2012; Muzhandu, et al. 2014; Faske and Starr. 2007; Mendoza, et al. 2008; Castillo, et al. 2013; Schrimsher, et al. 2011).

The current treated of nematicide development is with biological compounds applied to the seed. Therefore, the objective of this research was to determine the efficacy of selected new biological experimental compounds applied as seed treatments for the management of nematodes on soybean.

Materials and Methods

This research was conducted over a two-year period that encompassed four specific research studies and objectives. The development of a potentially uses of biological nematicide requires a series of steps. Step one is to the process was to identify a biological nematicide candidates. The two candidates of choice were the bacterial derived products heat-killed *Burkholderia rinojensis* variants and an experimental bacterial candidate (non-disclosed by Albaugh, LLC). The second step was to identify and screen secondary nematicidal products that could potentially be combined or stackedwith our bacterial products to increase the overall efficacy of a seed treatment nematicide. The third step was to combine *Burkholderia rinojensis* with SAR and bacterial metabolites (secondary nematicidal products) to increase nematicidal activity and consistency. Each of the research steps associated with one objective was evaluated on the *Heterodera glycines, Rotylenchulus reniformis,* and *Meloidogyne incognit*a which are the three economically important nematodes on soybeans.

In all tests soybean seeds were treated with a standard base fungicide package that included metalaxyl, thiabendazole and Tolclofos-methyl. All seeds were treated by Albaugh, LLC. The treatment list of the experimental biological compounds evolved over time as different variants of bacteria products, rates and combinations of products were evaluated to improve efficacy. All products were evaluated on soybean cyst nematode *H. glycines*, reniform nematode *R. reniformis*, and root-knot *M. incognit*a.

The first step *Burkholderia rinojensis* variant and rates. The two products of choice were evaluated at different rates two production variants (variants 1 and 2) (Table 3.1).

The second step Bacterial metabolite and SAR seed treatments. This step included several products that were not labeled for nematodes, but from previous literature had some indications that indicated probable nematicide activity (Table 3.5). These products/compounds systemic acquired resistance (SAR) products saponin and harpin protein based SAR products (non-disclosed by Albaugh, LLC).

Step three was experimental bacteria and *Burkholderia rinojensis* combinations. In this step, we encluded an additional bacterial nematicide candidate at three different rates and combinations of *Burkholderia rinojensis* variant 2 (BIOST^{TM} Nematicide) with the SAR compounds and the *Bacterial Metabolite* product (Table 3.9). In this step was required the impact of stacking different modes of action for early season seed treatment nematicide activity.

In our 2016 study (2016 Seed Treatment Comprehensive Study), many of the treatments and combinations were reexamined again to verify the previously results (Table 3.13). The standard nematicide seed treatment were included in these studies included *Pasteuria nishizawae*, *Bacillus firmus* Votivo, Avicta, and ILeVo.

Inoculum production of *Heterodera glycines* Cysts, Eggs, and Juveniles

H. glycines [NL1-Rhg/HG-type 7/race 3] previously planted in a greenhouse and maintained on Williams 82 (/PI 518671) was as inoculum in all tests. (Klink et al. 2005; Pant et al. 2014). The cysts were dislodged from the roots of 50 days old plants using strong water and. Cysts were suspended in water and immediately poured through the 20-pore sieve nested on a 100-pore sieve. (Mclean, K. S., et al. 1990). Cyst were counted on graded Petri dishes using a stereo-microscope at 40X magnification. (Debora C. Ladner, et al, 2008). Eggs were released from the cysts using a modified cyst crusher for 1

minute (Mclean, K. S., et al. 1990). After crushing, eggs were passed through a 200-um pore sieve to remove broken cysts and debris nested on a 500-um pore sieve. *H. glycines* second stage juveniles were extracted from the soil using gravity screening. The soil was collected on a 325 um pore sieves then processed further by sucrose centrifugal flotation for 1 munite. (Jenkins. W. R. 1964).

Rotylenchulus reniformis* and *Meloidogyne incognita

Rotylenchulus reniformis and *Meloidogyne incognita* were maintained in the greenhouse on cotton and corn respectively. The eggs for both nematodes were extracted from fresh roots by using NaOcl for 4 minutes with using 200 um pore sieves nested on 500 um pore sieves. (Mclean, K. S. 1993). Juveniles were extracted from the soil by sucrose centrifugal flotation. (Ayoub, S. M. 1980; Jenkins. W. R. 1964).

Methods for Greenhouse tests

In all tests seeds treated with biological treatments were sown (2 seeds/pot) in 15 cm diameter clay pots filled with 500 cm^3 of the sterilized soil-sand mixture (1:1, v/v). A suspension of 2500 eggs (*H. glycines* and *M. incognita*) or 2500 vermiform reniform nematode (*R. reniformis*) were pipetted into the pots at the time of planting. Two holes' depression (2.5 cm dia x 2.5 cm deep) were made around the seeds and 3ml of inoculum was pipetted onto the seeds. All experimental treatments were arranged in a RCBD with 5 replications and allowed to grow in the greenhouse maintained at approximately 25°C with artificial light of 12 hours/day. Plants were watered daily and received fertilizer weekly. At 60 days, the plants were harvest plant development and nematodes life stage development was recorded.

Plant Parameters and nematodes measurements

Plants parameters measurements included fresh weight, height of plants, number of nodes, number of seed pods, and root weight. Nematode population development was measured by the number of juveniles/ 500cm3 recovered from the soil, number of cysts on the roots, and number of eggs from the cyst. For the root-knot nematode the percent of the root system with galls was rated according to the following method. Root galling is recorded on a 0 – 5 scales, where 0 = no galling, 1 = 25% galling, 2 = 50% galling, 3 = 75% and 4 =100% galling. (Daykin and Hussey, 1985).

Root image acquisition and analysis

The plant roots systems for each treatment were scanned and acquired images were analyzed for cumulative root length (RCL), surface area (RSA), average root diameter (RAD), root volume (RV), number of tips (RNT), number of forks (RNF), and number of crossings (RNC) using winRHIZO Pro software (Version 2009c, Regent Instruments, Inc.). Roots were cut and separated from the stems and washed thoroughly but avoiding any major disturbance to the root system. The cleaned individual root systems were floated in 5 mm of water in a 0.3 × 0.2 m Plexiglas tray. Roots were then untangled and separated using a plastic paint brush to minimize root overlap. The tray was placed on top of a Dual Scan optical scanner (Regent Instruments, Inc., Quebec, Canada), linked to a computer. Greyscale root images were acquired by setting the parameters to ‘‘high’’ accuracy (resolution 800 by 800 dpi).

Statistical analysis

The data for plant measurements and nematode populations was analyzed using SAS Statistical Software System version 9.4. Data was subjected to analysis of variance (SAS Institute, 2011) using a randomized complete block design with 5 replications. Differences in treatments means were separate using Fisher`s Protected Least Significant Difference Test for all the results (SAS Institute, 2011).

Results

Burkholderia rinojensis variant and rates

Soybean cyst nematode (*Heterodera glycines*)

Two varaints of *Burkholderia rinojensis* were identified for use in this study. All varaints and rates produced significant effects to improve better on soybean plant development. These included above ground plant weight, height of plant, number of nodes, and weight of roots compared to the control treatment. *B. rinojensis* varaints 1 and 2 reduced the number of cyst, juveniles, and eggs of *H. glycines* compared to the control (Table 3.2). *B. rinojensis.* varaints 1(5 floz/cwt) and *B. rinojensis* varaints 2 (5 floz/cwt) application rate had similar results in reducing the reproductive factor number for nematode life stages when compared to the control. Most of the treatments were similar to abamectin which was used as a standard. (Table 3.2).

Reniform nematode (*Rotylenchulus reniformis*)

Burkholderia rinojensis varaints 1 and 2 no significant impact on plant growth and when used as a seed treatment to soil infested with *R. reniformis*. However, there was significant effect on weight of roots in the *B. rinojensis* varaints 2 at the (7 fl. oz).

Root weight was 12.2 gram compared to 5.3 gram in the control (Table 3.3). There were no adverse effects on plant growth from *Burkholderia rinojensis* varaints 1 and 2.

The *B. rinojensis* variants were significantly lower than the fungicide check. *R. reniformis*, used as a seed treatment numbers were reduced from 20703 juveniles and 23587 eggs in the control to 3397 juveniles per 500 cm3 soil and 4635 eggs in *B. rinojensis* at (7 fl. oz rate). Most of *B. rinojensis* variants treatments were similar to abamectin, except the (3floz rate) of *B. rinojensis.* variant 1 (Table 3.3).

Root-knot (*Meloidogyne incognita*)

There was no significant effect on plant development by *M. incognita.* No negative effects were recorded on soybean plant growth from any biological seed treatment (Table 3.4). All *B. rinojensis.* variants significantly reduced nematode reproduction when compared to the fungicide check, except *B. rinojensis.* variant 1 at (3 fl. oz/cwt). *B. rinojensis.* variant 2, was statistically similar to that of abamctin at all rates; however, *B. rinojensis.* variant 1 higher reproductive factor values were recorded for abamectin at 3, 5, 7, and 10 fl. oz/cwt. (Table 3.4). The same treatments (*B. rinojensis.* variant 1 at 3, 5, 7, and 10 fl. oz/cwt did significantly reduced nematode from 5144 juveniles and 24205 eggs in the control to 1373.2 juveniles, 5665 eggs, 1201.4 juveniles, and 7081.2 eggs respectively in these treatments. Treatments also reduced the number of galls on roots compared to the control. *B. rinojensis* at 7 and 10 fl. oz reduced number of galls from 3.2 in the control to 0.6 and 0.4, respectively. (Table 3.4).

***Bacterial metabolite* and SAR seed treatments**

Soybean cyst nematode (*Heterodera glycines*)-2015

Soybean plant growth was not affected by *H. glycines* were using biological seed treatments. Soybean plant weight, height, number of nodes, number of pods, and weight of roots were not significantly affected by *H. glycines* compared with the control treatments. (Table 3.6).

H. glycines populations were influenced by the biological seed treatments; *H. glycines* cyst counts were 566.28 cysts in the control compared with 154.44 and 60.06 in the bacterial metabolite and Abamectin treatments respectively. These two treatments also significantly reduced the number of juveniles and eggs per cysts compared with the control. All treatments, including the two nematicide standards (abamectin and fluopyram) had significantly lower reproductive factor values respectively compared to the fungicide control and the untreated seeds. (Table 3. 6). None of the SAR or bacterial metabolite products were statistically different from the two nematicide standards, but were statically different from the fungicide only control and the untreated control.

Reniform nematode (*Rotylenchulus reniformis*)

2015; *Rotylenchulus reniformis* produced no significantly reductions on plant weight, height, number of nodes, number of pods, and weight of roots when the biological seed treatments were used with the compared control (Table 3.7). Significantly fewer numbers of vermiform life stages of *R. reniformis* were recorded when the biological seed treatments were used. Saponin (0.2 fl. oz/cwt) reduced numbers of juveniles and vermiform adults from 8961 and 10815 respectively in the two control treatments to 1538.8 juveniles per 500 cm3 soil. All treatments, except the standard

products fluorpyram, significantly reduced nematode reproduction compared with the control. The biological treatments were not significantly different from the abamectin standard, but were significantly better than fluopyram. (Table 3.7).

Root-knot nematode (*Meloidogyne incognita*).

There were no negative effects of *M. incognita* on plant growth including weight of plants, height, number of nodes, and weight of roots with using the biological seed treatments. (Table 3.8).

All biological products and nematicide standards significantly reduced *M. incognita* reproduction compared with the untreated seeds and the fungicide check. (Table 3.8). There were no differences between biological products and the nematode standards (abamectin and fluopyram). *M. incognita* was reduced life stages development with the biological seed treatments. *M. incognita* juveniles were reduced from 141110 and 99395 juveniles in the two control treatments (fungicides only and untreated seeds) to 3012.6 and 3399 juveniles per 500 cm3 soil in the saponin (0.2 fl/oz) and bacterial metabolite treatments. Also, the same treatments significant in reducing the number of eggs and the average of galls compared with the controls.

Experimental Bacteria and *Burkholderia rinojensis* combination

Soybean cyst nematode (*Heterodera glycines*)-2015

There were no significantly effects on plant growth by *H. glycines* with using the biological seed treatments. ALB EXP Bacteria at (5, 10, and 15 floz/cwt), *B. rinojensis* varint 2 (5 floz/cwt), *B. rinojensis* varint 2 + bacterial metabolite; *B. rinojensis* varint 2 + Saponin; *B. rinojensis* varint 2 + Harpin) produced significantly higher plant weights and

plant heights, compared with the control (Table 3.10). The number of *H. glycines* cysts, juveniles, and eggs were reduced in most treatments with the biological seed products (Table 3.10). ALB EXP bacteria performed better at the higher application rates than the lower rate (5 floz/cwt). All treatments were statistically similar to the abamectin standard except *B. rinojensis* variant 2 treatment.

Reniform nematode (*Rotylenchulus reniformis*)-2015

There were no significant reduction on soybean plant growth and development by *R. reniformis* in the presence of the biological seed treatments. ALB EXP Bacteria 3 and *B. rinojensis* in (5 floz/cwt) reduced weight of roots. Roots weight was 8, 9.3 gram, compared with 5.4 gram in the control. The combination of harpin + *B. rinojensis* (5 floz/cwt) produced the great results on plant growth.

Most biological seed treatments reduce the number of *R. reniformis* juveniles and vermiform adult as well as eggs compared to control. The combination treatments (*B. rinojensis* varaint 2+ Harpin SAR), reduced juveniles and eggs number 5150 juveniles and 8755 in the control to 1806.6 juveniles per 500 cm3 soil and 3090 eggs. Most treatments (ALB EXP Bacteria at 5, 10, and 15 floz/cwt; *B. rinojensis* varint 2 at 5 floz/cwt; *B. rinojensis* varint 2 + bacterial metabolite*; B. rinojensis* varint 2 + Saponin; *B. rinojensis* varint 2 + Harpin) produced results that were similar to abamectin (Table 3.11).

Root-knot nematode (*Meloidogyne incognita*)-2015

There was no significant reduction on soybean growth and development by *M. incognita* in the presence of the biological treatments (Table 3.12).

Nematodes reproduction was significantly reduction in all the biological treatment compared to the fungicide control. Treatments also significantly reduced the number of juveniles and eggs were reduced from 12875 juveniles and 14935 eggs in the control to 3347.5 juveniles and 3862.4 eggs in ALB EXP Bacteria. and 2575 juveniles, and 3090 eggs in *B. rinojensis* 1 + bacterial metabolite treatments. The same treatments significantly reduced the average of number galls per root compared to the control (Table 3.12).

Soybean Cyst Nematode (*Heterodera glycines*)-2016

In the2016, there was no negative significant effects on the plant growth parameters resulted from plant weight, height, number of nodes, number of pods, or weight of roots by *H. glycines* infection in the presence of the biological seed treatments compared to control treatments. Most biological seed treatments lead to increase plants weight and roots weight compared to untreated seeds. *B. rinojensis* (5 and 2 floz/cwt) increased plants weight, plant height, number of nodes, and roots weight (Table 3.14). Bacterial metabolite + *B. rinojensis* differences were from the control.

The effects of *B. rinojensis* (5 and 2 floz/cwt) significantly reduced the number of cysts, juveniles, and eggs. (Table 3.14). The combination bacterial metabolite + *B. rinojensis* treatment significantly reduced the number of cysts, juveniles, and eggs compared to control treatments. *B. rinojensis* variant 1 and 2, the combination *B. rinojensis* + saponin, and ALB Experimental Bacteria at (3 floz/cwt) also had similar effects on nematode life stage development. All treatments were statistically similar to the abamectin standard except the low rate *B. rinojensis* treatment 2 and treatment 7.

Roots image acquisition and analysis with *H. glycines* did not reveal any negative significant effects on roots growth from biological seed treatments compared to control treatments. *B. rinojensis* treatment (5 and 2 fl. oz/ cwt) had greater root length, surface area of the root, average root diameter, root volume, number of tips, number of forks, and number of crossings compared to control treatment. (Table 3.15). Bacterial metabolite + *B. rinojensis* were significant different from the control treatment to improve roots growth and other treatments regarding. The number of tips, forks, and crossings were significant different compared to control treatment and the other seed treatments (Table 3.15).

Reniform Nematode (*Rotylenchulus reniformis*)

There were no negative effects on soybean by *R. reniformis* in the presence of the experimental compounds. *B. rinojensis* 1 and 2 + saponin (0.16 floz/cwt) improved plant growth which included plants height, number of nodes, and number of pods compared to the control treatments (fungicide treatment only and untreated seeds) (Table 3.16). Plant weights were greater with the biological seed treatments were used.

R. reniformis reproduction was reduced in all biological treatments, combination treatments and the three nematicide standards compared with the untreated seeds and the fungicide standard. (Table 3.16). The effect of biological seed treatments reduced number of Vermiform and juvenile's life stages were reduced from 15707.5 and 19570 juveniles in the control treatments to 2317.5 and 2832.5 juveniles where *B. rinojensis* at (3 and 2 fl. oz/ cwt) respectively. Also, the same treatments reduced the number of eggs from 20600 and 19364 eggs in the control treatments (fungicides treatment only and untreated seeds) to 2369 and 2575 eggs. Most of the biological seed treatments gave

similar results to abamectin which used as standard in this experiment Table (3.16). as well as other nematicide standards (*Pasteuria nishizawae and Bacillus firmus*). The numerically best treatment was the low rate *B. rinojensis* (3 floz/cwt) variant 2 (BIOST Nematicide).

Roots image acquisitions and analysis showed no negative effects on roots growth by *R. reniformis* in the presence of the biological seed treatments when compared with the control (Table 3.17). The effects on roots development was significant roots growth with *B. rinojensis* in rates (5 and 2 fl. Oz/ cwt) on root length, surface area of root, average root diameter, root volume, number of tips, number of forks, and number of crossings compared to the control. Roots from Saponin + *B. rinojensis* had significant more number of tips, forks, and crossings compared to the control.

Root-knot (*Meloidogyne incognita*)

There were no negative effects on soybean plants caused by *M. incognita* in the presence of the biological seed treatments (Table 3.18). Plants from *B. rinojensis* variant 1 and 2 had the highest of plant weights compared to the control, fungicides only and untreated seeds controls. Plant weight was 26.4 grams from plants in *B. rinojensis* variant 1 (5 fl/oz /cwt) compared to 10.4 and 14.4 grams in the controls. Plants from this treatment were significant taller and had more of pods compared with plants in other treatments.

All the biological treatments and nematicide standards significantly reduce nematode population compared with the untreated seeds and the fungicide control. All biological treatments were statistically similar to the nematicide standards. *B. rinojensis* at (3 fl. oz/ cwt) reduced number of juveniles from 17767.5 and 12102.5 in the control to

2060 juveniles per 500 cm3 soil. Also, the same treatment reduced the number of eggs from 21630 and 9888 eggs in the controls to 2163 eggs. The combination treatment *B. rinojensis* + bacterial metabolite reduced both juveniles and eggs compared to the control treatments. Most of treatments significantly reduced the number of galls on the roots compared to control treatments.

Roots image acquisitions and analysis (table 3.19) showed no significant effects on roots growth *M. incognita* in the presence from the biological seed treatments when compared with the control treatments (Table 3.19). Root length, surface area of root, average root diameter, root volume, number of tips, number of forks, and number of crossings were grater from plant that were treated with ALB-EXP bacterial compared with the control treatment. The number of root tips and forks were 5914.6, 19831.2 respectively compared to 2257.2 and 5289.8 in the control treatment.

Discussion

Our primary objective in this research was to identify a viable biological candidate that would be efficacious on the important nematodes in soybeans. In our first and third steps of study, we evaluated two production variants of *Burkholderia rinojensis* and an Experimental Bacterial product provided by Albaugh, LLC. All the biological products performed statistically better than the fungicide check in regard to reducing eggs and juveniles, as well as the overall nematode reproduction of *H. glycines*. In many cases, these variants and experimental's performed similar to the nematicide standard, abamectin. None of the biological candidates impacted the host plant development when challenged by *H. glycines, M incognita* and *R. renifomis.*

When comparing the variants of *B. rinojensis* on multiple nematodes, one of the variants was more consistent in regard to efficacy and performed better at lower rates. The *B. rinojensis* variant 2 was more efficacious and was more consistent across application rates and the three nematode speices we studied. *B. rinojensis* variant 1 rates (7, 10 floz/cwt) did not perform as well on Reniform or Root-knot nematodes as variant 2. *B. rinojensis* variant 2 was also not significantly different on nematode management from the abamectin treatment in two trials (Reniform or Root-knot nematodes), while the variant 1 was significantly less efficacious than the abamectin. *B. rinojensis* variant 1 (3, 5 floz/cwt) often failed to differentiate from *B. rinojensis* variant 2 in rates (3, 5 floz/cwt). *B. rinojensis* variants had a significantly less root-knot nematode reproduction value when compared to the fungicide control, except *B. rinojensis* variant 1 at (3 floz/cwt). *B. rinojensis* variant 2, at all rates, was statistically similar to that of the standard abamctin. *B. rinojensis* variant 1 had a significantly higher reproductive factor value to that of abamectin. With the *R. reniform* nematode, *B. rinojensis* variant treatments were statistically similar to the standard of abamectin, except *B. rinojensis* variant 1 at 3 floz rate (Table 3.3). Having a biological product that performs similar to the commercial nematicide standard at lower use rates is preferable in the industry. Since total slurry rates in soybeans are limited, usually less than 7 to 8 floz/cwt for chemicals and water, biological products that perform well at lower use rates (~3 to 4 floz/cwt) are desirable.

Currently, seed treatments that have been marketed to management of *H. glycines* are Avicta® (abamectin, Syngenta), and Clariva® (*Pasteuria nishizawae*, Syngenta), and VOTiVO® (Bacillus firmus, Bayer CropScience) have been shown activity against soybean cyst nematode (*H. glycines).* The possibility of a *Burkholderia sp*. that has been

shown activity against different pathogens as a biocontrol agent (Burkhead, K.D.et al 1994, Janisiewicz, W.J. et al. 1988). Lately, the isolation from the soil of *Burkholderia rinojensis* had been shown activity as the insecticidal properties to the new strain from Japan. All cell broth cultures of *Burkholderia rinojensis,* that have the name strain A396, has shown having toxicity effect on the beet armyworm (*Spodoptera exigua*) Hübner (Lepidoptera: Noctuidae) and also have seen impacted on two- different spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). (Cordova-Kreylos, A.L. et al. 2013). The selected *B. rinojensis* variant 2used in this study will be marketed by Albaugh LLC as BioST Nematicide 100. The active ingredient is heat-killed *B. rinojensis* and spent fermentation broth. There are a number of nematodes listed on the label including *H. glycines, M. incognita* and *R. reniformis* on soybeans. The literature describes the active ingredients as being a collection of enzymes and toxins that have nematocidal properties on the above nematode species via contact and ingestion.

