**MBI-3X Bio-Nematicide Plant Health Effects on Root Crops**

by

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## Abstract

MBI-3X is a new nematicide formulated from fermented and killed microbial broth. The broth contains large amounts of dead cells and an array of secondary metabolites in the fermentation supernatant with a corresponding array of potential effects on plants and agricultural pests. Some of these metabolites have been observed to have phytotoxic or herbicidal effects on plant tissue when applied at high rates. However, preliminary observations also suggest there may be positive plant health effects in certain species, particularly root crops. If MBI-3X is confirmed to promote plant health, this could be the result of fertilization by the nutrient broth, hormesis, or some unpredicted factor. Experiments were conducted to confirm the existence of plant health effects on selected commercially important underground plant structures such as radish (*Raphanus sativus*), sugar beet (*Beta vulgaris*), onion (*Allium cepa*), and other root crops. The nature of plant health effects was represented by collecting data on yield and phenology, above and below-ground biomass, root area and root size distribution. MBI-3X was found to have some bio-stimulant activities on some of the crops used, but results were inconsistent between repetitions. Most crops exhibited increase in fine root tip and lengths with the addition of MBI-3X. An extended hormesis curve was observed on the second repetition of narcissus. This may be due to secondary metabolites herbicidal effect at 100 times the standard field rate. Abiotic stressors, microclimate, and other factors may cause for the inconsistent results. MBI-3X did exhibit bio-stimulant tendencies with most of the crops.

Fertilization by the liquid nutrient broth can also be a contributing factor to the plant growth effect. MBI-3X was not confirmed to have plant health effects for the selected root crops.

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# Introduction

In 2000, the annual crop loss due to parasitic nematodes was $9.1 billion dollars in the United States. The number of available nematicides is in decline due to research costs and new environmental restrictions (Chitwood, 2003). The use of nematicides can present a variety of potential risks, such as the evolution of nematicide resistant parasitic nematodes, environmental contamination, and phytotoxicity to the crops themselves. Nematicides such as aldicarb and 1,2-Dibromo-3-Chloropropane can persist in groundwater for decades (Chitwood, 2003). Methyl bromide and other broad-spectrum fumigant based nematicides kill both parasitic and beneficial microorganisms in the soil. Nematicides that are non-fumigant can be effective as well. Aldicarb attacks a board range of parasitic nematicides; however, it is highly toxic. Bio-nematicides are natural product based nematicides (Asolkar et al., 2013). Bio-nematicides such as Ditera, a nematode-parastic fungus *Myrothecium Verrucaria* works well against plant parasitic nematodes, but not on free-living or mammalian-parasitic nematodes. The control is not perfect with Ditera, with *Meloidogyne incognita*, the eggs are unaffected. These products can change the complex population balances of the soil ecosystem, which can cause poor plant growth (Chitwood, 2003). Ntalli and Caboni's (2012) review on botanically derived nematicides noted that until research can clearly show the mode of action, target, and practical field application of the bio-nematicides product acceptance will continue to be a challenge. Newer nematicide research is focused on creating nematicides that are more targeted, effective, economically sustainable, and less environmentally harmful (Chitwood, 2003 and Ntalli and Caboni, 2012).

MBI-3X is a new nematicide formulated from fermented and killed microbial broth. The broth contains large amounts of dead cells and an array of secondary metabolites in the fermentation supernatant with a corresponding array of potential effects on plants and agricultural pests. Secondary metabolites can indirectly affect growth and development of plants (Asolkar et al., 2013). Some of these metabolites have been observed to have phytotoxic or herbicidal effects on plant tissue when applied at high rates (Asolkar et al., 2013). One of these secondary compounds is *Romidepsin*. At a rate of over 100 times the standard field rate of MBI-3X, this secondary compound can exhibit herbicidal effects (Louis Boddy, 2018). MBI-3X at standard field rate does not cause phytotoxicity to plants. However, preliminary observations also suggest there may be plant health effects in certain species, particularly in regard to root growth.

If MBI-3X is confirmed to promote plant health, this could be the result of a variety of processes, such as fertilization by the nutrient broth, hormesis, or bio-stimulant activity. The nutrient broth contains a large amount of nitrogen and other nutrients, such as calcium, iron, magnesium, phosphorus, and potassium that could act as a potential fertilizer for the plant (Louis Boddy, 2018). On the other hand, hormesis occurs when a low dose of a toxin, such as a pesticide, has a beneficial effect, such as increased plant growth. Hormesis is a biphasic dose response occurrence, which is defined by low dose stimulation and then a high dose inhibition (Calabrese, 2014). Growth stimulation could also be assisted by secondary metabolites in MBI-3X's microbial broth.

The objective of this study is to determine if MBI-3X has any positive plant health effects on root crops.

Literature Review

## *Meloidogyne incognita* life stages

Nematicides are used to kill parasitic nematodes that infect the root systems of plants. Nematodes are non-segmented worm-like invertebrates that can reside in the soil and in roots of infected plants. The Southern root knot nematode, *M. incognita*, for example, infects a broad range of agricultural crops, such as cotton (*Gossypium hirsutum*) and tomato (*Solanum lycopersicum*) (Abad et al., 2008). The nematode's life cycle consists of six stages: egg, four juvenile stages (J1, J2, J3, and J4) and an adult stage. It takes approximately 20 to 24 days to complete a life cycle. The life cycle can also be divided into two phases, parasitic and pre-parasitic (UC IPM, 2014). At J2, the nematode is mobile. The nematode enters the host plant and inserts itself with a stylet into the root's cell. Once the nematode enters the root it becomes sedentary, *M. incognita* is an obligate endoparasite. The nematode starts to live off the host root system and grow until it reaches adult stage. Every time it reaches another growth stage, it molts its outer layer and enlarges itself (Abad et al., 2008). The nematode injects secretions that induce the root cells to enlarge into giant cells (Trudgill and Blok, 2001). The female uses the stylet as a medium to collect nutrient from the plant host. The female restarts the cycle by releasing a large amount of gelatinous eggs out into the root's outer surface (Abad et al., 2008). Cells around the eggs enlarge and become gall shaped. Only J2 and the adult female stage can feed. Reproduction can be asexual or sexual (Trudgill and Blok, 2001).

**Nematode effects on agriculture**

Effects of nematodes on agriculture can range from 3 to 20% production losses, depending on the crop (Chitwood, 2003). *M. incognita* can infect a broad range of plant hosts, including many vegetable and tree crops, rose (*Rosa spp.*), and walnut.

Once crops are infected by nematodes there are few methods that can help alleviate the infestation. For example, fumigation can be used, but many fumigants, such as methyl bromide, are being phased out due to negative environmental impact to the ozone layer and compliance with the Clean Air Act (Extoxnet, 1993). Due to the removal of methyl bromide and other effective but environmentally unfriendly fumigants, growers are limited on strategies that can control nematode infestation and are also economically efficient (Radewald, 1987). Other fumigants currently being used are restricted-use pesticides, such as 1, 3-dichloropropene and chloropicrins and are less effective compared to methyl bromide. Other non-fumigant nematicides are mostly reversible acetylcholinesterase inhibitors, such as aldicarb and other carbamates and organophosphates (Chitwood, 2003). The new emerging nematicides are bio-nematicides, such as Ditera that utilized nematode-parasitic fungi to control targeted parasitic nematodes, such as *M. incognita* (Chitwood, 2003). Solarization is effective for killing nematodes in the top 30 cm of soil that is infected (UC IPM, 2014). This can be useful if the crop only grows shallow roots or if the nematode populations are not deep. Choosing crop cultivars that are resistant to nematodes can help reduce infection damage (UC IPM, 2014). Alternative *M. incognita* control methods that are more targeted, but still environmentally friendly and economically feasible are in development. In this study, MBI-3X is a bio-nematicide that targets *M. incognita* and is less harmful to the environment.

## Plant Health

Döring et al. (2012) argued that plant health is an underdeveloped concept. Since there are many conflicting definitions of what plant health is, they created guidelines to help define the concept. Plant health should be a technical term for plant hygiene. Plant health should not simply be determined just by a plant being free of disease, as it is more complex than being disease free. A plant health definition should provide insight for plant health management. Cook (2000) defined plant health management as the science and practice of assisting plants to achieve their full genetic potential by removing and understanding abiotic and biotic factors that would prohibit this potential.

One important group of plant health products is the bio-stimulants that often alleviate abiotic stressors, such as nutrient deficiency. Jardin (2015) defined a bio-stimulant as any substance that improves nutritional use efficiency of the targeted organism. This substance can contain microorganisms and may reduce the negative consequences of abiotic stresses. In this case improved plant health is not caused by increased concentration of nutrients from the broth or solution, but from the actual bio-stimulant itself. One class of bio-stimulants is seaweed extracts that promote plant growth. Khan et al. (2009) defined bio-stimulants as materials that are not fertilizers, but still promote plant growth in a small amount. Many components of seaweed extracts and their modes of action are still unknown, and could possibly have some synergistic activity when combined (Khan et al., 2009).

Plant- microbe symbiosis is a main theme of bio-stimulants. Hassan (2017) studied plant growth promoting endophytes isolated from felty germander (*Teucrium polium L.)* plants and

showed that plant microbe symbiosis can influence plant growth and improve soil quality damaged by pesticide and other agricultural uses. He inoculated bacterial and fungal endophyte extracts onto corn (*Zea mays*) in order to observe plants' reaction to the inoculants for the following growth parameters, like root length, shoot and root weights (fresh and dry). There was a significant increase in all the growth parameters compared to the control for the plants with a mixture of two bacterial endophytes. However, when compared to the plants with only an individual bacterial endophyte inoculant there was no difference. Hassan had similar results with fungal endophyte inoculates. With further genetic testing and analysis he concluded microbial endophytes promote plant growth in many ways. Directly, microbial endophytes enhanced plant growth by releasing plant hormones, such as indole acetic acid. Indirectly, they produce antimicrobial activities, such as degrading enzymes that inhibit pathogenic microorganisms (Hassan, 2017). In general, plants with microbial inoculants had larger biomass compared to non-inoculated controls.

Another plant health effect is hormesis, a biphasic dose response occurrence defined by low dose stimulation and then a high dose inhibition (Calabrese, 2014). In general, hormesis is a dose response relationship phenomenon (Edward and Baldwin, 2002). Synthetic herbicides can induce a hormesis effect on certain plant species (Cedergreen, 2008). Low doses of herbicide can stimulate plant growth (Cedergreen, 2008). Hormesis can occur in any type of toxin. Hormesis has been associated with pesticide drifts onto non-targeted organisms (Cedergreen, 2008).

Hormesis can have a potential effect on plant biomass. Cedergreen (2008) tested eight herbicides on hydroponic barley (*Hordeum vulgare)* plants. She found glyphosate and

metsulfuron-methyl at low dose (5-10% of field rate) was most consistent in inducing hormesis with 25% increase in barley biomass compared to control. However, the results observed in one repetition were not always repeated in another. Cedergreen determined that the experimental design used was not structured to reliably test for hormesis. The other six herbicides increased biomass, but not due to hormesis.

There are issues with studying hormesis. The results can be inconsistent among repetitions. Even if hormesis effect was found, the effect on biomass increase would be minimal. Increase in biomass can also be caused by other factors. Stress can force resource allocation to fruit or seed production. The application of pesticide could be uneven in the field. Thus, the hormesis effect is difficult to prove since there are other contributing factors that may affect the biomass increase (Cedergreen, 2008). Hormesis is therefore a controversial concept. For some researchers, it can be attributed to plant stress or as an experimental artifact (Cedergreen, 2008). The existence of hormesis can have positive and negative implications. It has the potential to increase crop production if it exists, and if managed correctly. However, Cedergreen (2008) noted that hormesis can cause increases in weed resistance to herbicides if spray drifts occurs too often.

Most of the literature for hormesis focuses on herbicide effects on crops. One such example is Brito et al. (2017), who studied hormesis effect of glyphosate on plants. They wanted to know if glyphosate had any hormesis effect, and whether or not glyphosate can be a growth stimulant at low dosages. Similar to Cedergreen (2008), the dose response was masked by many other factors, such as plant age, environmental condition variabilities, and pesticide dosages.

Brito et al. (2017) noticed that some biomass yield increases appeared under only field conditions. Even though MBI-3X is a bio-nematicide, one of the secondary metabolite is herbicidal in large concentration. In this case, MBI-3X only contain a minute amount of this secondary metabolites, thus the rates this study will be showing will still be in the low dose stimulation phase of the hormesis curve for these herbicidal metabolite (Figure 1).

Chelinho et al. (2017) examined a possible hormesis effect on non-target soil nematode communities with the bionematicide 1,4-napthoquinon. 1,4-napthoquinon, is commonly found in walnut (*Juglans spp.)* husk. 1,4-napthoquinon had a toxic effect on plants and non-target nematodes and soil organisms overall. However, the authors also noticed that at a low dosage there was growth stimulation in biomass for canola (*Brassica napus).* 1,4-napthoquinon concentration of less than 20 mg/kg of soil can act as a protectant to non-target plants and organisms; however, for thorough control of targeted plant parasitic nematodes, application rates would be much higher. They suggested a modification of the bionematicide application in the field.

MBI-3X's nutrient broth is another potential contributor to plant health effects on the targeted plant. Jiang's et al., 2018 studied a bio-fertilizer, Ning Shield, which controlled root knot nematodes and promoted plant growth on *Trichosanthes krilowii* (Jiang et al. 2018). Increase root mass was observed with addition of the bio-fertilizer. The bio-fertilizer was effective in controlling root knot nematode, and also significantly increases root biomass. Similar to MBI-3X, Ning Shield contained microbial compounds that can stimulate plant growth.

In general, nutrient broths contain the basic necessity for the targeted microorganism to grow in a liquid solution. It can have various ingredients, such as nitrogen and protein sources that assist with microorganism growth (Caprette, 2015). These substances once applied to a plant can act as fertilizer because some of the nutrients that promote microorganism growth also promote plant growth, such as nitrogen, sulfur and phosphorus.

This study will mainly focus plant health effect of MBI-3X on so-called root crops with varying underground structures, such as carrot (*Daucus carota*) and potato (*Solanum tuberosum*).

# Methods and Materials

## Root crop selection

Selected root crops were chosen for varying types of commercially important underground structures. Table 1 details the root crops chosen for this experiment. The following crops were chosen initially: radish, potato, carrot, onion, Narcissus *(Narcissus poeticus),* beet *(Beta vulgaris),* ginger *(Zingiber officinal),* and purple yam (*Dioscorea alata).* Ginger and yam were removed due to poor germination and difficulty obtaining starters.

## Planting Substrate

The planting substrate was placed in high heat resistant autoclave plastic bags. Substrate was autoclaved at 121 degrees Celsius for one hour, and was left to cool before incorporation of fertilizer. General substrate composition consisted of 22.5% silt loam (topsoil), 17.5% peat moss, 50% sand, 7.5% perlite, and 2.5% vermiculite. The soil series used for the topsoil component of the substrate was a Yolo County silt loam obtained locally outside of Woodland, California, and classified as fine-silty loam from the thermic family of Mollic Xerofluvents. This soil is well drained, and has moderate permeability (National Cooperative Soil Survey 2000).

