THESIS

CONTROL OF *MELOIDOGYNE CHITWOODI* (COLUMBIA ROOT-KNOT NEMATODE) BY MICROBIAL SOIL INOCULANTS IN POTATOES (*SOLANUM TUBEROSUM*)

Submitted by

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ABSTRACT

CONTROL OF *MELOIDOGYNE CHITWOODI* (COLUMBIA ROOT-KNOT NEMATODE) BY MICROBIAL SOIL INOCULANTS IN POTATOES (*SOLANUM TUBEROSUM*)

Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al) is a major pest in commercial potato production in the northwestern, United States of America. *M. chitwoodi* infestation is widespread throughout the potato (*Solanum tuberosum*) growing regions of the U.S and other areas of the world. *Meloidogyne* spp. causes severe crop damage and economic losses in a broad range of economically important crops. Traditionally, *M. chitwoodi* has been controlled by the applications of chemical-based soil fumigants and nematicides. Chemical based controls have shown good effect at controlling *M. chitwoodi,* but due to their human toxicity, possible damage to the environment, development of nematode resistance to chemical nematicides, decreased availability of labeled chemical nematicides and the high cost of chemical nematicides there is a need for alternative methods to control *M. chitwoodi.* Specific soil microorganisms have been found to be antagonistic and parasitic to *M. chitwoodi* and other *Meloidogyne* spp. in potatoes and several other crops. It has also been proposed that the use of soil microorganisms that are antagonistic and parasitic to plant parasitic nematodes are an essential component to long term sustainable Integrated Nematode Management (INM). Due to the agricultural need for the development of alternative control methods of *Meloidogyne* spp. in crop production worldwide two commercially available microbial soil inoculant products were tested under greenhouse and open-field conditions. The two commercially available microbial soil inoculant products that were tested are NemaRoot, which contains *Purpureocillium lilacinus* (formally known as *Paecilomyces lilacinus*) and BioFit N, which contains *Azotobacter chroccum*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus mycoides*, and *Trichoderma harzianum.* Previous findings have shown that *Bacillus subtilis, Bacillus megaterium, Trichoderma harzianum* and *Purpureocillium lilacinus* all have the ability to control *Meloidogyne* spp. to varying degrees in a number of diverse crops. The greenhouse experiments that were conducted for this research showed that NemaRoot was able to reduce *M. chitwoodi* root galling by 64% ($P < 0.001$), eggs by 74% ($P < 0.001$) to 91% ($P < 0.001$), second-stage juveniles in the substrate by 80% ($P < 0.001$), the reproductive factor by 67% ($P < 0.001$) to 80% ($P < 0.001$) and potato tuber damage by 77% ($P < 0.001$) to 82% ($P < 0.001$) in potatoes. The greenhouse experiments also showed that BioFit N was able to reduce *M. chitwoodi* root galling by 73% ($P < 0.001$, eggs by 81% ($P < 0.001$) to 97% ($P < 0.001$), second-stage juveniles in the substrate by 81% ($P < 0.001$), the reproductive factor by 82% ($P < 0.001$) to 87% ($P < 0.001$) and potato tuber damage by 78% ($P < 0.001$) to 78% ($P < 0.001$) in potatoes. The commercial open-field potato experiment showed that 2, 3 and 4 applications of BioFit N at a rate of 1.12 kg/ha per application were able to control *M. chitwoodi* tuber damage as well as 2 applications of Vydate (*Oxamyl*) at a rate of 2.2 L/ha per application. These results show that biocontrol of *M. chitwoodi* with microbial soil inoculants are an effective control method; especially, when used as a part of an Integrated Nematode Management (INM) strategy.

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Introduction

Columbia root-knot nematode (*Meloidogyne chitwoodi*; Golden, O'Bannon, Santo, & Finley, 1980) is a major soilborne pest throughout the world in cultivated crop production (Nicol, Turner, Coyne, den Nijs, Hockland, & Tahna Maafi, 2011). In 1974, an unknown root-knot nematode was first discovered in a Russet Burbank potato from Aberdeen, Idaho. Originally, this unknown root-knot nematode was classified as *Meloidogyne hapla* Chitwood. Then in 1980, Golden, O'Bannon, Santo, and Finley, classified *Meloidogyne hapla* Chitwood as a new species of *Meloidogyne*, which was then named *Meloidogyne chitwoodi. M. chitwoodi* was then also given the common name of Columbia root-knot nematode. However, old illustrations and specimens of *Meloidogyne* show evidence that *M. chitwoodi* may have been discovered as early as 1930. *M. chitwoodi* have a large host range, and they can reproduce on many families of plants, which greatly complicates its control. *M. chitwoodi* also causes major crop damage and economic losses to potato and carrot crops (European and Mediterranean Plant Protection Organization & Center for Agriculture and Bioscience International, 1997).

Meloidogyne spp. spends most of their life cycle as sedentary within the plant roots. *Meloidogyne* spp. has four juvenile stages before molting into an adult. The first-stage juveniles go through their first molt within the egg and emerge as second-stage juveniles. The second-stage juveniles are free living in the soil and infect the root by penetrating the zone of elongation. They then move intercellularly to the zone of differentiation. At this point, the second-stage juvenile *Meloidogyne* spp. triggers the formation of five to seven giant cells, which are commonly referred to as a root gall. Once the giant cell feeding site is established the

Meloidogyne spp. goes through three more molts to develop into their adult stage. Female *Meloidogyne* spp. remain sedentary within the root and lay their eggs on the outside surface of the roots in a gelatinous matrix. Male *Meloidogyne* spp. regain their ability to move once they reach the adult stage, when they then leave the root to live in the rhizosphere (Williamson & Hussey, 1996). *M. chitwoodi* adult males are most likely functionless as in other *Meloidogyne* spp. and reproduction is parthenogenetic (European and Mediterranean Plant Protection Organization & Center for Agriculture and Bioscience International, 1997).

M. chitwoodi eggs and second-stage juveniles can survive sub-freezing over wintering temperatures without any host roots being present. *M. chitwoodi* low temperature development threshold is 5°C and has an optimal developmental temperature of 25°C. The over wintered *M. chitwoodi* population takes 600 – 800 Celsius degree-days from the time plant roots are present for the first generation of eggs to hatch. For field grown potatoes, *M. chitwoodi* takes 950 – 1,100 Celsius degree-days from the time of potato seed piece planting for the first generation of eggs to hatch. Each additional generation during that growing season takes 500 – 600 Celsius degree-days to hatch (Table 1.1).

Table 1.1. *M. chitwoodi* Degree-Days Development

Host: Russet Burbank Potatoes	DD (°F)	DD (°C)
Air temperatures were used in degree-day calculations		
Tuber initiation:	810-900	450-500
Ow females produce egg masses:	1080-1440	600-800
Second generation hatch:	1710-1980	950-1100
Juveniles II in tubers (1st generation)	1778-2099	988-1166
Third generation hatch:	2700-2880	1500-1600
First generation time:	1800	1000
Subsequent generation time:	900-1080	500-600

Source: Pinkerton, Santo, and Mojtahedi (1991)

M. chitwoodi above ground symptoms may be difficult to detect and may appear similar to plants that have a damaged or unhealthy root system. In general, *Meloidogyne* spp. symptoms are plant stunting, lack of vigor, wilting and yield losses. There can also be secondary infections of the roots by pathogens due to the damage to the roots that is caused by the nematodes (Perry, Moens, & Starr, 2010). In potatoes, *M. chitwoodi* not only infest the plant roots, but they also infest the potato tubers. The infestation of the potato tubers causes raised bumps on the outside surface of the tuber, and brown spots in the upper 5.25 mm of the vascular tissue of the tuber (Elling, 2013). The potato tuber defects caused by *M. chitwoodi* can substantially lower the value of a crop, or make the crop unsellable for the fresh market, processing market and potato seed tuber market (Ingham, Zink, & David, 2002).

Traditionally, *M. chitwoodi* has been controlled with conventional soil fumigants, such as, Telone II (*1,3-dichloropropene*) and nematicides, such as, Vydate (*Oxamyl*). These and other conventional chemical controls can have good efficacy against *Meloidogyne* spp., but they pose a high risk to human health, can cause environmental damage and they have the potential to allow development of nematode resistance (Hafez & Sundararaj, 2007). Due to these factors, it has been recommended to employ an Integrated Nematode Management (INM) strategy to best control plant parasitic nematodes in crop production. INM is a sustainable approach that employs the combined use of biological, physical and chemical tools to control plant parasitic nematodes within their economic threshold for a specific crop (Hafez & Sundararaj, 2007; Roberts, 1993). Microbial soil inoculants have been shown to be effective as a biological control agent for the control of *Meloidogyne* spp. in several crops (Mohamed, Allam, & Barakat, 2012). Some non-pathogenic soilborne bacteria and fungi are antagonistic and parasitic against *Meloidogyne* spp. (Lamovšek, Urek, & Trdan, 2013). The plant growth promoting rhizobacteria, *Bacillus subtilis* and *Bacillus megaterium* are antagonistic towards many species of *Meloidogyne* spp. *Bacillus subtilis* and *Bacillus megaterium* and other rhizobacteria can produce toxic nematicidal compounds and induce plant defense responses (Lamovšek, Urek, & Trdan, 2013). These rhizobacteria can also physically prevent second-stage juvenile *Meloidogyne* spp. from penetrating the roots and lower the concentration of root exudate compounds that plant parasitic nematodes use to find plant roots in the rhizosphere (Lamovšek, Urek, & Trdan, 2013). Several soil fungi can produce nematicidal compounds and parasitize *Meloidogyne* spp. eggs and juveniles. Two of the most used soil fungi to control *Meloidogyne* spp. are *Purpureocillium lilacinus* (formerly known as *Paecilomyces lilacinus*) and *Trichoderma* spp. (Lamovšek, Urek, & Trdan, 2013).

Greenhouse Experiment 1 and Greenhouse Experiment 2 were conducted to test the efficacy of two microbial soil inoculants to control *M. chitwoodi* in potatoes (*Solanum tuberosum*). In these two experiments, a single species microbial soil inoculant labeled NemaRoot, comprised of *Purpureocillium lilacinus* ($2x10^9$ UFC/g) (https://www.innovakglobal.com/en/nemaroot-mexico/) was tested side by side for control of *Meloidogyne* spp. with a multi-species microbial soil inoculant labeled BioFit N, comprised of *Azotobacter chroccum* ($1x\ 10^5$ CFU/g), *Bacillus subtilis* ($1x\ 10^8$ UFC/g), *Bacillus megaterium* ($1x\ 10^6$ CFU/g), *Bacillus mycoides* ($1x\ 10^5$ CFU/g), and *Trichoderma harzianum* ($1x\ 10^6$ CFU/g) (https://www.innovakglobal.com/en/biofitn-eua/). The first aim of these greenhouse experiments was to determine if microbial soil inoculants could control the Reproductive Factor of *M. chitwoodi*, which is defined with the following equation [R_f = final population (P_f)/initial population (P_i)] (Brown, Mojtahedi, Santo, & Williamson, 1997), in potatoes (*Solanum tuberosum*). The second aim of the experiments was to test the hypothesis that a multi-species microbial soil inoculant could be more effective at controlling the Reproductive Factor of *M. chitwoodi* than a single species microbial soil inoculant. This is due to different microorganisms having the potential to control *M. chitwoodi* at different developmental life stages, and thus, allowing for greater overall control of *M. chitwoodi* (Lamovšek, Urek, & Trdan, 2013).

Materials and Methods

Greenhouse Experiment 1

In 2014, Greenhouse Experiment 1 was conducted on Silverton potato plantlets in 15.24 cm diameter pots with 1.50 L of a 2/3 sterilized sand and 1/3 sterilized vermiculite by volume substrate. There were two controls and two microbial inoculant treatments with 15 repetitions each laid out in a complete randomized block design within the greenhouse. Control 1 had no products applied to it and it was inoculated with zero *M. chitwoodi* eggs. Control 2 had no product applied to it and it was inoculated with 1,000 *M. chitwoodi* eggs at planting. Treatment 1 was inoculated with 1,000 *M. chitwoodi* at planting and treated with NemaRoot. Treatment 2 was inoculated with 1,000 *M. chitwoodi* at planting, and treated with BioFit N. Both NemaRoot and BioFit N treatments were applied at a rate of 2.0 g per 1.0 L of distilled water to their respective treatments at two-week intervals starting at planting. Each treatment received a total of four inoculations with their respective microbial inoculant product. The greenhouse environmental temperature was maintained between 16^{o}C - 25^{o}C. All controls and treatments were irrigated with Plant Marvel 14-4-14 **Cal-Mag Special** fertilizer at a rate of 100 ppm nitrogen, as needed. The experiment was grown in the greenhouse for 7 weeks for a total of 1,000 Celsius degree-days of *M. chitwoodi* development.

At the conclusion of the experiment all plants had their growing substrate carefully washed from the plant’s roots. Then all plant roots were evaluated for number of galls per root, number of *M. chitwoodi* eggs per root and the number of *M. chitwoodi* second-stage juveniles per 250 cc of substrate. *M. chitwoodi* second-stage juveniles were extracted from 250 cc of substrate taken from each plant pot according to the procedure laid out by Coyne, Nicol, and Claudius-Cole (2009), and counted under a dissecting microscope at 20 times magnification. *M. chitwoodi* eggs were extracted by using a procedure from Hussey and barker and counted under a dissecting microscope at 30 times magnification (Hussey & Barker, 1973). *M. chitwoodi* reproductive factor was then calculated for the controls and treatments with the following equation. Reproductive factor [R_f = final population (P_f)/initial population (P_i)] (Brown, Mojtahedi, Santo, & Williamson, 1997).