Another objective in this research was to evaluate secondary nematicidal combination which included bacterial metoblite and SAR products candidates that could be used as stand-alone nematicides or in combination with other nematicides (staking modes of action) for improved efficacy. When examination bacterial metabolite and SAR seed treatment, none of the bacterial metabolite and SAR products screened had an impact on plant development in the greenhouse screening including, soybean weight of plant, height of plant, number of nodes, number of pods, or weight of roots in soils infested with *H. glycines, M. incognita or R. reniformis when* compared with the fungicide only and untreated seeds control. The nematode results indicated that all biological seed treatments were statistically significant in their ability to reduce the eggs

and cyst of *H. glycines* (Table 3.6), reduce eggs and juveniles of *M. incognita* (Table 3. 7), and vermiform stages of *R. reniformis* (Table 3.8), compared with the untreated check. In most trials, the impact on nematode reproduction reproductive factor values were statistically similar to that of the nematicide standard abamectin reproductive factor value with *H. glycines, R. reniformis,* and *M. incognita*.

Saponin (SAR) and the bacterial metabolites were not statistically different from the fungicide/nematicide product fluopyram (Tables 3.6,3.7 and 3.8) in greenhouse soils infested with *H. glycines* or *M. incognita.* However, the biologicals performed better than fluopyram on *H. glycines* and *R. reniformis*. The SAR and bacterial metabolite was statistically different than the fluopyram on *R. reniformis*. Fluopyram is a fungicide that has been shown to have activity against nematodes and as a dehydrogenase inhibitor of fungi and effect on fungal respiration (Avenot and Michailides, 2010). Early testing has also shown have activity of fluopyram on the plant-parasitic nematodes *H. glycines*. (Zaworski, Edward R. 2014).

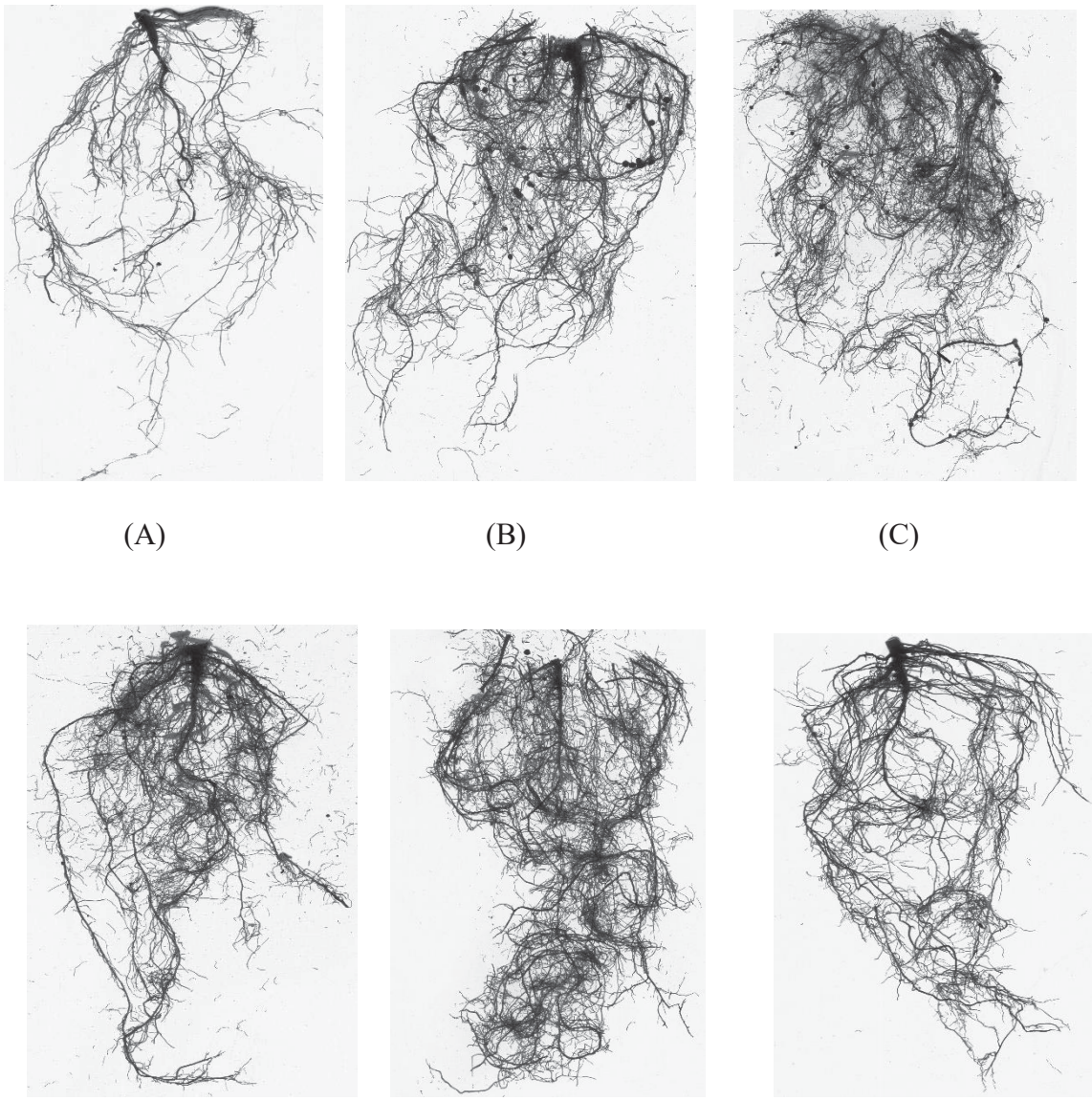
The combinations in these experiments were designed to see the broad range of plant protection. In combination, it is hard to determine if one chemical or biological agent is activity more effective than others treatment or if the products are interacting. However, for this reason we used seed treatment combinations in which these products will likely to be marketed. In soybeans, finding secondary nematicidal products that can be stacked with traditional or other biological offerings (ie *B. rinojensis* variant 2) may improve overall product performance on nematodes. Products with lower use rates would fit better as a companion nematicide (less than 1floz/cwt) than higher application rate products. The application rate of the SAR products tested in 2015 and 2016 were 0.1

and 0.2 floz/cwt, while the bacterial metabolite product application rate was 3 floz/cwt. If these products were combine with other nematicides on the market in soybeans, ILeVo at 2.13 floz/cwt, Avicta at 3 floz/cwt and *B. rinojensis* variant 2 at 3 floz/cwt, the lower use rate products may be a more desirable combination when stacking modes of action against nematodes.

In greenhouse studies that evaluated the combination of *B. rinojensis* variant 2 with saponin and a bacterial metabolite generally increased the efficacy of the seed treatment over the *B. rinojensis.* variant 2 used alone. Both the saponin and the bacterial metabolite numerically reduced the reproductive factor values over the *B. rinojensis* variant 2 (3 fl. oz/cwt) alone in studies with both *H. glycines* and *M. incognita*. No statistical or numerical differences were detected with the *R. reniformis.* These findings were repeated in 2016, in that the combination product (two modes of action) generally reduced reproductive factor values over the *Burkholderia rinojensis* variant 1 and the secondary nematicide compounds (SAR and Bacterial metabolite) applied as a solo nematicide product.

In 2016, (Table 3.15), we applied the methodology of examination root develops with *H. glycines* the WinRHIZO optical scanner (Regent Instruments, Inc.). This was detriment to be an efficient method that allow image analysis resulting from the different treatments and examining the root morphological traits. This technique has provided data easily analyzed by established software protocols for root characteristics and provided accurate screening. Therefore, this method was used for screening of root traits of soybean infected with *H. glycines* and treated with the biological seed treatment option. Plant roots optimize their root architecture to acquire water and essential nutrients from

soil. The number of root tips, forks, and crossings that have been shown playing a significant role on root architecture because they have potential to enhance penetration through soil layers, resulting in a positive effect on getting nutrients. (Figure 3.1). The root tips, forks, and crossings densities differed significantly with biological seed treatments compared to untreated seeds. So, when the plants grew well results from more nutrient from the soil. Also, roots will penetrate deep in the soil and improve plant growth as indicated from uses of the biological seed treatments. The increase in biomass may related to the modifications in phenotype which could include leaf and stem growth and rise the photosynthetic averages (Reddy et al., 1998; Reddy et al., 1995, 2004).



(A) (B) (C)

(D) (E) (F)

Figure 3.1 Root scanning of H. glycines effected roots from plants treated with biological seed treatments infected with soybean cyst nematode.

(A) Control treatment, (B) *Burkholderia renojensis (3 fl. oz/ cwt), (C) Burkholderia renojensis varaint 2, (D) Bacterial metabolite + Burkholderia renojensis, (E) Abamectin, (F) Pasteuria nishizawae.*

Reniform nematode (*Rotylenchulus reniformis*)

No negative effects on roots growth by *R. reniformis* with biological seed treatments compared to the untreated control. (Table 3.17). Roots images from *Burkholderia rinojensis* treatments measured in a grater number of tips, number of forks, and number of crossings compared to control treatment (Figure 3.2). On increase in the number of root tips leads in resulted to cotton growth. (Brand, et al. 2016). Root length, number of froks, and number of crossing are considered the best measurement to describe the multiple stree situations. (Brand, et al. 2016).

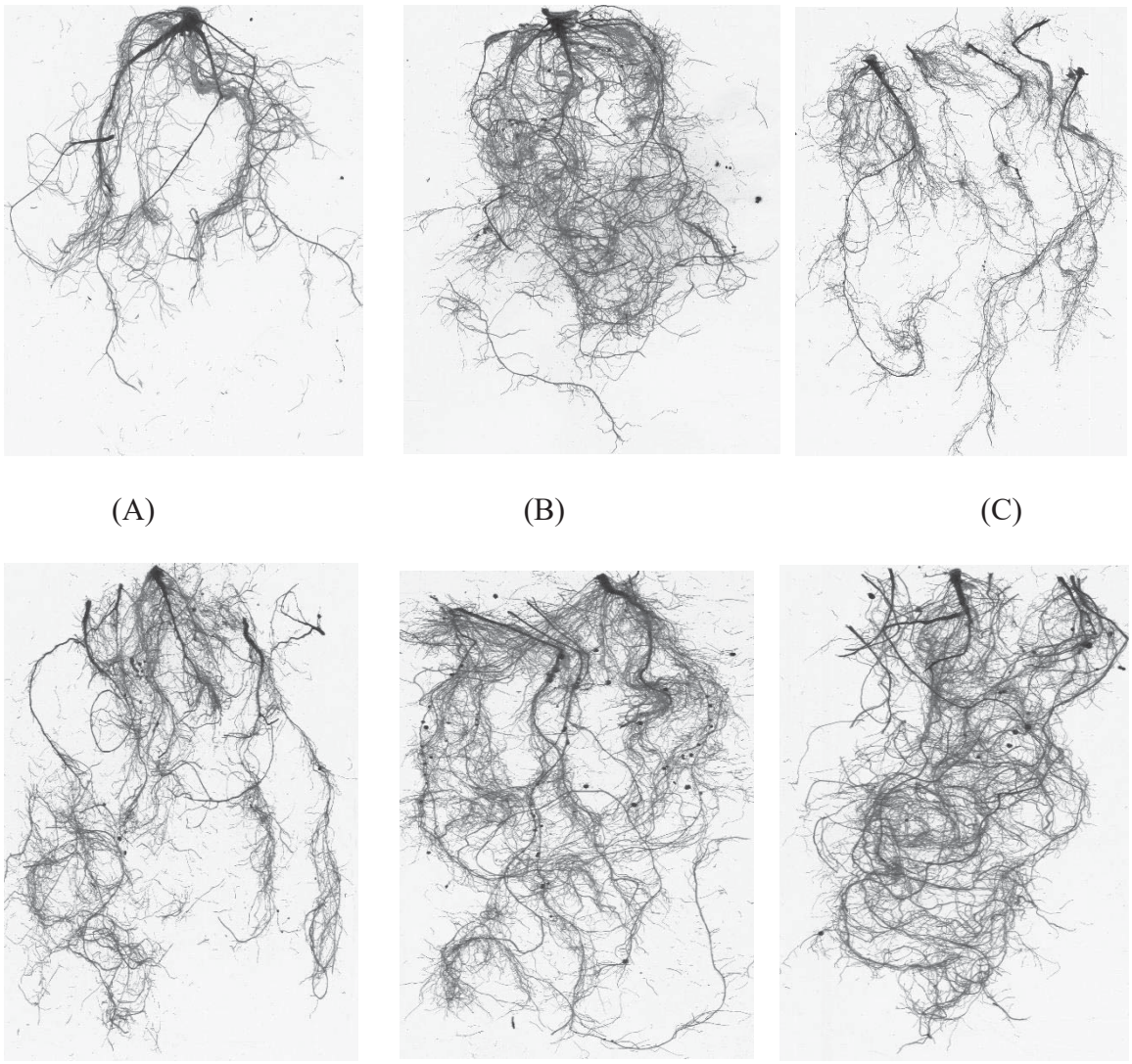


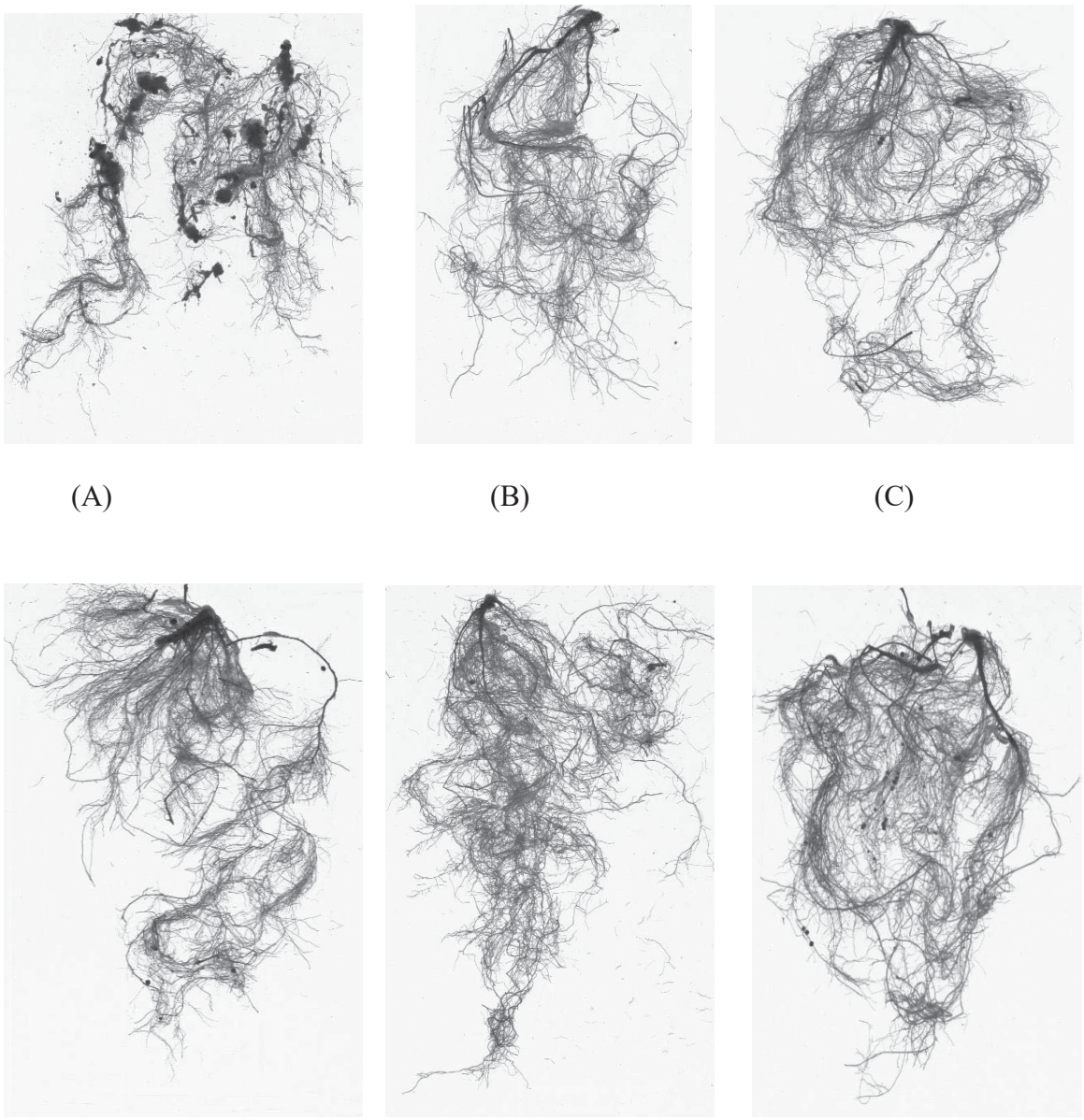
Figure 3.2 Root scanning of R. reniformis effected roots from plants treated with biological seed treatments infected with reniform nematode.

(A) Control treatment*, (B) Burkholderia renojensis* (2 fl. oz/ cwt), *(C) Burkholderia renojensis* (3 fl. oz/ cwt)*, (D)* Saponin + *Burkholdera rinojensis., (E)* Saponin alone, *(F)* Abamectin alone.

Root-knot nematode (*Meloidogyne incognita*).

M. incognita produced no negative effect on roots from plants with biological seed treatments compared to control treatments on roots growth (Table 3.19). The

number of root tips, forks, and crossings play an essential role on the root architecture for soil penetration and result in improved plant growth. Root tips, forks, and crossings densities were significantly improved with biological seed treatments compared to untreated seeds (Figure 3.3). According to Wijewardana, Chathurika., et al. (2015), high number of roots froks and crossings lead to improved roots growth system during uptake nutrient potential and water. Also, high numbers of tips have been shown to help corn plants to grow well and tolerate to unfavorite inveronmental casaes; in addition, that help plants to take water and essential nutrient when the roots be deeper. Wijewardana, Chathurika., et al. (2015). The long of roots have been resulted to extract the essential nutrients of the bed out soil profile. Barber., (1995).



(A) (B) (C)

(D) (E) (F)

Figure 3.3 Root scanning of *M. incognita* effected roots from plants treated with biological seed treatments infected with Root-knot nematode.

(A) Control treatment, (B) Burkholderia renojensis (5 fl. oz/cwt), (C) Saponin alone, (D) Abamectin alone, (E) ALB-EXP Bacteria 1, (F) Bacillus sp. alone.

Conclusions

Burkholderia rinojensis was identified of the biological candidate for management of nematodes on soybean. Two variants of *B. rinojensis* reduced the life stage development of *H. glycines*, *R. reniformis,* and *M. incognita* on soybean. None of the candidates impacted host plant growth development when infected with *H. glycines, M incognita* and *R. renifomis. B. rinojensis* variant 2 was the most overall consistent product in reducing the number of eggs and juveniles of all nematodes *B. rinojensis* variant 2 was also effect at a low use rate of 3 floz/cwt. *B. rinojensis* variant 2 was more efficacious at low rate (3 floz/cwt) compared with *B. rinojensis* variant 1 on most nematodes. The bacterial metabolite and SAR- Saponin treatments were used as secondary nematicidal candidates that may be used as stand-alone nematicide or in combination with other nematicides (staking modes of action) for improved efficacy. Both products reduced nematode reproduction and had no negative effect on plant growth. Saponin was effective at a lower rate compared to the bacterial metabolite. Combinations of *B. rinojensis* variant 2 with saponin and a bacterial metabolite generally increased efficacy over the *B. rinojensis* variant 2 alone. Future research will focus on the stacking of different biological modes of action, like SAR- Saponin, to enhance nematicidal activity.

Table 3.1 Burkholderia renojensis variant and seed application rates for *H. glycines, M. incognita* and *R. reniformis* management

Treatments	Product	Description
1	Fungicide check (Control)	Control
2	*Burkholderia var* 1 at 3 floz/cwt	Heat killed *Burkholderia spp* – production variant 1
3	*Burkholderia var* 1 at 5 floz/cwt	Heat killed *Burkholderia spp* – production variant 1
4	*Burkholderia var 1* at 7floz/cwt	Heat killed *Burkholderia spp* – production variant 1
5	*Burkholderia var* 1 at 10 floz/cwt	Heat killed *Burkholderia spp* – production variant 1
6	*Burkholderia var* 2 at 3 floz/cwt	Heat killed *Burkholderia spp* – production variant 2
7	*Burkholderia var* 2 at 5 floz/cwt	Heat killed *Burkholderia spp* – production variant 2
8	*Burkholderia var* 2 at 7floz/cwt	Heat killed *Burkholderia spp* – production variant 2
9	*Burkholderia var* 2 at 10 floz/cwt	Heat killed *Burkholderia spp* – production variant 2
10	Abamectin at 3 floz/cwt	Seed Treatment Nematicide Standard

All the treatments treated with fungicide as base treatment by Albuagh, LLC.

Table 3.2 Effect of *Burkholderia renojensis* seed treatments on soybean plant development and nematode life stages *Heterodera glycines*- 2015.

Plant Development						Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes/plant	Pods	Root Weight. /g	Cyst	Eggs	Juveniles/500cm3 soil	Reproductive Factors
Control	10.7 d	33.1 b	9.6ba	8.6bc	7.8bc	214.52 a	21630 a	8926 a	12.30 a
Burkholderia var 1, 3 fl. oz/cwt	19.3 bc	40.8 a	11.8ba	10bac	10.9bac	77.22cb	3605 b	2317.5cb	2.39 b
Burkholderia var 1, 5 fl. oz/cwt	19.1bc	40.2ba	10.8ba	8.4bc	9.5bc	75.074cb	4120b	3862.5cb	3.22 b
Burkholderia var 1, 7 fl. oz/cwt	22 bac	44.2 a	12.4 a	10.8bca	10.3ba	64.35cb	4978.2b	4120 b	3.66 b
Burkholderia var 1, 10 fl. oz/cwt	14.1 dc	38.4ba	8.6b	6.6 c	6.4 c	120.12b	3948.2b	2317.5cb	2.55b
Burkholderia var 2, 3 fl. oz/cwt	19.2bc	39a	9.8 ba	8.2 bc	6.8bc	111.42cb	4635b	3862.5cb	3.44 b
Burkholderia var 2, 5 fl. oz/cwt	27ba	44.9a	12.6a	14.8 a	14.6a	53.624c	3733.6b	2060 cb	2.33 b
Burkholderia var 2, 7 fl. oz/cwt	22.7 ba	44.3 a	10.8ba	12.2bac	11.6 ba	71.5 cb	4806.6b	2057.5cb	2.77 b
Burkholderia var 2, 10 fl. oz/cwt	18.8bdc	41.8 a	10.6ba	10.2bac	11.4 ba	96.524cb	5407.4b	3347.5 cb	3.54 b
Abamectin at 3 fl. oz/cwt	30.2 a	43.2 a	11 ba	14 ba	14.7 a	68.64cb	3604.8b	1802.5 c	2.19 b
P-Value	0.0018	0.0816	0.3245	0.1542	0.0127	0.0001	0.0010	0.0001	0.0001
L.S.D 0.05	8.3703	7.4517	3.2789	5.9873	4.9658	58.766	7795.5	2102.8	3.0525

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500.

Table 3.3 Effect of *Burkholderia renojensis* seed treatments on soybean plant development and (*Rotylenchulus reniformis*) life stage development- 2015.