Analysis of the substrate was done by Dellvalle Laboratory, Inc. on 03/21/2016 (Table 2). The substrate was slightly acidic at pH 6.1, but still within the acceptable range for most crops (The Gardener's Network, 2016). Electro-conductivity of substrate was high (6.06 dS/m), which reflects the high concentration of salt in the substrate. The soil was saline. However, once

substrate was placed in containers water was added to the soil until fully saturated. The excess water drained out of the holes at the bottom of the containers. The water should have washed off some of the salinity out of the substrate by the time the seeds germinated. Substrate was chosen because it had high concentration of sand, which was easier to wash off for root analysis. Table 2 showed the substrate contained a relatively low concentration of potassium, which was addressed through the incorporation of slow release fertilizer.

Osmocote Classic mini prill slow release fertilizer was incorporated into the soil mixture once the soil was cooled to room temperature. The fertilizer contained 19% nitrogen, 6% phosphate, 10% potassium, and 3.5% sulfur. The fertilizer was encased in a yellow, solid, mini pearl shaped sphere which slowly releases the fertilizer when the soil temperature reached 15 °C and above. Release was also affected by the amount of moisture within the soil. Warm moist conditions quicken the release of fertilizer. The fertilizer can last for 3-4 months when temperatures are around 20 °C. The amounts of Osmocote added per container were 73 g per 6.6 L, 30 g per 2.8 L, and 7.9 g per 0.5 L.

The supplemental fertilizer used was Miracle-Gro® Water Soluble All Purpose Plant Food. It contained 24% nitrogen, 8% phosphate, and 16% potassium. The supplemental fertilizer was only used when a majority of the plants in one crop planting began to exhibit nitrogen deficiency (chlorosis on leaves). A stock solution of 14.18 g of Miracle-Gro® to 3.79 L of water was prepared. Once thoroughly mixed, 50 mL of the stock solution was poured onto the base of the plant. There was a deliberate avoidance of the foliar section of the plant during the drench.

This was to mimic field conditions and ensure that there was complete coverage of the underground structures.

**Planting and Growing Conditions**

Plants were grown in a greenhouse located in Davis, California (38°32'31.8"N, 121°43'34.0"W). Greenhouse lights were turned on to ensure a minimum 14-hour day length if ambient light was below 18 kLUX. Relative humidity was maintained at 55% ± 5%. Dehumidification turned on when relative humidity went above 60%. Day/night temperature was kept in the range of 18.9 - 26.7 °C.

Container sizes were selected based on underground structure. Three types of round, black, plastic containers were used: 6.6 L, 2.8 L, and 0.5 L. Containers were lined with unbleached paper towels to prevent the planting substrate from seeping out. Once the container was filled with the fertilized substrate, two or more seeds were planted in the center of the container. The container was watered thoroughly and left to germinate. After seeds germinated plants were thinned down to only one plant per container. The plant that was closest to the center and most similar in height to the rest of the population was chosen.

**Experimental Design**

Each crop and cultivar constituted a single independent experiment, and each experiment was repeated once. The design used was a randomized complete block with five blocks. Each block had one container (replicate) per treatment. There were seven treatments; therefore, each block contained seven containers.

Table 1 shows the dates of planting, drenches, and harvest. It also provides the repetition and block numbers. For the second repetition, 2.8 L container crops, Pacemaker III beet, Red Ace beet, Dutch Master Narcissus, and Walla Walla Onion were increased to 10 blocks to raise the power of the experiment. Radish had 10 blocks for both repetitions. The exceptions were carrot and narcissus due to poor germination: carrot had five, and Dutch Master narcissus had seven blocks.

Blocks were arranged based on the size of the plants. Research Randomizer website was used to generate randomized numbers for blocking each crop experiment.

**Treatment rates and applications**

Treatments were based around the field rate of MBI-3X: 0, 0.5x, 1x, 2x, 3x, 4x, 5x. Treatment one was the control with distilled water. When smallest plants were at 2-3 true leaves, the first drench of MBI-3X was applied. A second drench was applied 21 days after the first application. Refer to Table 3 for concentrations of MBI-3X per container size. Concentrations of MBI-3X were determined based on the standard field application rate on MBI-3X nematicide label of 3.07 L per hectare, equivalent to the high end of the label rate (8 quarts per acre). The goal was to observe any changes based on different multiples or fractions of the standard field application rate of MBI-3X. A 50 mL carrier volume was chosen to mimic the field application of MBI-3X through drip tape, sprinkler, or other drench mechanism. 50 mL is roughly equivalent to 13,565 L per hectare; the volume was enough to saturate most of the underground structures in the pots. Calculations were based on the surface of each container (0.5 L, 2.8 L, and 6.6 L). Treatments were diluted into 50 mL solutions, with the balance being deionized water. After

each drench, observations of phytotoxicity were made after seven and 14 days, and at weekly intervals thereafter, unless no phytotoxicity had yet been observed.

**Harvest Protocol**

Harvest for each crop was timed according to the seed lot instructions and/or observed growing stages of each crop. Harvest date adjustment was made for the second repetition of narcissus because above ground plant structures were senescing earlier than expected. Above-ground biomasses was cut at soil height and evaluated for fresh and dry weights. Commercially important underground structures were evaluated for fresh weight. To provide additional information on root system health WinRHIZO (Regent Instruments Inc., STD4800 Scanner, 2014), root scanning software, was used to scan roots for fine root tip counts and length. The root length is the total added amount of linear root lengths within the given root diameter range. Repetition 1of carrot and both repetitions for radish were omitted from root scans due to early harvest before WinRHIZO was available. Fine root length in ranges 0.0 to 0.5 mm and 0.5 to 1.0 mm diameters were counted, and total linear length of those roots were summed up as the total length for that specified range. The results in the ANOVA are the average fine root length (cm) for the treatment. Fine root tip count in ranges 0.0 to 0.5 mm and 0.5 to 1.0 mm diameters were counted, and the total tips were combine as the total tip count for that specified range.

**Statistical Analysis Parameters**

Analysis was done using Minitab 17 software. The null hypothesis was, “there were no significant differences among treatments.” The alternative hypothesis was, “there was at least one treatment that was significantly different.” Normality and equal variance assumptions were

checked at the beginning of each analysis (Schilling, 2014). An ANOVA table was produced by running a general linear model and a residual table was created to determine possible outliers. Any observation with a standard residual greater than three was removed and the data was re-analyzed without the omitted outlier. Patino and Ferreira (2015) noted that confidence interval presents the imprecision or uncertainty around the effect size. The narrower the confidence interval is, the more certainty the effect size is representing the true population. In literature the standard confidence interval is 95%, but 90% or 99% are also acceptable (Patino and Ferreira, 2015 and Schilling, 2014 and Porcher, 2009). Thus, since the imprecision of plant health work is expected to be relatively greater than other more frequently traversed lines of research, the confidence interval was set at 90% for analyses.

The sample size for this experiment is small, so the margin of error will be greater. If the treatment P-value of ANOVA was greater than 0.10 ($P>0.10$), it was deemed not significant. If the treatment P-value was less than 0.10 ($P<0.10$), then a Fisher Protected LSD post hoc test was used for finding significant among treatment means. Plants that were incompletely formed, such as lacking measurable above or below-ground biomass, were excluded from analysis. Polynomial regression curves were used to find possible trends between repetitions. Since plant health is a dose response curve the results will most likely a curvilinear response; a polynomial regression curve was the preferred choice to show possible dose-rate trends. $R^2$, Fisher protected least significant difference, and coefficient of variation percentage values were used to assist with trend observations.

**Phytotoxicity Ratings and Statistical Analysis Parameters**

Phytotoxicity was rated on a scale of zero through five. Zero represented no phytotoxicity observed and five represented observed 100% foliar damage or no viable above surface structure. If there was partial foliar damage on a leaf, then a half point was given. However potato was treated as a percentage of leaves affected due to large mass size. Phytotoxicity evaluations included observations of chlorosis, necrosis, or any leaf/steam deformation the plant exhibited after treatment.

Phytotoxicity analysis was conducted as described for harvest data analysis.

# Results

## Phytotoxicity

Phytotoxicity analysis for all crops resulted in no significant effect. At probability level $p<0.10$ and confidence interval of 90%, MBI-3X did not exhibit significant phytotoxic effects on any of the crops used in this study.

## Pacemaker III beet experiment results

The first repetition yielded no significant p-values for harvestable plant parts, fine root tips and lengths counts (Table 4). However, the second repetition showed significance with total fine root length in the fine root diameter range 0.0 to 0.5 mm and fine root tips in both ranges (0.0 to 0.5 mm and 0.5 to 1.0 mm) (Table 5).

Growth parameters that exhibited significant p-values were compared between repetitions. A polynomial regression curve was used for trend analysis. Fine root tip in the range from 0.0 to 0.5 mm showed counts that were similar to the untreated control for all treatments (Figure 2). The second repetition showed a positive trend towards higher doses of MBI-3X (At five times the standard field rate with 1933 tip count average. The untreated control had the smallest count average (1108 tips). The $R^2$ value for repetition 2 was 0.88. A positive plant health trend was observed for the second repetition. The first repetition did not show any trend. There was no difference among the treatments compared to the untreated.

For fine root tip count in the range of 0.5 to 1.0 mm (Figure 3), the first repetition showed no significant trend. The error bars are very small for all rates for repetition one. The second repetition showed a positive trend towards higher doses of MBI-3X with 172 average tip counts for five times the standard field rate. The untreated control was the lowest compared to all other treatment at 103 tip counts. The $R^2$ value was 0.87. The second repetition had more root tips for both ranges compared to the first repetition. This was not observed for the fine root length.

**Red Ace beet experiment results**

Red Ace first repetition had significant results for fresh beet weight and both ranges of fine root tips (0.0 to 0.5 mm and 0.5 to 1.0 mm) (Table 6). The second repetition did not yield the same results (Table 7). There were no significant p-values for any of the growth parameters.

The first repetition had a U-shaped regression curve (Figure 4). The untreated control had the largest beet size out of all rates at 110.41 g. The downward slope trend continued until four times the standard field rate (34.49 g) and picked up at five times the standard field rate (75.37 g). The $R^2$ value was 0.64. The lowest beet weight was 34.49 g for four times the standard field rate. Underground structure phytotoxicity could not be ruled out for this study. However this negative impact to beet biomass was not observed in beets applied with five times the standard field rates of MBI-3X. This might be an outlier because the coefficient of variation on Table 6 was 59.48. The second repetition regression curve showed no significant differences among treatments.

For fine tip counts repetition comparisons at in the range 0.0 to 0.5 mm, repetition 1 had a high count average for the untreated control (Figure 5). Nonetheless, there was no real trend with either repetition. The $R^2$ value was 0.06 for the first repetition. The second repetition regression

curve fared worse with $R^2$ value of 0.03. Even with significant p-value for the first repetition, the regression curve showed no real trend. The untreated had the most fine root tip count.

Fine tip counts repetition comparisons in the range 0.5 to 1.0 mm (Figure 6) showed no trend for either repetition. $R^2$ values were very low for both repetitions.

**Sugar beet experiment results**

The first repetition had significant p-values for fine root tips in the range 0.0 to 0.5 mm (Table 8). Four times the standard field rate had the lowest count of 751 tips. The second repetition had significant p-value for fine root length in the range 0.5 to 1.0 mm (Table 9). The second repetition did not show the same significant result for the same growth parameter that repetition one had.

Fine root lengths in the range 0.5 to 1.0 mm repetition comparison did not show any consistent trends (Figure 7). Both repetitions had very low $R^2$ values (0.29 and 0.50 respectively). The second repetition had large error bars compared to the first. For the second repetition at rates four times the field rate had the most fine root length of 654.46 cm compared to the untreated.

The fine root tip count averages for 0.0 to 0.5 mm range comparison had better regression curves (Figure 8). However, both $R^2$ values were very low (0.42 and 0.69 respectively). First repetition did not show any discernable trend. The twice the standard field rate had the lowest fine root tip count compared to the untreated. The second repetition had a dosage curve that continued to increase up to four times the standard field rate.

**Scarlet Nantes carrot experiment results**

Both repetitions did not yield any significant results (Tables 10 and 11); therefore no regression curves were produced.

**New Baby and Dutch Master Narcissus experiment results**

The first repetition with New Baby narcissus had no significant results (Table 12). The second repetition with Dutch Master Narcissus had significant results (Table 13). There were significant p-values for fresh foliar, root, and foliar water weights. In both repetitions fine root length and tips did not yield any significant differences.

The foliar fresh weight repetitions comparison agreed with the ANOVA results (Figure 9). The first repetition showed no actual trend ($R^2$ at 0.06), while the second repetition showed a better regression curve ($R^2$ at 0.55) with twice the amount of standard field rate having the highest average fresh foliar weight (10.43 g).

Fresh root weight also showed no trend for the first repetition, but the second repetition showed a possible dosage response curve ($R^2$ at 0.53) (Figure 10). At lower doses of MBI-3X the root weight was above the untreated control of 8.31 g. At half the standard field rate had 10.43 g and the standard field rate had 14.24 g. After that the fresh root weights decreased as concentration of MBI-3X increased. Thus, for at least one repetition of fresh root weight there was a possible dose dependent response.

The foliar water weight repetitions comparison was very similar to the foliar fresh weight comparison (Figure 11). The second repetition had an $R^2$ value of 0.56.The regression curve showed an increase in foliar water weight from untreated control all the way up to twice the standard field rate. After that the weight decreased. There was a possible dosage curve for the

second repetition of narcissus. Based on the fresh water weight result, the dose response curve seen in foliar fresh weight was due to increase uptake of water with added MBI-3X. Significant result was not observed in the dry foliar weight. Another study of narcissus is needed to confirm this phenomenon of MBI-3X increasing foliar biomass by inducing uptake of water.

**Walla Walla onion experiment results**

The first repetition had significant results for fine root length in the ranges 0.0 to 0.5 mm and fine root tip counts for both ranges (0.0 to 0.5 mm and 0.5 to 1.0 mm) (Table 14). However on the second repetition, there were no significant results for any growth parameters (Table 15).

Onion did not show trends for fine root length averages comparison in the range 0.0 to 0.5 mm (Figure 12). Nonetheless, the first repetition did show a very slightly positive linear dose response with $R^2$ value of 0.79. Even with no significant results the second repetition at rates four and five times the standard field rate had the most fine root tip lengths compared to the untreated. The untreated control had the lowest length count average of 105.06 mm. Five times standard field rate had the highest average of 153.40 mm.

Fine root tip count averages in the range 0.0 to 0.5 mm repetitions comparison showed a positive regression curve only on the first repetition with $R^2$ value of 0.82 (Figure 13). As MBI-3X rates increased there was an increase in average for fine root tip count at this range. The second repetition showed no trend, and root tip counts were much lower compared to the first.