Greenhouse Experiment 2

In 2015, Greenhouse Experiment 2 was conducted on potato plants started from greenhouse grown certified pest and disease free Norkotah 8 mini seed tubers in 30.50 cm diameter pots with 8 L of 1/3 peat moss, 1/3 vermiculite and 1/3 sand by volume of substrate that was amended with 2.10 g of Osmocote Classic 14-14-14 per liter of substrate. There were three controls and six treatments with 20 repetitions each. Control 1 had no products applied to it and it was inoculated with zero *M. chitwoodi* eggs. Control 2 had no product applied to it and it was inoculated with 1,000 *M. chitwoodi* eggs at planting. Control 3 had no product applied to it and it was inoculated with 5,000 *M. chitwoodi* eggs at planting. Treatment 1 was inoculated with zero *M. chitwoodi* eggs at planting and treated with NemaRoot. Treatment 2 was inoculated with 1,000 *M. chitwoodi* eggs at planting and treated with NemaRoot. Treatment 3 was inoculated with 5,000 *M. chitwoodi* eggs at planting and treated with NemaRoot. Treatment 4 was inoculated with zero *M. chitwoodi* eggs at planting, and treated with BioFit N. Treatment 5 was inoculated with 1,000 *M. chitwoodi* eggs at planting, and treated with BioFit N. Finally, Treatment 6 was inoculated with 5,000 *M. chitwoodi* eggs at planting, and treated with BioFit N. The NemaRoot and BioFit N treatments were applied at the rate of 0.014 g of its respective microbial product with 1,573 ml of distilled water per plant to its respective treatments. This rate is the equivalent of 1.12 kg per hectare with 1.27 cm per hectare, which is the manufactures recommended field application rate. Each treatment was applied with its respective microbial product at three-week intervals starting at 10 days after planting. The experiment was grown in the greenhouse for 12 weeks to allow for the proper maturation time of the tubers of the Norkotah 8 cultivar for a total of 1,487 Celsius degree-days of *M. chitwoodi* development. The greenhouse environmental temperature was maintained between 16°C - 25°C. All controls and treatments were irrigated with Plant Marvel 14-4-14 **Cal-Mag Special** fertilizer at a rate of 100 ppm nitrogen from week one through week five of the experiments, as needed. All controls and treatments were also irrigated with Winfield, Gainer 10-20-30 fertilizer at a rate of 100 ppm nitrogen from week six through week ten of the experiments, as needed. For week eleven through week twelve the experiment was irrigated with tap water only, as needed.

At the end of the twelve-week experiment each of the eight blocks were harvested separately. Then the controls and treatments were evaluated for *M. chitwoodi* second-stage juveniles per 500 cc of substrate, *M. chitwoodi* eggs per 1.0 g of root, *M. chitwoodi* reproductive factor and *M. chitwoodi* tuber damage. The 500 cc samples of substrate from the experiment were shipped to Western Laboratories in Parma, ID for the quantification of *M. chitwoodi* second-stage juveniles. *M. chitwoodi* eggs were extracted by using a procedure from Hussey and Barker (1973) and counted under a dissecting microscope at 30 times magnification. *M. chitwoodi* reproductive factor was then calculated for the controls and treatments with the following equation. Reproductive factor [R_f = final population (P_f)/initial population (P_i)] (Brown, Mojtahedi, Santo, & Williamson, 1997). Tubers collected from the experiment were first incubated at 22.2°C for an eight-week period. Then three tubers per plant were randomly selected for *M. chitwoodi* tuber damage evaluation. The selected tubers were peeled by hand and *M. chitwoodi* infection sites were counted under a magnifying lamp (Pinkerton, Santo, Ponti, & Wilson, 1986).

Statistical Analysis

Both Greenhouse Experiment 1 and Greenhouse Experiment 2 utilized a randomized complete block design. A two-way ANOVA analysis and a Tukey Honest Significant Difference post-hoc test was performed on all data points collected across the treatment groups to determine statistical significances of each treatment and the control.

Results

Greenhouse Experiment 1

In this experiment *M. chitwoodi* root galling was reduced by 73% by the BioFit N treatment over the control ($P < 0.001$) and by 64% by the NemaRoot treatment over the control ($P < 0.001$) (Figure 1.1). There was no statistically significant difference found between the BioFit N and NemaRoot treatments for the *M. chitwoodi* root galling data set in this experiment ($P = 0.800$) (Figure 1.1).

After the *M. chitwoodi* galls per root were analyzed the *M. chitwoodi* eggs were extracted from the roots and counted. *M. chitwoodi* eggs per root were reduced by 97% by the BioFit N treatment over the control ($P < 0.001$), and the NemaRoot treatment reduced *M. chitwoodi* eggs per root by 91% over the control ($P < 0.001$) (Figure 1.2). There was no statistically significant difference found between the BioFit N and NemaRoot treatments for the *M. chitwoodi* eggs per root data set in this experiment ($P = 0.989$) (Figure 1.2).

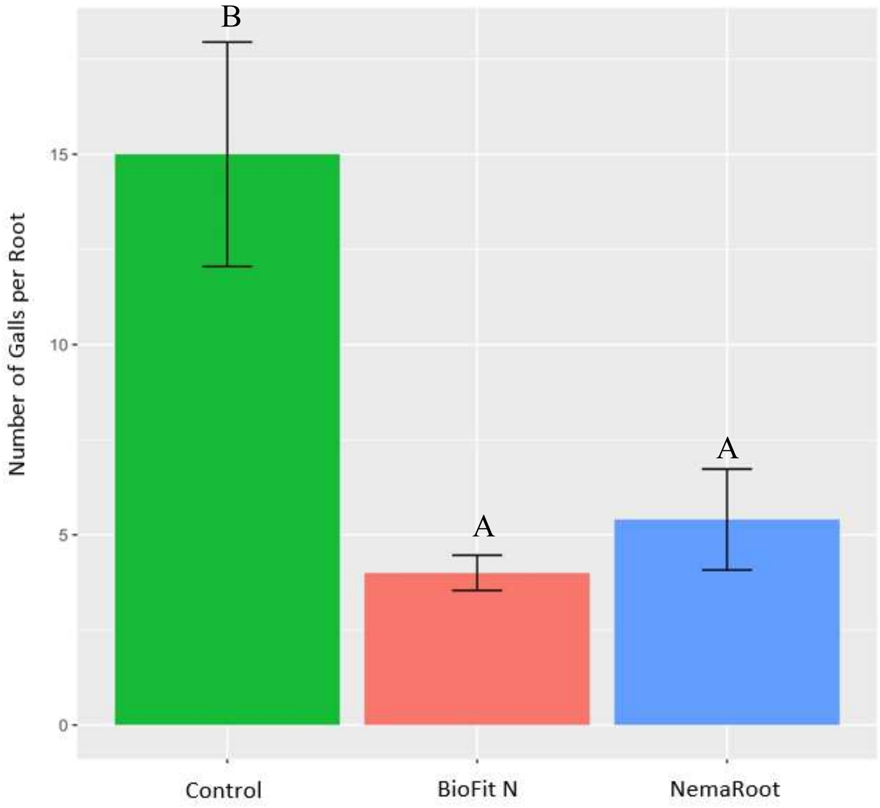

Figure 1.1. Root gall index.

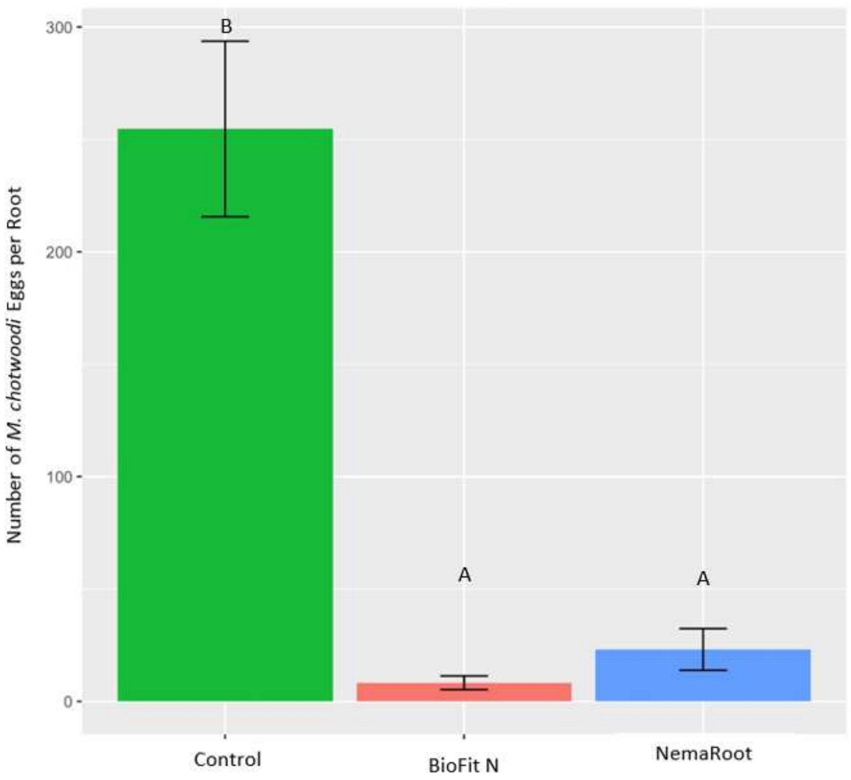

Figure 1.2. ***M. chitwoodi*** **eggs per roots.**

The *M. chitwoodi* second-stage juveniles (J2) were extracted from the substrate samples and counted. After counting the *M. chitwoodi* second-stage juveniles (J2) under a dissecting microscope it was found that the BioFit N treatment reduced *M. chitwoodi* second-stage juveniles (J2) by 81% over the control ($P < 0.001$) (Figure 1.3). The NemaRoot treatment reduced *M. chitwoodi* second-stage juveniles (J2) by 80% over the control ($P < 0.001$) (Figure 1.3). There was no statistically significant difference found between the BioFit N and NemaRoot treatments for the *M. chitwoodi* second-stage juveniles (J2) data set in this experiment ($P = 0.989$) (Figure 1.3).

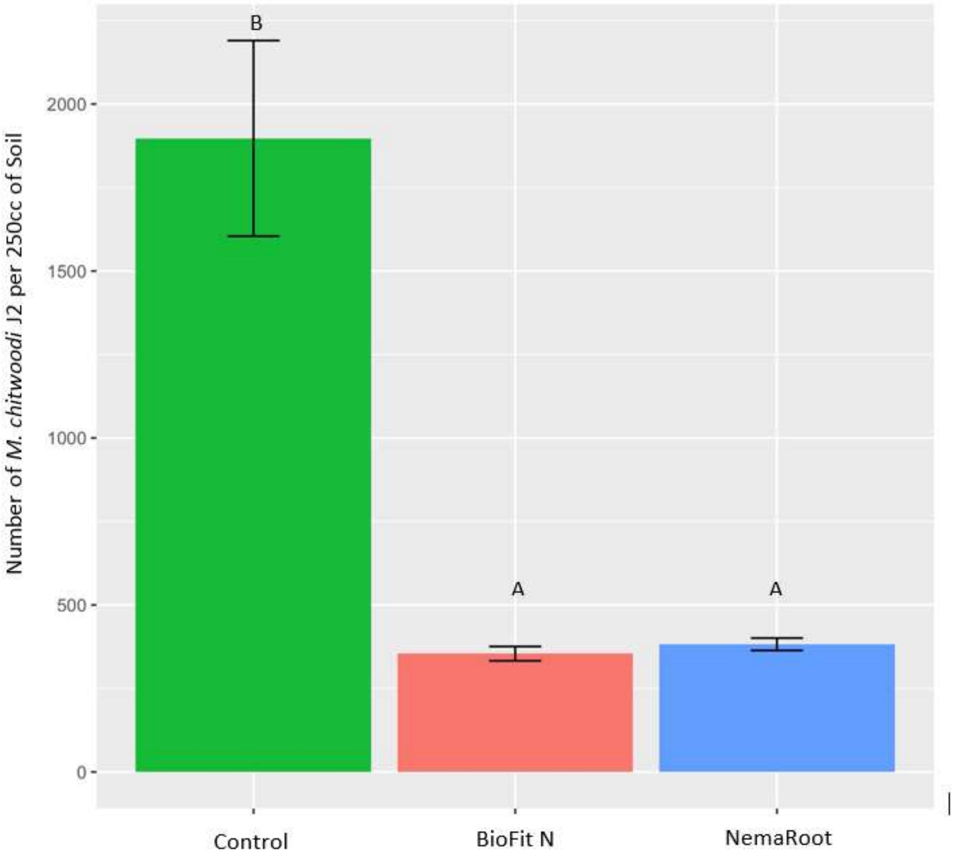

Figure 1.3. ***M. chitwoodi*** **second-stage juveniles (J2) per 250 cc of substrate.**

M. chitwoodi reproductive factor was then calculated for the controls and treatments. It was found that the BioFit N reduced *M. chitwoodi* reproductive factor by 82% over the control ($P < 0.001$) (Figure 1.4). The *M. chitwoodi* reproductive factor was reduced 80% by the NemaRoot treatment over the control ($P < 0.001$) (Figure 1.4). There was no statistically significant difference found between the BioFit N and NemaRoot treatments for the *M. chitwoodi* reproductive factor data set in this experiment ($P = 0.986$) (Figure 1.4).

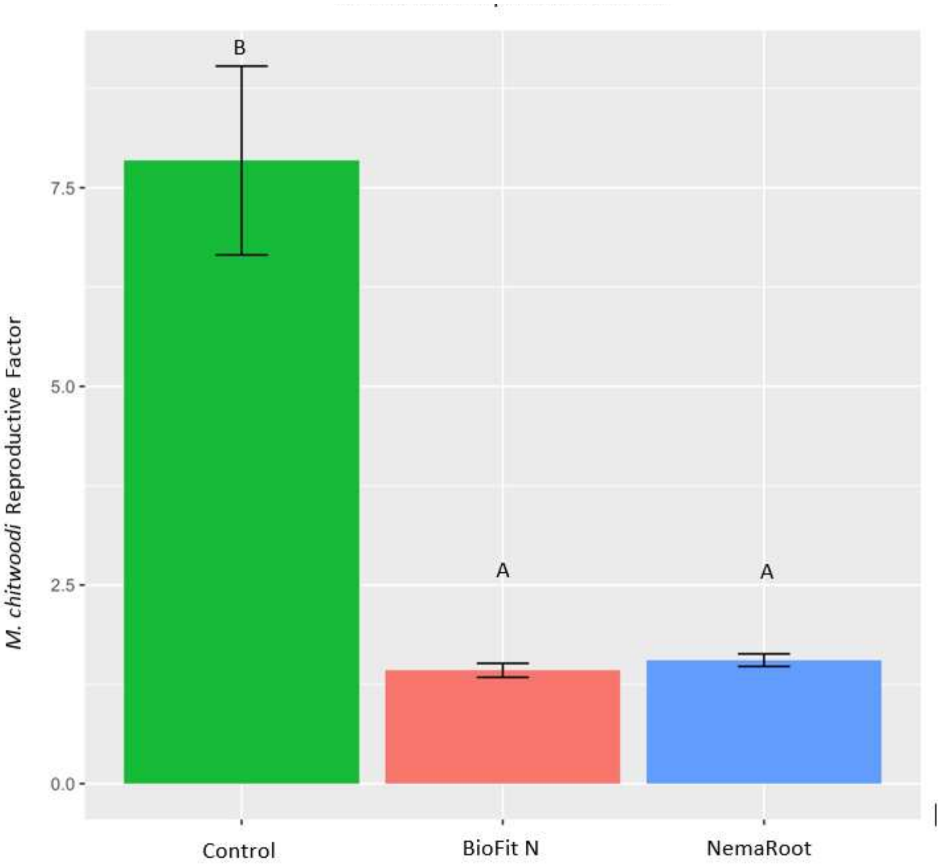

Figure 1.4. *M. chitwoodi* reproduction factor.