	Plant Development					Nematode Life Stages Development		
Treatments	Plant Weight / g	Plant Height /cm3	Nodes/ plant	Pods	Root Weight. /g	Juveniles/ 500cm3 soil	Eggs	Reproductive Factors
Control	14.3ba	42.4a	11.4bc	11.8ba	5.3bc	20703a	2358a	17.71a
Burkholderia var 1, 3 fl. oz/cwt	16.1b	47.2a	11.8bac	10.6ba	7.9bc	7107b	6180b	5.31b
Burkholderia var 1, 5 fl. oz/cwt	21.8ba	48.4a	14.8bac	13.8ba	10.8bac	4017b	5665b	3.87cb
Burkholderia var 1, 7 fl. oz/cwt	21.5ba	49.6a	13ba	12.6ba	12.2c	3397b	4635b	3.21cb
Burkholderia var 1, 10 fl. oz/cwt	17.8ba	45.2a	12.6ba	9.4b	6.9bc	4015b	5150b	3.66cb
Burkholderia var 2, 3 fl. oz/cwt	18.8ba	44.2a	10.8c	10.8ba	10.9bc	6178b	4506b	4.27cb
Burkholderia var 2, 5 fl. oz/cwt	16.8b	43.2a	13.8bac	9.4b	8.3bc	3727b	3605b	2.93cb
Burkholderia var 2, 7 fl. oz/cwt	26.1 ba	44.4a	15.2ba	14ba	15a	4325b	4120 b	3.37cb
Burkholderia var 2, 10 fl. oz/cwt	19.9ba	42.8a	12.4bac	10.4ba	11.6bac	3706b	3708b	2.96cb
Abamectin, 3 fl. oz/cwt	27.6a	43a	15.4a	16.6a	15.8a	2060b	3090b	2.06c
P-Value	0.381	0.455	0.215	0.527	0.007	0.0001	0001	0.0001
L.S.D 0.05	10.649	7.216	3.9023	6.9414	5.1408	5512.6	5438.7	2.7778

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = Reniform Eggs+ Reniform Juveniles at 60 days/ 2500 juveniles.

Table 3.4 Effect of *Burkholderia renojensis* seed treatments on soybean plant development and *Meloidogyne incognita* life stage development- 2015.

	Plant Development					Nematode Life Stages Development			
Treatments	Plant Weigh t / g	Plant Height /cm3	Nodes/ plant	Pods	Root Weigh t. /g	Juvenil es/500 cm3 soil	Eggs	Galls	Reprodu ctive Factors
Control	16.6b	44.4b	7.6b	11.2b a	11.5b	5144a	24205a	3.2a	11.74a
Burkholderia var 1, 3 fl. oz/cwt	27.4a	52.1ba	16.4a	14.4b a	15.9a	2703.6 b	6695cb	1.2b	3.75abd
Burkholderia var 1, 5 fl. oz/cwt	31.5a	53ba	15.6a	16.2b a	14.6ba	1373cb	5665cb	1.4b	2.81cbd
Burkholderia var 1, 7 fl. oz/cwt	27.3a	51.8ba	16.4a	14ba	14.4ba	2060cb	10300cb	0.8b	4.94cb
Burkholderia var 1, 10 fl. oz/cwt	25a	49.9ba	15.8a	15.4b a	14.7ba	1854cb	12875b	0.4b	5.89b
Burkholderia var 2, 3 fl. oz/cwt	29.9a	53.5a	16.4a	17.2a	14ba	1201cb	7081cb	1.2b	3.31cbd
Burkholderia var 2, 5 fl. oz/cwt	27.9a	45.26 ba	17.2a	17.4a	13.8ba	1287cb	5150cb	0.8b	2.57cd
Burkholderia var 2, 7 fl. oz/cwt	27.1a	47.6ba	15a	12.2b a	12.8ba	1888cb	5665cb	0.6b	3.02cbd
Burkholderia var 2, 10 fl. oz/cwt	25a	45.66 ba	15.2 a	9.2b	12.8ba	1673cb	8755cb	0.4b	4.17cbd
Abamectin, 3 fl. oz/cwt	31.7a	45.8ba	17.2a	17a	14.1ba	618c	2575c	0.8b	1.27d
P-value	0.036 0	0.281 4	0.0038	0.233 0	0.402 5	0.0067	0.0004	0.000 7	0.0001
L.S.D 0.05	10.70 8	8.974	4.3685	6.810 8	3.391	2028.7	8227.8	1.136 1	3.2342

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = RK Eggs+ RK Juveniles + RK Galls at 60 days/ 2500 eggs

Table 3.5 Bacterial metabolite and SAR-Saponin used as seed treatments for management of *H. glycines, M. incognita* and *R. reniformis*

Treatments	Product	Description
1	Fungicide check (Control)	Control
2	SAR1 - Saponin at 0.1 floz/cwt	SAR product with saponin
3	SAR1 - Saponin at 0.2 floz/cwt	SAR product with saponin
4	Bacterial Metabolite at 3 floz/cwt	Biostimulant Bacterial Metabolite
5	Abamectin at 3 floz/cwt (0.15 mg ai/seed)	Nematicide standard 1
6	Fluopyram at 2.3 floz/cwt (0.25mg ai/seed)	Nematicide standard 2
7	UTC	Untreated seed – no fungicides

All the treatments treated with fungicide as base treatment by Albuagh, LLC.

Table 3.6 Effect of Bacterial metabolite and SAR -Saponin seed treatments on soybean plant development and *Heterodera glycines* life stage development -2015.

Plant Development						Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes/ plant	Pods	Root Weight. /g	Cyst	Juveniles / 500 cm3 soil	Eggs	Reproductive Factors
Control	11.9ba	28.8a	10.4ba	6.2a	6.5bc	566.28 a	8343ba	56650a	26.22a
Saponin, 0.1fl. oz/cwt	13ba	27.8a	9.2ba	5.2a	11.2a	257.4b	3476dc	1133 b	6.02b
Saponin, 0.2 fl. oz/cwt	18.7ba	32.06a	10.8ba	6.4a	11.9a	154.44 cb	3862dc	8240 b	4.90b
Bacterial Metabolite, 3 fl. oz/cwt	19.4ba	32.26a	12.6a	8.2a	11.4a	145.86 cb	4326dc	5793 b	4.10b
Abamectin, 3 fl. oz/cwt	23a	33.7a	11ba	5.4a	14.2a	60.06c	2163d	3433 b	2.26b
Fluopyram, 2.3 fl. oz/cwt	17.3ba	29.4a	11ba	6a	10.8ba	137.28 cb	6798bc	6695 b	5.45b
Untreated seed	7.3b	26a	8.2b	2.4a	4.4c	600.6a	11742a	45835a	23.27a
P-Value	0.2189	0.5661	0.4437	0.6357	0.0027	0.001	0.0002	0.0001	0.0001
L.S.D 0.05	12.675	8.8442	4.088	5..957	4.6454	181.07	3770.3	23142	9.1142

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs.

Table 3.7 Effect of Bacterial metabolite and SAR -Saponin seed treatments on soybean plant development and *Rotylenchulus reniformis* juveniles and reproductive factor development -2015.

	Plant Development					Nematode Life Stages Development	
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight. /g	Juveniles/ 500 cm3 soil	Reproductive Factors
Control	12.2ba	31.6de	7.6b	8.6a	5.5a	8961ba	3.58ba
Saponin, 0.1fl. oz/cwt	16.5b	35.4dc	9.2bc	5.2ba	6.6ba	3708c	1.48c
Saponin, 0.2 fl. oz/cwt	25.4a	40.8bc	12.8a	9.2a	8.3a	1538.8c	0.61c
Bacterial Metabolite, 3 fl. oz/cwt	19.0b	45.2ba	10.6ba	6.2ba	8ba	3090c	1.23c
Abamectin, 3 fl. oz/cwt	18.4bc	48.2a	9.4bc	5.2ba	7.4a	1854c	0.74c
Fluopyram, 2.3 fl. oz/cwt	14.2c	27.2e	7.6c	5.4b	6.7b	5562ba	2.22ba
Untreated seed	10.3b	37dc	9.6bc	6.6a	5.9ba	10815a	4.32a
P-value	0.017	0.0001	0.0028	0.0526	0.4620	0.0006	0.0006
L.S.D 0.05	6.1447	6.7832	2.4678	4.583	3.0599	4345.9	1.7384

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Reniform Juveniles at 60 days/ 2500 juveniles.

Table 3.8 Effect of Bacterial metabolite and SAR -Saponin seed treatments on soybean plant development and *Meloidogyne incognita* life stage development -2015.

Plant Development						Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods/	Root Weigh t /g	Juvenil es/ 500 cm3 soil	Eggs	Galls/ plant	Reproduc tive Factors
Control	10.7d	39.4ba	10.8ba	5.6a	10.7a	10815a	141110a	3.4a	60.77a
Saponin, 0.1fl. oz/cwt	22.8a	34.8b	9b	4a	12.4a	3090bc	14420bc	2dc	7.00bc
Saponin, 0.2 fl. oz/cwt	22.9a	37.4ba	11.2ba	7.2a	15.5a	3012bc	50470bc	3dac	21.39bc
Bacterial Metabolite, 3 fl. oz/cwt	23a	40.2a	13.4a	7.4a	9.9a	3399bc	13390bc	1.4d	6.71bc
Abamectin, 3 fl. oz/cwt	20.5ba	39.8ba	11.8ba	7.6a	11.3a	1545c	3433.2c	0.2e	1.99c
Fluopyram, 2.3 fl. oz/cwt	18bc	35ba	12.4a	7.8a	15.4a	2060c	7210c	2.4dbc	3.70c
Untreated seed	13.6dc	38.4ba	13.8a	8.2a	8.7a	7416ba	99395a	3.6a	42.72ba
P-value	0.0001	0.2301	0.0891	0.4290	0.2825	0.0035	0.0170	0.001	0.0137
L.S.D 0.05	4.451	5.3416	3.2993	4.2547	6.4405	4329.3	87499	1.1379	36.276

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Juveniles + Galls at 60 days/ 2500 eggs.

Table 3.9 Experimental Bacteria and *Burkholderia renojensis* combinations used as seed treatments for management of *H. glycines, M. incognita* and *R. reniformis*

Treatments	Product	Description
1	Fungicide check (control)	Control
2	ALB EXP Bacteria at 5 oz/cwt	Experimental Bacterial M3
3	ALB EXP Bacteria at 10 oz/cwt	Experimental Bacterial M3
4	ALB EXP Bacteria at 15 oz/cwt	Experimental Bacterial M3
5	*Burkholderia spp*. Var 2 at 5 floz/cwt	Heat killed Burkholderia spp – production variant 2
6	*Burkholderia + Bacterial Metabolite*	Two modes of action Burkhoderia and Bacterial Metabolite
7	*Burkholderia + Saponin (SAR)*	Two modes of action Burkhoderia and Saponin
8	*Burkholderia + Harpin (SAR*	Two modes of action Burkhoderia and Harpin
9	Abamectin	Nematicide standard 1

All the treatments treated with fungicide as base treatment by Albuagh, LLC.

Table 3.10 Effect of Experimental Bacteria and *Burkholderia renojensis* combinations seed treatments on soybean plant development and *Heterodera glycines* life stages development-2015.

Plant Development						Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight /g	Cyst	Juveniles/ 500 cm3 soil	Eggs	Reproductive Factors
Control	12.6ba	26.4ba	14.8a	6.8a	4.6bac	308.8 a	1854a	10300a	4.98a
ALB EXP Bacteria, 5fl. oz/cwt	19.5a	32a	15.6a	9.4a	9.2a	120.1 b	1030bcd	4506.2bc	2.26bc
ALB EXP Bacteria,10 fl. oz/cwt	15.9ba	31.8ba	15.4a	9a	7.3bac	75.07 b	1545ba	3218.6c	1.93c
ALB EXP Bacteria, 15 fl. oz/cwt	18.3bc	31.6ba	13.2ba	5.8a	8.6c	94.38 b	824ecd	3218.6c	1.65c
Burkholderia spp. Var 2 ,5 fl. oz/cwt	13.7bac	33.4a	13.8ba	7a	6.4bac	137.2 b	1158.6bc	5150bc	2.57bc
Burkholderia + Bacterial Metabolite	18.9ba	33a	15.2a	7.2a	8.5ba	180ba	1545ba	8368.6ba	4.03ba
Burkholderia + Saponin	13.8bac	30.4ba	13ba	7.6a	6.7bac	77.22 b	686.4ecd	3433c	1.67c
Burkholderia + Harpin	19.7bc	27.8ba	13ba	6.8 a	5.9bc	68.64 b	515ed	3605bc	1.67c
Abamectin	17.4bc	36.3c	13.1ba	8.6a	7.6bc	51.48 b	309e	1957c	0.92c
P-Value	0.0296	0.0141	0.1449	0.4669	0.2375	0.0095	0.0001	0.0257	0.0039
L.S.D 0.05	5.8095	4.1144	3.3639	3.619	3.2744	130.85	604.4	4894.6	1.9668

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs.

Table 3.11 Effect of Experimental Bacteria and *Burkholderia renojensis* combinations seed treatments on soybean plant development and *Rotylenchulus reniformis* life stage and reproductive factors - 2015.

Plant Development						Nematode Life Stages Development		
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight . /g	Juveniles / 500 cm3 soil	Eggs	Reproductive Factors
Control	11.6c	33.4bcd	12b	9.8bac	5.4d	5150a	8755a	5.56a
ALB EXP Bacteria, 5fl. oz/cwt	16bac	30d	14.8ba	7.4bc	7.1bdc	1609.3c	3862.4cb	2.18cd
ALB EXP Bacteria, 10 fl. oz/cwt	14.1bc	31.4cd	16.3ba	7c	7.6bdac	4183.5ba	4506.2cb	3.47b
ALB EXP Bacteria, 15 fl. oz/cwt	14.9bac	33bcd	14.6ba	8.2bac	8bac	3090bc	3862.4cb	2.78cbd
Burkholderia spp. Var 2 ,5 fl. oz/cwt	17.4ba	35.2bcd	17.8a	11.2ba	9.3ba	2317.5c	4506.2cb	2.72cbd
Burkholderia + Bacterial Metabolite	16.3bac	36bc	15.6a	8.4bac	6.7dc	2575c	3433.2cb	2.40cbd
Burkholderia + Saponin	14.9bac	38.2da	17.2a	9.6bac	7.2bdc	2832.5bc	5150b	3.19cb
Burkholderia + Harpin	20a	42.4a	15.9a	11.4a	6.4dc	1806.6c	3090c	1.95d
Abamectin	18.5ba	37.2ba	17.2a	10.4bac	9.6a	1931.2c	2575c	1.80d
P-Value	0.1060	0.0011	0.0283	0.2145	0.0133	0.0014	0.0001	0.0001
L.S.D 0.05	5.3029	5.2713	3.5232	3.8266	2.2614	1652.9	2213.4	1.1025

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Reniform Eggs+ Reniform Juveniles at 60 days/ 2500 juveniles.

Table 3.12 Effect of Experimental Bacteria and *Burkholderia renojensis* combinations seed treatments on soybean plant development and *Meloidogyne incognita* life stage and reproductive factors -2015.

Plant Development						Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight. /g	Juveniles/ 500 cm3 soil	Eggs	Galls	Reproductive Factors
Control	6.6b	27c	12.2ba	4c	6.3c	12875a	14935a	3.6a	11.12b
ALB EXP Bacteria, 5fl. oz/cwt	16.9a	31.8bac	14.6ba	11a	7.1ba	4635b	3862b	1bcd	3.39b
ALB EXP Bacteria, 10 fl. oz/cwt	14.4a	31bc	14ba	8.8ba	9.8a	4640b	4506b	1.8bc	3.65b
ALB EXP Bacteria, 15 fl. oz/cwt	10.2ba	29.4c	11.8b	5bc	7.4bc	5922.5b	5278b	0.8bcd	4.48b
Burkholderia spp. Var 2 ,5 fl. oz/cwt	11.3ba	31.8bac	13ba	6.8ba	9.2ba	3605b	7210b	2.2ba	4.32b
Burkholderia + Bacterial Metabolite	17.6a	37.2a	14.8ba	9ba	6.7ba	2575b	3090b	2b	2.26b
Burkholderia + Saponin	17a	36.4ba	14.4ba	8.8ba	6.5ba	2832.5b	4120b	1.6bcd	2.78b
Burkholderia + Harpin	15.8a	32.2bac	14.6ba	9ba	7.7a	2317.5b	2832b	0.4cd	2.06b
Abamectin	16.6a	28c	15a	11a	6.4ba	3347.5b	3862b	0.2d	2.88b
P-Value	0.0472	0.0065	0.3448	0.228	0.0119	0.0027	0.0113	0.0011	0.0001
L.S.D 0.05	7.4357	5.4319	3.1564	4.3203	2.1262	4824	6242.6	1.4625	3.0314

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = Eggs+ Juveniles + Galls at 60 days/ 2500 eggs.

Table 3.13 Biological compounds and specific rates applied as seed treatments for the management *H. glycines, M. incognita* and *R. reniformis*

Treatments	Product	Description
1	Fungicide check (control)	Control
2	Burkholderia var 2 at 3 floz/cwt	Heat killed Burkholderia Var 2
3	Burkholderia var 2 at 5 floz/cwt	Heat killed Burkholderia Var 2
4	Burkholderia var 2C at 2 floz/cwt	Heat killed Burkholderia Var 2 concentrate
5	Burkholderia var 2C at 4 floz/cwt	Heat killed Burkholderia Var 2 concentrate
6	Burkholderia var 1 at 3 floz/cwt	Heat killed Burkholderia Var 1
7	Burkholderia var 1 at 5 floz/cwt	Heat killed Burkholderia Var 1
8	Burkholderia var 1C at 3 floz/cwt	Heat killed Burkholderia Var 1concentrate
9	Saponin at 0.16 floz/cwt	SAR product – Saponin
10	Bacterial Metabolite at 3 floz/cwt	Biostimulant – Bacterial Metabolite G
11	Burkholderia var 2 + Saponin at 0.16 floz/cwt	Two modes – Burkholderia and Saponin
12	Burkholderia var 2 + Bacterial Metabolite at 3 floz/cwt	Two modes – Burkholderia and Bacterial Metabol.
13	Abamectin	Seed Treatment Nematicide Standard
14	*Pasteuria nishizawae*	Seed Treatment Nematicide Standard
15	*Bacillus firmus*	Seed Treatment Nematicide Standard
16	ALB Experimental Bacteria 3 floz/cwt	Albaugh's Experimental Bacteria M3
17	Untreated Seed	Non-treated soybean seed

All the treatments treated with fungicide as base treatment by Albuagh, LLC.

Table 3.14 Effect of biological seed treatments on soybean plant development and *Heterodera glycines* life stage development- 2016.

Development	Plant					Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight. /g	Cyst	Juveniles/ 500 cm3 soil	Eggs	Reproductive Factors
Control	23.4c	45.4e	17.4c	9.6de	10ed	1441.44a	42230a	52730a	38.56a
*Burkholderia var 2, 3*fl. oz/cwt	41.8ba	64.2ba	31a	16.8bac	18.2bac	274.56cde	10557.5e	14523b	10.14c
Burkholderia var 2, 5 fl. oz/cwt	44.8ba	72.6a	32.2a	19.8ba	16.2bac	139cde	6617.5e	3502cb	4.10de
Burkholderia var 2 C, 2 fl. oz/cwt	49.6a	74.2a	28ba	17.8ba	20.4ba	152.74cde	8240ed	4738cb	5.25dce
Burkholderia var 2C, 4 fl. oz/cwt	35.2bac	67.8ba	26.4bac	15.2bdac	12.6edc	326.1cde	4892.5bc	7725cb	5.17dce
Burkholderia var 1, 3 fl. oz/cwt	37bac	74a	27bac	17bac	14edc	257.4cde	9785bcd	12051cb	8.83dce
Burkholderia var 1, 5 fl. oz/cwt	37bac	70ba	28ba	13.2bdec	16.2bac	386.1cd	9527.5bcd	12978cb	9.15dc
Burkholderia var 1C, 3 fl. oz/cwt	40.8ba	71ba	31.6a	17.6bac	17bac	317.46cde	7210b	7519cb	6.01dce
Saponin, 0.16 fl. oz/cwt	35.2bac	71.6ba	24bac	12.6dec	14.8ebdac	497.64cb	6437.5bcd	9270cb	6.48dce
Bacterial Metabolite, 3 fl. oz/cwt	43.2ba	70ba	29.2ba	15.4bdac	14edc	214.5de	9707.5e	8961cb	7.55dce
Burkholderia var 2 + Saponin	33.6bc	68.6ba	26.2bac	11.8dec	14.8ebdac	111.54e	5665e	6489cb	4.90dce
Burkholderia var 2 + Bacterial Metabolite	35bac	70.2ba	24.8bac	11.6dec	14.4ebdc	145.86de	5407.5e	3811c	3.74de
Abamectin	43.2ba	63.4bac	33a	21.4a	12.6edc	111.54e	3347.5e	3708c	2.86e
Pasteuria nishizawae	36bac	65ba	27.4bac	11.8dec	15.2bdac	145.86de	9707.5e	5047cb	5.96dce
Bacillus firmus	33bc	66.4ba	29ba	15.2bdac	14.2edc	120.12e	7210e	4738cb	4.82dce
ALB Experimental Bacteria	34bc	63.6ba	26.8bac	13.8bdac	13.9edc	265.98cde	5922.5ecd	10609cb	6.71dce
Untreated Seed	25.4c	52.4edc	20.8bc	8.4e	9e	720.6b	32445ba	44805a	31.18b
P-Value	0.057	0.0001	0.3509	0.0192	0.0353	0.0001	0.0001	0.0001	0.0001
L.S.D 0.05	15.026	11.083	10.124	6.685	6.1409	270.42	7584.2	10494	5.9929

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs.

Table 3.15 Effect of biological seed treatments on roots paramters measurement from *Heterodera glycines* infected soybean- 2016.

Treatments	Soybean Roots Parameters						
	Root of length (cm)	Surface area (cm2)	Average root diameter (mm)	Root volume (cm3)	Number of tips	Number forks	Number of crossings
Control	1635.606h	226.6175bac	0.46072bdac	2.5394ef	7086.6ahf	12811.2h	838.4f
*Burkholderia var 2, 3*fl. oz/cwt	3071.855b	434.2695a	0.45454ebdac	4.961ac	14090.8edf	29828.4a	1993.6a
Burkholderia var 2, 5 fl. oz/cwt	3693.864a	490.3604ba	0.42298ebdac	5.1814ba	36472a	32656a	1994.2a
Burkholderia var 2 C, 2 fl. oz/cwt	3009.721cb	462.9451hefdg	0.49094a	5.751a	13186.2egdf	28845.4ba	1865ba
Burkholderia var 2C, 4 fl. oz/cwt	2258.633gfed	318.4506bdc	0.44372ebdac	3.5878ed	8268.2eghf	18381.2gdfceh	1261fdec
Burkholderia var 1, 3 fl. oz/cwt	3099.399b	393.1735hig	0.40144edf	3.9842dc	23455.2cb	23049.2bc	1567.8bdac
Burkholderia var 1, 5 fl. oz/cwt	1788.577ch	264.0259efdc	0.47006bac	3.1074ed	6354.8gh	14818.2gf	988.8fe
Burkholderia var 1C, 3 fl. oz/cwt	2727.811cefh	357.1hefdg	0.42098edf	3.7402d	26576.2b	22322dc	1414.8bdec
Saponin, 0.16 fl. oz/cwt	2170.065gefh	319.0228bdc	0.47452bac	3.7662d	8422.8eghf	18653.2gdfceh	1194.8fdec
Bacterial Metabolite, 3 fl. oz/cwt	2991.537cb	372.661hefig	0.3957ef	3.7082ed	23458.2cb	21200.4dce	1381.2dec
Burkholderia var 2 + Saponin	1933.287gfh	293.9674efdg	0.48498ba	3.599ed	6346.8ah	16456.6gdfeh	1105.8fde
Burkholderia var 2 + Bacterial Metabolite	2952.647cebd	390.8048hi	0.455edf	4.15356ed	22364.2cd	20164.4gdfceh	1508.8fdec
Abamectin	2705.273gh	315.7823hefdg	0.05556ebdac	4.0648ed	14391.6eghf	18440.8gfeh	1464.2fdec
Pasteuria nishizawae	2784.35cbd	322.2248hegdg	0.36956f	2.9818ed	25216.4cb	19150gdfce	1449.8bdac
Bacillus firmus	2021.949gfh	281.1155hfi	0.4434ebdac	3.124ed	7661.4eghf	15989gfeh	1147.2fdec
ALB Experimental Bacteria	2458.538cebd	304.1508hefig	0.39434ef	2.9976ed	14284.6edf	15846.8gfeh	1242.6fdec
Untreated Seed	938.2703i	144.7337j	0.49188a	1.7984f	4006.6h	6425.8i	350.4g
P-Value	0.0001	0.00013	0.0009	0.00011	0.00016	0.00015	0.00013
L.S.D 0.05	580.85	81.169	0.0629	1.1798	7209.6	6275.1	470.95

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.