Fine root tip count averages in the range 0.5 to 1.0 mm repetitions comparison showed the positive regression curve only on the first repetition with $R^2$ value of 0.71 (Figure 14). As MBI-3X rates increased there was an increased average for fine root tip count at this range. The

second repetition showed no trend. The first repetition had a larger amount of root tips on average compared to the second, but the standard errors were larger in the first repetition compared to the second.

**Golden potato experiment results**

The first repetition had significant results for fine tip counts for both ranges (0.0 to 0.5 mm and 0.5 to 1.0 mm) (Table 16). The second repetition had significant results for fine root length in the range 0.0 to 0.5 mm, and fine tip counts in the range 0.0 to 0.5 mm (Table 17).

Fine root length average in the range 0.0 to 0.5 mm repetitions comparison exhibited positive trend in the second repetition with the $R^2$ value of 0.88 (Figure 15). With an increase in concentration of MBI-3X there was an increase in fine root length counts for the second repetition. The first did not show any significant difference among treatments. The average amount of root length in range 0.0 to 0.5 mm was more in second repetition compared to the first.

There was positive plant health trend for potato fine root tip count average between repetitions comparison in the range 0.0 to 0.5 mm (Figure 16). In the first repetition the untreated control had 4793 tip count. As the rate of MBI-3X increased, the root tip count decreased until twice the standard field rate with 3002 tip count. After the twice the standard field rate, the root tip count increased with the highest count of 5043 tips. However, even with the dose curve the $R^2$ value was relatively low at 0.48. The second repetition showed significant trend. The regression curved show a positive upward trend. With increase rates of MBI-3X the higher to fine root tip counts. $R^2$ value was 0.91. The second repetition showed a stronger dose response curve compared to the first repetition. As MBI-3X rates increased there were more fine tip

counts at this range. These two repetitions were the only ones that exhibited repeated significant for a growth parameter for any crop.

Fine root tip count average between repetitions comparison in the range 0.5 to 1.0 mm was different (Figure 17). The first repetition still showed no significant regression curve. The second repetition had a probable dose rate response curve with an $R^2$ value of 0.84. A half of the field rate had more root tips (1011 tips) compared to all other treatments. At standard field rate tip counts decreased, and continued to decrease as rates increased.

**Cherry Belle radish experiment results**

Radish edible root fresh weight was the only growth parameter with significant result (Table 18). There were no significant results for the second repetition of radish (Table 19). There was no trend observed for beet average weight repetitions comparison even with significant results for the first repetition (Figure 18).

## Discussion

The most prominent significant ANOVA results were for fine roots growth parameters for most of the crop experiments. In Hassan (2017) and Khan's et al. (2009) bio-stimulants studies, increase root growth and development was observed when bio-stimulants were added compared to the untreated control plants. For this MBI-3X study, fine root tips exhibited an increase in biomass for especially tips in range 0.0 to 0.5 mm as MBI-3X increased for Pacemaker III beet second and onion first repetition. The sugar beet exhibited a more hormesis-like dose response curve and the potato had a positive U shaped curve response.

Narcissus, radish, and Red Ace beet also showed biomass increase in non-fine root growth parameters, but these significant results were only observed in one repetition. The ANOVA results showed no consistency between repetitions of most crops. The only crop experiment that had both repetitions showing repeated significant ANOVA results was potato. Fine tip count in the range 0.0 to 0.5 mm was repeated for potato. A strong positive trend was observed for the second repetition, but not for the first. The first have a positive regression curve but the significant rates had less tips compared to the untreated control. Potential hormesis curves were only found in Narcissus second repetition. Nonetheless, trends were inconsistent, and MBI-3X plant health effects could not be confidently confirmed for root crops in this study.

Plant health effects were not consistently repeated between repetitions, which is not an unusual phenomenon, and has been previously reported in the literature (Cedergreen, 2008; Cassán and Díaz-Zorita, 2016). Seasonal differences can be the reason why some repetitions

were not exhibiting the same results. Some of the repetitions were not grown in the same season due to space and time restriction in the greenhouse. However, all other environmental settings were the same for both repetitions.

Seasonality was not an issue with Pacemaker III and Red Ace beet repetitions. These repetitions were done simultaneously, but in different locations within the greenhouse. This eliminated potential seasonal effect on harvest results. Nevertheless, those repetitions still exhibited different trends.

Belz and Cedergreen's (2010) study on effect of growing conditions on hormesis of the herbicide parathenin showed small changes to the growing condition such as temperature, nutrient, and light intensity can affect hormesis response in lettuce (*Lactuca sativa var. capitate)* on root growth. Schaecher (2017) noted that the microbiomes performance varied in different locations within and across the field. He noted that differences in soil and environmental conditions can affect the microorganisms' performance. Bio-stimulants can improve underperforming fields because soil microbiomes can be an indicator for soil yield potential.

For this MBI-3X study, microclimate seems to have played a role on divergent results between repetitions for plant health. Figure 19 diagramed the first repetition of Red Ace beet and second repetition Pacemaker III beet were on benches that were shaded by a wall, which partitioned the two greenhouses. The second repetition of Red Ace beet and first repetition Pacemaker III beet benches were in the sunnier and warmer location of the greenhouse. The second repetition of Red Ace beet and first repetition Pacemaker III beet did not exhibit any significant treatment response. The microclimate for first repetition of Red Ace beet and second

repetition Pacemaker III beet were in a slightly more shaded area of the greenhouse. They were near a wall partitioning two greenhouses compared to their counterparts which are at the center of the greenhouse with no wall shade. The minor reduction to natural sunlight can act as a potential stressor that triggered the plant roots to interact with MBI-3X microbial broth. MBI-3X may have been acting as a bio-stimulant to plants that were growing in suboptimal conditions. Plant and microbe symbiotic interaction is vital for plant development (Schaecher, 2017). With the addition of MBI-3X, the concentration of secondary metabolites near the roots are more readily available for the root to interact with in the presence of an abiotic stress, such as partial shading.

In this study, MBI-3X did not have any hormesis effect on root crops except for the second repetition of narcissus. The hormesis effect was not in the usual range for the standard hormesis curve (Calabrese, 2014). Usually the dosages are small percentage of a toxin, and not at full standard field rate, such as 10% or 15% (Cedergreen, 2008). Plant growth improved compared to the control up to double the amount of standard field rate for MBI-3X. In figure 1, the rates of MBI-3X used for this study are much lower than the kill rate of *Romidepsin*. The rates of MBI-3X must be very large in order to see herbicidal effects on the root crops. The rates used in this study were still within the hormesis zone of the dose curve of *Romidepsin*. MBI-3X contains a very small amount of *Romidepsin* at standard field rate. To show herbicidal effects, MBI-3X would need to be at least100 times more concentrated than the standard field rate used in this experiment (Louis Boddy, 2018). Based on this MBI-3X study's results, narcissus second repetition exhibited a response curve that was closely aligned to Calabrese's (2014) description of hormesis biphasic dose response. MBI-3X concentrations of half, at, twice, and triple standard

field rates had increasing yields. At four and five times the standard field rate of MBI-3X the yield quickly decreased.

Belz and Cedergreen (2010) also argued that hormesis was more likely to occur when the growth medium was suboptimal. In this study, soil nutrients were not a limiting factor. Fertilization through slow release prill was incorporated within the autoclaved soil. Macro and micronutrients were abundant and within normal growing standards (Table 2). Environmental condition is an important factor that can determine if hormesis will happen. They argued that hormesis was unlikely to happen in conditions that are optimal for growth and under extreme adverse conditions. Hormesis will appear somewhere in between these conditions. Expression of hormesis is therefore likely environmentally dependent.

Irrigation, lighting, and temperature were usually set to optimal growth conditions in the greenhouse. Small changes to the growing condition can affect plant health response (Belz and Cedergreen, 2010 and Schaecher, 2017). These seemingly minor changes can affect the ability for significant results in one repetition to be repeated in another (Cedergreen, 2008).

For plant health, Cassán and Díaz-Zorita (2016) argued inconsistent field results were a major obstacle to why many plant growth promoting compounds were not released commercially. In their case, *Azospirillum sp.* inoculation showed signs of increased plant biomass. The inoculant had a production response or plant growth response for 70% of the experiments that were reviewed. The experiments were set up in various locations, such as in Asia and South America. The inconsistent results were perhaps caused by the complex interactions between the modes of actions the microorganisms had on the plants. Multiple abiotic

stress conditions within the field prevented the presence of microorganisms. Cassán and Díaz-Zorita noted that the type of crop management methods and practices were closely related to the occurrence of abiotic limitation to plant growth, which can partially explain for inconsistent results.

Thus, MBI-3X was perhaps not able to act as a bio-stimulant for the second repetition of Red Ace beet and first repetition Pacemaker III beet because the crops were already located in optimal growing conditions. MBI-3X was more able to act as a bio-stimulant for the first repetition of Red Ace beet and second repetition Pacemaker III beet due to slightly suboptimal growing conditions.

Schaecher (2017) presented an argument that bio-stimulants interact with roots only when plants are stressed by specific stressors. For example, *Burkholderia phytofirmans* interacts with plant roots when the plant is in the cold, drought, or in light stress (Schaecher, 2017). In this study, the second repetition of narcissus had to endure longer and colder season to break dormancy of the bulbs, even though it was planted in the spring. There were less macro and micronutrients left in the soil than when initially started due to the long dormancy period. Consequently, the plants were more likely to accept MBI-3X as a bio-stimulant.

Cedergreen (2008) also stated that a small population size can mask plant health effects like hormesis, bio-stimulant, and bio-fertilizer. In a small population plant health effects can be hard to notice. Our study population was small due to space and resource limitations. Replicates/blocks were either five or ten plants each. A plant health study with larger population might show better results.

Besides hormesis, does MBI-3X improve plant health through other mechanisms? Schaecher (2017) noted the microbial products, such as MBI-3X, contained bacteria and fungi that acted as a crop protectant and enhancement. Microbial interaction is crop- and growth-condition specific. MBI-3X may be acting as a bio-stimulant because there were increased biomasses in some of the root crops. This was especially noticeable on fine root tips in range 0.0 to 0.5 mm. At the higher rates there was increase in root tip count. Pacemaker III beet second repetition for fine tip count in the range 0.0 to 0.5 mm showed an increase in yield as rates of MBI-3X increased compared to untreated control. This showed that MBI-3X can act as a bio-stimulant for some of the root crops. Referring back to Pacemaker III and Red Ace beets repetitions, MBI-3X appeared to act as a bio-stimulant by alleviating the abiotic stress of partial shading. However, trend was not repeated between repetitions. On the other hand, the nutrient broth MBI-3X contents could have acted as fertilizer by providing the plants with additional nutrient supplements. Similar to the bio-fertilizer, Ning Shield, MBI-3X did increase root growth parameters. However, MBI-3X cannot be called a bio-fertilizer. At standard field rate, the nutrient concentrations were too small to be useful for the target plants. Therefore, further studies are needed to fully confirm this notion of which components of MBI-3X is causing the plant health effects.

## Conclusion

The most noticeable growth parameter that continues to show up for the selected root crops that exhibited significant results was the increase in fine roots. However, MBI-3X did not show any consistent plant health effect on the root crops in this study.

Based on the results of this study MBI-3X does not appear to induce hormesis in these crops at the rates tested except for narcissus second repetition. The hormesis effect was not reflected on the previous repetition, so confidence in this effect for narcissus is very low. However, MBI-3X did exhibit bio-stimulant tendencies for some of the crops. Concentrations of nutrient from the nutrient broth at standard rate leave great doubt for MBI-3X as a bio-fertilizer. Abiotic stressors, such as day length, irrigations, seasonality, and even plant locations could have cause the inconsistent results. Microbial interactions are complex and contain multiple modes of actions that are sensitive to changes in environment (Cassán and Díaz-Zorita, 2016). This can inhibit or induce response depending on the situation. As a bio-stimulant, it seems MBI-3X could enhance growth of certain growth parameters in fields at not as optimal growth conditions, such as low microbial flora and nutrients (Scheacher, 2017). MBI-3X can ameliorate abiotic stress in underperforming fields and bring them up to standard by adding beneficial microorganisms and nutrient into the soil microbiomes. This can improve soil yield potential (Schaecher, 2017).

A larger sample size can increase the response results because plant health effects are hard to notice in small experiments (Cedegreen, 2008). Overall, there were signs of plant health

bio-stimulus from MBI-3X, but a larger sample size with more controlled conditions is required to adequately test for this effect. A breakdown of components of MBI-3X can simplify this endeavor.

Literature Cited

Abad, P. et al. 2008. Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotechnology. 26:909-915. doi:10.1038/nbt.1482.http://www.nature.com/nbt/journal/v26/n8/full/nbt.1482.html

Aktar, M.W, D. Sengupta , A. Chowdhury. 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicology. 2(1):1-12. doi:10.2478/v10102-009-0001-7

Asolkar, R.N., A.L. Cordova-Kreylos, P. Himmel , P.G. Marrone. 2013. Discovery and Development of Natural Products for Pest Management. American Chemical Society. 3:17-30. doi: 10.1021/bk-2013-1141.ch003

Belz, R. G., N. Cedergreen. 2010. Parthenin hormesis in plants depends on growth conditions. Environmental and Experimental Botany. 69:293-301. doi:10.1016/j.envexpbot.2010.04.010.

Brito, I.P., L. Tropaldi, C.A. Carbonari, E.D. Velini. 2017. Hormesis effects of glyphosate on plants. Pest Management Science. doi:10.1002/ps.4523

Calabrese, E. J. 2014. Hormesis: a fundamental concept in biology. Microbal Cell 1(5):145-149. doi: 10.15698/mic2014.05.145

Calabrese, E. J., and L. A. Baldwin. 2000. Radiation hormesis: the demise of a legitimate hypothesis. Hum. Exp. Toxicol. 19:76–84. doi: 10.1191/096032700678815611

Calabrese, E. J., L. A. Baldwin. 2003. Hormesis: The Dose-Response Revolution. Annu. Rev. Pharmacol. Toxicol. 43:175-97. doi: 10.1146/annurev.pharmtox.43.100901.140223.

Caprette, D. R. 2015. Laboratory Studies in Applied Microbiology. Rice University. http://www.ruf.rice.edu/~bioslabs/bios318/318manual.htm (March 19, 2018).