Greenhouse Experiment 2

M. chitwoodi damage to the potato tubers for the controls and the treatments were evaluated for both the 1,000 and 5,000 *M. chitwoodi* egg inoculum rates. It was found that the BioFit N treatments reduced *M. chitwoodi* tuber damage by 78% ($P < 0.001$) for the 1,000 *chitwoodi* egg inoculum rate (Figure 1.5) and by 78% (*P* < 0.001) for the 5,000 *chitwoodi* egg inoculum rate over their respective controls (Figure 1.6). NemaRoot treatments reduced *M. chitwoodi* tuber damage by 77% (*P* < 0.001) for the 1,000 *chitwoodi* egg inoculum rate (Figure 1.5) and by 82% (*P* <0.001) for the 5,000 *chitwoodi* egg inoculum rate over their respective controls (Figure 1.6). There was a Block effect at the 1,000 *M. chitwoodi* egg inoculation level, which means that the difference between these treatments would be greater or smaller depending on when the blocks were harvested. There was no statistically significant difference found between the BioFit N and NemaRoot treatments for *M. chitwoodi* tuber damage for these data sets for both the 1,000 (*P* = 0.990) and 5,000 (P = 0.884) *M. chitwoodi* egg inoculum rates (Figures 1.5 and Figure 1.6.).

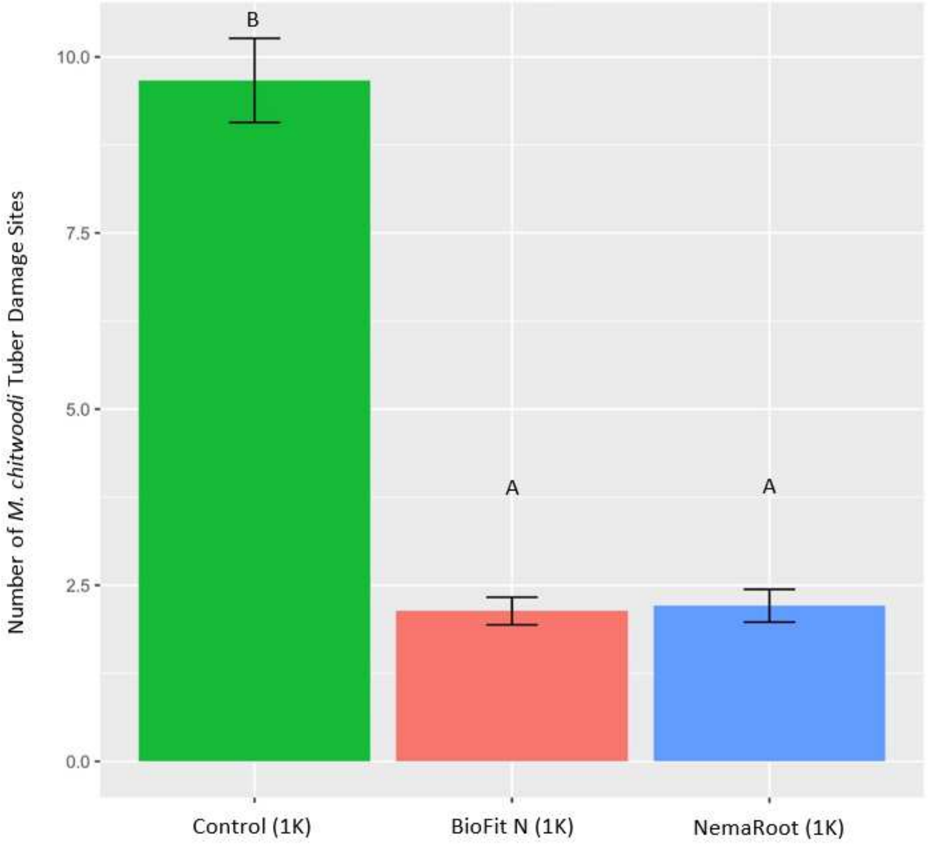

Figure 1.5. ***M. chitwoodi* tuber damage at the 1,000 *M. chitwoodi* egg inoculum rate.**

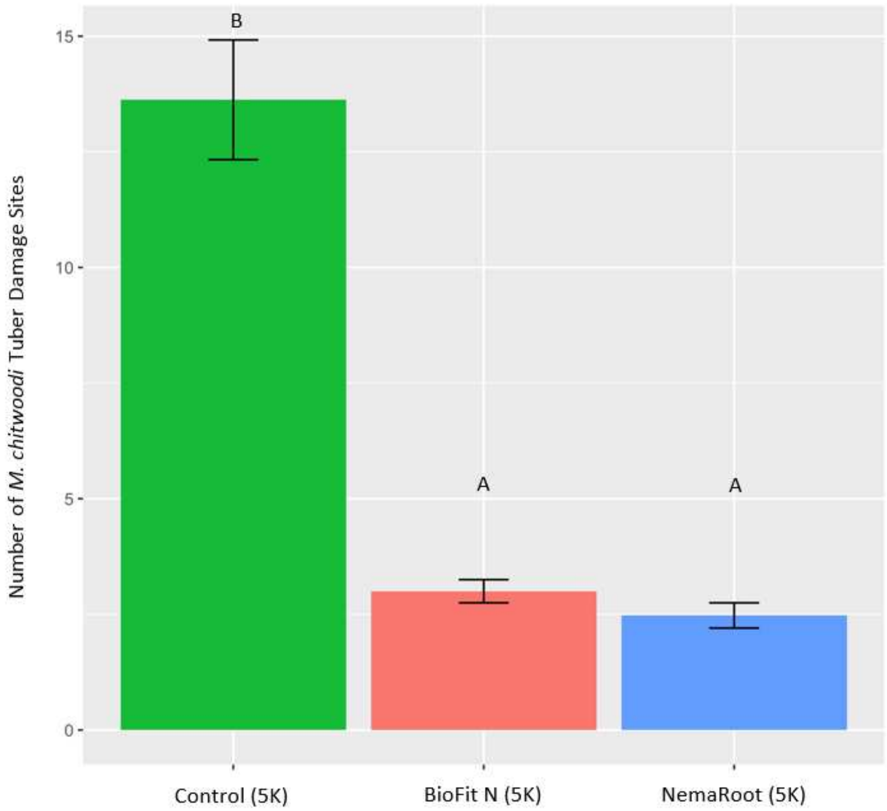

Figure 1.6. *M. chitwoodi* tuber damage at the 5,000 *M. chitwoodi* egg inoculum rate.

M. chitwoodi eggs per 1 gram of root for the controls and the treatments were evaluated for both the 1,000 and 5,000 *M. chitwoodi* egg inoculum rates. It was found that the BioFit N treatments reduced *M. chitwoodi* eggs per 1 gram of root by 83% ($P < 0.001$) for the 1,000 *chitwoodi* egg inoculum rate (Figure 1.7) and by 81% ($P <0.001$) for the 5,000 *chitwoodi* egg inoculum rate over their respective controls (Figure 1.8). NemaRoot treatments reduced *M. chitwoodi* eggs per 1 gram of root by 80% ($P < 0.001$) for the 1,000 *chitwoodi* egg inoculum rate (Figure 1.7) and by 74% (p-value <0.001) for the 5,000 *chitwoodi* egg inoculum rate over their respective controls (Figure 1.8). There was no statistically significant difference found between the BioFit N and NemaRoot treatments for the *M. chitwoodi* eggs per 1 gram of root data sets for both the 1,000 ($P = 0.916$) and 5,000 ($P = 0.371$) *M. chitwoodi* egg inoculum rates (Figure 1.7 and Figure 1.8).

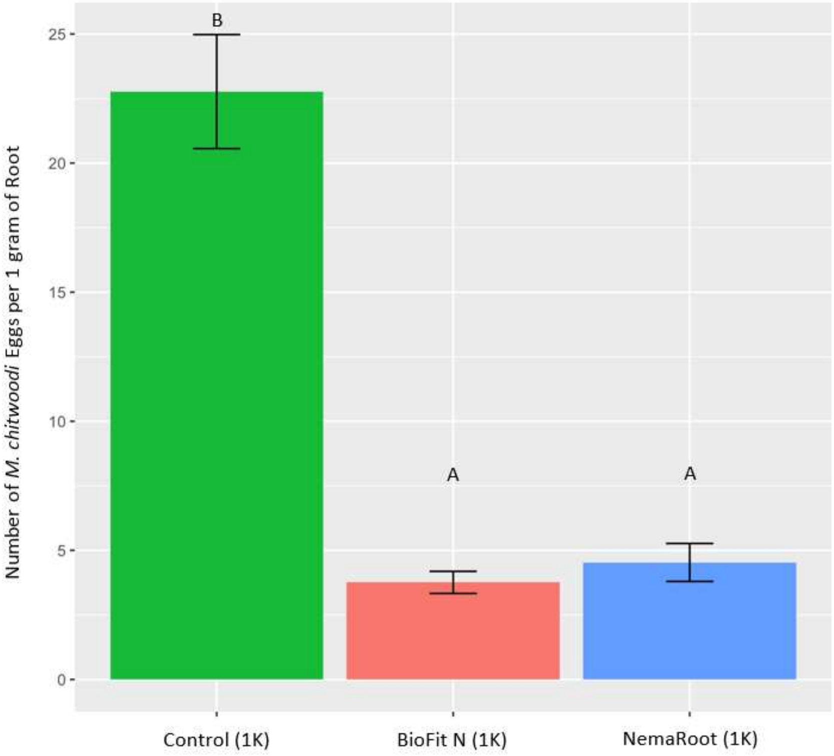

Figure 1.7. ***M. chitwoodi*** **eggs per 1 gram of root at the 1,000** ***M. chitwoodi*** **egg inoculum rate.**

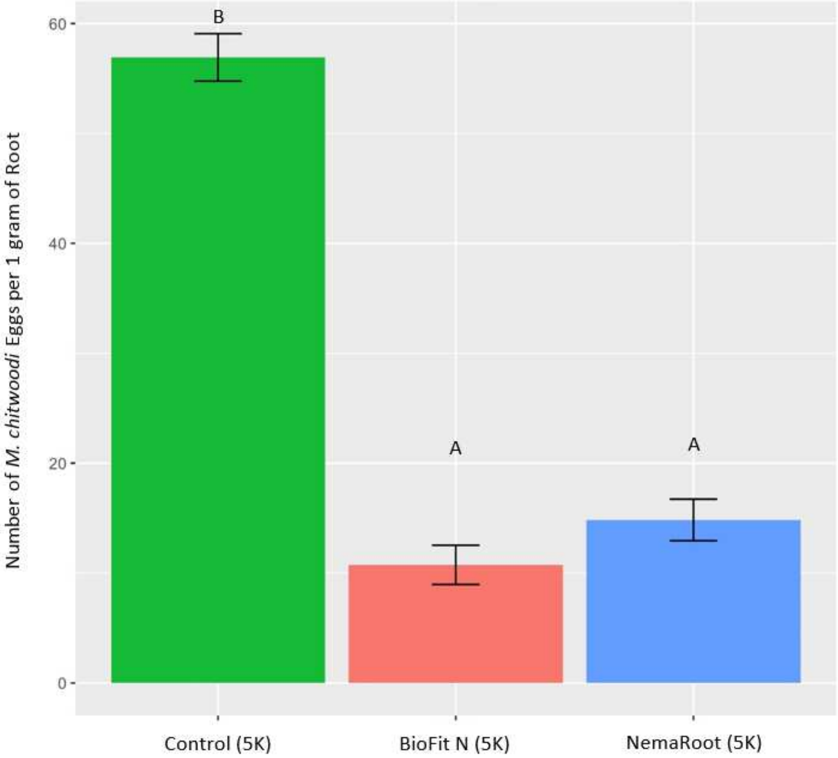

Figure 1.8. ***M. chitwoodi*** **eggs per 1 gram of root at the 5,000** ***M. chitwoodi*** **egg inoculum rate.**

M. chitwoodi reproductive factor for the controls and the treatments were evaluated for both the 1,000 and 5,000 *M. chitwoodi* egg inoculum rates. It was found that the BioFit N treatments reduced *M. chitwoodi* Reproductive Factor by 87% ($P < 0.001$) for the 1,000 *chitwoodi* egg inoculum rate (Figure 1.9) and by 81% ($P < 0.001$) for the 5,000 *chitwoodi* egg inoculum rate over their respective controls (Figure 1.10). NemaRoot treatments reduced *M. chitwoodi* Reproductive Factor by 80% ($P < 0.001$) for the 1,000 *chitwoodi* egg inoculum rate (Figure 1.9) and by 67% ($P < 0.001$) for the 5,000 *chitwoodi* egg inoculum rate over their respective controls (Figure 1.10).

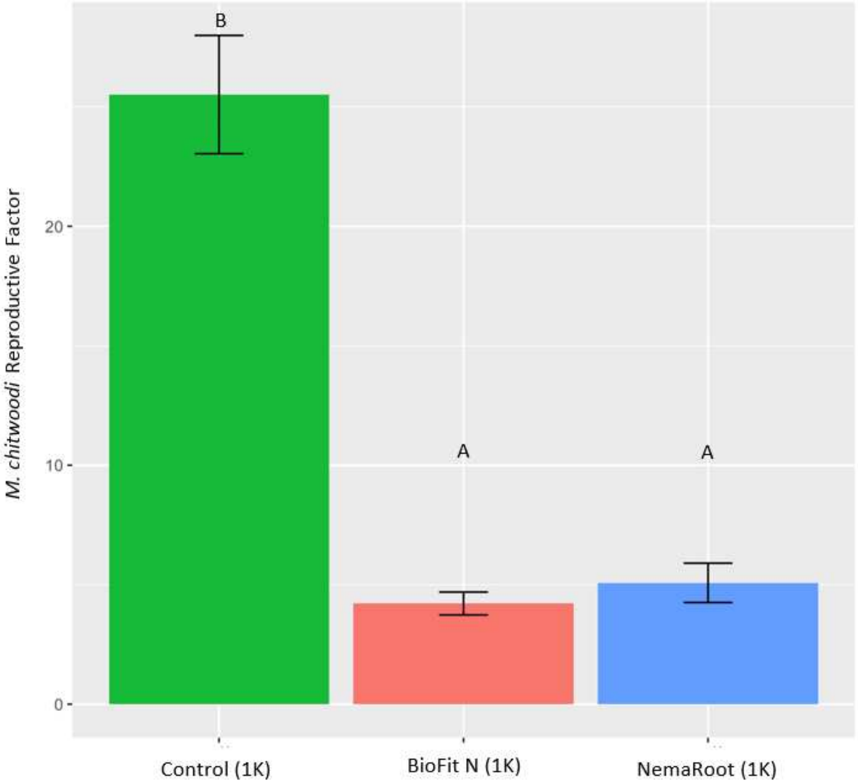

Figure 1.9. *M. chitwoodi* reproductive factor at the 1,000 *M. chitwoodi* egg inoculum rate.