Table 3.16 Effect of biological seed treatments on soybean plant development and *Rotylenchulus reniformis* life stage- 2016.

	Plant Development					Nematode Life Stages Development		
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight. /g	Juveniles/ 500 cm3 soil	Eggs	Reproductive Factors
Control	19.3bdc	49.8e	15.8hg	7.2ef	10.3bdac	15707.5a	20600a	14.52a
*Burkholderia var 2, 3*fl. oz/cwt	35.5ba	58.6ebdac	25.6ba	14.2bac	14.3a	2317.5c	2369b	1.87e
Burkholderia var 2, 5 fl. oz/cwt	28.7bdac	57ebdac	23.4ebda	12.4ebdac	12.5bac	3605c	2060b	2.26ed
Burkholderia var 2 C, 2 fl. oz/cwt	27.4bdac	60ebdac	20.2ebdhgc	10.4ebdf	10.9bdac	2832.5c	2575b	2.16ed
Burkholderia var 2C, 4 fl. oz/cwt	29.7bdac	63ebdac	20.6ebdhgc	11.2ebd	11.9bdac	4120c	3708b	3.13cebd
Burkholderia var 1, 3 fl. oz/cwt	28.4bdac	65.4ba	19ebdhgc	16.8edf	12.1bdac	4314c	4532b	3.53cebd
Burkholderia var 1, 5 fl. oz/cwt	29.3bdac	63.4bac	22.4ebda	11.2ebd	10.5bdac	3090c	4738b	3.13cebd
Burkholderia var 1C, 3 fl. oz/cwt	30.3bdac	64.2bac	21.6ebd	12.2ebdac	11.7bdac	4635c	5871b	4.20cebd
Saponin, 0.16 fl. oz/cwt	26bdac	57.8ebdac	18edhg	5f	10.5bdac	5147.5c	6798b	4.77cb
Bacterial Metabolite, 3 fl. oz/cwt	29.8bdac	65.6ba	19.8ebdhgc	7.6edf	10.3bdac	3954c	3296b	2.9ced
Burkholderia var 2 + Saponin	36.6a	65.8ba	24.2bac	17.2ebd	13.5ba	4377.5c	4223 b	3.44cebd
Burkholderia var 2 + Bacterial Metabolite	22dc	51.4ed	15.4h	14.8f	8.9bdc	5922.5cb	7622b	5.41b
Abamectin	31.9dac	61ebdac	24bdac	15.4ba	15.9bac	3347.5c	1957b	2.12ed
Pasteuria nishizawae	24.7bdac	65.6ba	16.4hg	9.2edf	9.7bdac	4120b	3399b	3.007ced
Bacillus firmus	27.4bdac	64.4bac	20ebdhgc	9.6edf	10.5bdac	4892.5c	3502b	3.35cebd
ALB Experimental Bacteria	29.2bdac	59.4ebdac	21.8ebdhgc	13.2bdac	11.7bdac	4732.5c	3914a	3.45cebd
Untreated Seed	18.4d	53edc	16.8hg	7.2ef	7.3d	19570a	19364a	15.57a
P-Value	0.490	0.0390	0.0023	0.0018	0.561	0.0001	0.0001	0.0001
L.S.D 0.05	12.898	11.857	6.0949	5.7423	5.0945	4347.4	4668	2.3009

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = Reniform Eggs+ Reniform Juveniles at 60 days/ 2500 juveniles.

Table 3.17 Effect of biological seed treatments on roots parameter measurments from with *Rotylenchulus reniformis* infected soybean - 2016.

Treatments	Soybean Roots Parameters						
	Root of length(cm)	Surface area (cm2)	Average root diameter (mm)	Root volume (cm3)	Number of tips	Number forks	Number of crossings
Control	1454.25ed	184.6593bdac	0.37826pbdgcf	1.646e	6530.4edc	8363.4ebdac	651ebdcf
*Burkholderia var 2, 3*fl. oz/cwt	2506.101a	317.3799a	0.40172edgf	3.1744ebdac	7825.6bc	16537.6bdac	1653.2a
Burkholderia var 2, 5 fl. oz/cwt	2363.422a	307.1432a	0.42186edgcf	3.3022bdac	7794.6bc	16329bdac	1321bdac
Burkholderia var 2 C, 2 fl. oz/cwt	2283.98ba	315.194a	0.4148edgf	3.9624a	6860edc	20552bdac	1616.2a
Burkholderia var 2C, 4 fl. oz/cwt	2428.527a	254.0805bdac	0.45398bdac	3.6676ba	7668.6bedc	14730.8a	1138ebdacf
Burkholderia var 1, 3 fl. oz/cwt	1916.7ebdac	272.3242bdac	0.41292edgf	2.6398ebdcf	8067.6bac	14921ebdac	1200.8ebdac
Burkholderia var 1, 5 fl. oz/cwt	2304.12a	279.5466bdac	0.38944g	2.7352ebdcf	9141.4ba	16872.6bdac	1400.6bac
Burkholderia var 1C, 3 fl. oz/cwt	2464.611a	290.4015bac	0.3947egf	3.0096ebdac	8022.2bac	18918.2bdac	1719.6a
Saponin, 0.16 fl. oz/cwt	2261.22ba	319.3368a	0.40112edgf	2.8714ebdac	7042edc	17288.6ba	1579.6a
Bacterial Metabolite, 3 fl. oz/cwt	2142.872bac	286.5992bac	0.40662edgf	2.966ebdac	7784bdc	16404.4bac	1342.6bdac
Burkholderia var 2 + Saponin	2421.159a	301.922ba	0.43804ebdgcf	3.6518bac	7758.6bdc	20459.4bdac	1607.4a
Burkholderia var 2 + Bacterial Metabolite	1974.39e	265.7934d	0.49894ebdac	2.5954f	7646.2g	12820.8a	1286.6f
Abamectin	2136.38ed	265.3207bdc	0.51426ba	3.55824ebdacf	8162.8edc	15827e	1352.8ef
Pasteuria nishizawae	1308.184e	199.9166bdc	0.44352ebdac	2.0312e	4205.2fg	10105.8ed	766.2edf
Bacillus firmus	1649.192ebdc	230.1575bdac	0.4227ebdgcf	2.4596edcf	5775.6fed	12563.4ebdc	905.4eddcf
ALB Experimental Bacteria	2086.012bdac	247.4985bdac	0.44786ebdac	3.2862bdac	6110.4fedc	17783.4bac	1461.2ba
Untreated Seed	1360.568e	187.0722d	0.47502bac	2.4612ebdcf	4311.6fg	11842.8fbdc	776.2ebdf
P-Value	0.001	0.01570	0.0021	0.0223	0.0001	0.0272	0.0006
L.S.D 0.05	642.96	106.72	0.0534	1.2078	2008.6	7105.2	611.23

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.

Table 3.18 Effect of biological seed treatments on soybean plant development and *Meloidogyne incognita* life stage development- 2016.

Plant Development						Nematode Life Stages Development			
Treatments	Plant Weight / g	Plant Height /cm3	Nodes	Pods	Root Weight. /g	Juveniles/ 500 cm3 soil	Eggs	Galls	Reproductive Factors
Control	10.4f	45e	16.2dc	3f	9.6b	17767.5a	21630a	3.8a	15.76a
*Burkholderia var 2, 3*fl. oz/cwt	16.8edf	49e	18.8bac	6.2edf	8.6b	2060b	2163cb	2bcd	1.69c
Burkholderia var 2, 5 fl. oz/cwt	15.6edf	46.2e	12d	2.6f	8.6b	4377.5b	7416cb	1.6ecd	4.71c
Burkholderia var 2 C, 2 fl. oz/cwt	19edbac	52.4ebd	19.2bac	5.4edf	7.2b	3090b	5253cbe	0.8ed	3.33c
Burkholderia var 2C, 4 fl. oz/cwt	23.4bdac	60.2bac	21bac	9.8bac	10.8ba	2832.5b	2472f	1.4ecd	2.12c
Burkholderia var 1, 3 fl. oz/cwt	21.4edbac	60.4bac	18.6bac	6.8ebd	7.6b	3605b	3399fe	1.2ecd	2.80c
Burkholderia var 1, 5 fl. oz/cwt	26.4a	61bac	18.2bdac	8.4bdac	11ba	3347.5b	2589.8fe	0.8ed	2.37c
Burkholderia var 1C, 3 fl. oz/cwt	19.2edbac	62.6ba	17.8bdac	8bdac	9.8b	2317.5b	3090fde	1.8becd	2.16c
Saponin, 0.16 fl. oz/cwt	26.6a	60bac	23.6a	9.8bac	9.4b	2575b	3708fda	1.4ecd	2.51c
Bacterial Metabolite, 3 fl. oz/cwt	23.8bac	63.6a	20.2bac	9.8bac	11ba	3605b	2472fe	1.4ecd	2.43c
Burkholderia var 2 + Saponin	23bdac	61bac	19bac	7.6ebdac	11ba	4377.5b	4326fde	2.2bc	3.48c
Burkholderia var 2 + Bacterial Metabolite	25.4ba	62.2ba	18.2bdac	9.2bac	11ba	2832.5b	5871cd	1.8becd	3.48c
Abamectin	24.8ba	60.4bac	16.8bdc	6.8ebd	12ba	2060.2b	2472fe	1.4ecd	1.81c
Pasteuria nishizawae	18.4ebd	53ebdac	17bdc	8.4bdac	18.4a	3862.5b	4944fde	1ecd	3.52c
Bacillus firmus	24.6bac	59.8bdac	21bac	10.8a	10.2ba	3347.5b	4635fe	0.6e	3.19c
ALB Experimental Bacteria	25.2ef	61.4bac	24a	7.4ebdac	9b	4120b	4017fde	0.8ed	3.25c
Untreated Seed	14.4f	51ed	22.2bac	4.2ef	13.4ba	12102.5a	9888a	3bf	8.79b
P-Value	0.0026	0.0009	0.0942	0.0001	0.8452	0.0178	0.0001	0.0001	0.0001
L.S.D 0.05	7.9063	10.705	6.5133	3.637	8.3379	7933.9	3069	1.2396	3.1994

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Juveniles + Galls at 60 days/ 2500 eggs.

Table 3.19 Effect of biological seed treatments on roots parameter measurments from *Meloidogyne incognita* infected soybean- 2016.

Treatments	Soybean Roots Parameters						
	Root of length (cm)	Surface area (cm2)	Average root diameter (mm)	Root volume (cm3)	Number of tips	Number forks	Number of crossings
Control	826.1493g	162.8655ed	0.3966bac	2.4142bc	2257.2fed	5289.8e	759.6ed
*Burkholderia var 2, 3*fl. oz/cwt	1251.067egf	189.0021edc	0.48112bc	2.2924bc	3064.8f	13754.6bdac	780.6ed
Burkholderia var 2, 5 fl. oz/cwt	2067.928ba	252.8823ebdac	0.4574bdc	2.8938bc	4302.4fcebd	17097.8bac	1247.6ebdac
Burkholderia var 2 C, 2 fl. oz/cwt	1869.527ebdacf	299.5171abc	0.49942bac	3.8526ba	5395.4cbd	17036.4bac	1204.2ebdac
Burkholderia var 2C, 4 fl. oz/cwt	1481.051ebdgcf	242.3354ebdac	0.51876ba	3.1598bac	3340.2fe	14018.2bdac	959.4ebdac
Burkholderia var 1, 3 fl. oz/cwt	1307.958edgf	196.2389ebdc	0.4703bc	2.3524bc	4029.4fced	10457.4ebdc	763.8ed
Burkholderia var 1, 5 fl. oz/cwt	1538.749ebdgcf	248.9445ebdac	0.51798ba	3.2126bac	3702fed	14124.2bdac	971ebdac
Burkholderia var 1C, 3 fl. oz/cwt	1416.919ebdgcf	224.6453ebdac	0.51874ba	2.8636bc	4187.2fced	12908.2ebdac	884ebdc
Saponin, 0.16 fl. oz/cwt	1939.227ebdac	285.666bdac	0.46062bdc	3.3582bac	6138b	17193bac	1267.6ebdac
Bacterial Metabolite, 3 fl. oz/cwt	1658.491ebdagcf	245.235ebdac	0.4679bc	2.8886bc	4061.6fced	14272.4bdac	1094.8ebdac
Burkholderia var 2 + Saponin	1324.262edgcf	219.4853ebdac	0.51014bac	2.919bc	4976cebd	12916.4ebdac	789.6edc
Burkholderia var 2 + Bacterial Metabolite	1465.628ebdgcf	234.8945ebdac	0.49932bac	3.014bac	4684.6fcebd	13660.8bdac	905.8ebdc
Abamectin	1646.622ebdagcf	264.7308ebdac	0.50456bac	3.4016bac	4267fcebd	17824.8ba	1091.2ebdac
Pasteuria nishizawae	1189.514gf	160.9095e	0.43118dc	1.7402c	4010.8fed	9239.4edc	695e
Bacillus firmus	1869.412ebdacf	330.7195a	0.56042a	4.6774a	4930.4fcebd	19332.6a	1149.4ebdac
ALB Experimental Bacteria	2102.195ba	318.9493ab	0.4828bac	3.9006ba	5914.6cb	19831.2a	1410.4bac
Untreated Seed	1233.096ebdagcf	234.6142ebdac	0.30016d	3.299bac	3362fe	7848ed	703.6ebdc
P-Value	0.0194	0.1536	0.0389	0.2087	0.0001	0.0095	0.1692
L.S.D 0.05	727.04	124.34	0.0792	1.6749	1890.7	8174.4	624.33

Data are means of the 5 replicates for each treatment at 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.

References

Agrios, G. N. 2005. Plant Pathology. Academic Press. New York, 1969, 1-629.

Askary, T. H. 2015. Limitation, research needs, and future prospects. P. 446 - 454. In Biocontrol agents of phytonematodes. Eds. Askary, T. H., and Martinelli, P. R. P. CAB International. Wallingford, OX, UK.

Ayoub, S. M. 1980. Plant nematology-an agricultural training aid. Pp. 81, Sacramento, CA, USA, Nema Aid Publication.

Avenot, H. F. and Michailides, T. J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Protection 29:643-651.

Barker, K. F. 1987. Evolving concepts of biological control of plant pathogens. Annual Review of Phytopathology 25:67-85.

Burkett-Cadena, M., Kokalis-Burelle, N., Lawrence, K. S., Van Santen, E., and Kloepper, J. W. 2008. Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biological Control. 47:55 - 59.

Barber, S.A. 1995. Soil nutrient bioavailability: A mechanistic approach. 2nd ed. John Wiley & Sons, New York.

Brand, David., Chathurika, Wijewardana., Wei, Gao., and K. Raja. Reddy. 2016. Interactive effects of carbon dioxide, low temperature, and ultraviolet-B radiation on cotton seedling root and shoot morphology and growth Front. Earth Sci. 10(4): 607–620.

Burkhead, K.D.; Schisler, D.A.; Slininger, P.J. 1994. Pyrrolnitrin production by biological-control agent Pseudomonas cepacia B37W in culture and in colonized wounds of potatoes. Appl. Environ. Microbiol., 60, 2031–2039.

Burkholder, W. H. 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology 40**:**115–117.

Caballero-Mellado, J., Onofre-Lemus, J., Estrada-de, los. Santos. P., and Martinez-Aguilar, L. 2007. The tomato rhizosphere, an environment rich in nitrogen-fixing *Burkholderia* species with capabilities of interest for agriculture and bioremediation. Appl. Environ. Microbiol. **73:**5308–5319.

Caballero-Mellado, J., Martínez-Aguilar, L., Paredes-Valdez, G., Estrada-de, los. Santos. P. 2004. *Burkholderia unamae* sp. nov., an N2-fixing rhizospheric and endophytic species. Int. J. Syst. Evol. Microbiol. 54:1165–1172.

Cassida, L., Falkinham, J., and Cain, C. 2004. Non-obligate predatory bacterium *Burkholderia casidae* and uses thereof. US patent US 6,689,357 B2.

Castillo, J. D., Lawrence, K. S., and Kloepper, J. W. 2013. Biocontrol of the reniform nematode by *Bacillus firmus* GB-126 and *Paecilomyces lilacinus* 251 on cotton. Plant Disease. 97:967 - 76.

Cheng, A. C., and Currie, B. J. 2005. Melioidosis: epidemiology, pathophysiology, and management. Clin. Microbiol. Rev. 18:383–416.

Chen, W.M., de Faria, S. M., James, E. K., Elliott, G.N., Lin, K.Y., Chou, J. H., Sheu, S.Y., Cnockaert, M., Sprent, J. I, and Vandamme, P. 2007. *Burkholderia nodosa* sp. nov., isolated from root nodules of the woody Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. Int. J. Syst. Evol. Microbiol. **57:**1055–1059.

Coenye, T., and Vandamme, P**.** 2003. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environ. Microbiol. **5:**719– 729.

Cordova-Kreylos, A.L., Fernandez, L.E., Koivunen, M.; Yang, A., Flor-Weiler, L., and Marrone, P.G. 2013. Isolation and characterization of *Burkholderia rinojensis sp. nov*., a non-Burkholderia cepacian complex soil bacterium with insecticidal and miticidal activities. Appl. Environ. Microbiol. 79, 7669–7678.

Cronin D, Moenne-Loccoz Y, Fenton A, Dunne C, Dowling D.N., & O'gara F .1997a. Role of 2, 4-diacetylphloroglucinol in the interaction of the biocontrol *Pseudomonas* strain F113 with the potato cyst nematode *Globodera rostochiensis*. Appl Environ Microbiol 63: 1357–1361.

Daykin, M. E., and R. S. Hussey. 1985. Staining and histopathological techniques in nematology. Pp. 39-48 in K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advancedtreatise in *Meloidogyne*, Vol. II Methodology, Raleigh: North Carolina State University Graphics.

Debora C. Ladner1, Paul B. Tchounwou1* and Gary W. Lawrence2. 2008. Evaluation of the Effect of Ecologic on Root Knot Nematode, *Meloidogyne incognita*, and Tomato Plant, *Lycopersicon esculenum*. Environmental Toxicology Research Laboratory, College of Science, Engineering and Technology, Jackson State University, Jackson, Mississippi 39217, USA, Department of Entomology and Plant Pathology, Mississippi State University, MS 39762, USA.

Denning, D. W.; Hope, W. W. Trends Microbiol. 2010, 18, 195− 204.

Duddington, C. L. 1951. Two new predacious hyphomycetes. Transactions of the British Mycological Society 34:598-603.

Eilenberg, J., Hajek, A., and Lomer, C. 2001. Suggestions for unifying the terminology in biological control. BioControl. 46:387 - 400.

Ennouri, K., Ben, Khedher., S, Jaoua, S., and Zouari, N. 2013. Correlation between delta-endotoxin a proteolytic activity produced by *Bacillus thuringiensis* var. *kurstaki* growing in an economic production medium. Biocontrol Sci. Technol. [Epub ahead of print.] doi:10.1080/09583157 .2013.791364.

Faske, T. R. and Starr, J. L. 2007. Cotton root protection from plant-parasitic nematodes by abamectin-treated seed. Journal of Nematology 39:27-30.

Gallagher LA, Manoil C. 2001. *Pseudomonas aeruginosa* PAO1 kills Caenorhabditis elegans by cyanide poisoning. J Bacteriol. 2001; 183:6207–6214. doi: 10.1128/JB.183.21.6207-6214.2001. [PMC free article][PubMed] [Cross Ref]

Haas, D. Defago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol. 2005; 3:307–319. doi: 10.1038/nrmicro1129.

Henderson, S.T., and Johnson, T.E. 2001. Daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. Curr Biol. 2001; 11:1975–1980. doi: 10.1016/S0960-9822(01)00594-2. [PubMed] [Cross Ref]

Janisiewicz, W.J.; Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with *Pseudomonas cepacia*. Phytopathology 78, 1697–1700.

Jenkins, W.R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Dis Rep 48:692.

Kerry, B. R. 1987. Biological control. Pp. 233-263 in Brown, R. H., and Kerry, B. R., eds. Principles and practice of nematode control in crops. Academic Press, New York, USA.

Klink. V.P., Alkharouf. N., MacDonald. M. and Matthews. B.F. 2005. Laser capture microdissection (LCM) and analysis of Glycine max (soybean) syncytial cells formed by the soybean cyst nematode *Heterodera glycines*. Plant Mol. Biol. 59: 969-983.

Keren-Zur, M., Antonov, J., Bercovitz, A., Feldman, K., Husid, A., Kenan, G., Markov, N., and Rebhun, M., 2000. *Bacillus firmus* formulations for the safe control of root-knot nematodes. In: Proceedings of the Brighton Crop Protection Conference on Pests and Diseases. Vol. 2A. 47 - 52.

Knudsen, G. R, and Spurr, H. W. 1987. Field persistence and efficacy of 5 bacterial preparations for control of peanut leaf spot. Plant Dis. 71:442–445.

Leahy, J. G., Byrne, A. M., and Olsen, R. H. 1996. Comparison of factors influencing trichloroethylene degradation by toluene-oxidizing bacteria. Appl. Environ. Microbiol. 62:825–833.

Lessie, T. G., Hendrickson, W., Manning, B. D., and Devereux, R. 1996. Genomic complexity and plasticity of *Burkholderia cepacia*. FEMS Microbiol. Lett. 144:117–128.

Mahamuni, Shrikumar. Vijaykumar. 2015. Antifungal Trait of *Burkholderia gladioli* Strain VIMP02 (JQ811557). International Journal of Science and Research (IJSR) ISSN. Volume 4 Issue 8, (Online): 2319-7064.

Mclean, K.S. 1993. The interrelationship of *heterodera glycines* and *fusarium solani* in Sudden Death Syndrome of soybeans. Mississippi State University. July. 1993.

Mclean, K.S, G, W. Lawrence., and K, W. Roy. 1990. Sudden death syndrome: scanning electron and light microscopic observations of soybean roots inoculated with Fusarium solani from A. phytopathology 80: 1043. (Abstract).

Mendoza, A. R., Kiewnick, S., and Sikora, R. A. 2008. In vitro activity of *Bacillus firmus* against the burrowing nematode Radopholus similis, the root-knot nematode *Meloidogyne incognita* and the stem nematode *Ditylenchus dipsaci*. Biocontrol Science and Technology. 18:377 - 389.