Cassán F., M. Díaz-Zorita. 2016. The Contribution of the Use of *Azospirillum sp*. in Sustainable Agriculture: Learnings from the Laboratory to the Field. In: Castro-Sowinski S. (eds) Microbial Models: From Environmental to Industrial Sustainability. Microorganisms for Sustainability, vol 1. Springer, Singapore. 293-321. doi:10.1007/978-981-10-2555-6_14

Cedergreen, N. 2008. Herbicides can stimulate plant growth. Weed Research, 48: 429-438. doi:10.1111/j.1365-3180.2008.00646.x

Chelinhoa, S., et al. 2017. Toxicity of the bionematicide 1,4-naphthoquinone on non-target soil organisms. Chemosphere. 181: 579-588. doi:10.1016/j.chemosphere.2017.04.092

Chitwood, D.J. 2003. Nematicides. Encyclopedia of Agrochemicals. 3:1104–1115, John Wiley & Sons, New York, NY; http://handle.nal.usda.gov/10113/43874

Cook, J.R. 2000. Advances in Plant Health Management in the Twentieth Century. Annual Review of Phytopathology 38:1, 95-116. doi:10.1146/annurev.phyto.38.1.95

Delaplane, K.S. 1996. Pesticide Usage in the United States: History, Benefits, Risks, and Trends. The University of Georgia, Athens. http://ipm.ncsu.edu/safety/factsheets/pestuse.pdf

Döring, T. F., M. Pautasso, M.R. Finckh, and M.S. Wolfe. 2012. Concepts of plant health – reviewing and challenging the foundations of plant protection. Plant Pathology. 61:1–15. doi:10.1111/j.1365-3059.2011.02501.x

Everris. 2016. Osmocote Classic mini prill. http://everris.us.com/product/3-4-mo-19-6-10-mini-prill (July 11, 2016).

Extension Toxicology Network EXTOXNET. 1993. Methyl bromide, Cooperative Extension Offices of Cornell University, Michigan State University, Oregon State University, and University of California at Davis.

http://pmep.cce.cornell.edu/profiles/extoxnet/haloxyfop-methylparathion/methyl-bromide-ext.htmL. (October 21, 2015)

Hassan, S.E.D., 2017. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium L.* Journal of Advanced Research. 8(6): 687-695. doi:10.1016/j.jare.2017.09.001.

Jiang, C. H., et al. 2018. Evaluation of root-knot nematode disease control and plant growth promotion potential of biofertilizer Ning shield on *Trichosanthes kirilowii* in the field. Brazilian Journal of Microbiology. 49:232-239. doi: 10.1016/j.bjm.2017.08.009.

Jungk, A. 2001. Root hairs and acquisition of plant nutrients from soil. J. Plant Nutr.Soil Sci. (2001), 164: 121-129. doi: 1436-8730/01/0204-0121

Khan, W., U. P. Rayirath, S. Subramanian, et al. 2009. Seaweed Extracts as Biostimulants of Plant Growth and Development. Journal of Plant Growth Regulation. 28: 386-399. doi:10.1007/s00344-009-9103-x

Minitab. 2013. MINITAB 17. Minitab, State College, PA.

Mitkowski, N., and G. S. Abawi. 2003. Root-knot nematodes. Plant Health Instructor. 10: 194. doi: 10.1094/PHI-I- 2003-0917-01. http://www.apsnet.org/edcenter/intropp/lessons/Nematodes/Pages/RootknotNematode.aspx\

National Cooperative Soil Survey. 2000. Official Series Edition Yolo Series. https://soilseries.sc.egov.usda.gov/OSD_Docs/Y/YOLO.htmL (July 12, 2016).

Ntalli, N.G., and P. Caboni. 2012. Botanical Nematicides: A Review. Journal of Agricultural and Food Chemistry. 60(40): 9929-9940. doi: 10.1021/jf303107j

Patino, C. M., J.C. Ferreira. 2015. Confidence intervals: a useful statistical tool to estimate effect sizes in the real world. Jornal Brasileiro de Pneumologia. 41 (6):565-566. doi:10.1590/s1806-37562015000000314

Penn State College of Agricultural Sciences. 2015 Phytotoxicity – Plant Diseases, Penn State Extension, Penn State. http://extension.psu.edu/pests/plant-diseases/all-fact-sheets/phytotoxicity (October 21, 2015)

Plant Protection 1/135 (4). 2014 Phytotoxicity assessment. European and Mediterranean Plant Protection Organization Bulletin. 44: 265–273. doi: 10.1111/epp.12134

Porcher, R. 2009. Reporting Results of Orthopaedic Research: Confidence Intervals and p Values. Clinical Orthopaedics and Related Research. 467(10): 2736–2737. http://doi.org/10.1007/s11999-009-0952-1

Radewald, J. D., M. V. McKenry, P. A. Roberts, B. B. Westerdahl. 1984. The importance of soil fumigation for nematode control. California Agriculture. November-December: 16-17. https://ucanr.edu/repositoryfiles/ca4111p16-63044.pdf

Regent Instruments Inc. 1996. WinRHIZO. Regent Instruments, Canada http://regent.qc.ca/assets/winrhizo_about.html

Report on Plant Disease 1101. 1993. Root Knot Nematodes. University of Illinois Extension, College of Agriculture, Consumer and Environmental Sciences. http://extension.cropsciences.illinois.edu/fruitveg/pdfs/1101.pdf

Schaecher, S. 2017.Microbes in Agriculture Battling the Perceptions of Snake Oil. Presentation presented at: 2nd Partnerships in Biocontrol, Biostimulants & Microbiome. Global Engage, Philadelphia, Pennsylvania. 2–3 Nov. 2017.

Schilling, M.W. 2014. CHEMICAL ANALYSIS Sampling and Statistical Requirements. In: C. Devine, M. Dikeman, editors. Encyclopedia of Meat Sciences Second Edition. Elsevier, Amsterdam, Netherlands 187 – 192. doi: 10.1016/B978-0-12-384731-7.00051-9

Scotts Company LLC. 2016. Miracle-Gro® Water Soluble All Purpose Plant Food. http://www.miraclegro.com/smg/goprod/miracle-gro-plant-food/prod70342?locale=en_US (July 11,2016)

The Gardener's Network. 2016. Soil pH Levels for Vegetables. http://www.gardenersnet.com/atoz/phlevel1.htm (July 18, 2016)

Trudgill, D. L., and V. C. Blok. 2001. Apomictic, polyphagous root-knot nematodes: Exceptionally successful and damaging biotrophic root pathogens. Annu. Rev. Phytopathol. 39:53-77. doi: 10.1146/annurev.phyto.39.1.53

University of California, Davis Integrated Pest Management. 2014. Definition of Integrated Pest Management, University of California Integrated Pest Management, University of California. http://www.ipm.ucdavis.edu/GENERAL/ipmdefinition.htmL(October 19, 2015)

University of California, Davis Integrated Pest Management. 2014. Nematode Management, University of California Integrated Pest Management, University of California. http://www.ipm.ucdavis.edu/GENERAL/ipmdefinition.htmL (October 19, 2015)

Urbaniak G. C. and Plous S. 1997-2016. Research Randomizer. https://www.randomizer.org/ (July 11, 2016)

William-Linera, W. and J.J. Ewel. 1984. Effect of autoclave sterilization of a tropical andept on seed germination and seedling growth. Plant and Soil. 82: 263-268. Revised 1984. https://people.clas.ufl.edu/jackewel/files/williams-linera-1984-plant-and-soil.pdf

# Appendix

Tables

**Table 1.** Important dates and information of each crop used.

| Cultivar | Crop | Repetition | Pot size | Blocks | Root structures | Important Dates | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Planting | First Drench | Second Drench | Harvest |
| Pacemaker III | Beet | 1‡ | 2.8 L | 10 | Beet | 05/16/2016 | 06/01/2016 | 06/22/2016 | 07/05/2016 |
| | | 2 | 2.8 L | 10 | Beet | 05/20/2016 | 06/08/2016 | 06/29/2016 | 07/12/2016 |
| Red Ace | Beet | 1§ | 2.8 L | 10 | Beet | 05/17/2016 | 06/01/2016 | 06/22/2016 | 07/11/2016 |
| | | 2¶ | 2.8 L | 10 | Beet | 05/23/2016 | 06/08/2016 | 06/29/2016 | 07/14/2016 |
| Sugar | Beet | 1 | 6.6 L | 5 | Beet | 02/04/2016 | 02/26/2016 | 03/18/2016 | 05/03/2016 |
| | | 2# | 6.6 L | 5 | Beet | 05/09/2016 | 05/25/2016 | 06/10/2016 | 07/25/2016 |
| Scarlet Nantes | Carrot | 1†† | 2.8 L | 5 | Modified tap root | 01/21/2016 | 02/17/2016 | 03/09/2016 | 04/12/2016 |
| | | 2‡‡ | 2.8 L | 5 | Modified tap root | 05/16/2016 | 06/08/2016 | 06/29/2016 | 07/29/2016 |
| New Baby | Narcissus | 1 | 2.8 L | 5 | Bulb | 01/21/2016 | 02/17/2016 | 03/09/2016 | 05/04/2016 |
| Dutch Master | Narcissus | 2§§ | 2.8 L | 7 | Bulb | 05/16/2016 | 01/04/2017 | 01/25/2017 | 03/30/2017 |
| Walla Walla | Onion | 1 | 2.8 L | 5 | Bulb | 01/15/2016 | 02/17/2016 | 03/09/2016 | 05/03/2016 |
| | | 2¶¶ | 2.8 L | 10 | Bulb | 04/24/2016 | 5/18/2016 | 06/08/2016 | NA† |
| | | 2## | 2.8 L | 10 | Bulb | 12/09/2016 | 01/04/2017 | 01/25/2017 | 04/08/2017 |
| Golden | Potato | 1 | 6.6 L | 5 | Tuber | 01/27/2016 | 02/12/2016 | 03/04/2016 | 04/25/2016 |
| | | 2 | 6.6 L | 5 | Tuber | 04/21/2016 | 05/04/2016 | 05/25/2016 | 08/21/2016 |
| Cherry Belle | Radish††† | 1 | 0.5 L | 10 | Radish edible root | 01/27/2016 | 02/12/2016 | NA | 03/04/2016 |
| | | 2 | 0.5 L | 10 | Radish edible root | 03/08/2016 | 03/22/2016 | NA | 04/10/2016 |
| NA | Ginger‡‡‡ | 1 | 2.8 L | 5 | Rhizome | NA | NA | NA | NA |

**Table 1.** Continued

† NA, Not applicable.

‡ Missing data for fresh root weight for treatment 7 replicates 10 due to entry error.

§ Adjusted harvest date since the 9th is on a weekend.

¶ Some plants exhibited fungal growth.

# Plants were infested with aphids. However, aphids did not affect plant growth.

†† Omitted from root scans.

‡‡ Due to poor germination ten replicates became only five.

§§ Bulbs were dormant until shorter day length and cooler weather arrived. There are only 7 replicates due to poor germination.

¶¶ There was unusual high rate of mortality. It might be seasonally affected because previous planting in the colder season did not exhibit such mortality rate. This repetition was repeated in late December 2016.

## This was the do over for repetition 2 of onion in colder season.

††† Matured too fast for a second drench. Radish was omitted from root scans.

‡‡‡ Ginger were all pre-emergent drenches because poor germination. Ginger was not repeated because of germination difficulties.

**Table 2.** Bioanalysis of soil substrate used for the study.

| Minerals and pH of soil | Quantity/Value |
|---|---|
| pH | 6.10 |
| Total Salts | 6.06 dS/m |
| Calcium | 34.60 meq/l |
| Magnesium | 20.80 meq/l |
| Sodium | 11.40 meq/l |
| Alkali | 1.90% |
| Boron | 0.80 meg/l |
| Nitrate-N | 28.00 mg/kg |
| Phosphate-P | 49.00 mg/kg |
| Potassium | 49.00 mg/kg |
| Zinc | 2.60 mg/kg |
| Manganese | 36.90 mg/kg |
| Iron | 12.90 mg/kg |
| Copper | 3.10 mg/kg |

**Table 3.** MBI-3X concentrations (mL) based on container sizes**.**

| | | Container sizes | | | | | |
|---|---|---|---|---|---|---|---|
| | | Large 7L | | Medium 2.8L | | Small 0.5L | |
| **Treatment** | **Percentage rates from standard field rate of MBI-3X** | **Sample amount (mL)** | **Water amount (mL)** | **Sample amount (mL)** | **Water amount (mL)** | **Sample amount (mL)** | **Water amount (mL)** |
| 1 | 0% | 0.00 | 50.00 | 0.00 | 50.00 | 0.00 | 50.00 |
| 2 | 50% | 0.03 | 49.97 | 0.02 | 49.98 | 0.01 | 49.99 |
| 3 | 100% | 0.07 | 49.93 | 0.03 | 49.97 | 0.02 | 49.99 |
| 4 | 200% | 0.14 | 49.86 | 0.07 | 49.93 | 0.03 | 49.97 |
| 5 | 300% | 0.20 | 49.80 | 0.10 | 49.90 | 0.04 | 49.96 |
| 6 | 400% | 0.27 | 49.73 | 0.14 | 49.86 | 0.06 | 49.94 |
| 7 | 500% | 0.34 | 49.66 | 0.17 | 49.83 | 0.07 | 49.93 |

**Table 4.** Average weights and fine root lengths and tips of Pacemaker III Beet Repetition 1 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh Weight (g)** | **Beet Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 112.20 | 94.72 | 14.71 | 10.26 | 101.94 | 250.84 | 167.69 | 873 | 62 |
| 2 | 113.99 | 92.55 | 17.01 | 11.31 | 102.68 | 245.20 | 174.82 | 807 | 66 |
| 3 | 103.19 | 105.63 | 13.72 | 10.14 | 93.05 | 255.98 | 191.09 | 946 | 68 |
| 4 | 91.19 | 75.31 | 13.11 | 9.94 | 81.25 | 237.39 | 184.39 | 838 | 79 |
| 5 | 84.47 | 80.09 | 12.10 | 8.95 | 75.52 | 246.04 | 181.53 | 830 | 72 |
| 6 | 96.92 | 79.11 | 11.25 | 9.89 | 87.03 | 222.95 | 159.30 | 864 | 72 |
| 7 | 90.89 | 92.46 | 11.98 | 9.21 | 81.69 | 243.30 | 175.85 | 896 | 72 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV%[‡‡] | 37.00 | 48.44 | 53.65 | 31.94 | 38.21 | 48.19 | 34.32 | 43.27 | 34.12 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡] Additive sum of fine root length in the range provided in centimeters.

[§] Fine tip counts in the range provided.