There was no statistically significant difference found between the BioFit N and NemaRoot treatments for the *M. chitwoodi* Reproductive Factor data sets for both the 1,000 ($P = 0.915$) and 5,000 ($P = 0.370$) *M. chitwoodi* egg inoculum rates (Figure 1.9 and Figure 1.10).

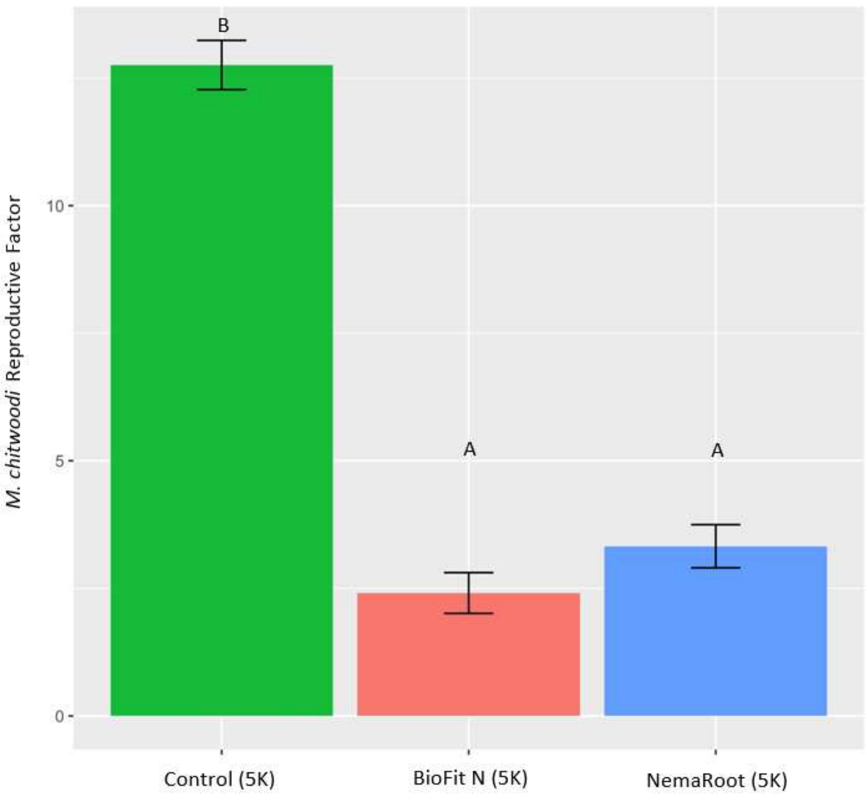

Figure 1.10. *M. chitwoodi* reproductive factor at the 5,000 *M. chitwoodi* egg inoculum rate.

Discussion

The results from these greenhouse experiments show that BioFit N, which is composed of *Azotobacter chroccum* 1x 10^5 CFU/g, *Bacillus subtilis* 1x 10^8 UFC/g, *Bacillus megaterium* 1x 10^6 CFU/g, *Bacillus mycoides* 1x 10^5 CFU/g, and *Trichoderma harzianum* 1x 10^6 CFU/g and NemaRoot (*Purpureocillium lilacinus* 2x10^9 UFC/g) were able to reduce the number of *M. chitwoodi* eggs on the roots, second-stage juveniles in the soil substrate, *M. chitwoodi* Reproductive Factor, root galling and *M. chitwoodi* tuber damage in potatoes (*Solanum tuberosum*). Another important result of these experiments is that BioFit N and NemaRoot were able to control *M. chitwoodi* potato tuber damage within the economic damage threshold for the sale of potato tubers in the U.S. wholesale fresh eating potato tuber market, which is defined as less than 6 *M. chitwoodi* individual damage sites per tuber (Pinkerton, Santo, Ponti, & Wilson, 1986). Whereas the tubers from the untreated controls scored above the *M. chitwoodi* economic tuber damage threshold for the sale of potato tubers in the U.S. wholesale fresh eating potato tuber market. These results also show great potential for the control of *M. chitwoodi* by microbial soil inoculants that contain microorganisms that are parasitic and antagonistic to *M.* chitwoodi, especially when used within an INM control strategy.

The findings of the two greenhouse experiments are supported by similar experiments that have investigated the control of *M. chitwoodi* and other *Meloidogyne* spp. by the application of *Bacillus subtilis, Bacillus megaterium, Trichoderma harzianum* and *Purpureocillium lilacinus* (formally known as *Paecilomyces lilacinus*) (Khalil, Allam, & Barakat, 2012; Padgham & Sikora, 2006; Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012). *Bacillus subtilis* and other similar rhizobacteria have been shown to act as plant-growth promoters. *Bacillus subtilis* and rhizobacteria have the ability to control *Meloidogyne* spp. through nematode antagonism and by increasing plant defense and resistance responses in the host plant (Lamovšek, Urek, & Trdan, 2013). Previous experiments have shown that *Bacillus subtilis* can reduce *M. incognita* egg populations on the roots by 72% and second-stage juveniles in the soil by 60% in tomato plants (*Solanum lycopersicum*) grown under greenhouse conditions (Khalil, Allam, & Barakat, 2012). Additionally, *Bacillus subtilis* has the ability to reduce egg hatching and to cause mortality of 50% in *M. javanica.* The same study found that *Bacillus subtilis* was able to reduce root galling caused by *M. chitwoodi,* and an increase in shoot length, shoot weight, root length and root weight in cowpea (*Vigna unguiculata subsp. Unguiculata*) and mash bean (*Vigna mungo* L.) (Dawar, Tariq, & Zaki, 2008). The Rhizobacteria, *Bacillus megaterium* has been found to reduce root penetration and gall formation by 40% and egg hatching by 60% on *M. graminicola* in rice (*Oryza sativa*) (Padgham & Sikora, 2006). *Bacillus megaterium* has also reduced the population of *M. chitwoodi* in potatoes (*Solanum tuberosu*) by 50%, and to have the ability to control *M. exigua* in coffee (*Coffea* spp.) and *M. javanica* in sunflower (*Helianthus* spp.) (Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012). *Bacillus megaterium* can produce nematicidal compounds that significantly inhibit egg hatching and reduces juvenile infection of *M. incognita* (Huang, Xu, Ma, Zhang, Duan, & Mo, 2010). *Trichoderma* harzianum has the ability to parasitize eggs and larvae of *Meloidogyne* spp. by breaking down the cuticle layer with enzymes, such as, chitinases, glucanases and proteases. *Trichoderma harzianum* produces toxic nematicidal compounds and reproduces within the nematode egg and juveniles host body (Sharma & Pandey, 2009). *Trichoderma harzianum* has been found to decrease *M. incognita* root galling by 70% to 97%, and second-stage juveniles by 21% to 98% in tomato plants (*Solanum lycopersicum*) (Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012). *Trichoderma harzianum* also reduced *M. incognita* root galling by up to 39% and parasitization of females by up to 51% in eggplants (*Solanum melongena*) (Rao, Parvatha Reddy, & Nagesh, 1998). Other studies have shown that *Trichoderma harzianum* can reduced *M. incognita* root galling by an average of 24% and egg masses by an average of 28% in okra (*Abelmoschus esculentus*) (Mukhtar, Arshad Hussain, & Zameer Kayani, 2013). *Purpureocillium lilacinus* was first discovered by Jatala et al. at the International Potato Center in Lima, Peru in 1979 (as cited in Khalil, Allam, & Barakat, 2012). *Purpureocillium lilacinus* has been shown to have great potential as a biological control of plant parasitic nematodes due to its ability to parasitize *Meloidogyne* spp. eggs and because it can colonize soil organic matter in addition to the rhizosphere. *Purpureocillium lilacinus* fungi is parasitic to *Meloidogyne* spp. eggs by direct hyphal penetration of the eggshell. It is believed that *Purpureocillium lilacinus* parasitism is connected with its ability to produce the enzyme serine protease, which has been shown to have nematicidal properties. The enzyme serine protease degrades *Meloidogyne* spp. eggshells and can prevent egg hatching (Sharma & Pandey, 2009). In the laboratory *Purpureocillium lilacinus* has been shown to infest and destroy *M. incognita* eggs within 5 days (Khalil, Allam, & Barakat, 2012). *Purpureocillium lilacinus* also produces a peptidal antibiotic P-168 that allows it to have antimicrobial properties against other fungi, yeast, and gram-positive bacteria (Mukhtar, Arshad Hussain, & Zameer Kayani, 2013). It has been found that *Purpureocillium lilacinus* reduced *M. incognita* root galling by 88%, egg masses by 77% and second-stage juveniles by 76% in tomato plants (*Solanum lycopersicum*) (Mohamed, Arshad Hussain, & Zameer Kayani, 2012). Four different isolates of *Purpureocillium lilacinus* have also been tested for their ability to control *M. javanica* in tomato plants (*Solanum lycopersicum*), which resulted in a reduction in the reproduction factor by 65%, 44%, 42% and 29% (Sabet, Olia, Sharifnabi, & Tehrani, 2013). Additionally, one pre-plant application of *Purpureocillium lilacinus* has been found to reduce *M.* incognita root galling by 66% and egg masses by 74% in tomato plants (*Solanum lycopersicum*), and it allowed for sufficient control to obtain commercially acceptable yields (Seid, Fininsa, Mekete, Decraemer, & Wesemael, 2015). *M. incognita* control in okra (*Abelmoschus esculentus*) by the application of *Purpureocillium lilacinus* has been reported to reduce egg masses by 30% and to reduce root galling by 27% (Mukhtar, Arshad Hussain, & Zameer Kayani, 2013).

The findings of these greenhouse experiments were able to show that microbial soil inoculants that contain *Bacillus subtilis, Bacillus megaterium, Trichoderma harzianum* and *Purpureocillium lilacinus* are able to control *M. chitwoodi* within the economic threshold for potato (Solanum tuberosum) crop production under greenhouse conditions. Previous findings support the efficacy of using microbial soil inoculants that contain *Bacillus subtilis, Bacillus* *megaterium, Trichoderma harzianum* and *Purpureocillium lilacinus* is able to provide effective control of *Meloidogyne* spp. within the economic threshold for a variety of agricultural crops; especially when INM approaches are utilized. These experiments did not show statistically significant results that a multi-species microbial soil inoculant (BioFit N) was able to control *M. chitwoodi* more effectively than a single species microbial soil inoculant (NemaRoot). Although, many of the results did show a trend suggesting that a multi-species microbial soil inoculant (BioFit N) may be able to provide greater control of *M. chitwoodi* than a single species microbial soil inoculant (NemaRoot). This is most likely due to the microbes in BioFit N having the potential to control *M. chitwoodi* at multiple developmental life stages. Future research is still needed to provide the best INM practices for specific agricultural crops that address *Meloidogyne* species present, environmental conditions, cropping system and the technology of the crop producers (Seid, Fininsa, Mekete, Decraemer, & Wesemael, 2015).

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CONTROL OF *MELOIDOGYNE CHITWOODI* (COLUMBIA ROOT-KNOT NEMATODES) IN COMMERCIALLY GROWN RUSSET NORKOTAH POTATOES WITH BIOFIT N, A MICROBIAL SOIL INOCULANT, IN THE SAN LUIS VALLEY, COLORADO

Introduction

Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden, O'Bannon, Santo, & Finley, 1980) is a major pest in commercial potato production in the northwestern, United States of America. *M. chitwoodi* infestation is widespread throughout the potato growing regions of California, Colorado, Idaho, Oregon, Utah and Washington (Nyczepir, O'Bannon, Santo, & Finley, 1982; Pinkerton & McIntyre, 1987; Santo, O'Bannon, Finley, & Golden, 1980). *M. chitwoodi* have also been detected in Argentina, Mexico, South Africa, and Turkey (Elling, 2013). In 2014, Colorado planted 24,373 ha of potatoes with 21,943 ha planted in the San Luis Valley of Colorado. The total Colorado potato crop value in 2014 has been calculated to be worth $214,802,000 (USDA NASS Mountain Regional Office, 2015). In the U.S. it is estimated that *M. chitwoodi* have the potential to inflict potato crop losses up to $40 million per year if not controlled. Potato farmers in the U.S. spend approximately $20 million per year in nematode soil fumigants and chemical controls; with some nematicide chemicals costing more than $741 per hectare (Suszkiw, 2009). Worldwide, yearly crop losses due to plant parasitic nematodes have been estimated at $173 billion; with estimated yearly U.S. losses of $13 billion (Elling, 2013).

M. chitwoodi reproduce on potato roots and tubers, which can allow for its spread to uninfected fields and distant potato farming areas by the transfer of infested soil and infested seed potato tubers. *M. chitwoodi* females are found in the outer 5.25 mm vascular ring of the tuber. Potato tuber damage consist of nematode-induced blemishes that lower tuber market value or make the crop unsellable when tuber damage is 10% or greater. In the fresh eating potato tuber export market and in all seed potato tubers there is a zero tolerance for infested tubers by *M. chitwoodi*; which enhances the economic impact of this pest. This is due to *M. chitwoodi* being designated as a quarantine pest by regulatory agencies in many countries (Elling, 2013). *M. chitwoodi* also has a very large host range, which can make it difficult to control by crop rotation alone. *M. chitwoodi* can feed and reproduce on approximately 3,000 plant species to include vegetables, legumes, cereals, grasses, bushes, tree fruits and ornamentals. *M. chitwoodi* also reproduce at high rates on oats (*Avena sativa*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*); which are common rotational crops in commercial potato production (Mojtahedi, Santo, & Wilson, 1988).

M. chitwoodi eggs and second-stage juveniles can survive subfreezing soil temperatures and overwinter in crop fields in the northwestern U.S. *M. chitwoodi* have a low temperature developmental threshold of 5^{o}C, and an optimal developmental temperature of 20^{o}C to 25^{o}C. Below 5^{o}C *M. chitwoodi* do not develop and go into a dormant state; where they can survive the winter until next season's crop is planted. The overwintered viable female eggs, once they hatch and the overwintered second-stage juvenile female *M. chitwoodi* population infest roots, develop into adult females within the roots and produce eggs masses at 600^{o}C to 800^{o}C degree-days after planting. The second-stage juveniles from the second-generation hatch from their eggs 950^{o}C to 1,100^{o}C degree-days after planting and then infest both roots and young tubers during the tuber bulking stage between 988^{o}C to 1,166^{o}C degree-days after planting. The third generation of *M. chitwoodi* females are then able to re-infest roots and tubers at 1,500^{o}C to 1,600^{o}C degree-days after planting (Table 2.1 and Figure 2.1) (Pinkerton, Santo, & Mojtahedi, 1991).