Muzhandu, R. T., Chinheya, C. C., Dimbi, S. and Manjeru, P. 2014. Efficacy of abamectin for the control of root knot nematodes in tobacco seedling production in Zimbabwe. African Journal of Agricultural Research 9:144-147.

Nierman, W. C., DeShazer, D., Kim, H.S., Tettelin, H., Nelson, K. E., Feldblyum, T., Ulrich, R.L., Ronning, C. M., Brinkac, L. M., Daugherty, S. C., Davidsen, T. D., Deboy, R.T., Dimitrov, G., Dodson, R. J., Durkin, A. S., Gwinn, M. L., Haft, D. H., Khouri, H., Kolonay, J. F., Madupu, R., Mohammoud, Y., Nelson, W. C., Radune, D., Romero, C. M., Sarria, S., Selengut, J., Shamblin, C., Sullivan, S. A., White, O., Yu, Y., Zafar, N., Zhou, L. W., and Fraser, C. M. 2004. Structural flexibility in the *Burkholderia mallei* genome. Proc. Natl. Acad. Sci. U. S. A. 101:14246–14251.

Parke, J. L., and Gurian-Sherman, D**.** 2001. Diversity of the *Burkholderia cepacian* complex and implications for risk assessment of biological control strains. Annu. Rev. Phytopathol. 39**:**225 258.

Pant, S.R., Matsye, P.D., McNeece, B.T., Sharma, K., Krishnavajhala, A., and Lawrence, G.W. (2014) Syntaxin 31 functions in Glycine max resistance to the plant parasitic nematode *Heterodera glycines* Plant Mol. Biol. 85: 107-121.

Qiao, K., Liu, X., Wang, H. Y., Xia, X. M., Ji, X. X. and Wang, K. Y. 2012. Effect of abamectin on root-knot nematodes and tomato yield. Pest Management Science 68:853-857.

Reddy, A. R., Reddy, K. R., Hodges, H. F. 1998. Interactive effects of elevated carbon dioxide and growth temperature on photosynthesis in cotton leaves. Plant Growth Regul, 26(1): 33–40.

Reddy, K. R., Kakani, V. G., Zhao, D., Koti, S., and Gao, W. 2004. Interactive effects of ultraviolet-B Radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. Photochem Photobiol, 79(5): 416–427.

Reddy, V. R., Reddy, K. R., Hodges, H. F. 1995. Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpira-tion, and water use efficiency. Field Crops Res, 41(1): 13–23.

Schrimsher, D. W., Lawrence, K. S., Castillo, J., Moore, S. R. and Kloepper, J. W. 2011. Effects of *Bacillus firmus* GB-126 on the soybean cyst nematode mobility in vitro. Phytopathology 101: S161.

Sikora, R. A. 1992. Management of the antagonistic potential in agricultural ecosystems for the biological of plant parasitic nematodes. Annual Review of Phytopathology 30:245-270.

Stirling, G. R. 2014. Biological control of plant parasitic nematodes, 2nd Edition. Wallingford. CAB International. Wallingford, OX, UK.

Stirling, G.R. 1991. Biological control of plant-parasitic nematodes. Wallingford, UK, CAB International. 282 pp.

Van Gundy, S. D. 1985. Biological control of nematodes: status and prospect in agricultural IPM systems. Pp. 467-477 in Biological Control in Agricultural IPM Systems. Academic Press, London.

Vicente, M. F.; Basilio, A.; Cabello, A.; Pelaez, F. Clin. Microbiol. Infect. 2003, 9, 15−32.

Wijewardana, Chathurika; Matthew, Hock., Brien, Henry., and K. Raja, Reddy. 2015. Screening Corn Hybrids for Cold Tolerance using Morphological Traits for Early-Season Seeding. crop science, vol. 55: 851-867.

Zhang, W., and Sulz, M. 2006. Chickweed bioherbicides US patent US 7,141,407 B2.

Zaworski, Edward. R. 2014. "Effects of ILeVO® on soybean sudden death syndrome and soybean cyst nematode" *Graduate Theses and Dissertations.* Paper 14261.

CHAPTER IV

ABILITY OF BIOLOGICAL SEED TREATMENTS TO REDUCE THE SOYBEAN CYST NEMATODE (HETERODERA GLYCINES) AND THE INCIDENCE OF SUDDEN DEATH SYNDROME (*FUSARIUM VIRGULIFORME)*

Abstract

Experiments were conducted in the greenhouse at the R.R. Foil Plant Science Research Center at Mississippi State University to examine the ability of biological seed treatments to reduce the Soybean Cyst Nematode *(Heterodera glycines)* and the incidence of Sudden Death Syndrome (*Fusarium virguliforme)* the causal agent of Sudden Death Syndrome (SDS) on soybean. Treatments included soil inoculated with *H. glycines* alone*, Fusarium virguliforme* alone*, and H. glycines + F. virguliforme* combinations and non-inoculated control. Seed applied products were received from and treated by Albaugh, LLC. Seeds were planted in 500 cm3 of a steam sterilized sand: soil mix (1:1/ V: V) in 10 cm dia clay pots. Seeds were placed into one 2.54 cm depression in each pot with the addition of 2500 eggs of *H. glycines,* and 1 gram of *F. virguliforme* for treatment with *H. glycines* and/or SDS. Treatments also included seeds treated the seed treatment nematicides standards Abamectin, Fluopyram, Clariva, and and fungicide only control. Treatments were arranged as a factorial in a randomized complete block design with five replications. Tests ran for 60 days. Parameter included effects on plant growth, nematode life stage development and the incidence of SDS. No negative

effects on soybeans were recorded from any biological seed treatments. Treatments with *Burkholderia renojensis* variant 2 and combination with Harpin and Saponin (SAR) significantly reduced the number of *H. glycines* cyst, juveniles, and eggs over the control. *Burkholderia renojensis,* SAR products, and *Bacterial metobilate* were statistically similar to the standards abamectin, fluopyram, and Clariva. Foliar disease was more severe in the treatments that included *H. glycines* + *F. virguliforme* than *F. virguliforme* alone.

Introduction

Sudden Death Syndrome (SDS) is a devastating fungal disease of soybeans. It was first observed in 1971 in Arkansas by H.J. Walters, who repored plants with symptoms exhibiting interveinal chlorotic lesions (Roy et al. 1997). In 1982, Hirrel named the disease "Sudden Death Syndrome "(SDS). In 2010, soybean losses were estimated at 4.7 million metric tons in the United States due to this disease (Bradley and Koenning, 2014). The causal agent of SDS is *Fusarium virguliforme* (Aoki et al., 2005) in the United States. However, other *Fusarium* species have been associated with this disease in the South America. Currently, SDS has been identified in most soybean producing states (Tylka and Marett, 2014) including Indiana, Iowa, Tennessee, Arkansas, Kentucky, Illinois, Michigan, Minnesota, Mississippi, Nebraska, South Dakota, Wisconsin, and Missouri (Chilvers and Brown-Rytlewski, 2010; Anderson and Tenuta, 1998; Yang and Rizvi, 1994; Ziems et al., 2006; Kurle et al., 2003; Tande et al., 2014; Roy et al., 1989; Rupe, 1989; Jardine and Rupe, 1993). McLean and Lawrence (1993) established that *F. virguliforme* was colonized associated with the *Heterodera glycines* and was cabable of colonizing the cysts and eggs.

SDS starts with infection of the soybean roots via germinating chlamydospores, which are the overwintering structure. Chlamydospores produce mycelium, which infect the plant roots (McLean and Lawrence, 1995). Plants infected at the time of planting develop the worst foliar symptoms, while older plants are less susceptible to infection (Gongora-Canul and Leandro, 2011). After infection, symptoms develop as discoloration of the roots and blue spore masses can sometimes be seen on the taproot. (Luo et al., 1999; Roy et al., 1997).

Foliar symptoms consist of interveinal chlorotic lesions, which may eventually become necrotic. Foliar symptoms are the result of a toxin (i.e., FvTox1) produced in the roots (Brar et al., 2011; Jin et al., 1996), and moves through the vascular system to the leaves. Environmental factors influence *F. virguliforme* infection and disease development. The optimum temperature for infection is 15-17˚C (Scherm and Yang, 1996; Gongora-Canul and Leandro, 2011); however, not for the development of foliar symptoms which in 22-25˚C (Gongora-Canul and Leandro, 2011; Scherm and Yang, 1996). Most management incorporates host resistance and cultural practices. These include delayed planting, tillage, and crop rotation with non-host plants. Delaying planting reduces the severity of SDS (Wrather et al., 1995; Hershman et al., 1990; De Bruin and Pederson, 2008). However, short-term crop rotations, with corn has been proven ineffective at reducing SDS on soybean (Xing and Westphal, 2009; Westphal and Xing, 2011). Long-term rotations with multiple crops can reduce SDS (Abdelsamad et al. 2012). Sudden Death Syndrome not only causes significant damage by itself, it also interacts with the soybean cyst nematode (*H. glycines*) (McLean and Lawrence, 1995; Xing and Westphal, 2006, Gao et al., 2006; Roy, 1989; Xing and Westphal, 2009). The

presence of *Heterodera glycines* in a field will lead to a greater severity of SDS (McLean and Lawrence, 1995. Lawrence, et al 1988).

Heterodera glycines is involved in a disease complex with SDS (Lawrence, G. W. et al 1988). Until recently, there were no fungicide seed treatments available to manage Sudden Death Syndrome (Weems et al., 2011). Currently, numerous compounds are being examined for efficacy for management of the *Heterodera glycines* including Headsup, Thiabendazole, ILeVo).

Recently, Heads Up has been summarized as biological control agent for SDS on soybean for several years in the United States. In some states, this product has been available in the market as a seed treatment. Heads Up is fungicide having activity to suppress symptoms of SDS and improved the yield for soybean plant. Heads Up is an environmental friendly and low-cost alternative that makes the Heads Up a great product for management of SDS. (Navi and Yang. 2016).

Thiabendazole is fungicide consider as broad- spectrum systemic fungicide that has shown activity against several fungal diseases that belong to Ascomycotina, Deuteromycotina, and Basidiomycotina. This product is related to benzimidazole fungicides as (2-Thiazol-4-yl) benzimidazole. Thiabendazole has shown superior activity against *Fusarium spp.* (L. V. Edgington, et al. 1971; H, J. Robinson. et al. 1964).

Fluopyram is fungicide product have been used against SDS on soybean since 2015 and also has shown some activity for *Heterodera glycines.* The product has been applied as a seed treatment. (Avenot and Michailides. 2010; Zaworki, Edward. R. 2014).

***Burkholderia sp*. as Antifungal agent**

Burkholderia sp. plays a role as a biocontrol agent related to enzymes that are produced by this genus of bacteria. These enzymes are included lipolytic, proteolytic, and hemolytic which have activity as toxin or antibiotics. (Vial, L., et al. 2007). Some strains of *Burkholderia species* have been produced some of antifungal products, that can be used as antibiotics for pathogens as management. (Chiarini, A. et al. 2006). The strain of *Burkholderia contaminans* has been shown activity as antifungal when compared with wild type strain. (Gu, Ganyu., et al. 2009). The biological effect of *Burkholderia sp.* has been activity fungi; therefore, *Burkholderia sp.* has continued to develop as an antifungal factor since 1996. (Casida, L.E., et al. 1993; Gross, H., et al. 2009). According to Wang, X. Q., et al (2015) strains of *Burkholderia pyrrocinia* have been identified from rhizosphere of the tobacco and has shown significant effects as antifungal activities plant and animal pathogens. The compounds produced antibiotics by *Burkholderia pyrrocinia* and secondary metabolites. *Burkholderia sp.* has been shown activity in the atmospheric nitrogen fixation, has potential uses as biocontrol, and also stimulus plant growth through antibiotic and secondary metabolites. (Caballero-Mellado, et al. 2004; Leathy, et al. 1996; Zuniga, et al. 2013). Some species of *Burkholderia* have shown significant management effects for seedling, damping off, on cotton caused by *Rhizoctonia solani*. (Yu, et al. 2007). *Burkholderia renojensis* has been described as a biocontrol agent against mites and other insect pests. (Cordova-Kreylos, et al. 2013). These management presented provides for using the potential *Burkholderia renojensis* as a possible biocontrol product for Sudden Death Syndrome and soybean cyst nematode management.

The objective of this study is to determine the efficacy of selected biological experimental compounds, including *Burkholderia renojensis,* applied as seed treatments for the management of the soybean cyst nematode and the reduction *F. virguliforme* infection on soybean.

Materials and Methods

Isolation and identification of *F. virguliforme*

The isolate of *Fusarium virguliforme* used in this study was isolated from SDS-symptomatic soybean roots from Mississippi fields. The roots were washed in running tap water for 5 minutes, lateral and taproots were cut into 3-5 mm sections with cortical and vascular tissues separated. The sections were surface disinfected for 5 second in 70% ethyl alcohol and 1 minute in 0.1% sodium hypochlorite and then were aseptically placed on potato-dextrose agar (PDA) amended with streptomycin sulfate (100 mg/L). *Fusarium virguliforme* grown on PDA plates for 7-10 days. (Mclean, K. S., and Lawrence, G. W. 1992, 1993). The selected isolate originated from cortical taproot tissue, and produced the characteristic blue pigment with scant aerial mycelium. This isolate of SDS was identified according the morphological characterstics of the organism using specific keys for classification and taxonomy.

Relationship between *F. virguliforme* and Soybean Cyst nematode

H. glycines [NL1-Rhg/HG-type 7/race 3] previously produced in the greenhouse and maintained on Williams 82 ($_{/PI\ 518671)}$ was used as inoculum (Klink et al. 2005; Pant et al. 2014). Light brown to tan cysts were dislodge from the roots of 45 to 50 day old plants with a strong water spray and collect on nested sieves with pore sizes of 20 Um

and 100 Um. Cyst are suspended in water then immediately poured through the 20-pore sieve nested on a 100-Um pore sieve. (Mclean, K. S., et al. 1990). Extracted cyst were counted on graded Petri dishes using a stero-microscope at 40X magnification. (Debora C. Ladner, et al, 2008). Eggs were released from the cysts using a modified seinhorst cyst crusher for 1 minute (Mclean, K. S., et al. 1990). The resultant suspension was passed through a 200-um pore sieve nested on a 500-um pore sieve to remove broken cysts and debris. *Heterodera glycines* second stage juveniles were extracted from soil using gravity screening and centrifugal flotation.

The tests were included (*Burkholderia renojensis* variant 2) with four rates (Table 1), bacterial metabolite and SAR seed treatment study (Table 4), *B. renojensis* combination with the SAR compounds and the bacterial metabolite product (Table 7), and seed Treatment Comprehensive Study (Table 10). *Pasteuria nishizawae* spores (Clariva), *Bacillus firmus* spore suspension (Votivo), and the chemical abamectin (Avicta). All were included as standard treatment seed also include the seed treatment fungicides Thiabendazole 4L ST, Metalaxyl, and Rhizolex. Seeds were treated with the appropriate experimental biological compounds and rates by Albaugh LLC. Biological compounds were examined at various rates and in combination with other nematicidal compounds for their effect on managing the soybean cyst nematode and subsequent reduction of the incidence of *Fusarium virguliforme.* All biological seed treatments were used in a study that included *F. virguliforme* + *Heterodera glycines, Heterodera glycines* alone, *F. virguliforme* alone and an untreated control. Tests were conducted in the greenhouse located on the R.R Plant Foil Science research center at North Farm, Mississippi State University.

All tests are planted into 15 cm dia. clay pots filled with an autoclaved freestone fine sandy loam sand: soil mix (1:1, v/v). Plants are grown first by sowing 2 seeds directly in pots filled with 500 cm3 of the sterilized soil-sand mixture under greenhouse conditions and infested with 2500 eggs of Soybean Cyst and 1 gram of *F. virguliforme* produced on corn culture.

Measurements and Parameters

Plants parameters measured included: fresh top weight, height of plants, number of nodes, number of seed pods, weight of seed pods, and root weights. Foliar SDS disease severity was rated at 60 days using a 0-7 scales, where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death (McLean and Lawrence, 1993). Nematode population development was measured by the number of juveniles/ 500cm3 soil, number of cyst, and number of eggs at 60 days after planting.

Root image acquisition and analysis

Root systems were scanned to acquired images and analyzed for the cumulative root length (RCL), surface area (RSA), average root diameter (RAD), root volume (RV), number of tips (RNT), number of forks (RNF), and number of crossings (RNC) using winRHIZO Pro software (Version 2009c, Regent Instruments, Inc.). Roots were excised and separated from the stems then washed thoroughly avoiding any disturbance. Individual root systems were floated in 5 mm of water in a 0.3 × 0.2 m Plexiglas tray prior to scanning. Roots were then untangled and separated to minimize root overlap. The

tray was placed on a dual Scan optical scanner (Regent Instruments, Inc., Quebec, Canada), linked to a computer. Greyscale root images were acquired with parameters set to ‘‘high’’ accuracy (resolution 800 by 800 dpi).

Statistical analysis

The data for plant and nematode population were analyzed using SAS statistical test system version 9.4 (Cary, NC). Data was subjected to analysis of variance (SAS Institute, 2011) using a factorial arrangement of treatments in randomized complete block design with 5 replications. PROC MIXED and differences in treatment means were separate using Fisher`s Protected Least Significant Difference Test (SAS Institute, 2011).

Results

Identification the isolation of *Fusarium virguliforme*

Fusarium virguliforme isolated from soybean roots that were infected with *F. virguliforme* showing characteristic symptoms of Sudden Death Syndrome. *F. virguliforme* was grown on PDA produced of the character blue-pigmented growth and was identified according to the morphology the organism with using specific key for classification and taxonomy for *F. virguliforme.* Figure (4.1). The culture isolated from the symptoms at plates produced microconidia, macroconidia, chlamydospores, and conidophores all characteristic of *F. virguliforme* Figure (4.2).

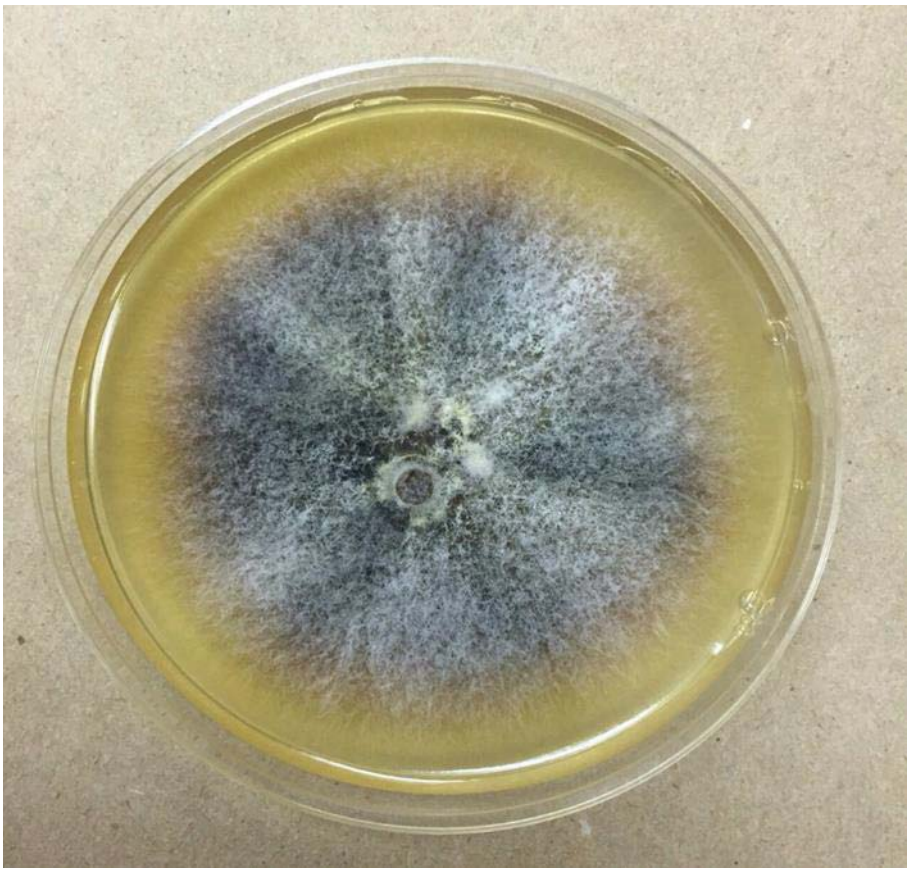


Figure 4.1 Isolates of *Fusarium virguliforme.*

Cultured from the soybean roots infested with *Fusarium virguliforme* and *Heterodera glycines.* (Photos by Weasam Aljaafri).

A *B* *C*

Figure 4.2 Characteristics growth structures of *F. Virguliforme* identified from infected soybean plants under microscope.

A-Conidiophore of *F. Virguliforme* (600X), *B*-Chlamedospores of *F. Virguliforme* (600X), *C*-Microconidia and Macroconidia of *F. Virguliforme.* (600X). (Photos by Weasam Aljaafri).

***Burkholderia renojensis* inoculum rates**

Four rates of *Burkholderia renojensis* product were examined for effects on plant growth development and reduce of Sudden Death Syndrome development and *Heterodera glycines.* There were no negative effects any of biological seed treatments on soybean plant development, in soil infested with *Heterodera glycines* alone, *Fusarium virguliforme* alone or in the non-treated (no pathogens) control. However, plant was growth development in the *F. virguliforme* + *H. glycines* combination compared to untreated seeds with *B. renojensis varaints* 2. Plant weights were 16.8, 16.9, 22.8 grams in *B. renojensis* varaint 2 (5 floz/cwt) with the untreated control, *H. glycines* alone, and *F. virguliforme* alone respectively compared to 14.8 grams in the *F. virguliforme* + *H. glycines* combination. Although the same treatment was significant with untreated seeds (10.6, 14.3, 12.5, and 6.6 gram) in the untreated control, *H. glycines* alone, and *F. virguliforme* alone, and *F. virguliforme* + *H. glycines* combination respectively. Also, there was no effects on the number of pods and weight of pods by any of biological seed treatments compared to the control when the treatments were included both of *F. virguliforme* + *H. glycines* together resulting in a reduced number of pods and pod weights. (Table 4.2).

The *B. renojensis* varaint 2 significantly reduced the number of cysts, and eggs, and juveniles of *H. glycines* compared to the control (Table 4.3). *B. renojensis* varaint 2 results similar to abamectin. *H. glycines* population numbers were affected by the present of *F. virguliforme* included cyst numbers were 386 and 107 per 500 cm3 soil in the *H. glycines* alone and *F. virguliforme* + *H. glycines* combination treatments respectively in the *B. renojensis* variant 2 (5 floz/cwt) treatments compared to 823, and 429 in *H.*

glycines alone and *F. virguliforme* + *H. glycines* combination treatments respectively in untreated seeds. Also, the same treatment reduced the reproductive factor from 1.01, and 0.81 in the *H. glycines* alone and *F. virguliforme* + *H. glycines* combination treatments to 9.72, and 3.03 with *H. glycines alone and F. virguliforme* + *H. glycines* combination in the untreated seed treatments. (Table 4.3).