[¶] Significance of treatment effects according to analysis of variance**.**

[#] Diameter range in millimeter.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

**Table 5.** Average weights and fine root lengths and tips of Pacemaker III Beet Repetition 2 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh Weight (g)** | **Beet Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 87.31 | 72.94 | 17.81 | 8.60 | 78.71 | 213.20 | 135.66 | 1108 | 103 |
| 2 | 72.64 | 66.29 | 16.30 | 7.49 | 65.15 | 253.18 | 191.89 | 1435 | 129 |
| 3 | 87.71 | 84.01 | 17.86 | 8.42 | 79.29 | 238.90 | 154.61 | 1181 | 114 |
| 4 | 91.72 | 75.03 | 16.85 | 7.86 | 80.92 | 225.37 | 163.52 | 1330 | 136 |
| 5 | 86.45 | 81.91 | 16.88 | 8.66 | 77.79 | 261.91 | 155.94 | 1577 | 145 |
| 6 | 104.33 | 92.39 | 19.96 | 10.19 | 94.14 | 243.27 | 133.48 | 1703 | 146 |
| 7 | 102.40 | 92.20 | 22.27 | 9.70 | 92.70 | 318.38 | 180.76 | 1933 | 172 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | ** | ** |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | 475.51 | 39.71 |
| CV%[‡‡] | 30.88 | 44.92 | 44.23 | 29.11 | 31.44 | 53.77 | 42.46 | 36.11 | 39.39 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 6.** Average weights and fine root lengths and tips of Red Ace Beet Repetition 1 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh Weight (g)** | **Beet Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)‡ #** | **0.50<.L.<=1.00 (mm)‡ #** | **0<.T.<=0.50 (mm)§#** | **0.50<.T.<=1.00 (mm)§#** |
| 1 | 77.96 | 110.41 | 13.21 | 9.17 | 68.79 | 169.48 | 115.89 | 1052 | 108 |
| 2 | 71.59 | 74.76 | 12.41 | 8.83 | 62.76 | 136.27 | 100.91 | 618 | 67 |
| 3 | 59.74 | 62.35 | 8.32 | 7.13 | 52.61 | 98.60 | 83.27 | 710 | 75 |
| 4 | 64.86 | 59.94 | 9.75 | 7.46 | 57.39 | 138.33 | 106.00 | 749 | 99 |
| 5 | 63.28 | 68.82 | 7.88 | 8.18 | 55.10 | 128.06 | 90.22 | 947 | 105 |
| 6 | 54.70 | 34.49 | 6.36 | 6.42 | 48.29 | 110.88 | 96.53 | 746 | 70 |
| 7 | 62.15 | 75.37 | 9.08 | 7.57 | 54.59 | 108.95 | 85.28 | 815 | 95 |
| p¶ | NS† | ** | NS | NS | NS | NS | NS | ** | ** |
| $LSD_{0.10}$†† | NS | 27.12 | NS | NS | NS | NS | NS | 226.17 | 23.75 |
| CV%‡‡ | 34.19 | 59.48 | 63.96 | 32.42 | 34.79 | 60.06 | 44.62 | 49.82 | 42.28 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 7.** Average weights and fine root lengths and tips of Red Ace Beet Repetition 2 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh Weight (g)** | **Beet Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 70.69 | 73.25 | 11.59 | 7.31 | 63.39 | 222.67 | 170.76 | 1008 | 129 |
| 2 | 69.55 | 79.39 | 11.26 | 7.44 | 62.11 | 221.12 | 151.55 | 988 | 110 |
| 3 | 69.07 | 64.57 | 11.29 | 6.94 | 62.13 | 268.02 | 229.05 | 1101 | 133 |
| 4 | 67.94 | 72.60 | 10.31 | 7.23 | 60.70 | 243.46 | 174.50 | 1031 | 127 |
| 5 | 75.45 | 68.63 | 11.38 | 7.58 | 67.87 | 283.49 | 188.17 | 1055 | 121 |
| 6 | 58.97 | 74.36 | 7.24 | 5.88 | 53.09 | 160.64 | 141.31 | 850 | 121 |
| 7 | 69.96 | 76.20 | 11.79 | 7.05 | 62.91 | 262.01 | 204.49 | 1129 | 132 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV%[‡‡] | 42.74 | 56.13 | 58.84 | 36.35 | 43.90 | 52.54 | 50.50 | 38.08 | 34.41 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡] Additive sum of fine root length in the range provided in centimeter.

[§] Fine tip counts in the range provided.

[¶] Significance of treatment effects according to analysis of variance.

[#] Diameter range in millimeter.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

**Table 8.** Average weights and fine root lengths and tips of Sugar Beet Repetition 1 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh weight (g)** | **Beet Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Fresh - Dry Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 423.72 | 162.22 | 9.81 | 30.38 | 393.35 | 143.98 | 118.34 | 1542 | 151 |
| 2 | 375.95 | 199.51 | 12.54 | 31.14 | 344.81 | 147.11 | 108.53 | 1416 | 130 |
| 3 | 447.61 | 239.56 | 13.78 | 32.03 | 415.59 | 187.03 | 140.82 | 1722 | 149 |
| 4 | 316.70 | 148.06 | 7.71 | 24.84 | 291.85 | 78.30 | 54.02 | 751 | 81 |
| 5 | 435.53 | 207.47 | 9.89 | 33.88 | 401.65 | 122.31 | 93.83 | 1201 | 121 |
| 6 | 413.06 | 232.60 | 11.27 | 31.89 | 381.16 | 125.10 | 103.23 | 1166 | 120 |
| 7 | 387.29 | 189.22 | 10.55 | 30.56 | 356.73 | 134.53 | 104.63 | 1198 | 124 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | ** | NS |
| $LSD_{0.10}$[††] | NS | 526.29 | NS | NS | NS | NS | NS | 526.29 | NS |
| CV%[‡‡] | 28.59 | 46.73 | 51.27 | 26.59 | 28.97 | 44.21 | 45.79 | 42.30 | 33.18 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 9.** Average weights and fine root lengths and tips of Sugar Beet Repetition 2 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh weight (g)** | **Beet Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 382.70 | 430.88 | 41.90 | 38.04 | 344.65 | 686.90 | 447.96 | 2259 | 149 |
| 2 | 390.54 | 431.98 | 42.89 | 36.73 | 353.82 | 472.94 | 327.95 | 2279 | 171 |
| 3 | 443.21 | 442.38 | 38.15 | 40.99 | 402.23 | 580.64 | 360.10 | 2432 | 176 |
| 4 | 398.04 | 473.83 | 39.85 | 37.75 | 360.29 | 787.07 | 529.83 | 2858 | 221 |
| 5 | 412.26 | 461.34 | 45.39 | 39.87 | 372.39 | 764.77 | 526.86 | 2938 | 213 |
| 6 | 389.03 | 417.21 | 36.39 | 39.74 | 349.30 | 730.95 | 654.46 | 3313 | 208 |
| 7 | 383.59 | 382.79 | 28.29 | 34.39 | 349.20 | 678.58 | 467.81 | 2310 | 154 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | [**] | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | 153.68 | NS | NS |
| CV%[‡‡] | 19.73 | 16.51 | 38.06 | 20.96 | 20.36 | 53.58 | 50.48 | 42.73 | 51.50 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡] Additive sum of fine root length in the range provided in centimeter.

[§] Fine tip counts in the range provided.

[¶] Significance of treatment effects according to analysis of variance.

[#] Diameter range in millimeter.

[††] $LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡] CV%, Coefficient of variation percentage

**Table 10.** Average weights of Scarlet Nantes Carrot Repetition 1 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | |
|---|---|---|---|---|---|---|
| | **Foliar Fresh weight (g)** | **Carrot Fresh Weight (g)** | **Fresh Root Weight (g)** | **Length of carrot (cm)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** |
| 1 | 8.43 | 12.62 | 4.21 | 8.32 | 1.82 | 6.61 |
| 2 | 10.18 | 14.07 | 9.80 | 9.38 | 2.17 | 6.41 |
| 3 | 13.00 | 14.36 | 15.49 | 9.50 | 2.95 | 10.05 |
| 4 | 9.26 | 8.83 | 18.27 | 8.53 | 2.31 | 6.95 |
| 5 | 12.30 | 10.89 | 19.98 | 7.88 | 2.41 | 9.88 |
| 6 | 8.30 | 3.73 | 1.57 | 6.25 | 1.33 | 4.64 |
| 7 | 12.33 | 20.51 | 16.84 | 9.20 | 2.24 | 10.09 |
| p[‡] | NS[†] | NS | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS |
| CV%[‡‡] | 62.02 | 73.93 | 99.22 | 28.64 | 60.19 | 71.44 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡]Significance of treatment effects according to analysis of variance.

[§] Root scan was not used because crop was harvested before the implementation of WinRhizo.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

**Table 11.** Average weights and fine root lengths and tips of Scarlet Nantes Carrot Repetition 2 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh Weight (g)** | **Carrot Fresh Weight (g)** | **Fresh Root Weight (g)** | **Length of Carrot (cm)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 18.76 | 32.41 | 14.30 | 10.70 | 3.53 | 15.23 | 214.86 | 242.58 | 454 | 85 |
| 2 | 20.25 | 37.91 | 18.35 | 8.35 | 3.98 | 16.27 | 178.34 | 138.94 | 476 | 38 |
| 3 | 20.19 | 35.36 | 15.12 | 9.84 | 3.96 | 16.23 | 195.48 | 163.38 | 493 | 41 |
| 4 | 12.06 | 15.44 | 12.03 | 8.28 | 2.34 | 9.72 | 165.30 | 162.91 | 450 | 75 |
| 5 | 13.32 | 10.03 | 17.20 | 5.98 | 2.62 | 10.71 | 131.44 | 167.85 | 498 | 73 |
| 6 | 12.67 | 24.86 | 17.16 | 8.00 | 3.48 | 14.75 | 217.76 | 202.38 | 643 | 66 |
| 7 | 20.23 | 32.18 | 15.63 | 9.70 | 3.63 | 16.60 | 162.24 | 182.76 | 410 | 52 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV%[‡‡] | 80.54 | 89.93 | 87.39 | 50.80 | 81.03 | 80.63 | 66.90 | 57.25 | 50.72 | 54.02 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡] Additive sum of fine root length in the range provided in centimeter.

[§] Fine tip counts in the range provided.

[¶] Significance of treatment effects according to analysis of variance.

[#] Diameter range in millimeter.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

**Table 12.** Average weights and fine root lengths and tips of New Baby Narcissus Repetition 1 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh weight (g)** | **Bulb Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 2.08 | 7.15 | 1.98 | 0.32 | 1.76 | 94.49 | 80.59 | 2023 | 151 |
| 2 | 0.89 | 6.22 | 0.95 | 0.15 | 0.74 | 93.72 | 65.29 | 1959 | 169 |
| 3 | 1.63 | 8.70 | 1.58 | 0.27 | 1.36 | 96.24 | 70.77 | 2142 | 166 |
| 4 | 1.19 | 5.40 | 1.22 | 0.17 | 1.02 | 74.76 | 63.46 | 1717 | 122 |
| 5 | 2.51 | 8.84 | 2.13 | 0.38 | 2.13 | 99.22 | 128.86 | 2085 | 176 |
| 6 | 0.84 | 6.91 | 0.83 | 0.13 | 0.71 | 94.66 | 53.09 | 2092 | 137 |
| 7 | 1.24 | 6.18 | 1.50 | 0.19 | 1.05 | 112.77 | 84.71 | 2535 | 173 |
| p[¶] | NS† | NS | NS | NS | NS | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV%[‡‡] | 84.42 | 41.91 | 97.15 | 86.36 | 84.28 | 35.91 | 73.39 | 36.91 | 29.34 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡] Additive sum of fine root length in the range provided in centimeter.

[§] Fine tip counts in the range provided.

[¶] Significance of treatment effects according to analysis of variance.

[#] Diameter range in millimeter.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

**Table 13.** Average weights and fine root lengths and tips of Dutch Master Narcissus Repetition 2 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh Weight (g)** | **Bulb Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Number of bulbs** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 5.84 | 17.30 | 8.31 | 0.61 | 1.17 | 5.23 | 44.61 | 172.62 | 751 | 37 |
| 2 | 7.69 | 17.62 | 10.43 | 0.83 | 1.57 | 6.86 | 37.60 | 97.36 | 734 | 47 |
| 3 | 9.78 | 20.98 | 14.24 | 1.03 | 1.57 | 8.75 | 49.36 | 154.62 | 813 | 35 |
| 4 | 10.43 | 21.23 | 12.32 | 1.04 | 1.29 | 9.39 | 48.14 | 135.74 | 744 | 45 |
| 5 | 7.75 | 16.63 | 9.46 | 0.77 | 1.00 | 6.98 | 31.18 | 119.08 | 608 | 33 |
| 6 | 6.39 | 15.83 | 8.63 | 0.81 | 1.43 | 5.70 | 32.24 | 127.84 | 660 | 41 |
| 7 | 6.48 | 13.27 | 7.70 | 0.72 | 1.00 | 5.76 | 32.72 | 114.66 | 675 | 37 |
| p[¶] | ** | NS[†] | ** | NS | NS | ** | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | 3.00 | NS | 3.70 | NS | NS | 2.32 | NS | NS | NS | NS |
| CV%[‡‡] | 40.18 | 33.91 | 42.96 | 41.61 | 47.80 | 40.29 | 45.04 | 56.86 | 40.99 | 38.02 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 14.** Average weights and fine root lengths and tips of Walla Walla Onion Repetition 1 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh weight (g)** | **Bulb Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 23.56 | 63.71 | 2.85 | 2.30 | 21.25 | 105.06 | 206.35 | 2116 | 211 |
| 2 | 41.41 | 76.84 | 4.23 | 3.90 | 37.51 | 120.05 | 251.48 | 2438 | 229 |
| 3 | 37.73 | 94.43 | 3.40 | 3.20 | 34.53 | 130.88 | 215.67 | 2694 | 243 |
| 4 | 30.11 | 74.04 | 2.76 | 2.81 | 27.30 | 115.92 | 204.56 | 2341 | 209 |
| 5 | 20.28 | 49.35 | 3.54 | 2.12 | 18.16 | 130.07 | 208.01 | 2828 | 250 |
| 6 | 28.01 | 58.65 | 2.42 | 2.59 | 25.42 | 149.88 | 182.50 | 3019 | 254 |
| 7 | 29.03 | 73.94 | 1.81 | 3.17 | 25.86 | 153.40 | 155.35 | 3244 | 278 |
| p[¶] | NS[†] | NS | NS | NS | NS | ** | NS | ** | ** |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | 27.51 | NS | 426.36 | 32.12 |
| CV%[‡‡] | 64.96 | 69.95 | 76.61 | 58.69 | 65.93 | 25.12 | 58.65 | 24.81 | 17.15 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 15.** Average weights and fine root lengths and tips of Walla Walla Onion Repetition 2 at different rates of MBI-3X bio-nematicide.

| Treatment | Growth Parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Foliar Fresh weight (g)** | **Bulb Fresh Weight (g)** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 33.84 | 43.35 | 5.19 | 2.68 | 31.15 | 136.19 | 202.47 | 525 | 69 |
| 2 | 37.57 | 50.71 | 3.98 | 2.82 | 34.74 | 116.00 | 221.24 | 526 | 79 |
| 3 | 43.64 | 58.21 | 7.16 | 3.36 | 40.27 | 147.46 | 251.14 | 763 | 97 |
| 4 | 41.27 | 53.64 | 4.64 | 3.05 | 38.22 | 108.97 | 203.41 | 524 | 71 |
| 5 | 35.08 | 58.21 | 4.39 | 2.70 | 32.38 | 103.94 | 180.39 | 492 | 64 |
| 6 | 45.85 | 65.18 | 5.67 | 3.32 | 42.53 | 142.47 | 233.83 | 653 | 88 |
| 7 | 48.76 | 63.91 | 7.26 | 3.56 | 45.19 | 180.12 | 234.19 | 652 | 81 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV%[‡‡] | 57.15 | 51.25 | 85.16 | 54.11 | 57.46 | 64.67 | 46.16 | 53.26 | 41.03 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡] Additive sum of fine root length in the range provided in centimeter.