Table 2.1. ***M. chitwoodi*** **Degree-Day Development.**

Host: Russet Burbank Potatoes	DD (°F)	DD (°C)
Air temperatures were used in degree-day calculations		
Tuber initiation:	810-900	450-500
Ow females produce egg masses:	1080-1440	600-800
Second generation hatch:	1710-1980	950-1100
Juveniles II in tubers (1st generation)	1778-2099	988-1166
Third generation hatch:	2700-2880	1500-1600
First generation time:	1800	1000
Subsequent generation time:	900-1080	500-600

Source: Pinkerton, Santo, and Mojtahedi (1991)

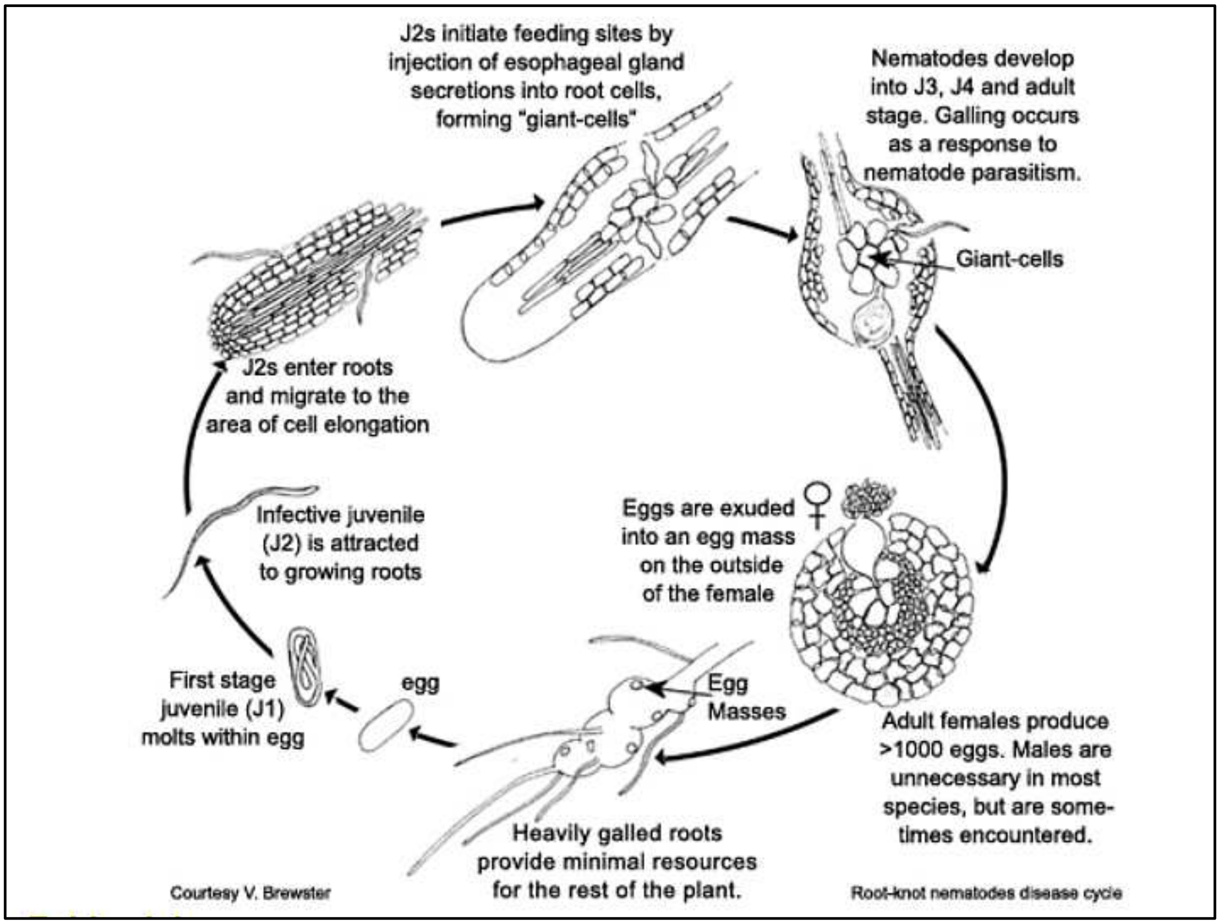

Figure 2.1. Basic life cycle of ***Meloidogyne spp.*** **(Diagram courtesy of G. Abawi and V. Brewster).**

An Integrated Nematode Management (INM) strategy is recommended as a sustainable approach to manage and control plant parasitic nematodes in a potato cropping system due to many factors. The main factors that INM is recommended are the human toxicity of nematode control chemicals, possible damage to the environment, development of nematodes resistance to chemical nematicides, decreased availability of labeled chemical nematicides and the high cost of chemical nematicides. INM utilizes biological, physical, and chemical control strategies to manage plant parasitic nematodes in commercial potato production (Hafez & Sundararaj, 2007). As part of an INM approach it has been shown that specific microorganisms within the soil and rhizosphere can provide crop protection from plant parasitic nematodes (Khalil, Allam, & Barakat, 2012). Certain beneficial soil bacteria and fungi have been shown to have antagonistic and parasitic action on plant parasitic nematodes. Non- pathogenic bacteria, such as, *Bacillus subtilis* and *Bacillus megaterium* antagonize plant parasitic nematodes by producing compounds that are toxic to nematodes, inducing plant resistance, lowering compounds in the rhizosphere that attract plant parasitic nematodes to plant roots and by acting as a physical barrier within the rhizosphere that prevent juveniles from penetrating the roots (Lamovšek, Urek, & Trdan, 2013). Some fungi, such as, *Trichoderma* spp. have been shown to parasitize nematodes, and to produce toxic compounds that are harmful to nematode development. *Trichoderma* spp. conidia parasitize the juvenile cuticle and eggshell of *Meloidogyne* spp. *Trichoderma* spp. has also been shown to hinder plant parasitic nematode root penetration, and to have the ability to improve plant growth (Lamovšek, Urek, & Trdan, 2013).

Soil testing for *M. chitwoodi* to accurately analyze field populations can be difficult to determine in a commercial field setting due to naturally occurring variations of *M. chitwoodi* populations. It should also be noted that only a few second-stage *M. chitwoodi* female juveniles and/or a few viable *M. chitwoodi* female eggs from the overwintering population are needed to produce high nematode population during a given growing season. At minimum, one composite soil sample derived from 20 soil core samples should be used for every 2 ha of a crop field to determine *M. chitwoodi* populations of a given field (Ferris, Mullens, & Foord, 1990; Hafez, 2003).

During the potato growing season of 2015, BioFit N, a commercially available multi-species microbial soil inoculant was tested on a 55.5 ha commercial production potato field in the San Luis Valley of Colorado. The aim of this experiment was to test the ability of a commercially available microbial soil inoculant to control *M. chitwoodi* potato tuber damage within the economic threshold for the U.S. fresh eating potato tuber market. A secondary aim of this chapter was to compare BioFit N's efficacy with that of Vydate (*Oxamyl*) to provide information for the development of INM best practice strategies for commercial potato tuber producers in the San Luis Valley of Colorado.

Materials and Methods

Filed Conditions and Experiment Evaluation

BioFit N is a commercially available multi-species microbial soil inoculant product comprised of *Azotobacter chroccum* (1x 10^5 CFU/g), *Bacillus subtilis* (1x 10^8 UFC/g), *Bacillus megaterium* (1x 10^6 CFU/g), *Bacillus mycoides* (1x 10^5 CFU/g), and *Trichoderma harzianum* (1x 10^6 CFU/g) (https://www.innovakglobal.com/en/biofitn-eua/). The efficacy of BioFit N at three different application frequencies over the growing season (4 applications, 3 applications and 2 applications) to control *M. chitwoodi* in a commercial potato cultivation setting was compared to the efficacy of two applications of Vydate (*Oxamyl*) at three different pre-planting levels of *M. chitwoodi* second-stage juvenile (360 J2, 120 J2 and 60 J2). The field experiment was conducted during the potato growing season of 2015 in the San Luis Valley of Colorado on the VanTreese

Farm, Field 3; which is a 55.5 ha commercial production field that is in a standard potato barley rotation under conventional crop production management. Fourth generation Norkotah 3 seed potatoes were planted on May 6th, 2015, and 7th, 2015 with a row spacing of 86.4 cm and an in-row spacing of 35.6 cm.

On May 11th, 2015, twenty-five soil core samples were taken per 1.6 ha plot from Field 3. The 25 soil core samples per 1.6 ha plot were put together into one composite soil sample per plot; where 500 cc of composite soil per 1.6 ha plot was labeled and collected in soil collection bags onsite and stored according to the University of Idaho's 'Sampling Procedure to Diagnose Nematode Infestation' document (Hafez, 2003).

The composite soil samples were then shipped to Western Laboratories in Parma, ID for the quantification of *M. chitwoodi* second-stage juveniles. Plots 3, 4 and 5 were selected in the Northwest quadrant of Field 3 for use as the Vydate control. Plots 7, 8 and 10 were selected in the Southwest quadrant of Field 3 for the BioFit N 4 application treatment. Plots 19 and 23 were selected in the Southeast quadrant of Field 3 for the BioFit N 3 application treatment. Lastly, plot 13 was selected in the Northeast quadrant of Field 3 for the BioFit N 2 application treatment (Table 2.2 and Figure 2.2).

Table 2.2. VanTreese Field 3 Columbia Root-Knot Nematode Second-Stage Juveniles at Planting

Treatment	Rate per Application	Grid Sample Plot Number	*M. chitwoodi* Starting J2 Population
Vydate – 2 applications	2.2 L/ha	3	360
Vydate – 2 applications	2.2 L/ha	4	120
Vydate – 2 applications	2.2 L/ha	5	60
BioFit N – 4 applications	1.12 Kg/ha	7	120
BioFit N – 4 applications	1.12 Kg/ha	8	360
BioFit N – 4 applications	1.12 Kg/ha	10	60
BioFit N – 3 applications	1.12 Kg/ha	19	120
BioFit N – 3 applications	1.12 Kg/ha	23	60
BioFit N – 2 applications	1.12 Kg/ha	13	60

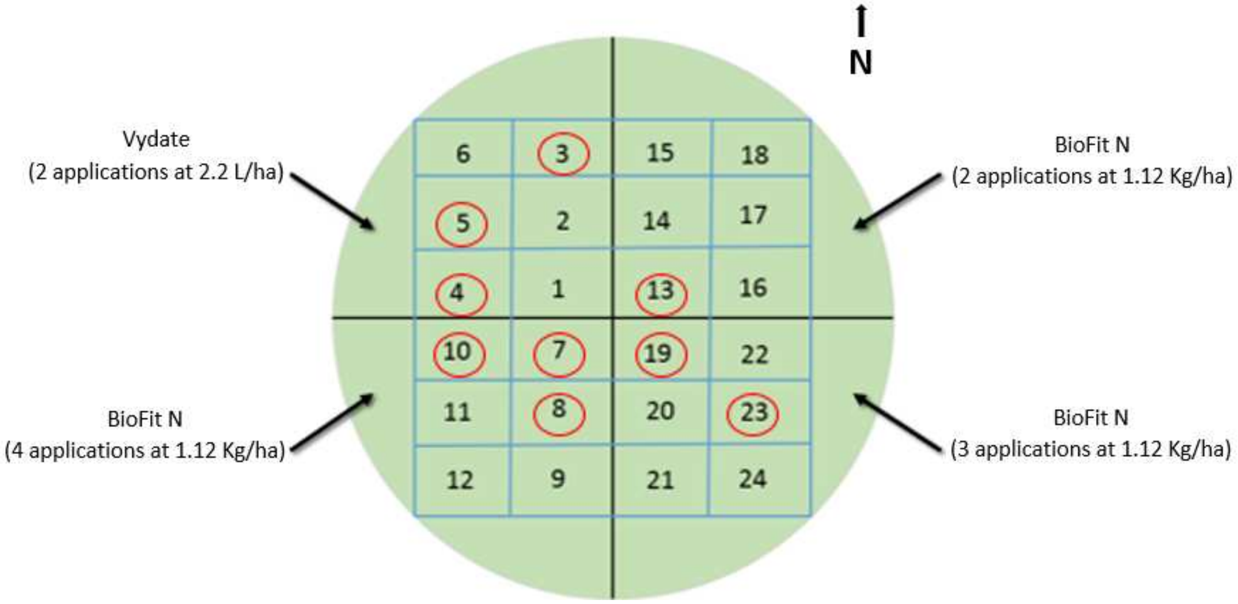

Figure 2.2. VanTreese field 3 Columbia root-knot nematode grid sample layout. (Numbers above indicate grid sample plot number, and red circles indicate experimental plots).

Vydate (*Oxamyl*) was applied two times over the growing season to the Northwest quadrant of Field 3 on June 22nd, 2015, at 536.8°C degree-days and on July 26th, 2015, at 943.3°C degree-days, at a rate of 2.2 L/ha through the center pivot irrigation system (Ingham, 2015). BioFit N was applied to the Southwest, Southeast and Northeast quadrants of Field 3 on June 6th, 2015, at 299.4°C degree-days and on June 24th, 2015, at 567.3°C degree-days. BioFit N was then applied to the Southwest and Southeast quadrant of Field 3 on July 25th, 2015, at 932.8°C degree-days and to the Southwest quadrant of Field 3 on August 15th, 2015, at 1,146.1°C degree-days (Ingham, 2015). The Southwest quadrant of Field 3 received four applications of BioFit N at 1.12 kg/ha through the center pivot irrigation system with 1.3 cm of water/ha. The Southeast quadrant of Field 3 received three applications of BioFit N at 1.12 kg/ha through the center pivot irrigation system with 1.3 cm of water/ha. The Northeast quadrant of Field 3 received two applications of BioFit N at 1.12 kg/ha through the center pivot irrigation system with 1.3 cm of water/ha.

On September 1st, 2015, all potato plants in Field 3 were vine killed by one application of Reglone Desiccant (*Diquat ion*) at an application rate of 1.2 L/ha. On September 9th, 2015, four rows were randomly selected that were 4.6 meter long per plot 3, 4, 5, 7, 8, 10, 13, 19 and 23 in Field 3. Each row was dug by a single row potato digger tractor implement and then hand dug to ensure all tubers per row were unearthed. Each row was then collected and bagged into separate potato sacks large enough to hold all the tubers from each row in one sack. All tubers collected for this experiment were then stored for one week in a secure potato storage shed. On September 16th, 2015, and 17th, 2015 all tubers from each row were evaluated for tuber weight and external tuber damage and defects. Then the ten largest tubers per row were cut in half for evaluation of interior tuber damage and defects. Then fifteen tubers per row were collected for future evaluation of *M. chitwoodi* tuber damage. The tubers collected for evaluation of *M. chitwoodi* tuber damage were then incubated at 22.2°C for 8 weeks. After 8 weeks of incubation at 22.2°C, five tubers per row were randomly selected for *M. chitwoodi* tuber damage evaluation. Tubers were peeled by hand and *M. chitwoodi* infection sites were counted under a magnifying lamp (Pinkerton, Santo, Ponti, & Wilson, 1986).