Sudden Death Syndrome symptoms developed only in the pots that included the *F. virguliforme* SDS symptoms developed at 60 days after planting. Foliar leaf symptoms were significantly more severe in treatments that included the *F. virguliforme* + *H. glycines* combination compared to *F. virguliforme* alone. Foliar symptoms weredisease effects increased in the treatments that included *F. virguliforme,* the *B. renojensis* varaint 2 (5, and 10 floz/cwt) of 0.8, 3.2, 1, and 3.2 in the treatments *F. virguliforme* alone and *F. virguliforme* + *H. glycines* combination compared to 2.8 and 4.8 in untreated seeds (Figure 4.3). There were no significant differences in SDS symptoms with any of *B. renojensis* variant 2 and the nematicide standard abamectin. All rates of *B. renojensis* variant 2 and the nematicide standard abamectin were significantly different from the fungicide only check and the untreated control seed when inoculated with either the *F. virguliforme* alone or the combination with *H. glycines.*

Bacterial metabolite and SAR seed treatments

Soybean plant growth was not affected by *H. glycines* when using the biological seed treatments compared to the control (Table 4.5). There was a significant effect on number of pods and weight of pods between the untreated control, *H. glycines* alone, *F. virguliforme* alone, and *F. virguliforme* + *H. glycines* combination. Most of the

biological seed treatments in the *F. virguliforme* + *H. glycines* combination no produced effects compared to the control (Table 4.5).

At 60 days, the numbers *H. glycines* were reduced by most of the biological seed treatments compared to the untreated control. In the treatment, bacterial metabolite (3 floz/cwt) cysts were 240.24, and 92.67 in *H. glycines* alone and *F. virguliforme* + *H. glycines* combination respectively compared to 849 and 463.32 cysts in the untreated seeds. Also, the same treatment of bacterial metabolite (3 floz/cwt) reduced the number of eggs and juveniles of *H. glycines* and lower of the reproductive factor 6.17 and 2.32 compared to 23.52 and 6.66 in the control treatment. In addition, all the other treatments, including the two nematicide standards (abamectin and fluopyram) had significantly lower reproductive factor values (6.33, 1.06, 6.65, and 2.12) in the *H. glycines* alone and *F. virguliforme* + *H. glycines* combination respectively compared to the fungicide control and the untreated seeds (Table 4.6).

Symptoms of the Sudden Death Syndrome developed only in pots that included *F. virguliforme alone* and *F. virguliforme* + *H. glycines* combinations. Foliar leaves symptom ratings at 60 days after planting were significantly more severe in the *F. virguliforme* + *H. glycines* combination treatments compared to *F. virguliforme* alone. Most of the treatments significant reduced foliar symptoms with *F. virguliforme alone* compared to untreated seeds (Figure 4.4).

***Burkholderia renojensis* Combinations**

In this test, we were looking at combinations of *B. renojensis* with SAR products and bacterial metabolite and their affects on plant growth and nematode life stage development. Plant growth with the *B. renojensis* combinations had no effects on *H.*

glycines and SDS with the biological seed treatments. The treatment *B. renojensis* + bacterial metabolite had a higher weight and plant height compared to the untreated control. (Table 4.8). Plants growth measurement with the *B. renojensis* combinations had no significantly impacted on *H. glycines* and SDS when using the biological seed treatments on height of plants, number of nodes, number of pods, weight of pods, and weight of roots compared to control treatments (fungicide treatment and untreated seeds) (Table 4.8).

The number of cysts, juveniles, and eggs of *H. glycines* were reduced in most treatments with biological seed treatments in the (an untreated control, *H. glycines* alone, and *F. virguliforme* alone and *F. virguliforme* + *H. glycines* combination) compared to the untreated seed control (Table 4.9). All treatments were significantly different than the fungicide check. All treatments were also statistically similar to the abamectin standard except the *B. renojensis* variant 2 treatment (Table 4.9).

Foliar leaves symptoms were significantly more severe in the pots that included both pathogens *F. virguliforme* + *H. glycines* combination treatments compared to *F. virguliforme alone* treatment. The symptoms of the SDS developed 60 days after planting. Foliar symptoms were lower in *the B. renojensis* varaint 2 (5 floz/cwt) with *F. virguliforme alone* and *F. virguliforme* + *H. glycines* combination compared with the untreated treatments. The other treatments had not reduced the severity of foliar disease symptoms in the treatments that included *F. virguliforme* + *H. glycines* combination. There were different numbers of the foliar disease symptoms in all of the treatments compared to untreated treatment (Figure 4.5).

2016 experiment the Seed Treatment Comprehensive

There were repeated in 2016 from previous year, statistically, there was no significant effect on the weight of plants, height, number of nodes, number of pods, weight of pods or weight of roots by *H. glycines* and SDS when using the biological seed treatments compared to the control (fungicides only and untreated seeds). *B. renojensis* (5 and 2 floz/cwt) significantly increased weight of plants, plant height, number of nodes, number of pods, weight of pods, and weight of plants in the untreated control, *H. glycines* alone, and *F. virguliforme* alone (Table 4.11). Also, the combinations of bacterial metabolite and *B. renojensis* were significantly different from the control treatment in regard to plant development. *F. virguliforme* + *H. glycines* combination had a significant effect on weight of plants compared to untreated control, *H. glycines* alone, and *F. virguliforme* alone; although, statistically, the treatments with *F. virguliforme* + *H. glycines* combination were not significant on plant development compared to untreated seeds.

The results of this test, showed similar results to the standards Abamectin and *Pasteuria nishizawae,* on plant growth including weight of plants, height, number nodes, number of pods, weight of pods, and weight of roots with using biological seed treatments (Table 4.11).

The effects of treatments on nematode development, significant reduced the number of cysts, eggs, and juveniles (Table 4.12) In addition, the treatment for combination between bacterial metabolite and *B. renojensis* also significantly reduced the number of cysts, juveniles, and eggs compared to the untreated treatments. All treatments were similar to the abamectin and *Pasteuria nishizawae* standards. *B.*

renojensis variant 2 (5 floz/cwt) reduced the number of cysts from 257.4 and 145.86 cysts in the *H. glycines* alone and *F. virguliforme* + *H. glycines* combination respectively compared to 1432.86 and 1252.68 cysts in the *H. glycines* alone and *F. virguliforme* + *H. glycines* combination in the control. The same treatment reduced number of eggs and juveniles per 500 cm3 soil compared to the control treatments. *B. renojensis* variant 2 (5 floz/cwt) lowered the reproductive factor from 22.70 and 16.30 in the control treatment with *H. glycines* alone and *F. virguliforme* + *H. glycines* combination respectively to 2.76, and 1.16 with *H. glycines* alone and *F. virguliforme* + *H. glycines* combination. In addition, the number of cysts, eggs, and juveniles were lower number in the treatments with *F. virguliforme* + *H. glycines* combination compared to *H. glycines* alone.

Foliar leaves symptoms were significantly more severe in pots that included *F. virguliforme* + *H. glycines* combinations compared to *F. virguliforme* alone. Foliar symptoms were higher in the treatments in the *F. virguliforme* + *H. glycines* combinations. Most of the treatments did not significantly effect severity of disease symptoms between *F. virguliforme alone and F. virguliforme* + *H. glycines* combination treatments even though these treated significantly reduced disease severity compared to control (Figure 4.6).

In 2016, (Table 4.13), the roots image acquisition and analysis with (*H. glycines and* SDS disease did not reveal any significant effect from biological seed treatments with untreated control, *H. glycines* alone, and *F. virguliforme* alone compared to control treatments. *B. renojensis* at (5 floz/cwt) for root length, surface area of the root, average root diameter, root volume, number of tips, number of forks, and number of crossings were higher compared to the control (Table 4.13). Bacterial metabolite and *B. renojensis*

were significant different from the control and other treatments regarding the number of tips, forks, and crossings.

Discussion

Fusarium virguliforme was isolated from the soybean roots that were showing symptoms of Sudden Death Syndrome. On PDA, the fungus produced characteristic of mycelium with blue-pigmented, grayish white or bluish color on PDA medium, thn we knew we had a positive a *F. virguliforme*. Microconidia with 30 to 65 um in the length and the width be 6 to 8 um, the chlamydospores have been seen single or double and terminal with the macroconidia or the hyphae. (Figure 4.1, 4.2). (Rupe, J. C., and G. J. Weidemann. 1986; Mclean, K. S., and Lawrence, G. W. 1993, 1995).

All the biological products performed better than the control reducing cysts, eggs, and juveniles, as well as overall nematode reproduction. Also, reduction in *H. glycines* population were indicated differences in number of cysts, eggs, and juveniles in the treatments with the *H. glycines* alone compared with the *F. virguliforme* + *H. glycines* combination (Tables (4.3 and 4.9). This reduction in the life stages of *H. glycines* when both *Fusarium virguliforme* and *H. glycines* are present on soybeans has been reported in the literature (Mclean, K.S., and G. W. Lawrence. 1992). *F. virguliforme* has been shown to parasites on soybean cyst nematode and prevent nematode from produce syncytium on the feeding site of soybean roots. (Hirrel, M. C. 1985; Lawrence, G. W. et al. 1988; Roy, K.W. et al. 1988; Roy, K. W. et al. 1989; Rupe, J. C. 1988, 1989; Mclean, K.S., and G. W. Lawrence. 1992).

In many of the cases, the *Burkholderia renojensis* and experimentals performed similar to the nematicide standard, abamectin and fluopyram. None of the biological

candidates had a negative impacted plant development when challenged by *H. glycines,* and Sudden Death Syndrome(SDS) (Tables 4.2 and 4.8). Currently, seed treatments that are marketed for management of *H. glycines* are Avicta® (abamectin, Syngenta), Clariva® (*Pasteuria nishizawae*, Syngenta), and VOTiVO® (Bacillus firmus, Bayer CropScience). The possibility of *Burkholderia sp* as biocontrol agents against various plant pathogens have been recorded (Burkhead, K.D.et al 1994, Janisiewicz, W.J. et al. 1988). Lately, the insecticidal properties that were recorded in Japan for the new strain of *B. rinojensis* isolated from soil has new been identified as biological agent (Cordova-Kreylos, A.L. et al. 2013). The selected *B. rinojensis* varian*t* 2 formulation production we estimated will be marketed by Albaugh LLC as BioST Nematicide 100 and it will include several important nematodes on its label, including soybean cyst nematode, root-knot, and reniform nematode.

SDS foliar leaf symptoms were significantly more severe in pots that included *F. virguliforme* + *H. glycines* combination treatments compared to *F. virguliforme* alone. The symptoms of the SDS developed after 60 days after planting of soybean in the greenhouse condition. Foliar symptoms were increased in the treatments that included *F. virguliforme* (Figure 4.3). Foliar symptoms were decreased in the presence of *B. rinojensis* variant 2 (5 floz/cwt) in the *F. virguliforme* alone and *F. virguliforme* + *H. glycines* combination compared to the untreated control. Other treatments were not significant in reducing the severity of foliar symptoms of the disease (Figure 4.5).

None of the bacterial metabolite and SAR seed treatment products screened had no impact on plant development including soybean weight of plant, height of plant, number of nodes, number of pods, number of pods, weight of pods, and weight of roots in

soils infested with *H. glycines and F. virguliforme,* untreated control, *H. glycines* alone, *F. virguliforme* alone, and *F. virguliforme* + *H. glycines* combination when compared with fungicide only and untreated seeds (Table 4.5). All biological seed treatments significantly reduced the cysts, eggs and juveniles of *H. glycines* (Table 4.6), over the untreated check. In most trials, the impact on *H. glycines* reproduction were statistically similar to that of the nematicide standard abamectin and floupyram. Fluopyram is a fungicide that have been shown activity against Sudden Death Syndrome (Avenot and Michailides, 2010). It is sold as ILeVO® (fluopyram, Bayer CropScience Co.) as a new seed treatment available in the markest since 2015 for soybean. Fluopyram reduced Sudden Death Syndrome foliar symptoms when compared to control plants. (Mueller et al., 2011). Early testing for this product also has been shown activity to reduce plant-parasitic nematodes included *H. glycines*.

Saponin (SAR) and the bacterial metabolites were not significantly different from the fungicide/nematicide product fluopyram (Table 4.5) in greenhouse soils infested with *H. glycines* and *F. virguliforme.* The combinations treatments were designed to see the broad range of protection when combining multiple modes of action on either nematodes or diseases. However, in combination, it is sometimes difficult to determine if one chemical or biological agent is active. In soybeans, finding secondary nematicidal products that can be stacked with traditional or other biological products like *B. rinojensis* variant 2 could improve overall product performance on nematodes. Products with lower use rates would fit better as a companion nematicide (less than 1floz/cwt) than higher application rate products. The application rate of the SAR products tested in 2015 and 2016 were 0.1 and 0.2 floz/cwt, while the bacterial metabolite product application

rate was 3 floz/cwt. If these products were combine with other nematicides on the market in soybeans, ILeVo at 2.13 floz/cwt, Avicta at 3 floz/cwt and *B. rinojensis* variant 2 at 3 floz/cwt, the lower use rate products may be a more desirable combination when stacking modes of action against nematodes.

In greenhouse studies that evaluated the combination of *B. rinojensis* variant 2 with saponin and a bacterial metabolite efficacy of the seed treatment was increased over the *B. rinojensis* variant 2 used alone. Both saponin and the bacterial metabolite numerically reduced nematode reproduction factor values over the *B. rinojensis* variant 2 alone in both *H. glycines* study.

SDS foliar leaf symptoms were significantly more severe in the pots that included both pathogens *F. virguliforme* + *H. glycines* combination treatments compared to *F. virguliforme* alone. Most of the treatments significantly reduced the foliar symptoms with *F. virguliforme* alone to untreated seeds. The treatment with SAR- saponin product and bacterial metabolite were numerically different in the number between *F. virguliforme alone and F. virguliforme* + *H. glycines* combination for foliar disease symptoms severity, however, both treatments were significantly different compared to untreated seeds (Figure 4.4). Foliar symptoms were increased in the treatments that included *F. virguliforme* + *H. glycines* combination; however, most of the treatments had no effect on severity of disease symptoms between *F. virguliforme alone and F. virguliforme* + *H. glycines* combination treatments even though were significantly affected to reduce disease compared to control treatments (Figure 4.6). *B. rinojensis* has shown activity as a biological agent against mite and insect pests. (Cordova-Kreylos, Ana. Lucia. 2013). *B. cepacian* produces antifungal substances that shown activity against *Colletotrichum*

gloeosporioides. (Kadir, K. et al. 2008). Some of *Burkholderia sp.* have been used to control seedling damping off disease on cotton incited by *Rhizoctonia solani* (Yu et al. 2007). Soybean cyst nematode (*H. glycines)* has often been associated with Sudden Death Syndrome (SDS) disease complex. Also, *F. virguliforme* has been shown to be a parasite on soybean cyst nematode and prevent nematode from producing the feeding site of soybean roots (Hirrel, M. C. 1985.; Lawrence, G. W. et al. 1988; Roy, K. W. et al. 1988.; Roy, K. W. et al. 1989.; Rupe, J. C. 1988.; Rupe, J. C. 1989). Sciumbato and Keeling in 1984 found *H. glycines* in all the fields that were showing symptoms of Sudden Death Syndrome (Sciumbato, G. L., and B. L. Keeling. 1985). Hirrel, was unable to creater the associated the *H. glycines* nematode with the incidence of Sudden Death Syndrome symptoms (Hirrel, M. C. 1986). Although, Hirrel found that most severe incidence of symptoms of disease was association with higher number (50-75 cysts/ pint of soil) of *H. glycines.* The high populations of *H. glycines* were also association with severe Sudden Death Syndrome in other states (Rupe, J. C. 1988). Rupe in (1988) at harvest found *H. glycines* populations were positively connected to symptoms of SDS in the fields, however, it was not untill 1988 which the role of *H. glycines* in SDS was clarified (Rupe, J. C. 1988).

In 2016, the same treatments (Table 4.13), discussed earlier were analyzed using WinRHIZO optical scanner (Regent Instruments, Inc.). The WinRHIZO optical scanner is an efficient method that allow image analysis and examination of the root morphological traits. This technique provides data that could be easily analyzed by established software protocols in a method of simple and rapid accurate screening of root characteristics. Therefore, this method was utilized for screening of root traits of soybean

grown under *H. glycines* and *F. virguliforme* infections. Plant roots optimize their root to acquire essential nutrients and water. Number of root tips, forks, and crossings have been shown significant roles on root structure because they have potential to encourage penetration through soil layers, that leads to good effects to getting water and essential nutrients for plant. In this study, length of roots, surface area, average diameter of roots, root volume, root tips, forks, and crossings densities differed significantly with biological seed treatments especially in the treatments (untreated, *H. glycines* alone, *F. virguliforme* alone) compared to untreated seeds. However, there was numerically differences between treatment with *H. glycines* and *F. virguliforme* combinations and untreated, *H. glycines* alone, *F. virguliforme* alone) although were significant compared to untreated seeds. The increase in biomass that could be because of modifications in phenotype, increase in leaf and stem growth and increase in the photosynthetic rates (Reddy et al., 1998; Reddy et al., 1995, 2004). That also could be related to the possibility of *B. rinojensis* as biocontrol agent against various plant pathogens have been recorded (Burkhead, K.D.et al 1994; Janisiewicz, W.J. et al. 1988). The high number of cyst leads to high number of root tips that what Tatalovice found it (Tatalovice, 2013). Tatalovice, in 2012 found that when enough soil moisture was available *F. virguliforme* can penetrate into the vascular tissue of the plants more frequently in the present of *H. glycines* more than in the absent of *H. glycines*. Other findings, ssuggest that *F. virguliforme* penetrates the roots of the plants more frequently close to the roots cap (Navi and Yang, 2008). Also, the number of roots tips can be decreased with lower number of cyst of *H. glycines* alone infection and more number of roots tips with present of *H. glycines* that could be less severity of SDS (Zaworski, Edward. R. 2014). Having two pathogens inciting disease at the same

location (root tip), may be another reason why less SCN cysts were associated with plants infected by *H. glycines* and *F. virguliforme* (competition).

Conclusion

The objective of this study was to identify a viable biological candidate that would be efficacious on soybean cyst nematode (*Heterodera glycines)* and Sudden Death Syndrome *(Fusarium virguliforme).* In this study, we evaluated four rates of *Burkholderia rinojensis* and an EXP bacterial product provided by Albaugh, LLC. All the biological products performed statistically better than the fungicide check in regard to reducing cysts, eggs, and juveniles, as well as the overall nematode reproduction. Also, differences in number of cysts, eggs, and juveniles in the treatments with *H. glycines* alone was higher numbers than *F. virguliforme* + *H. glycines* combination. SDS foliar leaves symptoms were significantly more severe in the pots that included both pathogens *F. virguliforme* + *H. glycines* combination compared to *F. virguliforme* alone treatment. The symptoms of the SDS were developed after 60 days after planting of soybean in the greenhouse condition. In the bacterial metabolite and SAR seed treatment study, none of the products screened did not impact plant development in greenhouse screening, soybean weight of plant, height of plant, number of nodes, number of pods, number of pods, weight of pods, and weight of roots in soils infested with *H. glycines and F. virguliforme* when compared with control. The nematode results indicated that all biological seed treatments were statistically significant in their ability to reduce the cysts, eggs per gram and juveniles of *H. glycines* compared to untreated. In greenhouse studies that evaluated the combination of *B. rinojensis* variant 2 with saponin and a bacterial metabolite generally increased the efficacy of the seed treatment over the *B. rinojensis*

variant 2 used alone. For instance, both the saponin and the bacterial metabolite numerically reduced reproductive factor values over the *B. rinojensis* variant 2 alone in both *H. glycines* study. These findings were repeated in the 2016 comprehensive study, in that the combination (two modes of action) generally reduced reproductive factor values over the *B. rinojensis* variant 2 and the secondary nematicide compounds applied as a solo nematicide product. Most of these biological controls have shown similar results to the several standard nematicide seed treatment products were also included in many of these studies as a positive nematicide check, including *Pasteuria nishizawae* spores (Clariva), *Bacillus firmus* spore suspension (Votivo), the chemical abamectin (Avicta) and fluopyram (ILeVo). Future research should focus on using different modes of action (fungicides and nematicides) that would promote both sustainable and economical protection in reducing both SDS and SCN.

Table 4.1 *Burkholderia renojensis* variant 2 and rates used for management soybean cyst nematode (*Heterodera glycines)* and Sudden Death Syndrome *(Fusarium virguliforme).*

Product	Description
1- Fungicide check	Control
2- *B. renojensis* varinat 2 at 3 floz/cwt	Heat killed *B. renojensis* – production variant 2
3- *B. renojensis* varinat 2 at 5 floz/cwt	Heat killed *B. renojensis* – production variant 2
4- *B. renojensis* varinat 2 at 7floz/cwt	Heat killed *B. renojensis* – production variant 2
5- *B. renojensis* varinat 2 at 10 floz/cwt	Heat killed *B. renojensis* – production variant 2
6- Abamectin at 3 floz/cwt	Seed Treatment Nematicide Standard
7- Untreated seed	Untreated seed – no fungicides

All the treatmnts were treated with fungicide as base treatment.

Table 4.2 Effect of *Burkholderia renojensis* variant 2 seed treatments on plants inoculated with *H. glycines and Fusarium virguliforme* - 2015.

Treatments	Plant/ Weight g	Plant/Height cm	Number of nodes	Number of pods	Pod Weight g	Roots/ Weight g
1-Untreated	15.7	47.6	23.2	14.2	10.3	7.6
1-Cyst alone	12.6	36.6	20.6	8.6	7.4	4.5
1-Fusarium alone	15.1	47	18.8	14.6	9.4	5.6
1 -C + F	7.3	32.6	13.8	8.2	6.4	4.2
2-Untreated	15.5	40.4	23.6	13.6	13.8	7.6
2-Cyst alone	11.9	35.6	20.6	9.8	11.3	5.7
2-Fusarium alone	15.8	47.4	19.4	13.4	8.4	9.7
2-C + F	15.2	39.8	18.8	8.6	9.4	5.26
3-Untreated	16.8	38.4	20.4	10.4	9.9	7
3-Cyst alone	16.9	39.4	22.8	15.2	19.7	7.9
3-Fusarium alone	22.8	51.8	22.2	19	13.7	9.6
3-C + F	14.8	37.2	15.2	11.4	9.7	5.9
4-Untreated	17.2	38.6	22	12.6	13.8	7.9
4-Cyst alone	14.1	40.4	19.9	12.4	14.3	6.8
4-Fusarium alone	19.6	49.2	21	19	10.5	9.5
4-C + F	12.4	38.2	16.6	11.4	9.7	5.2
5-Untreated	19	41.2	27.6	17.6	18.3	9.2
5-Cyst alone	14.1	43.4	17.8	13.2	16.1	7.6
5-Fusarium alone	15.7	44.2	19.4	12.4	7.5	8.8
5-C + F	13.4	35.4	16	10.2	7.8	7.16
6-Untreated	16.7	41.2	27.4	16.8	18.4	8.4
6-Cyst alone	13.5	38.4	19.2	13.2	11.1	6.9
6-Fusarium alone	23.6	55	25	21.4	13.5	11.1
6-C + F	15.4	43.4	14.6	11.8	12.7	6.8
7-Untreated	10.6	38.2	12.6	9.2	6.6	6.1
7-Cyst alone	14.3	36.6	12.8	11.2	6.5	5.2
7-Fusarium alone	12.5	41.6	14.4	9.4	5.9	4.9
7-C + F	6.6	32.6	7.6	5.6	4.8	4.2
P-Value	0.0001	0.0224	0.0001	0.0409	0.0001	0.0001
L.S.D 0.05	3.0823	5.82	2.9387	4.0298	3.2587	1.3108

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

Table 4.3 Effect of *Burkholderia renojensis* variant 2 seed treatments on *H. glycines* life stage development on soybean inoculated with *H. glycines and Fusarium virguliforme* - 2015.