[§] Fine tip counts in the range provided.

[¶] Significance of treatment effects according to analysis of variance.

[#] Diameter range in millimeter.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

**Table 16.** Average weights and fine root lengths and tips of Golden Potato Repetition 1 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh weight (g)** | **Tuber Fresh Weight (g)** | **Tuber counts** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 527.48 | 552.83 | 22.80 | 103.92 | 57.51 | 469.97 | 506.65 | 383.06 | 4793 | 364 |
| 2 | 520.07 | 565.88 | 22.40 | 126.21 | 59.68 | 460.40 | 466.98 | 376.53 | 3609 | 258 |
| 3 | 491.81 | 499.98 | 21.80 | 124.65 | 56.30 | 435.51 | 473.65 | 382.51 | 4554 | 323 |
| 4 | 568.16 | 468.07 | 23.00 | 127.30 | 68.62 | 499.54 | 376.75 | 299.65 | 3403 | 267 |
| 5 | 483.94 | 503.80 | 17.80 | 108.97 | 56.83 | 427.12 | 410.52 | 304.40 | 3783 | 258 |
| 6 | 506.78 | 488.48 | 20.40 | 85.53 | 65.76 | 441.02 | 375.03 | 313.37 | 3002 | 212 |
| 7 | 537.35 | 543.24 | 19.00 | 118.62 | 62.32 | 475.03 | 622.71 | 455.19 | 5043 | 365 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | NS | NS | ** | ** |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | NS | NS | 1224.48 | 93.72 |
| CV%[‡‡] | 22.37 | 17.48 | 26.19 | 32.10 | 19.61 | 23.20 | 31.83 | 33.32 | 31.29 | 32.20 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 17.** Average weights and fine root lengths and tips of Golden Potato Repetition 2 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh weight (g)** | **Tuber Fresh Weight (g)** | **Tuber counts** | **Fresh Root Weight (g)** | **Foliar Dry Weight (g)** | **Foliar Water Weight (g)** | **0<.L.<=0.50 (mm)[‡ #]** | **0.50<.L.<=1.00 (mm)[‡ #]** | **0<.T.<=0.50 (mm)[§#]** | **0.50<.T.<=1.00 (mm)[§#]** |
| 1 | 1098.40 | 188.57 | 26.20 | 190.14 | 128.77 | 969.63 | 1799.54 | 1706.31 | 7828 | 896 |
| 2 | 995.20 | 330.85 | 28.60 | 201.44 | 117.71 | 877.49 | 2332.14 | 2234.68 | 8563 | 1011 |
| 3 | 1089.80 | 267.47 | 24.40 | 197.83 | 136.09 | 953.71 | 2281.74 | 1992.93 | 8728 | 926 |
| 4 | 1006.80 | 165.22 | 20.20 | 176.42 | 106.33 | 900.47 | 2172.21 | 1929.55 | 8234 | 897 |
| 5 | 904.00 | 326.01 | 29.20 | 161.86 | 100.04 | 803.96 | 2309.98 | 1915.56 | 8537 | 796 |
| 6 | 1079.60 | 171.89 | 23.00 | 172.49 | 118.19 | 961.41 | 3082.88 | 1629.84 | 10012 | 601 |
| 7 | 997.20 | 134.05 | 27.40 | 216.22 | 124.26 | 872.94 | 3503.16 | 1739.40 | 11977 | 640 |
| p[¶] | NS[†] | NS | NS | NS | NS | NS | ** | NS | ** | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS | NS | 775.14 | NS | 2437.82 | NS |
| CV%[‡‡] | 21.16 | 73.04 | 40.72 | 32.06 | 22.02 | 21.63 | 38.40 | 39.24 | 29.28 | 43.06 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡ Additive sum of fine root length in the range provided in centimeter.

§ Fine tip counts in the range provided.

¶ Significance of treatment effects according to analysis of variance.

# Diameter range in millimeter.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 18.** Average weights of Cherry Belle Radish Repetition 1 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh weight (g)** | **Radish Edible Root Fresh Weight (g)** | **Fresh root weight (g)** | **Foliar Dry weight (g)** | **Foliar Water Weight (g)** |
| 1 | 12.26 | 30.65 | 1.08 | 1.38 | 10.88 |
| 2 | 11.89 | 25.15 | 1.06 | 1.33 | 10.56 |
| 3 | 11.37 | 29.49 | 1.08 | 1.30 | 10.07 |
| 4 | 12.14 | 26.61 | 0.92 | 1.40 | 10.74 |
| 5 | 12.61 | 25.90 | 1.62 | 1.48 | 11.13 |
| 6 | 10.63 | 21.75 | 0.93 | 1.25 | 9.38 |
| 7 | 10.91 | 24.45 | 0.87 | 1.29 | 9.61 |
| p[‡] | NS† | ** | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | 4.93 | NS | NS | NS |
| CV%[‡‡] | 28.38 | 40.80 | 62.16 | 23.89 | 29.26 |

** Significant at P <0.10 probability level.

† NS, no significant effect.

‡Significance of treatment effects according to analysis of variance.

§ Root scan was not used because crop was harvested before the implementation of WinRhizo.

††$LSD_{0.10}$, Least Significant Difference at α is 0.10

‡‡CV%, Coefficient of variation percentage

**Table 19.** Average weights of Cherry Belle Radish Repetition 2 at different rates of MBI-3X bio-nematicide.

| | Growth Parameters | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Foliar Fresh weight (g)** | **Radish Edible Root Fresh Weight (g)** | **Fresh root weight (g)** | **Foliar Dry weight (g)** | **Foliar Water Weight (g)** |
| 1 | 17.98 | 23.73 | 6.10 | 1.77 | 16.21 |
| 2 | 18.24 | 29.12 | 6.35 | 1.77 | 16.86 |
| 3 | 17.47 | 29.19 | 5.28 | 1.76 | 15.71 |
| 4 | 21.38 | 26.12 | 8.27 | 2.00 | 18.61 |
| 5 | 18.72 | 26.06 | 5.18 | 1.85 | 16.86 |
| 6 | 17.82 | 22.29 | 6.04 | 1.76 | 16.06 |
| 7 | 17.71 | 23.31 | 6.24 | 1.83 | 15.88 |
| p[‡] | NS[†] | NS | NS | NS | NS |
| $LSD_{0.10}$[††] | NS | NS | NS | NS | NS |
| CV%[‡‡] | 28.96 | 38.47 | 54.38 | 21.24 | 31.09 |

[**] Significant at P <0.10 probability level.

[†] NS, no significant effect.

[‡]Significance of treatment effects according to analysis of variance.

[§] Root scan was not used because crop was harvested before the implementation of WinRhizo.