Statistical Analysis

A two-way ANOVA analysis and a Tukey Honest Significant Difference post-hoc test was performed on Total Tuber Yield, Market Tuber Yield that is defined as tubers weighing 113 g and greater (> 4 oz.), Tuber Yield of tubers sized between 113 g to 227 g (4 oz. to 8 oz.), Tuber Yield of tubers sized between 227 g and greater (> 8 oz.) and *M. chitwoodi* Tuber Damage.

Results

For Total Tuber Yield there was no statistically significant difference found between any of the treatments from the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) levels of 360 and 60 (Figure 2.3). In the plots with a starting population of *M. chitwoodi*

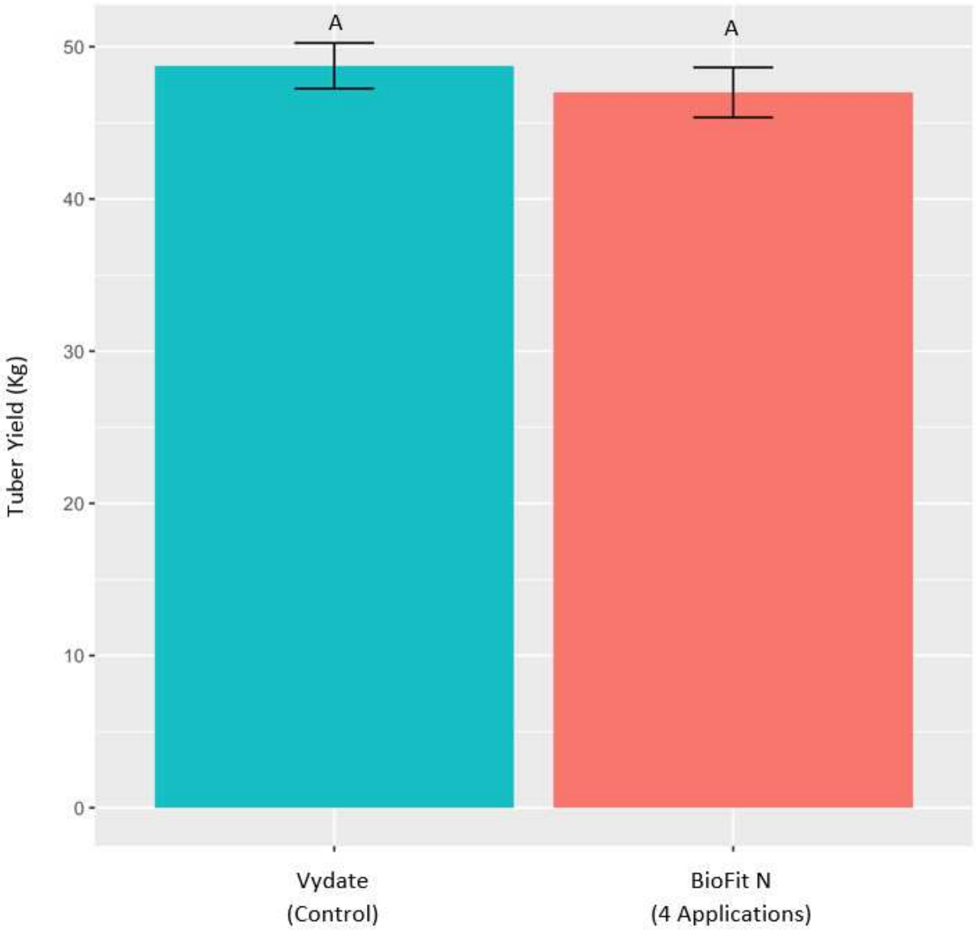

Figure 2.3. Total tuber yield for plots with starting populations of 360 *M. chitwoodi* J2.

second-stage juvenile (J2) level of 120 the Vydate control had a 14% greater total yield than the BioFit N 4 application treatment (*P* = 0.033) (Figure 2.4). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 60 there was no statistically significant difference found between the Vydate control and the BioFit N 4 application treatment, the BioFit N 3 application treatment, and the BioFit N 2 application treatment (Figure 2.5).

For Market Yield, which is defined as tubers weighing 113 g and greater (> 4 oz.), there was no statistically significant difference found between the control and any of the treatments from the plots with a starting populations of *M. chitwoodi* second-stage juvenile (J2) levels of 360 (Figure 2.6). In the plots with a starting population of *M. chitwoodi* second-stage juvenile

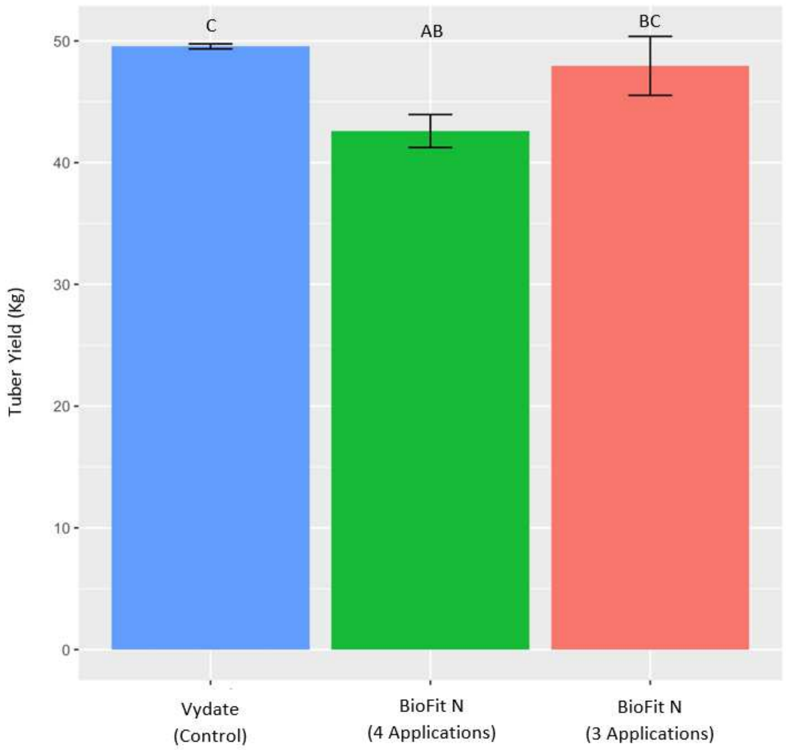

Figure 2.4. Total tuber yield for plots with starting populations of 120 *M. chitwoodi* J2.

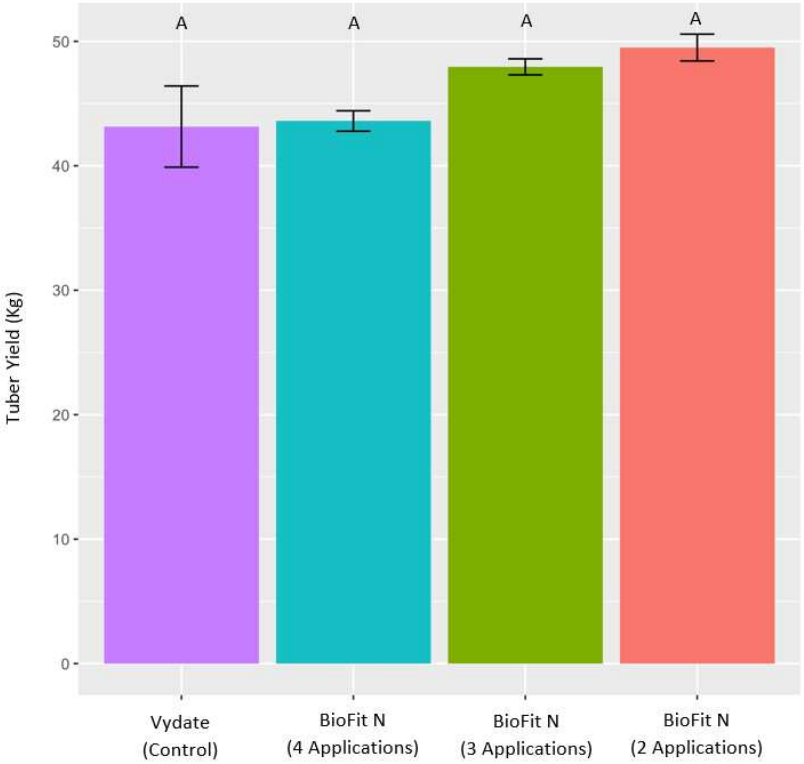

Figure 2.5. Total tuber yield for plots with starting populations of 60 *M. chitwoodi* J2.

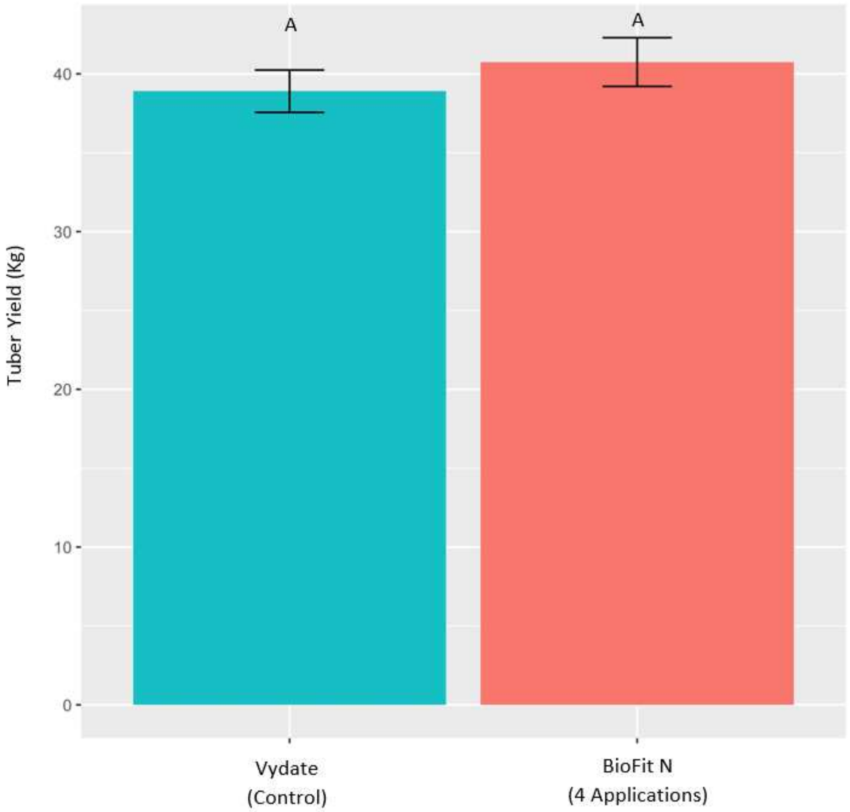

Figure 2.6. Market Tuber Yield for Plots with Starting Populations of 360 *M. chitwoodi* J2.

(J2) level of 120 the Vydate control had a 24% greater market yield than the BioFit N 4 application treatment ($P = 0.016$) (Figure 2.7). There was no statistically significant difference found between the other treatments from the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 120 (Figure 2.7). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 60 the BioFit N 2 application treatment had a 20% greater market yield than the Vydate control (P = 0.019) (Figure 2.8).

For Tuber Yield of tubers sized between 113 g to 227 g (4 oz. to 8 oz.) the Vydate control had a 23% greater total yield than the BioFit N 4 application treatment from the plots with a starting populations of *M. chitwoodi* second-stage juvenile (J2) levels of 360 ($P = 0.042$) (Figure 2.9). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 120 the BioFit N 3 application treatment had a 38% greater yield than the Vydate control

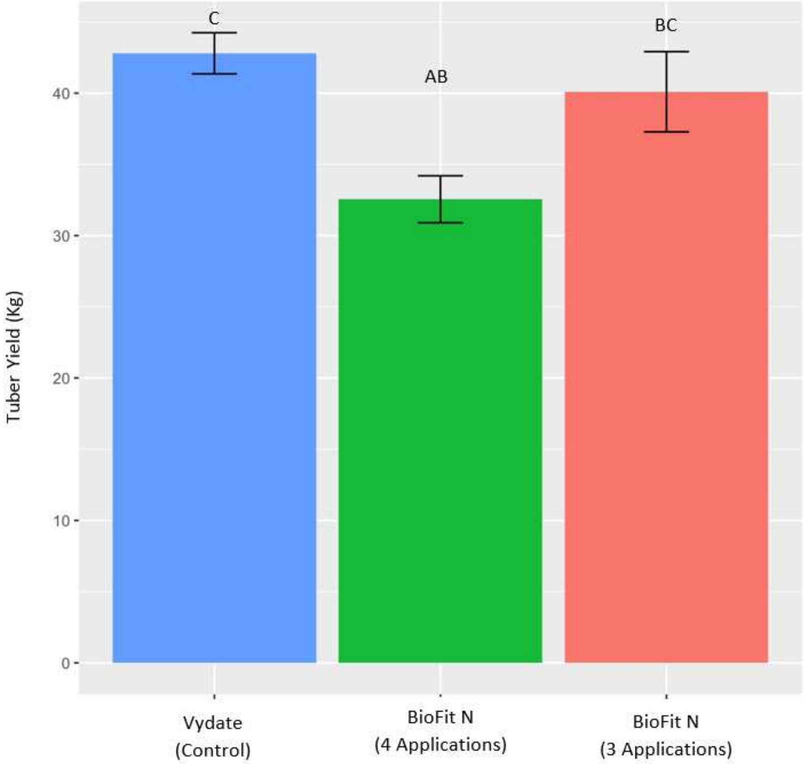

Figure 2.7. Market tuber yield for plots with starting populations of 120 *M. chitwoodi* J2.