Treatments	Cyst/plant	Eggs/ plant	Juveniles/500 cm3 soil	Reproductive Factors
1-Cyst alone	806.52	12108.57	6384.8	7.719
1-C + F	253.98	4597.039	2258	2.84
2-Cyst alone	368.94	1686.175	2884	1.97
2-C + F	171.6	2824.62	552	1.41
3-Cyst alone	386.1	1283.946	1648	1.32
3-C + F	107.24	1140.029	384	0.65
4-Cyst alone	368.94	2249.852	1464	1.63
4-C + F	143	1717.809	736	1.03
5-Cyst alone	334.62	1290.789	2317.44	1.57
5-C + F	64.34	1522.962	264	0.74
6-Cyst alone	334.62	1011.885	1287.44	1.05
6- C + F	42.9	651.8815	492	0.47
7-Cyst alone	823.68	12169.55	11330	9.72
7-C + F	429	5987.733	1176	3.03
P-Value	0.0001	0.0002	0.0001	0.0001
L.S.D 0.05	161.1	4612.8	2172.3	1.8806

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs + Cyst + Juveniles at 60 days/ 2500 eggs.

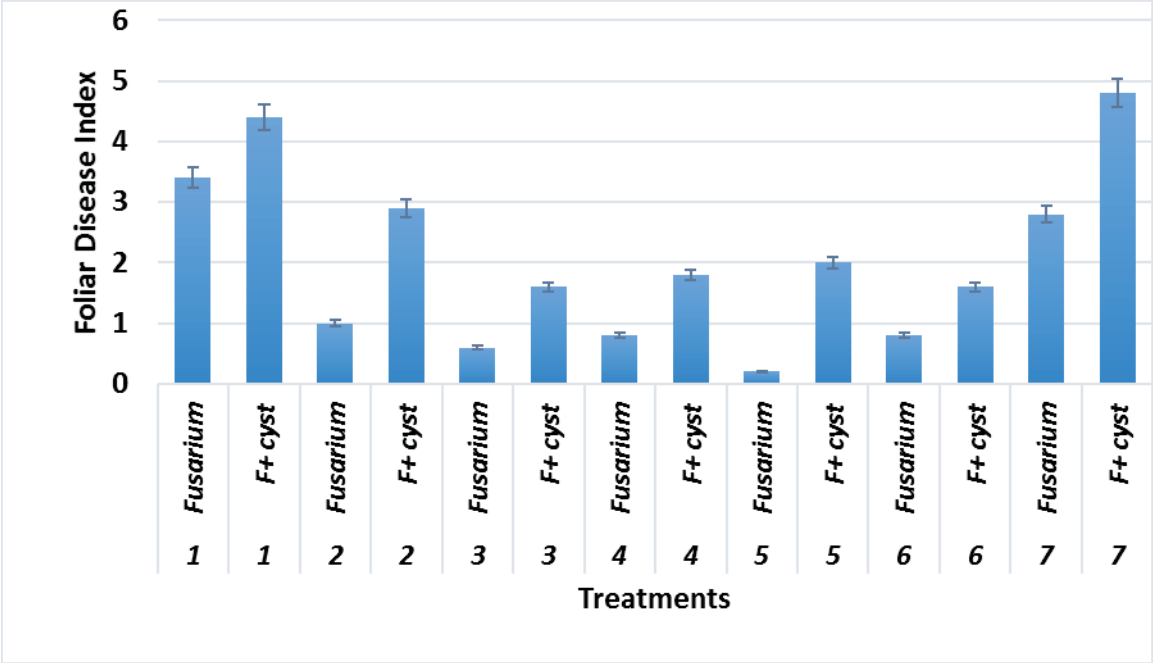


Figure 4.3 Foliar disease index rating in 2015 test with biological seed treatments.

Foliar SDS disease severity was recorded at 60 days after harvest using a 0-7 scales, where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death. Data are means of the 5 replicates for each treatment after 60 days. The means compared by using Fisher`s protected least significant difference test at 0.05.
P-Value = 0.0001, L.S.D 0.05 = 1.3961

Table 4.4 Bacterial metabolite and SAR seed treatment used for management soybean cyst nematode (*H. glycines)* and Sudden Death Syndrome *(Fusarium virguliforme).*

Product	Description
1- Fungicide Control	Control
2- SAR1 - Saponin at 0.1 floz/cwt	SAR product with saponin
3- SAR1 - Saponin at 0.2 floz/cwt	SAR product with saponin
4- Bacterial Metabolite at 3 floz/cwt	Biostimulant Bacterial Metabolite
5- Abamectin at 3 floz/cwt (0.15 mg ai/seed)	Nematicide standard 1
6- Fluopyram at 2.3 floz/cwt (0.25mg ai/seed)	Nematicide standard 2
7- Untreated seed	Untreated seed – no fungicides

All the treatmnts were treated with fungicide as base treatment.

Table 4.5 Effect of Bacterial metabolite and SAR seed treatment seed treatments on soybean plants inoculated with *H. glycines and Fusarium virguliforme* - 2015.

Treatments	Plant/ Weight g	Plant/Height cm	Number of nodes	Number of pods	Pod/Weight g	Roots/ Weight g
1-Untreated	18.9	43	20.8	12.8	13.9	7.7
1-Cyst alone	12	38.8	11.4	7	8.68	3.6
1-Fusarium alone	18	50	17.2	13.6	8	5.7
1 -C + F	10.9	26.4	9.6	5	3.9	6.1
2-Untreated	21.2	47.59	25.4	15	15.3	8.3
2-Cyst alone	18.6	46.2	23.4	13.8	15.4	8.12
2-Fusarium alone	21	57.8	20.6	15.2	8.4	6.3
2-C + F	17.3	35.6	12.8	6.6	4.5	5.9
3-Untreated	19	48.6	23.8	15	15.5	8.8
3-Cyst alone	15.8	47.6	18.8	9.8	10.1	6.4
3-Fusarium alone	20.2	60.6	17	13.8	7.6	5.3
3-C + F	20.4	34.2	10.4	5.2	4.6	7.1
4-Untreated	18.6	54.8	23.8	12.8	15.8	8
4-Cyst alone	17	46.6	17.2	10	11.3	7.4
4-Fusarium alone	17.6	47.6	17.4	9.2	5	7.6
4-C + F	16.2	35.72	11.8	9	5.8	7.5
5-Untreated	17.4	46	21.6	13	15.6	6.6
5-Cyst alone	15.4	42	20.8	10.8	12.8	8
5-Fusarium alone	24	53.4	24.4	12	7.8	8.1
5-C + F	15	30	9.8	4.4	3.5	5.8
6-Untreated	14.8	48.8	21.8	13.6	19.2	8.4
6-Cyst alone	14	39.4	17.8	7	9.4	8.29
6-Fusarium alone	27	58	28.8	19.6	12.8	8.9
6-C + F	14.6	26.6	8.4	3.6	3	5
7-Untreated	17.5	41.4	20.6	15.2	16.2	7.2
7-Cyst alone	8.2	34.8	9.6	3.6	4.8	4.2
7-Fusarium alone	18.6	52.4	13.8	7.6	4.6	6.4
7-C + F	10.3	29.5	9.2	3.8	2.9	4.6
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0493
L.S.D 0.05	2.3879	3.6117	2.2764	2.6053	2.4911	2.2228

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

Table 4.6 Effect of Bacterial metabolite and SAR seed treatment on *H. glycines* life stage development on soybean inoculated with *H. glycines and Fusarium virguliforme* - 2015.

Treatments	Cyst/plant	Eggs/ plant	Juveniles/500 cm3 soil	Reproductive Factors
1-Cyst alone	849	15415.67	34505	23.52
1-C + F	463.32	3092.229	11586.5	6.66
2-Cyst alone	223.08	3283.738	12102.5	10.73
2-C + F	214.5	1268.543	3798	3.92
3-Cyst alone	197.34	2192.067	14152.5	5.30
3-C + F	188.76	1585.747	3218	3.39
4-Cyst alone	240.24	1934.602	18282.5	6.17
4-C + F	92.67	867.5668	3347.5	2.32
5-Cyst alone	265.98	2082.889	16480	6.33
5-C + F	57.2	513.1887	1887.9	1.06
6-Cyst alone	203.22	2020.762	18540	6.65
6- C + F	94.38	1316.929	3862.5	2.12
7-Cyst alone	712.14	8240	32175	14.22
7-C + F	223.08	1952.095	9270	3.66
P-Value	0.0001	0.0001	0.0001	0.0001
L.S.D 0.05	140.04	2023	8077.7	4.1069

Data are means of the 5 replicates for each treatment 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.
Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs.

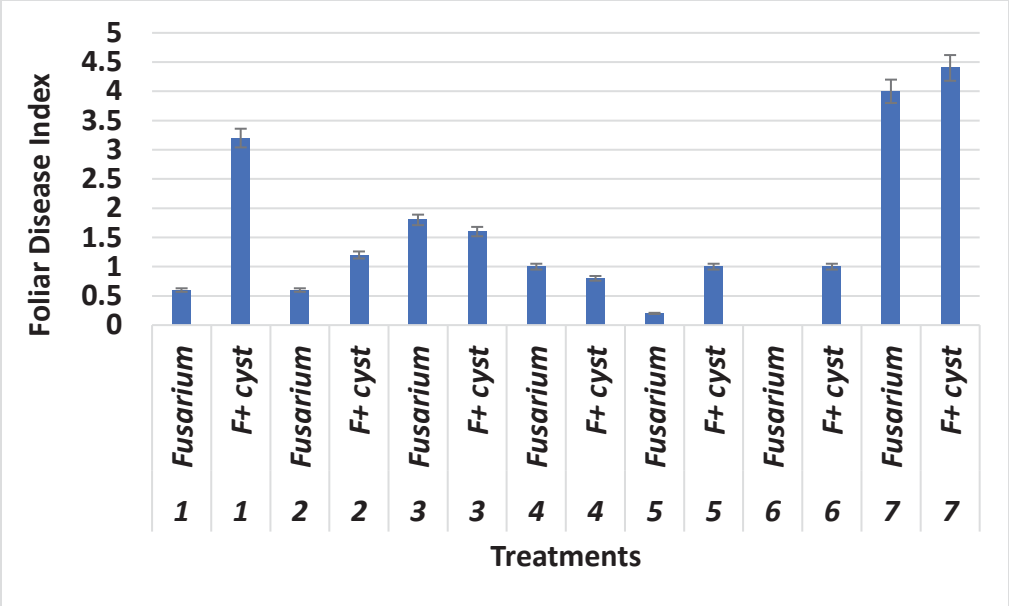


Figure 4.4 Foliar disease index rating in 2015 test with biological seed treatments.

Foliar SDS disease severity was recorded at 60 days after harvest using a 0-7 scale, where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death. Data are means of the 5 replicates for each treatment after 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.
P-Value = 0.0012, L.S.D 0.05 = 1.5369

Table 4.7 Experimental Bacteria and *Burkholderia renojensis Combination* seed treatment used for management soybean cyst nematode (*H. glycines)* and Sudden Death Syndrome *(Fusarium virguliforme)*-2015.

Product	Description
1- Fungicide	Control
2- *B. renojensis* Var 2 at 5 floz/cwt	Heat killed *B. renojensis* – production variant 2
3- *B. renojensis* + Bacterial Metabolite	Two modes of action *B. renojensis* and Bacterial Metabolite
4- *B. renojensis* + Saponin (SAR)	Two modes of action *B. renojensis* and Saponin
5- *B. renojensis* + Harpin (SAR	Two modes of action *B. renojensis* and Harpin
6- Abamectin	Nematicide standard 1
7- Untreated seeds	Untreated seed – no fungicides

All the treatments were treated with Fungicide as base treatment.

Table 4.8 Effect of Experimental Bacteria and *B. renojensis* Combination seed treatment seed treatments on soybean plants inoculated with *H. glycines and Fusarium virguliforme* - 2015.

Treatments	Plant/ Weight g	Plant/Height cm	Number of nodes	Number of pods	Pod/Weight g	Roots/ Weight g
1- Untreated	18	40.4	24.6	10.6	11.1	7.4
1-Cyst alone	10.6	30.4	9.6	6.8	5.2	5.42
1-Fusarium alone	17.4	56	18.2	13.8	6.8	6.8
1 -C + F	7.1	27.8	11.8	6	4.4	5.1
2- Untreated	18.8	44.4	26.8	15.6	15.8	9.4
2-Cyst alone	17.2	46	20.8	13.4	14.4	8.1
2-Fusarium alone	13	58.8	16	10.2	8.6	7.6
2-C + F	11.9	37.6	15.8	9.6	7.4	6.3
3- Untreated	13.8	44.6	24.2	14	12.3	6.7
3-Cyst alone	16.6	43.8	19	13.4	12.1	7.5
3-Fusarium alone	16	56.2	20.2	12.2	7.9	7.1
3-C + F	12.7	48	16.8	13	6.2	5.96
4- Untreated	21.4	44.2	33	17.8	21.1	9.2
4-Cyst alone	14.4	41.2	16.6	9	9.5	6.2
4-Fusarium alone	22.6	60.2	21.8	18	9.4	8.1
4-C + F	14	46	16.4	9.6	6.4	5
5- Untreated	14.8	42.6	21.8	11.4	12	9.1
5-Cyst alone	12.8	39	21	8.6	7.1	7.3
5-Fusarium alone	22	64.8	26.2	16.8	8	7.5
5-C + F	12	40.8	18.4	12	6.8	5.8
6- Untreated	19.4	38.2	28.8	18.2	19.3	9.7
6-Cyst alone	16.1	42.8	18.4	12.6	13.2	7.2
6-Fusarium alone	24	57	21.6	15.6	11.2	8.8

Table 4.8 (Continued)

6-C + F	14.5	41	18.6	13.8	12.4	6.8
7-Untreated	10.5	39	11.8	5.8	8	6
7-Cyst alone	11.7	37.4	12.6	9.2	6.5	5.4
7-Fusarium alone	10.7	39.2	13.2	8.4	5.1	4.8
7-C + F	11.8	32.6	11.4	6.6	5.2	3.6
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0010
L.S.D 0.05	2.6222	5.5055	2.819	2.5591	2.6947	1.3957

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

Table 4.9 Effect of Experimental Bacteria and *B. renojensis Combination* seed treatment on *H. glycines* life stage development on soybean inoculated with *H. glycines and Fusarium virguliforme* - 2015.

Treatments	Cyst/plant	Eggs/ plant	Juveniles/500 cm3 soil	Reproductive Factors
1-Cyst alone	574.86	7980.196	30282	15.53
1-C + F	288.3	1562.955	11793.5	5.45
2-Cyst alone	137.28	1313.187	12772	5.68
2-C + F	139.42	1781.416	3347.7	2.10
3-Cyst alone	248.82	1494.436	11536	5.31
3-C + F	57.2	975.9428	1737.5	1.10
4-Cyst alone	197.34	1241.886	10300	4.69
4-C + F	128.7	1571.41	2253.1	1.58
5-Cyst alone	248.82	1130.275	7210	3.43
5-C + F	75.054	839.2647	2896.5	1.52
6-Cyst alone	154.44	827.3017	7416	3.35
6- C + F	85.8	813.9161	1931.2	1.13
7-Cyst alone	772.2	7390.406	30282	15.37
7-C + F	453.04	2557.165	9630.5	5.05
P-Value	0.0001	0.0001	0.0001	0.0001
L.S.D 0.05	178	1559.3	6866.2	3.0026

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs.

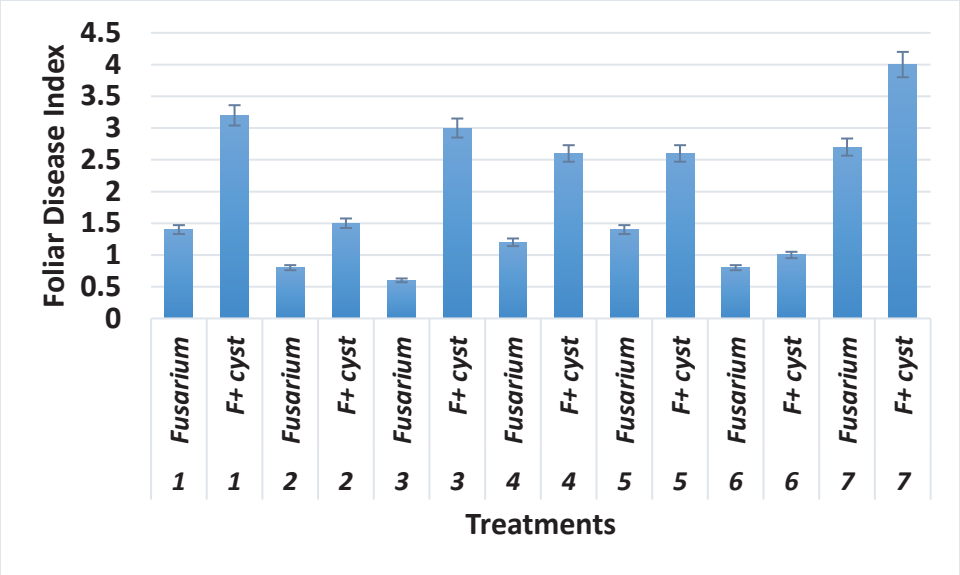


Figure 4.5 Foliar disease index rating in 2015 test with biological seed treatments.

Foliar SDS disease severity was recorded at 60 days after harvest using a 0-7 scales, where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death Data are means of the 5 replicates for each treatment after 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.
P-Value =0.0439, L.S.D 0.05 = 1.5424

Table 4.10 2016 Seed Treatment Comprehensive Study seed treatment used for management soybean cyst nematode (*H. glycines)* and Sudden Death Syndrome *(Fusarium virguliforme)*-2016.

Product	Description
1- Fungicide	Control
2- *B. renojensis* var 2 at 3 floz/cwt	Heat killed *B. renojensis* Var 2
3- *B. renojensis* var 2 at 5 floz/cwt	Heat killed *B. renojensis* Var 2
4- Saponin at 0.16 floz/cwt	SAR product – Saponin
5- Bacterial Metabolite at 3 floz/cwt	Biostimulant – Bacterial Metabolite G
6- *B. renojensis*+ Saponin at 0.16 floz/cwt	*B. renojensis* and Saponin
7- *B. renojensis*+ Bacterial Metabolite at 3 floz/cwt	*B. renojensis* and Bacterial Metabol.
8- Abamectin	Seed Treatment Nematicide Standard
9- *Pasteuria nishizawae*	Seed Treatment Nematicide Standard
10- Bacillus firmus	Seed Treatment Nematicide Standard
11- Untreated Seed	Non-treated soybean seed

All the treatments were treated with Fungicide as base treatment.

Table 4.11 Effect of Seed Treatment Comprehensive Study on soybean plants inoculated with *H. glycines and Fusarium virguliforme* - 2016.

Treatments	Plant/ Weight g	Plant/Height cm	Number of nodes	Number of pods	Pod/Weight g	Roots/ Weight g
1-Untreated	15.9	48.8	19.4	16.2	16.1	8.4
1-Cyst alone	14	52	19	12.6	9.7	4.1
1-Fusarium alone	19	60.4	25.2	15	6.9	7.8
1-C + F	12.1	45	14.2	7.4	5.3	5.6
2-Untreated	17.2	47.8	25.4	18.6	18.1	8.7
2-Cyst alone	19	56.6	25.5	16.2	10.7	6.6
2-Fusarium alone	26	73.2	25.6	14.12	7.3	7.7
2-C + F	16.2	50	17.4	11	8.3	9.6
3-Untreated	16.5	52.6	20	14.2	16.5	9
3-Cyst alone	21.4	59.2	22.6	14	9.3	7.1
Fusarium alone	20.6	68.8	24.4	10.6	5.5	7.4
3-C + F	16.1	55.6	18.2	10.6	7.7	8.09
4-Untreated	19.5	63.8	27.6	19.8	18.2	9.3
4-Cyst alone	21.8	60.4	23	14.8	8.2	7.6
4-Fusarium alone	18.8	75.6	25.6	9.2	5.8	7.7
4-C + F	14.5	55.6	19.8	9.8	6.8	8.2
5-Untreated	16.5	59.8	21.8	22.8	20.9	8
5-Cyst alone	22.9	72.2	21.2	12.4	8.1	7.5
5-Fusarium alone	18.6	76.6	20.4	11.8	6.8	6.2
5-C + F	19.2	58	21.8	13	9.5	8.6
6-Untreated	19.1	62	24.2	18.8	20.1	9.5
6-Cyst alone	25.4	66.2	25.8	18.6	11.2	7.9
6-Fusarium alone	23.7	84.4	31.2	11	7.7	7.3
6-C + F	14.8	42.6	20.4	8	5.4	8.5
7-Untreated	16.9	60.6	19.6	15.6	17.2	9.8
7-Cyst alone	22.6	73.2	21	15.8	8.5	8.9
7-Fusarium alone	26.4	84	26.6	9.8	5.4	7.5
7-C + F	17.4	56.4	20.6	11.8	6.5	6.7
8-Untreated	17.7	51.2	18.4	14.1	13.7	9.3
8-Cyst alone	19.2	69.6	20.8	12	6.6	7.3
8-Fusarium alone	19.5	64	25.6	9	5.5	8.7
8-C + F	15.4	54.8	20.6	10.2	7.3	6.7
9-Untreated	19.3	51.2	19.6	12.6	13.7	9.8
9-Cyst alone	19.5	69.8	23.2	10.2	5.5	6.5
9-Fusarium alone	26.8	82.6	29.8	12.4	7.1	8.89

Table 4.11 (Continued)

9-C + F	15.3	50.6	19.2	10.8	7.8	7.2
10-Untreated	17.6	50.2	22	15.4	14.9	9.8
10-Cyst alone	27.8	71.8	28.4	18.8	12	8.8
10-Fusarium alone	23.6	80	26	10.8	6.1	9.4
10-C + F	18.9	58	24	14.4	9.6	9.3
11-Untreated	12.7	41.2	17.8	9	9.9	7.9
11-Cyst alone	13.3	56.4	17.2	8.2	3.4	3.9
11-Fusarium alone	13.8	52.6	17.8	7	3	3.9
11-C + F	6.8	32.6	12.8	4.6	3	3.2
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L.S.D 0.05	2.9194	5.5337	3.0214	3.5199	2.6281	1.0194

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

Table 4.12 Effect of Seed Treatment Comprehensive Study on *H. glycines* life stage development on soybean inoculated with *H. glycines and Fusarium virguliforme* - 2016.