[††]$LSD_{0.10}$, Least Significant Difference at α is 0.10

[‡‡]CV%, Coefficient of variation percentage

Figures

**Figure 1.** *Romidepsin* projected hormesis curve.

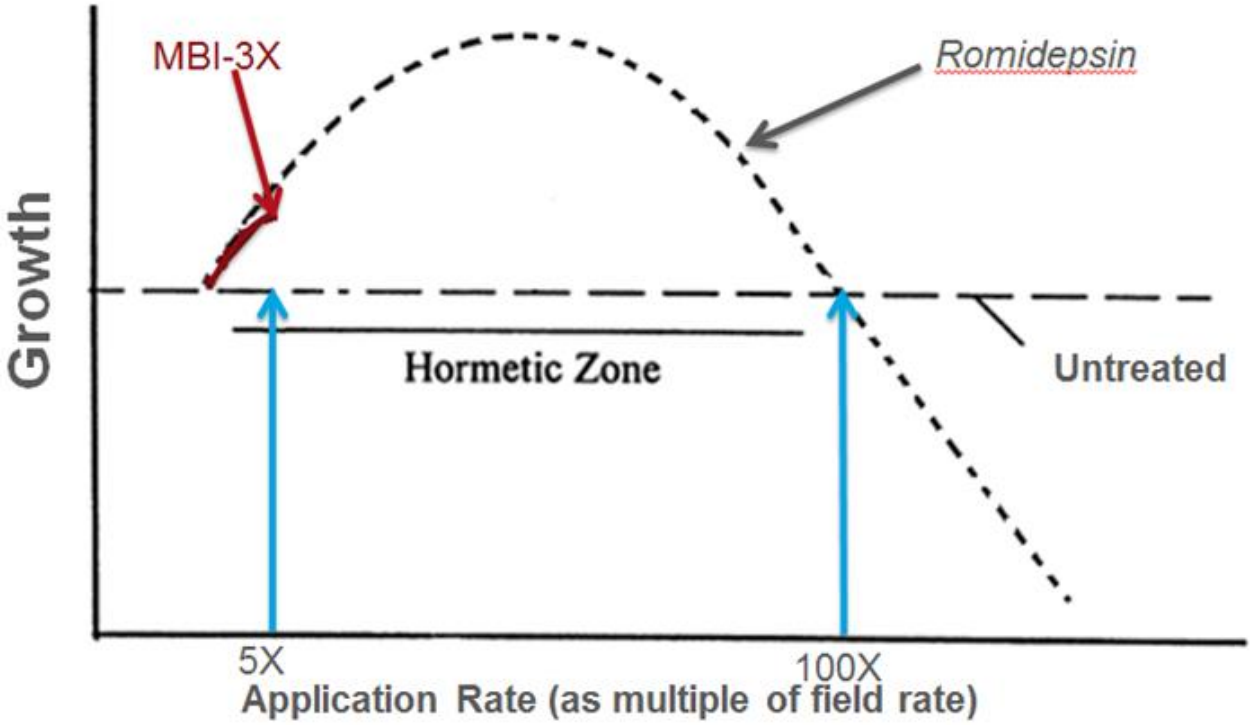


**Figure 2.** Pacemaker III Beet fine root tip counts in 0.0 to 0.5 mm range.

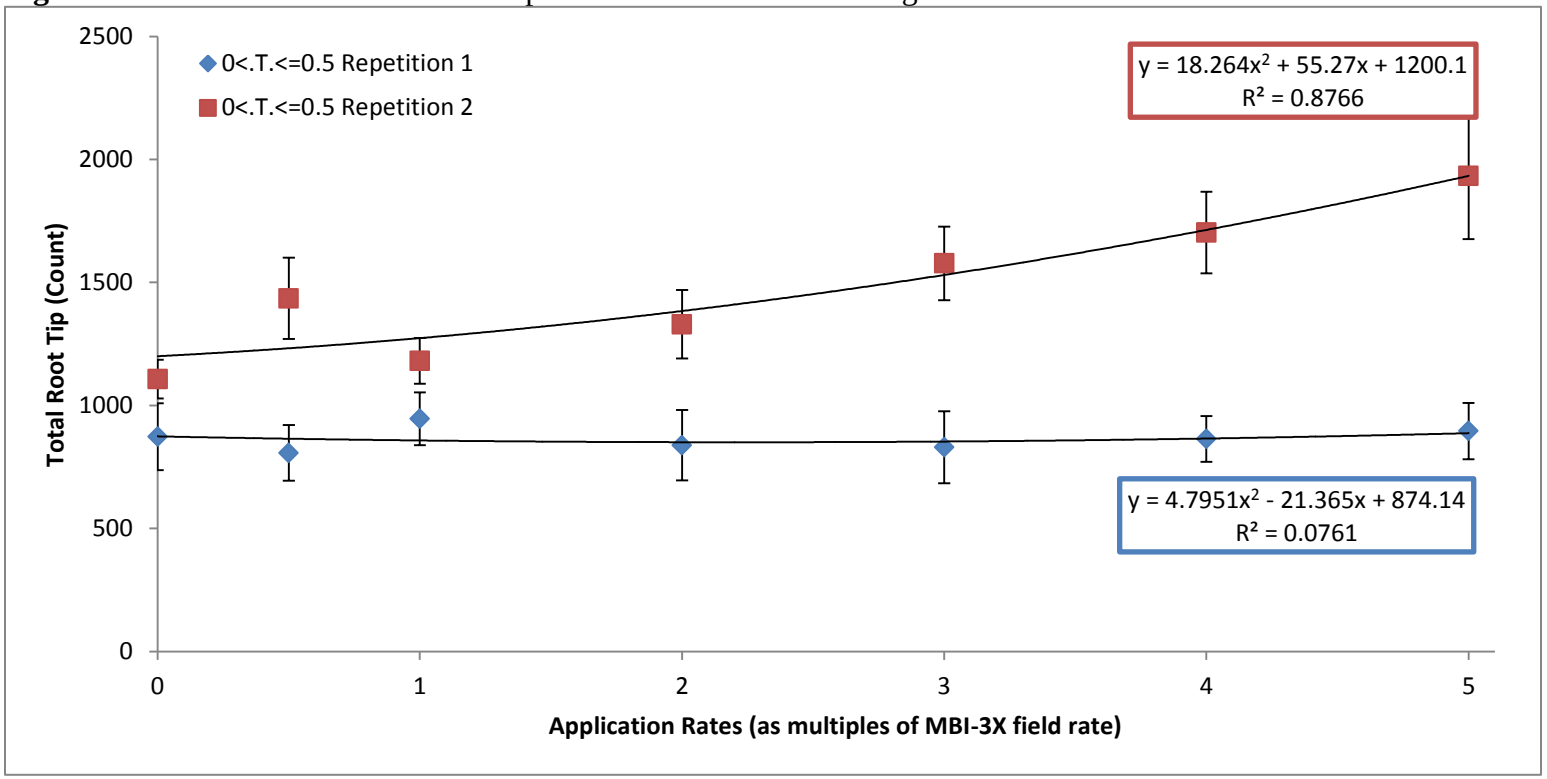


**Figure 3.** Pacemaker III Beet of fine root tip count in 0.5 to 1.0 mm range.

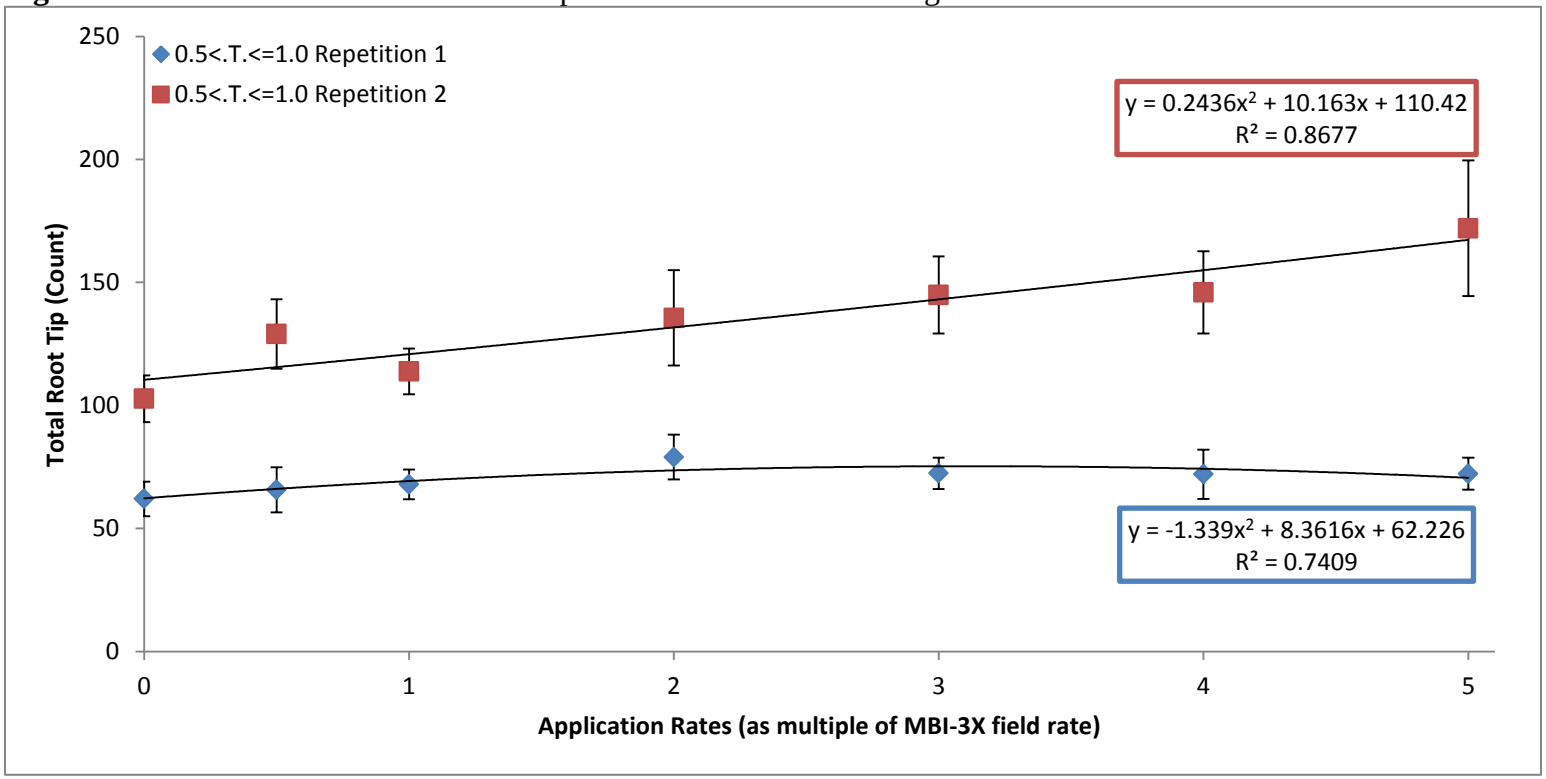


**Figure 4.** Red Ace Beet fresh beet weight.

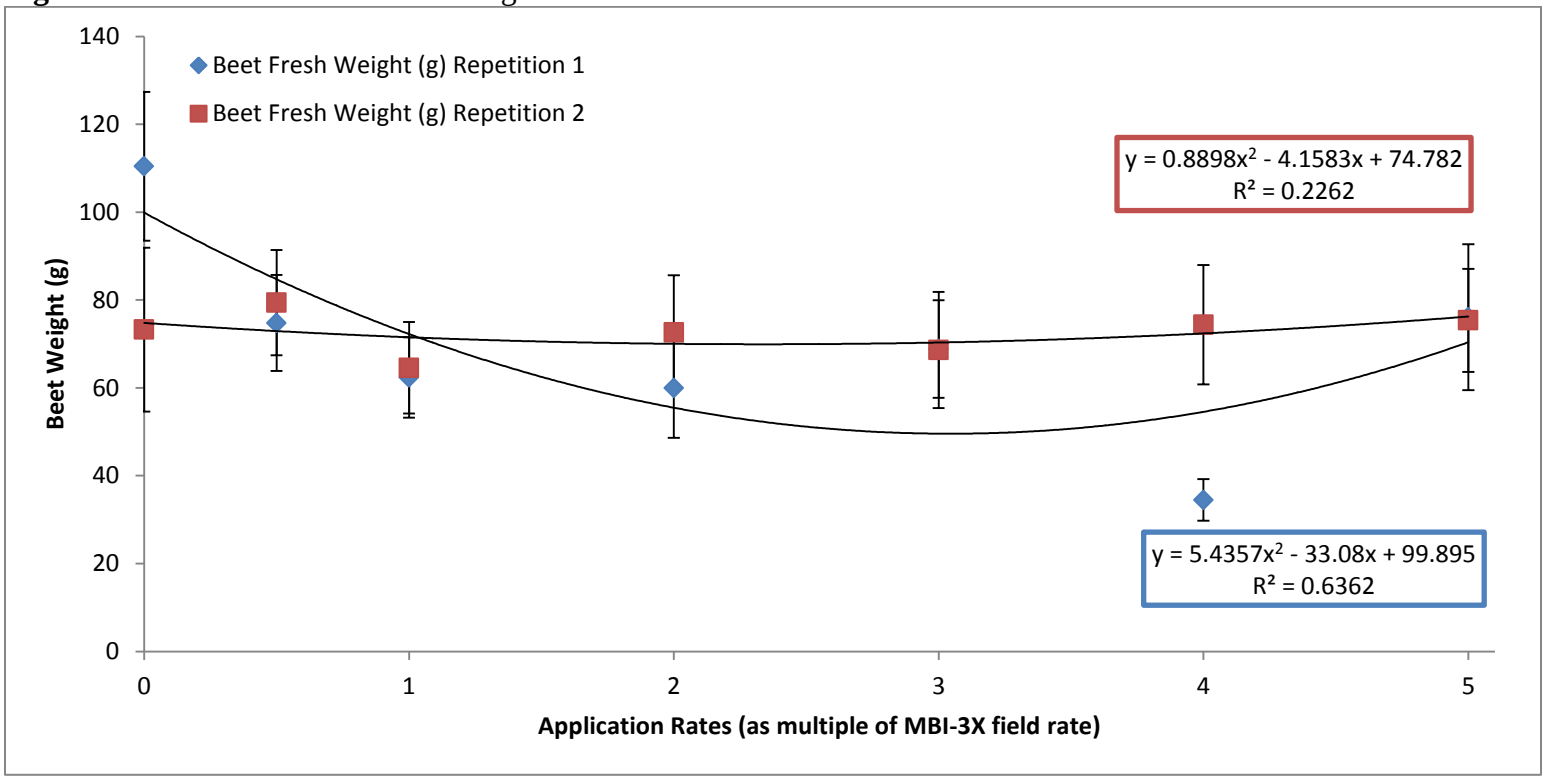


**Figure 5.** Red Ace Beet fine root tip counts in 0.0 to 0.5 mm range.

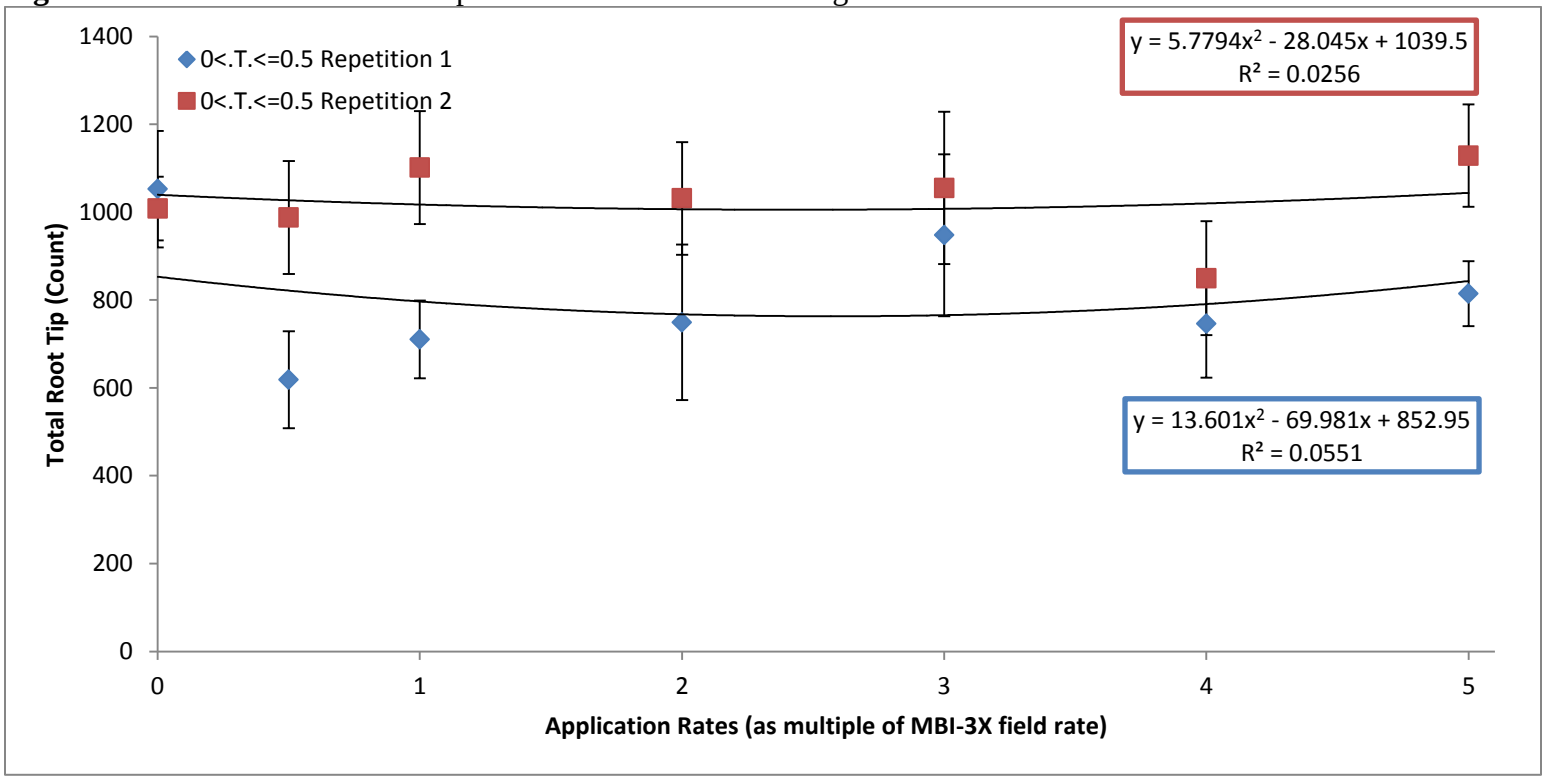