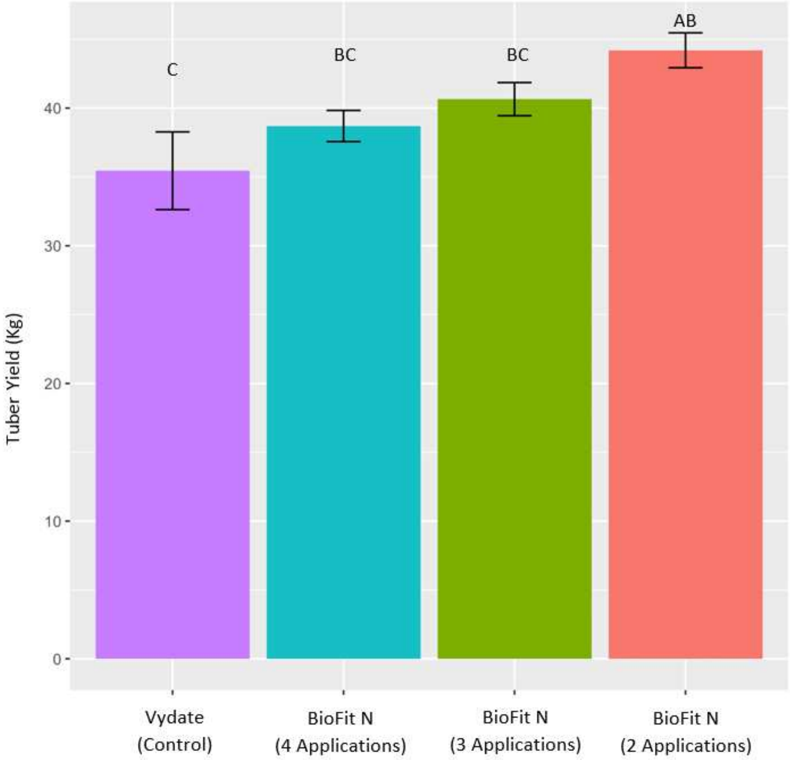

Figure 2.8. Market Tuber Yield for Plots with Starting Populations of 60 *M. chitwoodi* J2

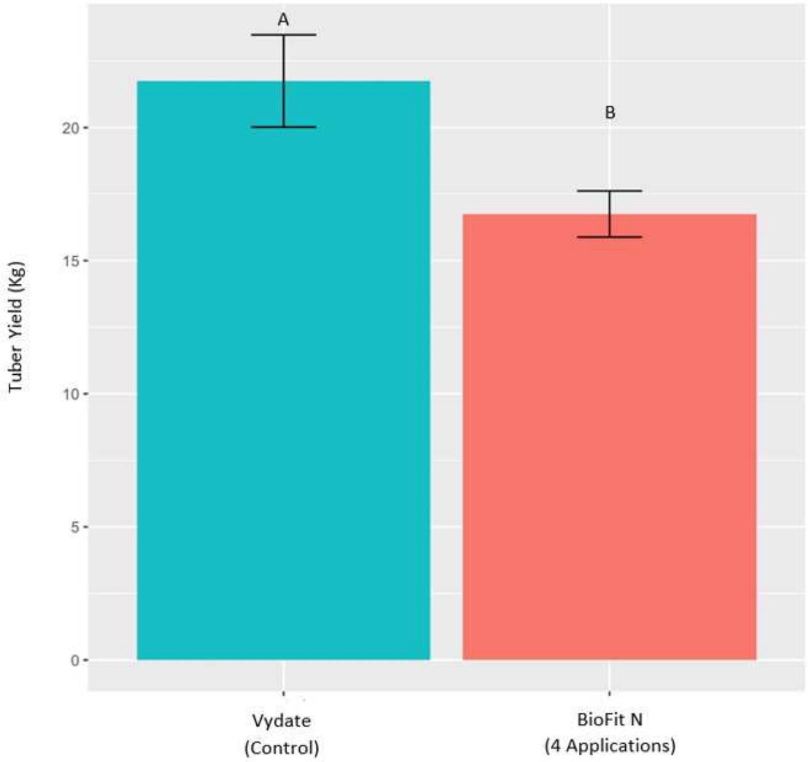

Figure 2.9. 113g to 227g (4oz. to 8oz.) Tuber Yield for Plots with Start Populations of 360 *M. chitwoodi* J2.

(*P* = 0.002) and the BioFit N 3 applications treatment also had a 25% greater yield than the BioFit N 4 application treatment (*P* = 0.024) (Figure 2.10). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 60 there was no statistically significant difference found between any of the treatments and the control (Figure 2.11).

For Tuber Yield of tubers sized between 227 g and greater (> 8 oz.) the BioFit N 4 application treatment had a 29% greater total yield than the Vydate control from the plots with a starting populations of *M. chitwoodi* second-stage juvenile (J2) levels of 360 (*P* = 0.029) (Figure 2.12). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 120 the Vydate control had a 44% greater yield than the BioFit N 4 application treatment

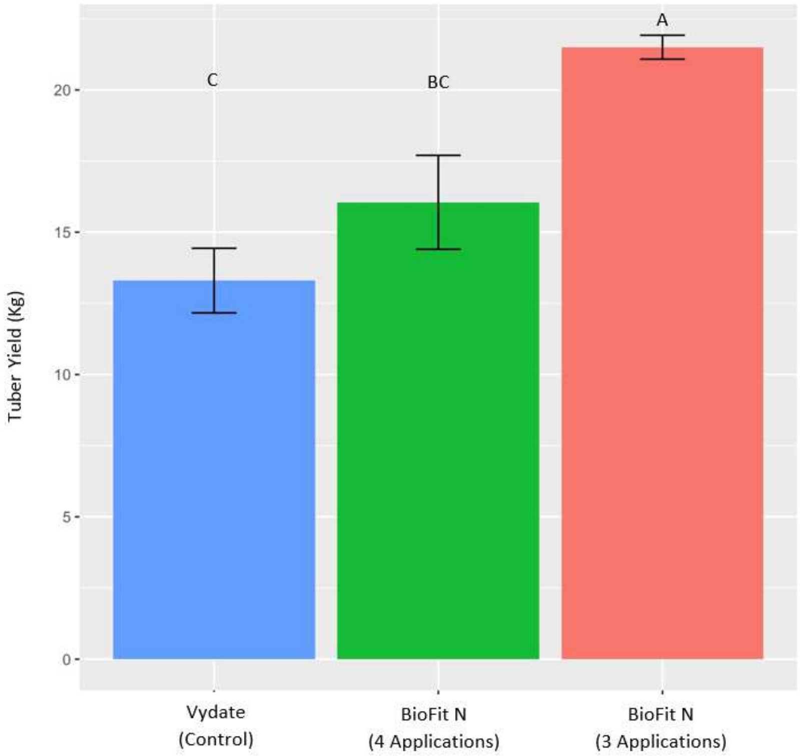

Figure 2.10. 113g to 227g (4oz. to 8oz.) Tuber Yield for Plots with Start Populations of 120 *M. chitwoodi* J2.

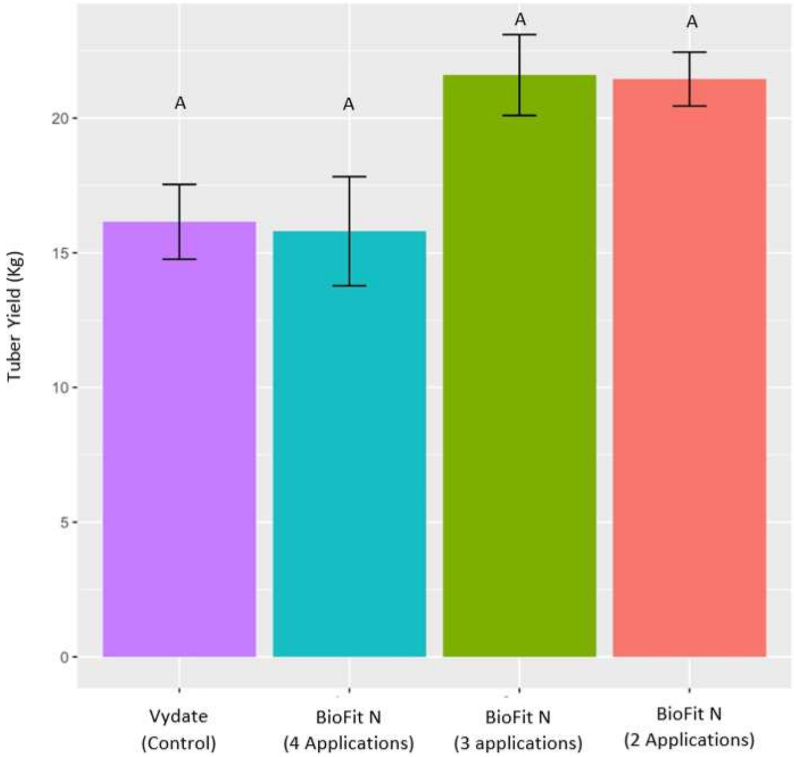

Figure 2.11. 113g to 227g (4oz. to 8oz.) Tuber Yield for Plots with Start Populations of 60 *M. chitwoodi* J2.

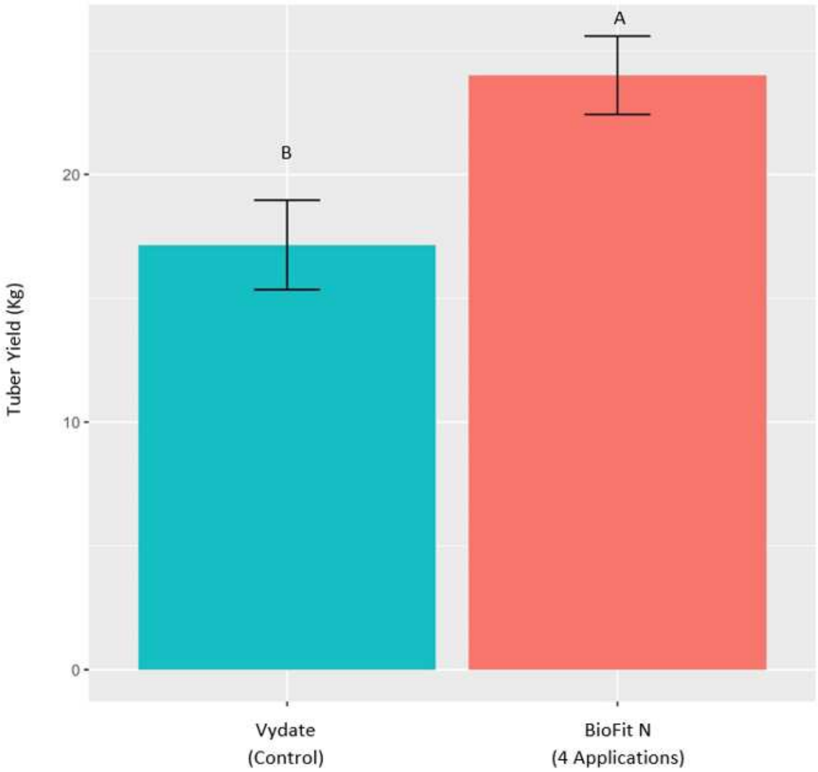

Figure 2.12. 227 g and greater (> 8 oz.) Tuber Yield for Plots with Start Populations of 360 *M. chitwoodi* J2.

($P = 0.015$) and the Vydate control also had a 37% greater yield than the BioFit N 3 applications treatment ($P = 0.037$) (Figure 2.13). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 60 there was no statistically significant difference found between any of the treatments and the control (Figure 2.14).

For *M.* chitwoodi Tuber Damage there was no statistically significant difference found between The Vydate control and the BioFit N 4 application treatment from the plots with a starting populations of *M. chitwoodi* second-stage juvenile (J2) levels of 360 (Figure 2.15). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 120 there was no statistically significant difference found between the Vydate control and any of the BioFit N treatments for *M. chitwoodi* Tuber Damage (Figure 2.16). In the plots with a starting population of *M. chitwoodi* second-stage juvenile (J2) level of 60 there was no statistical

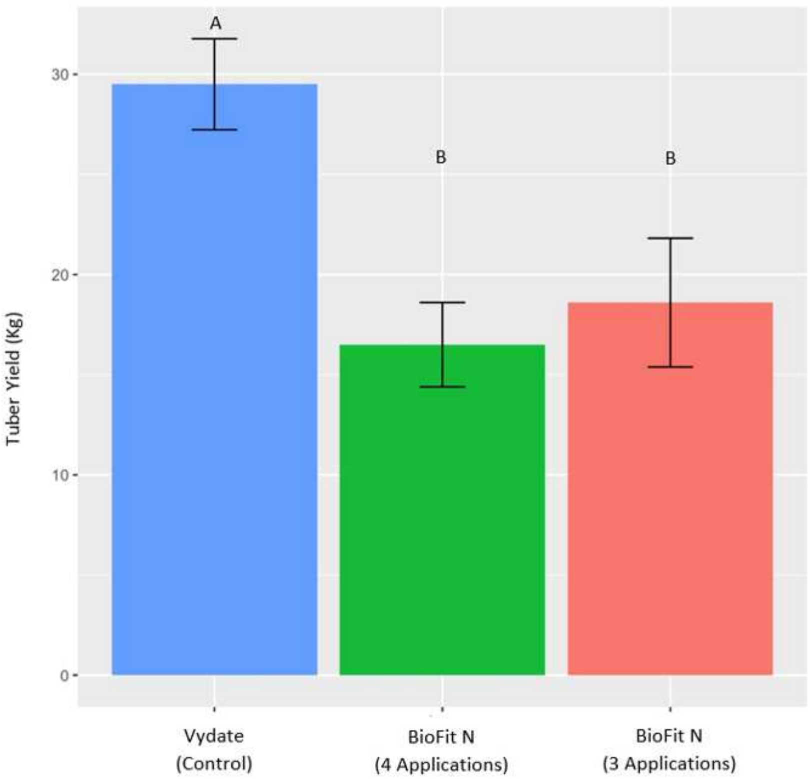

Figure 2.13. 227 g and greater (> 8 oz.) Tuber Yield for Plots with Start Populations of 120 *M. chitwoodi* J2.

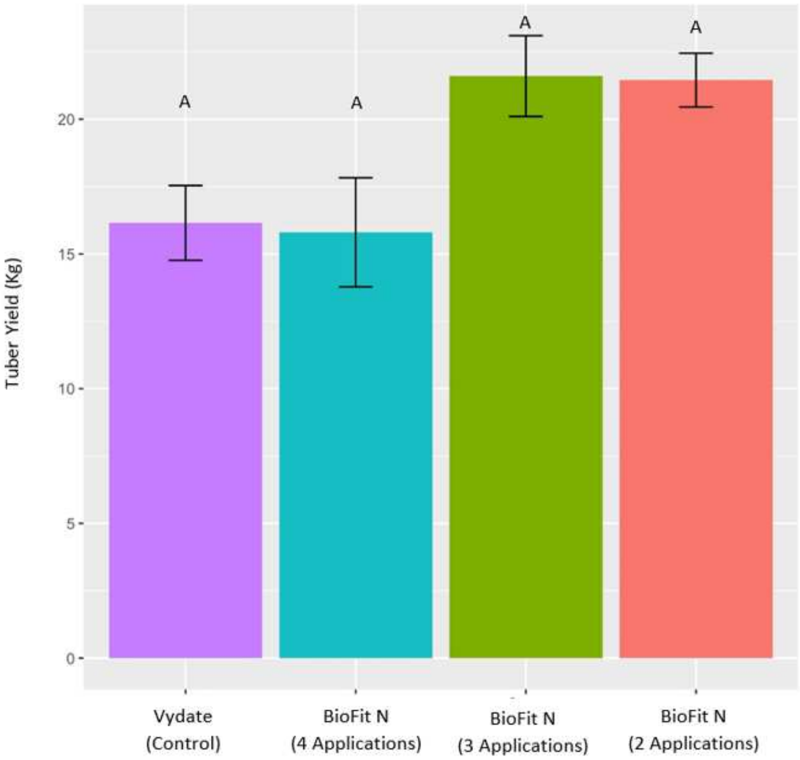

Figure 2.14. 227 g and greater (> 8 oz.) Tuber Yield for Plots with Start Populations of 60 *M. chitwoodi* J2.