Treatments	Cyst/plant	Eggs/ plant	Juveniles/500 cm3 soil	Reproductive Factors
1-Cyst alone	1432.86	33089.9	22248	22.70
1-C + F	1252.68	23041.17	16480	16.30
2-Cyst alone	411.84	4260.959	7004	4.67
2-C + F	154.44	641.6877	2369	1.26
3-Cyst alone	274.56	4508.682	5356	4.05
3-C + F	214.5	1021.668	2781	1.60
4-Cyst alone	411.84	1858.645	5150	2.96
4-C + F	223.08	887.9256	1751	1.14
5-Cyst alone	171.6	1563.899	4429	2.46
5-C + F	343.2	2158.408	4429	2.77
6-Cyst alone	420.42	1825.183	4223	2.58
6-C + F	205.92	3467.163	7931	4.64
7-Cyst alone	223.08	1414.185	1957	1.43
7-C + F	240.24	2098.121	5459	3.11
8-Cyst alone	197.34	1351.053	2163	1.48
8-C + F	128.7	1406.393	1648	1.27
9-Cyst alone	214.5	1848.91	2781	1.93
9-C + F	94.38	1631.434	3502	2.09
10-Cyst alone	274.56	1281.601	2575	1.65
10-C + F	120.12	897.1069	2884	1.56
11-Cyst alone	1209.78	41438.05	23587	26.49
11-C + F	1149.72	34452.03	28325	25.57
P-Value	0.0001	0.0001	0.0001	0.0001
L.S.D 0.05	243.35	5768.4	4892.5	3.8305

Data are means of the 5 replicates for each treatment at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs.

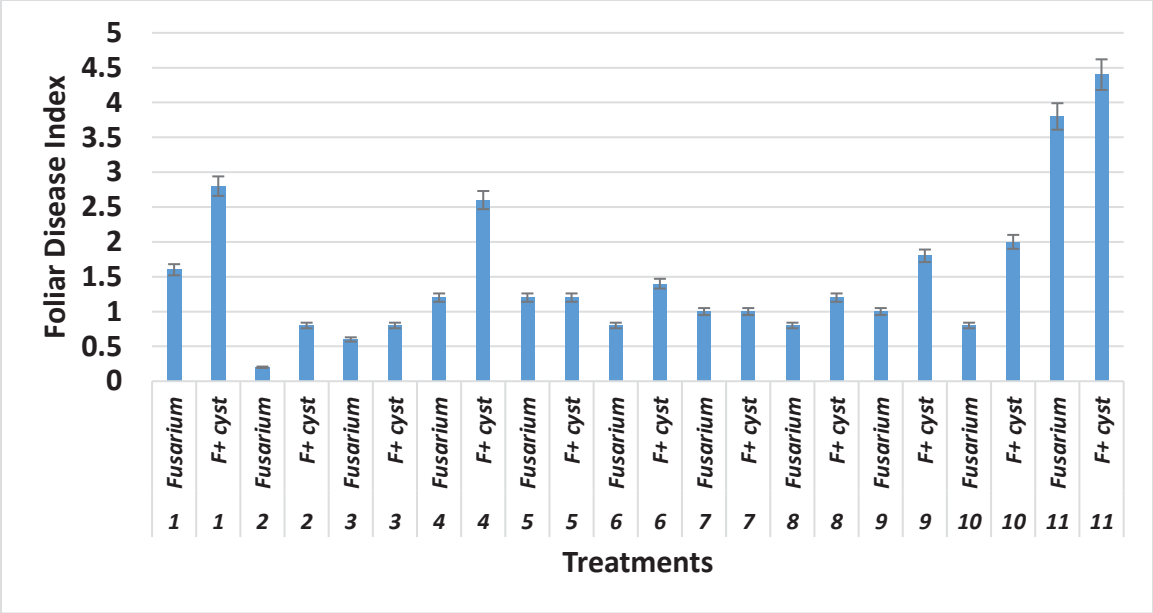


Figure 4.6 Foliar disease index rating in 2015 test with biological seed treatments.

Foliar SDS disease severity was recorded at 60 days after harvest using a 0-7 scales, where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death Data are means of the 5 replicates for each treatment after 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.
P-Value = 0.0003, L.S.D 0.05 = 1.2294.

Table 4.13 Effect of Seed Treatment Comprehensive Study on *Roots soybean* development by using WinRhizo inoculated with *H. glycines and Fusarium virguliforme* - 2016.

Treatments	Length(cm)	SurfArea(cm2)	AvgDiam(mm)	RootVolume(cm3)	Tips	Forks	Crossings
1-Untreated	2864.005	337.2408	0.38026	3.1808	8652.6	15356.6	2098
1-Cyst alone	895.8615	130.2826	0.46146	1.518	1999	6623.2	599.2
1-Fusarium alone	1580.411	204.6474	0.41456	2.1102	6069.6	10380	932.4
1-C + F	1266.383	186.9191	0.358332	1.39936	2484.8	5403.6	448.4
2-Untreated	2830.168	318.5235	0.3683	2.8958	12390.2	27201	2755.2
2-Cyst alone	1628.884	206.6882	0.40488	2.0908	4493.2	11709.8	1102.4
2-Fusarium alone	2079.93	267.6583	0.42218	2.7896	8068.2	14288.4	1417.8
2-C + F	1511.163	192.5786	0.38846	2.6602	6600.4	11702	914
3-Untreated	2707.973	310.1028	0.36938	2.8442	9765.6	27365.2	3073.8
3-Cyst alone	1661.617	229.0368	0.44008	2.5156	4270	13368.4	1176.2
Fusarium alone	1729.719	229.7667	0.42186	2.4352	5128.4	13725.8	1190.8
3-C + F	1649.607	222.4487	0.4043	2.2246	5385.4	12192.4	1048.8
4-Untreated	1565.947	224.4886	0.4571	2.567	4775.4	13581.2	1079.4
4-Cyst alone	1665.083	221.1263	0.42668	2.343	4974.2	13986.2	1266.8
4-Fusarium alone	1687.313	220.0915	0.41574	2.3038	5462	13635.2	1215.2
4-C + F	1780.386	245.8412	0.44514	2.7152	6224.8	15686.2	1233.2
5-Untreated	2400.572	300.6181	0.40032	3.0356	10731.8	22575	2074.2
5-Cyst alone	1815.533	237.8603	0.41994	2.507	6103.6	14164.8	1297.4
5-Fusarium alone	2046.976	239.4424	0.3809	2.2498	7757.6	14829	1526.6
5-C + F	1180.811	152.3071	0.41152	1.5674	5694.4	6697.8	509.8
6-Untreated	1937.269	270.6719	0.44768	3.0218	5162	18830.4	1608.6
6-Cyst alone	1901.023	245.0017	0.40856	2.5142	5486.8	15424.4	1564.2
6-Fusarium alone	1711.366	231.3839	0.44894	2.5296	3993.4	14377.2	1320
6-C + F	1071.553	187.4683	0.56072	2.6532	2095.8	8399.2	524.8
7-Untreated	1907.709	280.8034	0.4685	3.324	4629.4	19851.2	1653.6
7-Cyst alone	1980.575	235.3999	0.37852	2.232	7703.2	14749.4	1486.4
7-Fusarium alone	1742.004	202.2218	0.39374	1.9868	6623.2	14194.6	1339.8
7-C + F	1169.077	194.087	0.54846	2.6466	3694.4	8030.8	565.8
8-Untreated	1516.602	194.5979	0.40778	1.9902	3200.4	12326.2	1245.2
8-Cyst alone	1405.243	171.8764	0.38718	1.6744	3732.6	11221	1143.8
8-Fusarium alone	1366.244	196.2521	0.47568	2.3054	3649.6	10184.2	849.2
8-C + F	1154.361	174.1379	0.48096	2.1238	3944.2	8575	657.2
9-Untreated	1368.674	177.6895	0.42226	1.8584	4619.2	9754	872.6
9-Cyst alone	1492.008	185.2501	0.39724	1.8394	5123.4	10803	1025.8
9-Fusarium alone	1846.146	251.3629	0.43706	2.7402	5267.2	13818.8	1286.8
9-C + F	1462.516	202.1788	0.45716	2.2914	5102.6	10315.2	871.8
10-Untreated	1760.16	222.3565	0.41362	2.2966	6246.4	17338	1724
10-Cyst alone	1821.299	252.0771	0.45966	2.824	5344.4	16859.2	1781.8
10-Fusarium alone	2007.08	262.43	0.42254	2.7662	4928.2	15923.8	1789.2
10-C + F	1562.937	211.2252	0.44252	2.68654	4198.4	12444.4	1280.6

Table 4.13 (Continued)

11-Untreated	1587.109	218.5024	0.4164	2.6252	7523.2	9317	1239
11-Cyst alone	912.2734	122.834	0.42462	1.3242	2677.8	6655.6	648.2
11-Fusarium alone	805.8306	120.4797	0.44134	1.4644	2387.8	5966.6	635.8
11-C + F	674.0725	108.2647	0.3875	1.4192	1735.8	4124.4	295.8
P-Value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
L.S.D 0.05	302.94	34.751	0.036	0.3951	1427.3	3396.9	373.92

Data are means of the 5 replicates for each treatment measured at 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

References

Abdelsamad, N., Mbofung, G. C., Robertson, A. E., Liebman, M. and Leandro, L. F. 2012. Long-term crop rotations suppress soybean sudden death syndrome in Iowa. Phytopathology 102:1.

Anderson, T. R. and Tenuta, A. U. 1998. First report of *Fusarium solani* f. sp. *glycines* causing sudden death syndrome of soybean in Canada. Plant Disease 82:448.

Aoki, T., O'Donnell, K., and Scandiani, M. M. 2005. Sudden death syndrome of soybean in South America is caused by four species *of Fusarium: Fusarium brasiliense sp. nov., F. cuneirostrum sp. nov., F. tucumaniae, and F. virguliforme*. Mycoscience 46:162-183.

Avenot, H. F. and Michailides, T. J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Protection 29:643-651.

Bradley, C. A. and Koenning, S. R. 2014. Soybean disease loss estimates graph- 2009-2011. http://unitedsoybean.org/wp-content/uploads/Soybean-Disease-Loss-Estimates-Graph-2009-2011.pptx

Brar, H. K., Swaminathan, S., and Bhattacharyya, M. K., 2011. The *Fusarium virguliforme* toxin FvTox1 causes foliar sudden death syndrome-like symptoms in soybean. Mol. Plant-Microbe Interact. 24:1179-1188.

Burkhead, K.D.; Schisler, D.A.; Slininger, P.J. 1994. Pyrrolnitrin production by biological-control agent Pseudomonas cepacia B37W in culture and in colonized wounds of potatoes. Appl. Environ. Microbiol., 60, 2031–2039.

Caballero-Mellado, J., Martínez-Aguilar, L., Paredes-Valdez, G., and Estrada-de los Santos P. 2004. *Burkholderia unamae* sp. nov., an N2-fixing rhizospheric and endophytic species. Int. J. Syst. Evol. Microbiol. 54:1165–1172.

Casida, L. E., Jr. U.S. Patent 1993. 5, 232, 850.

Chiarini, A. Bevivino, C. Dalmastri, S. Tabacchioni, and P. Visca, 2006. Burkholderia cepacia complex species: health hazards and biotechnological potential, Trends Microbiol. 14: 277–286.

Chilvers, M. I. and Brown-Rytlewski, D. E. 2010. First report and confirmed distribution of soybean sudden death syndrome caused by *Fusarium virguliforme* in southern Michigan. Plant Disease 94:1164.

Cordova-Kreylos, A.L., Fernandez, L.E., Koivunen, M.; Yang, A., Flor-Weiler, L., and Marrone, P.G. 2013. Isolation and characterization of *Burkholderia rinojensis* sp. nov., a non-Burkholderia cepacian complex soil bacterium with insecticidal and miticidal activities. Appl. Environ. Microbiol. 79, 7669–7678.

De Bruin, J. L. and Pedersen, P. 2008. Soybean seed yield response to planting date and seeding rate in the upper Midwest. Agronomy Journal 100:696-703.

Debora C. Ladner1, Paul B. Tchounwou1* and Gary W. Lawrence2. 2008. Evaluation of the Effect of Ecologic on Root Knot Nematode, Meloidogyne incognita, and Tomato Plant, Lycopersicon esculenum. 1Environmental Toxicology Research Laboratory, College of Science, Engineering and Technology, Jackson State University, Jackson, Mississippi 39217, USA2Department of Entomology and Plant Pathology, Mississippi State University, MS 39762, USA.

Gao, X., Jackson, T. A., Hartman, G. L. and Niblack, T. L. 2006. Interactions between the soybean cyst nematode and Fusarium solani f. sp. glycines based on greenhouse factorial experiments. Phytopathology 96:1409-1415.

Gross, H.; Loper, J. E. Nat. Prod. Rep. 2009, 26, 1408−1446.

Gongora-Canul, C. C. and Leandro, L. F. S. 2011. Effect of soil temperature and plant age at time of inoculation on progress of root rot and foliar symptoms of soybean sudden death syndrome. Plant Disease 95:436-440.

Gu, Ganyu., Leif, Smith. B., Nian, Wangc., Hui, Wang., Shi, -En. Lu. 2009. Biosynthesis of an antifungal oligopeptide in Burkholderia contaminans strain MS14. Biochemical and Biophysical Research Communications 380: 328–332.

H. J. R. Robinson., H. F. Phares, and O. E. Grassle, J. Invest. 1964. Dermatol. 42: 479.

Hershman, D. E., Hendrix, J. W., Stuckey, R. E., Bachi, P. R. and Henson, G. 1990. Influence of planting date and cultivar on soybean sudden-death syndrome in Kentucky Plant Disease 74:761-766.

Hirrel, M. C. 1983. Sudden death syndrome of soybean: A disease of unknown etiology. (Abstr.) Phytopathology 73:501-502.

Hirrel, M. C. 1986. Disease severity and yield loss comparisons of soybean maturity groups affected with Sudden Death Syndrome. Proceedings of the Southern soybean disease workers 13: 61. (Abstract).

Hirrel, M. C. 1985. Sudden Death Syndrome of soybean: Assessment of cause and severity. Proceedings of the southern soybean disease workers 14:78 (Abstr).

Janisiewicz, W.J.; Roitman, J. 1988. Biological control of blue mold and gray mold on apple and pear with Pseudomonas cepacia. Phytopathology 78, 1697–1700.

Jardine, D. J. and Rupe, J. C. 1993. First report of sudden death syndrome of soybeans causes by *Fusarium solani* in Kansas. Plant Disease 77:1264.

Jin, H., Hartman, G. L., Nickell, C. D. and Widholm, J. M. 1996. Characterization and purification of a phytotoxin produced by *Fusarium solani*, the causal agent of soybean sudden death syndrome. Phytopathology 86:277-282.

Kadir, J., M. A. Rahman., T. M. Mahmud., R. Abdul. Rahman., and M. M. Begum. 2008. Extraction of Antifungal Substances from *Burkholderia cepacia* with Antibiotic Activity against *Colletotrichum gloeosporioides* on Papaya (*Carica papaya*). International Journal of Agriculture and Biology ISSN Print: Vol: 10, 1560–8530; ISSN Online: 1814–9596.

Klink. V.P., Alkharouf. N., MacDonald. M. and Matthews. B.F. 2005. Laser capture microdissection (LCM) and analysis of Glycine max (soybean) syncytial cells formed by the soybean cyst nematode Heterodera glycines. Plant Mol. Biol. 59: 969-983.

Kurle, J. E., Gould, S. L., Lewandowski, S. M., Li, S. and Yang, X. B. 2003. First report of sudden death syndrome (*Fusarium solani* f. sp. *glycines*) of soybean in Minnesota. Plant Disease 87:449.

Lawrence, G. W., K. W. Roy, and K. S. Mclean. 1988. Soybean cyst nematode associations with sudden death syndrome of soybeans. Phytopathology 78: 1514. (Abstract).

Leahy, J. G., Byrne, A.M., Olsen, R. H. 1996. Comparison of factors influencing trichloroethylene degradation by toluene-oxidizing bacteria. Appl. Environ. Microbiol. 62:825–833.

L. V. Edgington., K. L. Khew., and G. L. Barron. 1971. Phytopatholgy 61: 42-44.

Luo, Y., Myers, O., Lightfoot, D. A. and Schmidt, M. E. 1999. Root colonization of soybean cultivars in the field by *Fusarium solani* f. sp. *glycines*. Plant Disease 83:1155- 1159.

McLean, K. S. and Lawrence, G. W. 1992. Effect of *Heterodera glycines* on the severity of Sudden Death Syndrome. Journal of Nematology 24: 606. (Abstract).

McLean, K. S. and Lawrence, G. W. 1993. Interrelationship of Heterodera glycines and Fusarium solani in sudden death syndrome of soybean. Journal of Nematology 25:434-439.

McLean, K. S. and Lawrence, G. W. 1995. Development of Heterodera glycines as affected by Fusarium solani, the causal agent of sudden death syndrome of soybean. Journal of Nematology 27:70-77.

Muclean, K.S, G, W. Lawrence., and K, W. Roy. 1990. Sudden death syndrome: scanning electron and light microscopic observations of soybean roots inoculated with Fusarium solani from A. phytopathology 80: 1043. (Abstract).

Mueller, T. A., Knake, R. P. and Riggs, J. L. 2011. Control of *Fusarium virguliforme* (sudden death syndrome) with a seed treatment. Phytopathology 101: S124.

Navi, S.S. and Yang, X. B. 2008. Foliar symptom expression in association with early infection and xylem colonization by *Fusarium virguliforme* (formerly *F. solani* f. sp. *glycines*), the causal agent of soybean sudden death syndrome. Plant Health Progress; doi:10.1094/PHP-2008-0222-01-RS.

Navi S, Yang X. Summary of 11-year field trials of using HeadsUp - a Saponin plant protectant to manage soybean diseases. 2016. APS annual meeting, July 30 - Aug 3, Tampa, Florida, U.S.A.

Pant, S.R., Matsye, P.D., McNeece, B.T., Sharma, K., Krishnavajhala, A., Lawrence, G.W. (2014) Syntaxin 31 functions in Glycine max resistance to the plant parasitic nematode Heterodera glycines Plant Mol. Biol. 85: 107-121.

Reddy, A. R., Reddy, K. R., Hodges, H. F. 1998. Interactive effects of elevated carbon dioxide and growth temperature on photosynthesis in cotton leaves. Plant Growth Regul, 26(1): 33–40.

Reddy, K. R., Kakani, V. G., Zhao, D., Koti, S., and Gao, W. 2004. Interactive effects of ultraviolet-B Radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. Photochem Photobiol, 79(5): 416–427.

Reddy, V. R., Reddy, K. R., Hodges, H. F. 1995. Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpira-tion, and water use efficiency. Field Crops Res, 41(1): 13–23.

Roy, K. W., G. W. Lawrence, and K. S. Mclean. 1988. Pathogenicity testing of *Fusarium solani* from soybean. Proceedings of the Southern Soybean Disease Workers 15: 61. (Abstract).

Roy, K. W., Lawrence, G. W., Hodges, H. H., McLean, K. S. and Killebrew, J. F. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation of *Heterodera glycines* to disease severity. Phytopathology 79:191-197.

Roy, K. W., Rupe, J. C., Hershman, D. E., and Abney, T. S. 1997. Sudden death syndrome of soybean. Plant Dis. 81:1100-1111.

Rupe, J. C. 1988. Soybean Sudden Death Syndrome cultivar testing procedures. Proceedings of the Southern Soybean Disease Workers 15: 70. (Abstract).

Rupe, J. C. 1989. Frequency and pathogenicity of *Fusarium solani* recovered from soybeans with sudden-death syndrome. Plant Disease 73:581-584.

Rupe, J. C., and G. J. Weidemann. 1986. Pathogenicity of a *Fusarium sp.* isolated from soybean plants with Sudden Death Syndrome. Phytopathology 76: 1080 (Abstr).

Scherm, H. and Yang, X. B. 1996. Development of sudden death syndrome of soybean in relation to soil temperature and soil water matric potential. Phytopathology 86:642-649.

Sciumbato, G. L., and B. L. Keeling. 1985. Sudden Death Syndrome of soybean in Mississippi in 1984. Proceedings of the Southern Soybean Disease Workers 12: 64. (Abstract).

Tande, C., Hadi, B., Chowdhury, R., Subramanian, S. and Byamukama, E. 2014. First report of sudden death syndrome of soybean caused by *Fusarium virguliforme* in South Dakota. Plant Disease 98:1012.

Tatalovic, N., Tylka, G. L. and Leandro, L. F. 2012. Influence of watering on the dynamics of *Heterodera glycines* and *Fusarium virguliforme* interaction in soybean roots. Phytopathology 102:118.

Tatalovic, N., Tylka, G. L. and Leandro, L. F. 2013. Effect of watering regime and *Fusarium virguliforme (*Fv) infection on location of soybean cyst nematode (SCN) syncytia in soybean roots. Phytopathology 103:143.

Tylka, G. L. and Marett, C. C. 2014. Distribution of the soybean cyst nematode, *Heterodera glycines*, in the United States and Canada: 1954 to 2014.Plant Health Progress. 15.2: 13-15.doi:10.1094/PHP-BR-14-0006.

Wang, X.Q., A.X. Liu1, A. Guerrero, J. Liu, X.Q. Yu, P. Deng, L. Ma, S.M. Baird,

L. Smith, X.D. Li., and S.E. L. 2015. Occidiofungin is an important component responsible for the antifungal activity of Burkholderia pyrrocinia strain Lyc2. Journal of Applied Microbiology ISSN. 1364-5072.

Weems, J. D., Zhang, G. R., Ames, K. A., Haudenshield, J. S., Hartman, G. L., Bond, J. P. and Bradley, C. A. 2011. Effect of fungicide seed treatments on *Fusarium virguliforme* and sudden death syndrome of soybean. Phytopathology 101: S246.

Westphal, A. and Xing, L. J. 2011. Soil suppressiveness against the disease complex of the soybean cyst nematode and sudden death syndrome of soybean. Phytopathology 101:878-886.

Wrather, J. A., Kendig, S. R., Anand, S. C., Niblack, T. L. and Smith, G. S. 1995. Effects of tillage, cultivar, and planting date on percentage of soybean leaves with symptoms of sudden death syndrome. Plant Disease 79:560-562.

Vial L, Groleau MC, Dekimpe V, Deziel E. 2007. *Burkholderia* diversity and versatility: an inventory of the extracellular products. J. Microbiol. Biotechnol. 17:1407–1429.

Xing, L. J. and Westphal, A. 2006. Interaction of *Fusarium solani* f. sp. *glycines* and *Heterodera glycines* in sudden death syndrome of soybean. Phytopathology 96:763-70.

Xing, L. J. and Westphal, A. 2009. Effects of crop rotation of soybean with corn on severity of sudden death syndrome and population densities of *Heterodera glycines* in naturally infested soil. Field Crops Research 112:107-117.

Yang, X. B. and Rizvi, S. S. A. 1994. First report of sudden death syndrome of soybean in Iowa. Plant Disease. 78:830.

Yu, X.Q., Xi, L.J., Liu, Y.G., Li, G.W., Lan, Y.F., Zhu, X.P. and Li, X.D. (2007) Physio-biochemical characterization and molecular identification of Burkholderia cepacia isolate Lyc2 as a PGPR to cotton seedlings. Acta Phytopathologica Sinica 37, 426–432.

Zaworski, Edward. R. 2014. Effects of ILeVO® on soybean sudden death syndrome and soybean cyst nematode. Iowa State University.

Ziems, A. D., Giesler, L. J. and Yuen, G. Y. 2006. First report of sudden death syndrome of soybean caused by Fusarium solani f. sp. glycines in Nebraska. Plant Disease 90:109- 109.

Zuniga, A., Poupin, M.J., Donoso, R., Ledger, T., Guiliani, N., Gutierrez, R.A. and Gonzalez, B. (2013) Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of Arabidopsis thaliana by Burkholderia phytofirmans PsJN. Mol Plant Microbe Interact 26, 546–553.