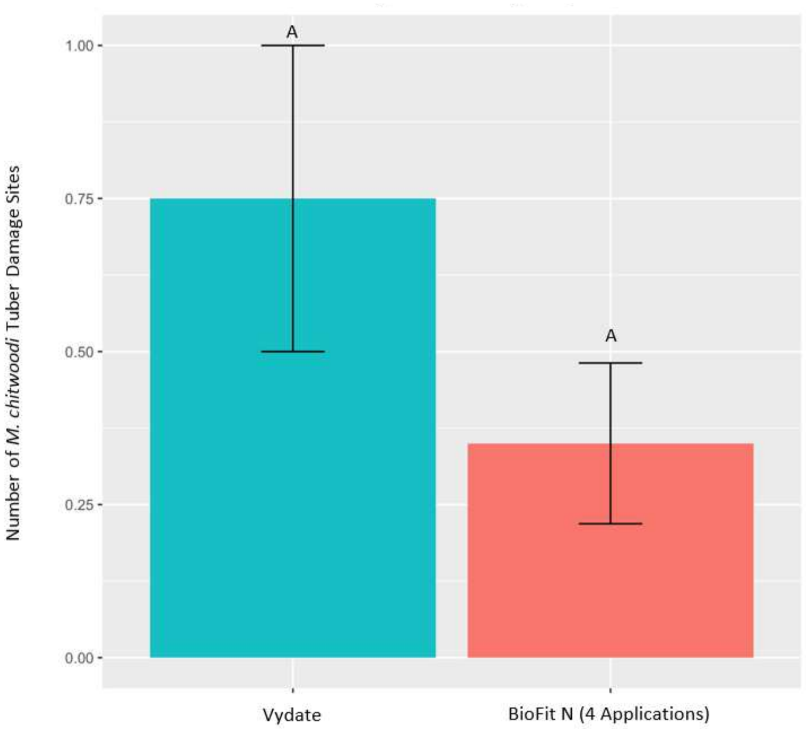

Figure 2.15. ***M. chitwoodi*** **Tuber Damage at the Starting J2 Population Level of 360.**

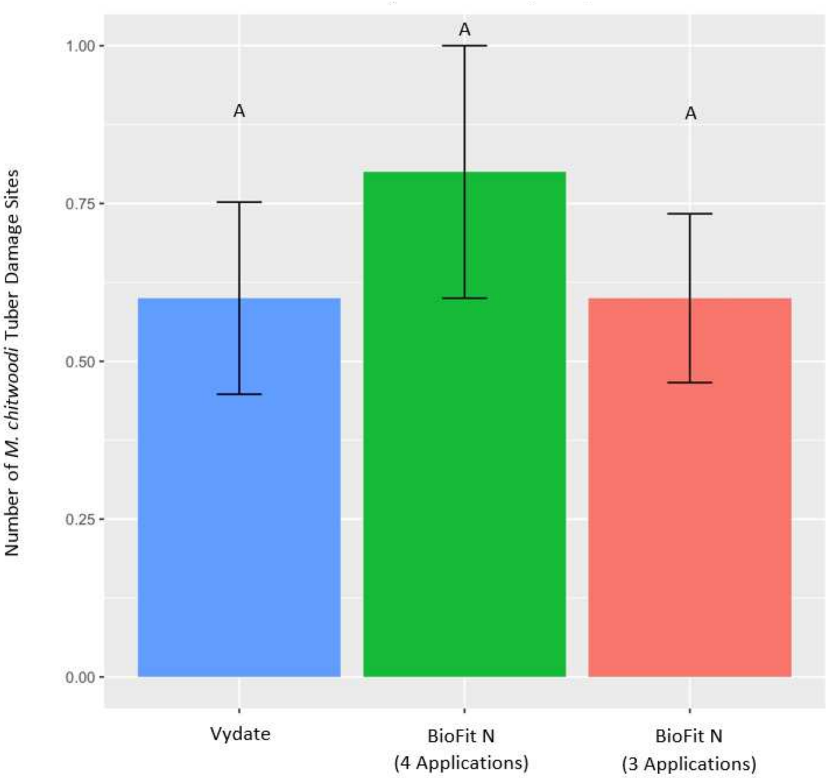

Figure 2.16. ***M. chitwoodi*** **Tuber Damage at the Starting J2 Population Level of 120.**

difference found between any of the Vydate control and the BioFit N treatments for *M. chitwoodi* Tuber Damage, but there was an 88% reduction of *M. chitwoodi* Tuber Damage between the BioFit N 2 application treatment and the BioFit N 3 application treatment ($P = 0.048$) (Figure 2.17).

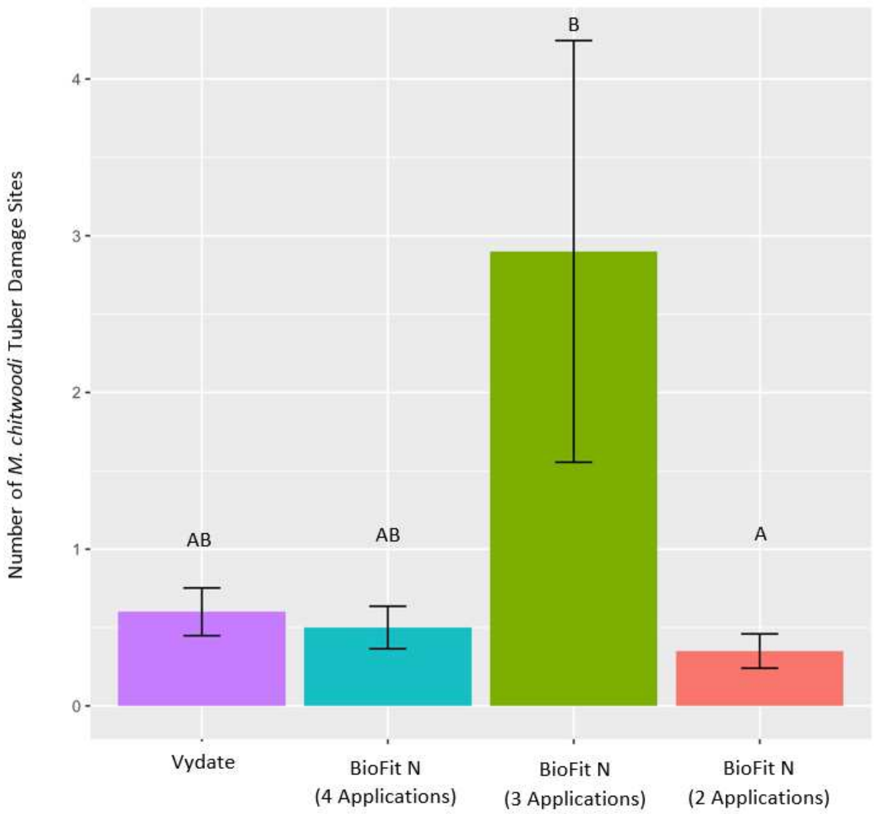

Figure 2.17. ***M. chitwoodi*** **tuber damage at the starting J2 population level of 60.**

Discussion

M. chitwoodi and other *Meloidogyne* spp. cause extreme crop damage and economic losses in potato crops worldwide if not controls throughout the growing season. Traditionally chemical soil fumigants and chemical nematicides have been used to control plant parasitic nematode populations and crop damage they cause. But in recent years new methods of plant parasitic nematode control are greatly needed due to many traditional controls being highly toxic, development of nematodes resistance to chemical nematicides, decreased availability of labeled chemical nematicides and the high cost of chemical nematicides (Hafez & Sundararaj, 2007) The control of *M. chitwoodi* and other *Meloidogyne* spp. is essential for world food security as the potato tuber crop is the largest non-grain food commodity in the world (Fiers, Edel-Hermann, Chatot, Hingrat, Alabouvette, & Steinberg, 2012). The results of this commercial potato field experiment showed that all of the BioFit N treatments and the Vydate control at all starting field population levels of *M. chitwoodi* second-stage juveniles-controlled *M. chitwoodi* potato tuber damage within the economic damage threshold for the sale of potato tubers in the U.S. wholesale fresh eating potato tuber market, which is defined as less than 6 *M. chitwoodi* individual damage sites per tuber (Figure 2.15, Figure 2.16, and Figure 2.17) (Pinkerton, Santo, Ponti, & Wilson, 1986). These findings show real promise for the control of *M. chitwoodi* tuber damage by a multi-species soil inoculants that contain *Bacillus subtilis, Bacillus megaterium* and *Trichoderma harzianum*. This is especially important for commercial potato farmers due to the extremely limited supply of Vydate (*Oxamyl*) that is currently available to farmers. Also, the use of the soil fumigant Telone (1,3-dichloropropene) is not economically viable for most commercial potato production operations (Kleczewski, 2015). Another, benefit of utilizing multi-species microbial soil inoculants that contain *Bacillus spp.* and *Trichoderma spp.* have the ability to suppress some soil borne diseases and they have been shown to increase overall plant health (Han, Cheng, Yoon, Song, Rajkarnikar, Kim, Yoo, Yang, & Suh, 2005; Howell, 2003). The potato yield data showed varying results with the Vydate control and the BioFit N treatments performing differently among the plots with different starting levels of *M. chitwoodi* second-stage juvenile (J2) populations. This suggest that the experiment sample size may have to be increased in future experiments to better determine what true effect the microorganisms in BioFit N has on potato tuber yield compared to that of Vydate.

The results in this experiment are supported by previous findings that show *Bacillus subtilis, Bacillus megaterium* and *Trichoderma harzianum* are able to control some root-knot nematodes species (*Meloidogyne* spp.) in a number of crops (Khalil, Allam, & Barakat, 2012; Padgham & Sikora, 2006; Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012). *Bacillus subtilis* and other similar rhizobacteria have been shown to act as plant-growth promoters. *Bacillus subtilis* and rhizobacteria have the ability to control *Meloidogyne* spp. through nematode antagonism and by increasing plant defense and resistance responses in the host plant (Lamovšek, Urek, & Trdan, 2013). *Bacillus subtilis* has been shown to reduce *M. incognita* egg masses by 72% and juveniles in the soil by 60% in tomato plants (*Solanum lycopersicum*) under greenhouse conditions (Khalil, Allam, & Barakat, 2012). *Bacillus subtilis* has also been found to reduce egg hatching and to cause mortality of 50% in *M. javanica.* Additionally, the same study found that *Bacillus subtilis* was able to produce significant reductions in root galling caused by *M. chitwoodi,* and a significant increase of shoot length, shoot weight, root length and root weight on cowpea (*Vigna unguiculata subsp. Unguiculata*) and mash bean (*Vigna mungo* L.) (Dawar, Tariq, & Zaki, 2008). *Bacillus megaterium* has been shown to reduce the root penetration and gall formation by 40% and egg hatching by 60% on *M. graminicola* on rice (*Oryza sativa*) (Padgham & Sikora, 2006). In previous studies, *Bacillus megaterium* has been found to reduce the population of *M. chitwoodi* in potatoes (*Solanum tuberosum*) by 50%, and to have the ability to control *M. exigua* on coffee (*Coffea* spp.) and *M. javanica* on sunflower (*Helianthus* spp.) (Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012). Other previous experiments have found that *Bacillus megaterium* produces nematicidal compounds that significantly inhibit egg hatching and reduces juvenile infection of *M. incognita* (Huang, Xu, Ma, Zhang, Duan, & Mo, 2010). *Trichoderma harzianum* parasitizes eggs and larvae of *Meloidogyne* spp. by breaking down the cuticle layer with enzymes, such as, chitinases, glucanases and proteases. *Trichoderma harzianum* then produces toxic nematicidal compounds and reproduces within the nematode egg and juveniles host body (Sharma & Pandey, 2009). *Trichoderma harzianum* has shown to have the ability to decrease *M. incognita* root galling by 70% to 97%, and second-stage juveniles by 21% to 98% in tomato plants (*Solanum lycopersicum*) (Radwan, Farrag, Abu-Elamayem, & Ahmed, 2012), and to reduce root galling by up to 39% and parasitization of females by up to 51% in eggplants (*Solanum melongena*) (Rao, Parvatha Reddy, & Nagesh, 1998). Other studies have also found that *Trichoderma harzianum* reduced *M. incognita* root galling by an average of 24% and egg masses by an average of 28% in okra (*Abelmoschus esculentus*) (Mukhtar, Arshad Hussain, & Zameer Kayani, 2013).

The findings in previous studies, and the findings of this experiment show that multi-species microbial soil inoculants, consisting of microorganisms that are antagonistic and parasitic to plant parasitic nematodes, have the ability to control *M. chitwoodi* and other *Meloidogyne* spp. within the nematode crop damage thresholds for a broad range of cultivated crops. Multi-species microbial soil inoculants also have the potential to target control of the different life stages of plant parasitic nematodes, which could greatly increase their efficacy. Also, the use of multi-species microbial soil inoculants that contain *Bacillus spp.* and *Trichoderma spp.* have the ability to suppress some soil borne diseases and they have been shown to increase overall plant health, which can allow for plants to better cope with plant parasitic nematode damage (Han, Cheng, Yoon, Song, Rajkarnikar, Kim, Yoo, Yang, & Suh, 2005; Howell, 2003). Additionally, more research needs to be done to identify which microorganisms that are antagonistic and parasitic to plant parasitic nematodes can best colonize and persist in the rhizosphere, endorhiza and the geocaulosphere (stolons and tubers) to allow for the greatest control (Diallo, Crépin, Barbey, Orange, Burini, & Latour, 2011). More focused research also needs to be done on fully integrating microbial controls of *Meloidogyne* spp. within commercial crop production settings to be able to develop the most effective INM strategies for commercial crop producers worldwide (Hafez & Sundararaj, 2007; Roberts, 1993).

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CONCLUSION

The findings of previous research on this topic, and the findings of the greenhouse and open-field experiments to control *M. chitwoodi* with NemaRoot and BioFit N in potatoes (Solanum tuberosum) indicate that microbial soil inoculants that contain *Bacillus subtilis, Bacillus megaterium, Trichoderma harzianum* and *Purpureocillium lilacinus* have the ability to control *M. chitwoodi* and *Meloidogyne* spp. within economic crop damage thresholds. Future research is still needed to provide the best Integrated Nematode Management (INM) practices for specific agricultural crops that address *Meloidogyne* species present, environmental conditions, cropping system and the technology of the crop producers. Additionally, research that focusing on the integration of microbial controls of *Meloidogyne* spp. with all other commercially available plant parasitic nematode controls still need to be investigated in a commercial crop production setting to be able to provide the best INM strategies for commercial crop producers worldwide.