**Figure 6.** Red Ace Beet fine root tip counts in 0.5 to 1.0 mm range.

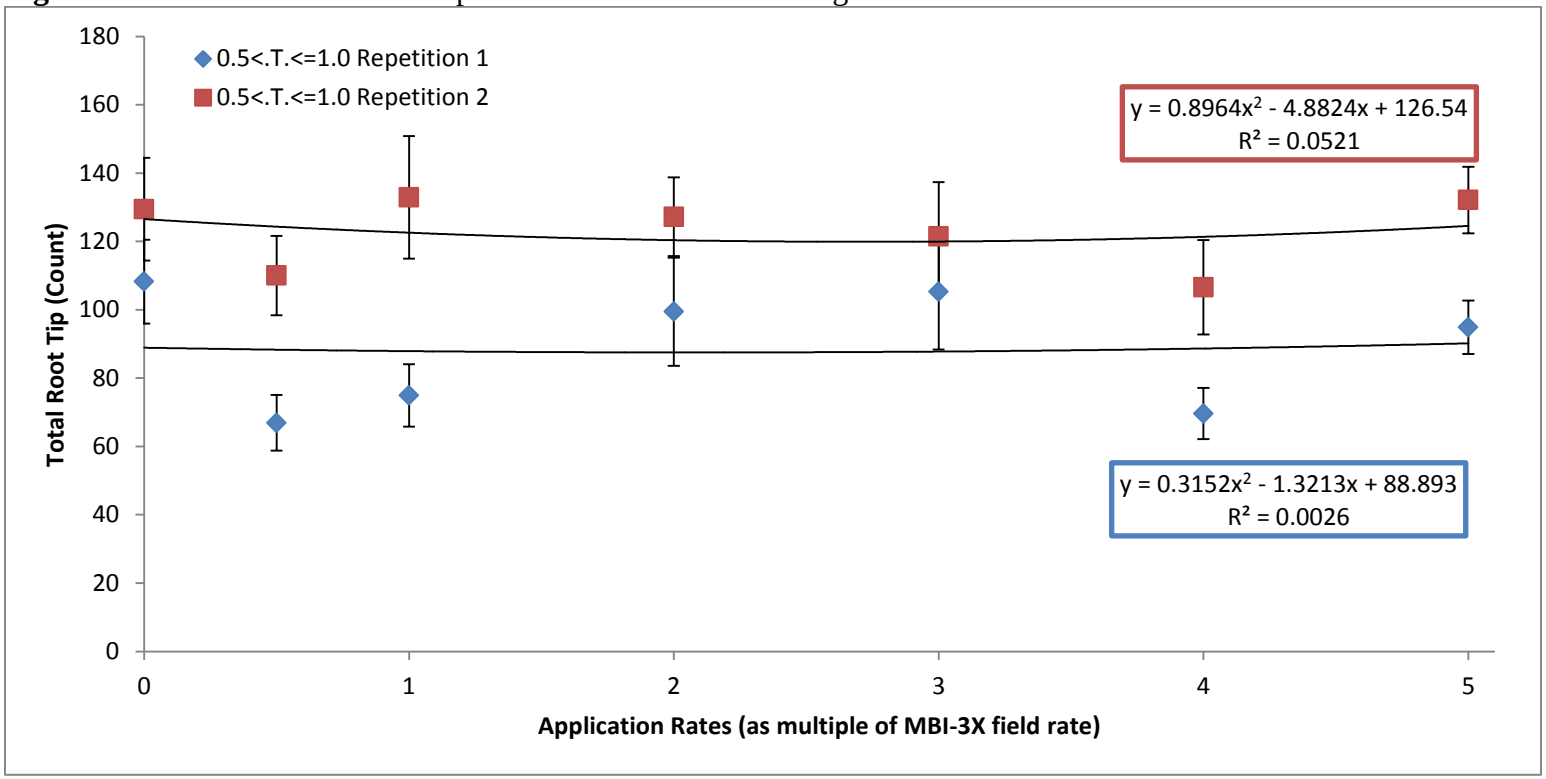


**Figure 7.** Sugar Beet fine root length in 0.5 to 1.0 mm range.

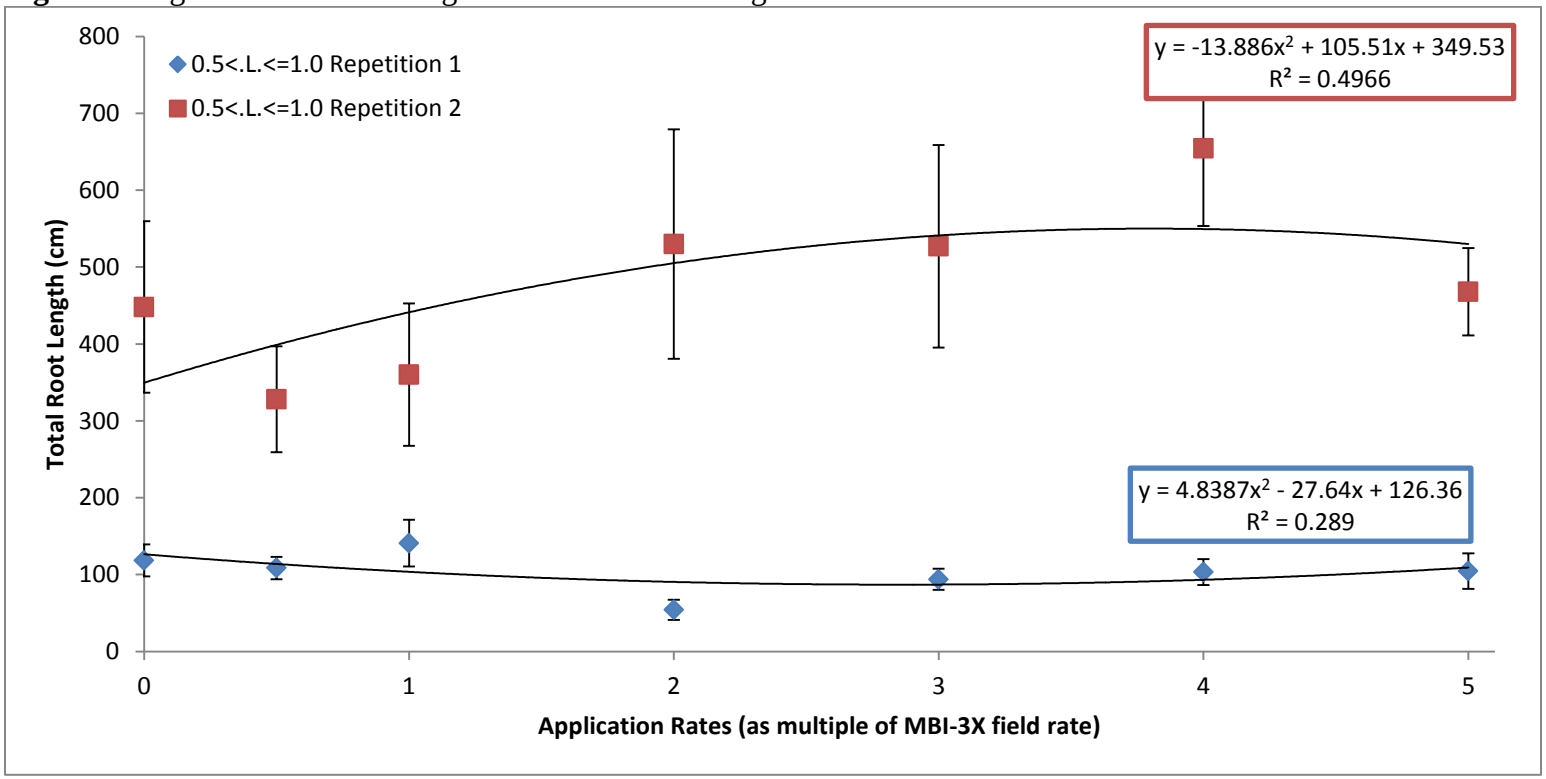


**Figure 8.** Sugar Beet fine root tip counts in 0.0 to 0.5 mm range.

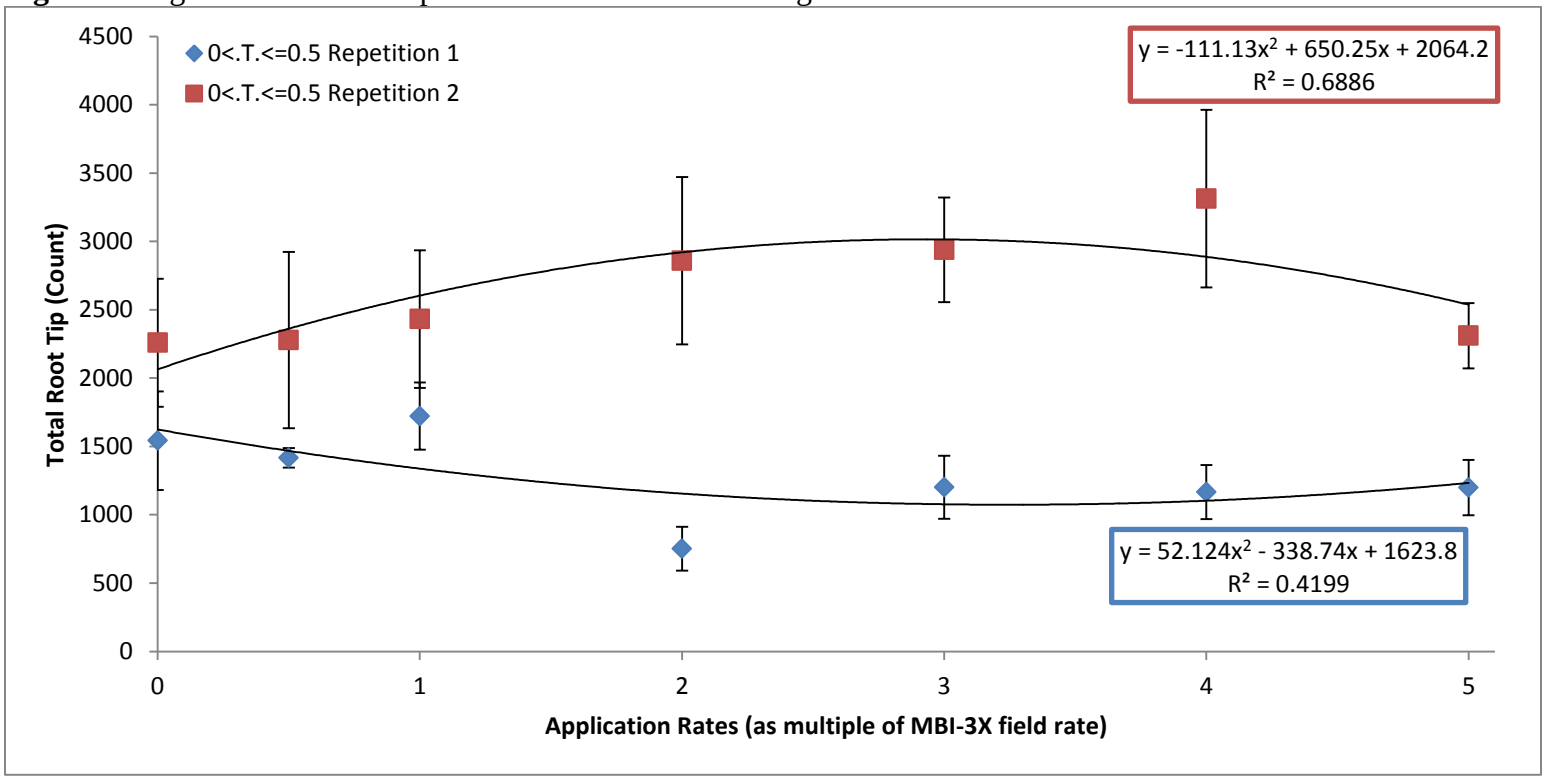


**Figure 9.** Narcissus fresh foliar weight.

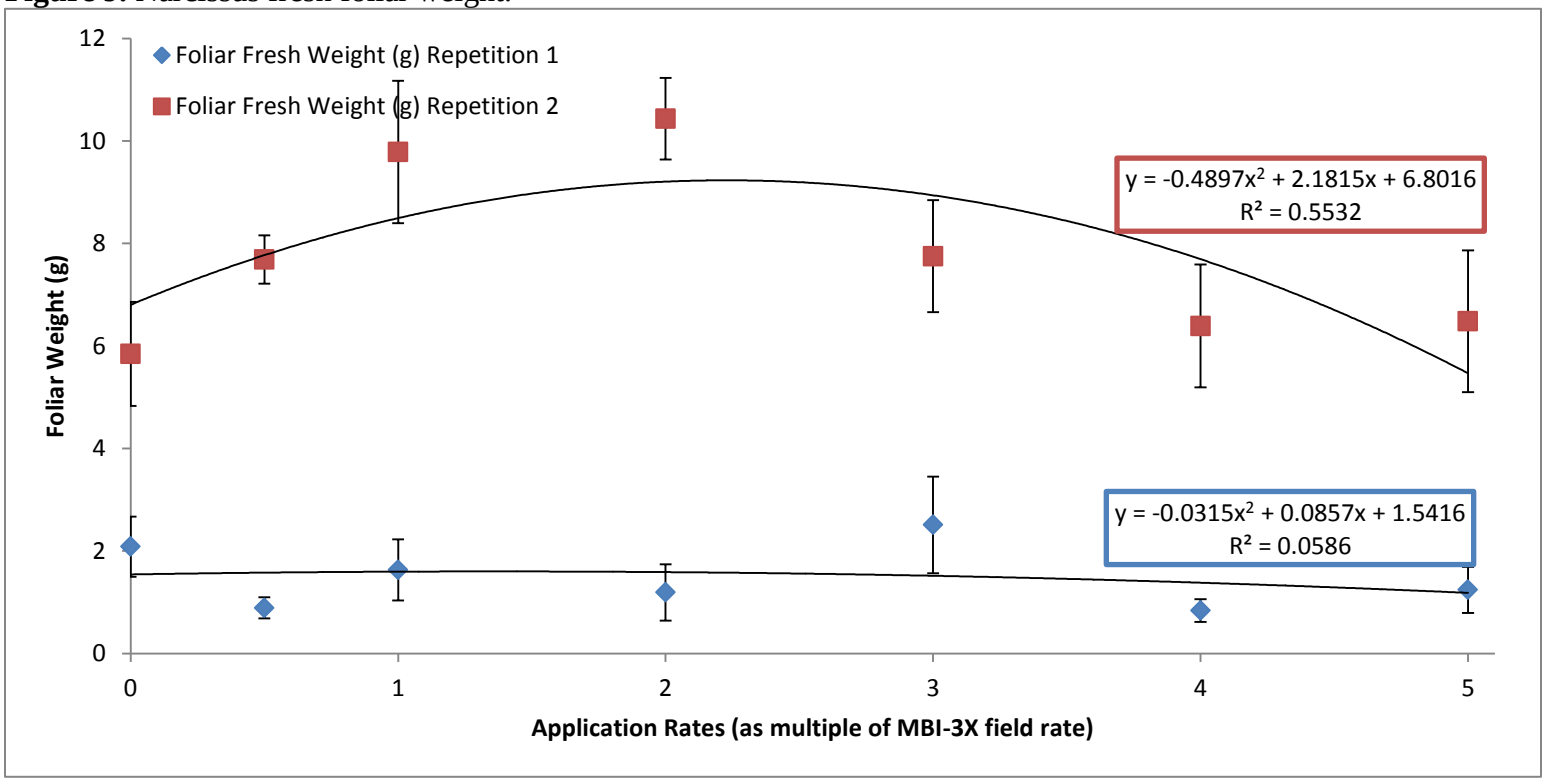


**Figure 10.** Narcissus fresh root weight.

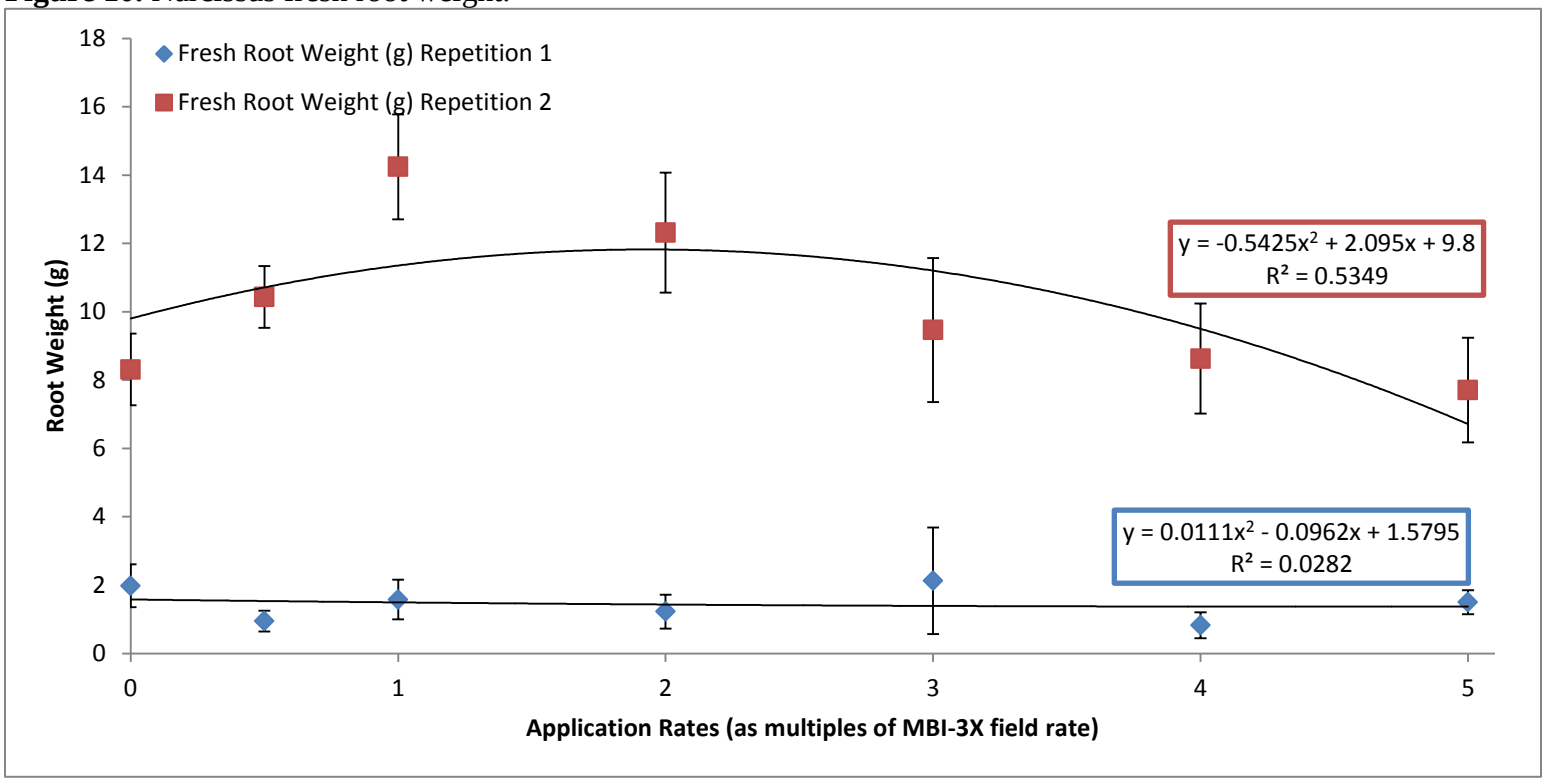


**Figure 11.** Narcissus foliar water weight.

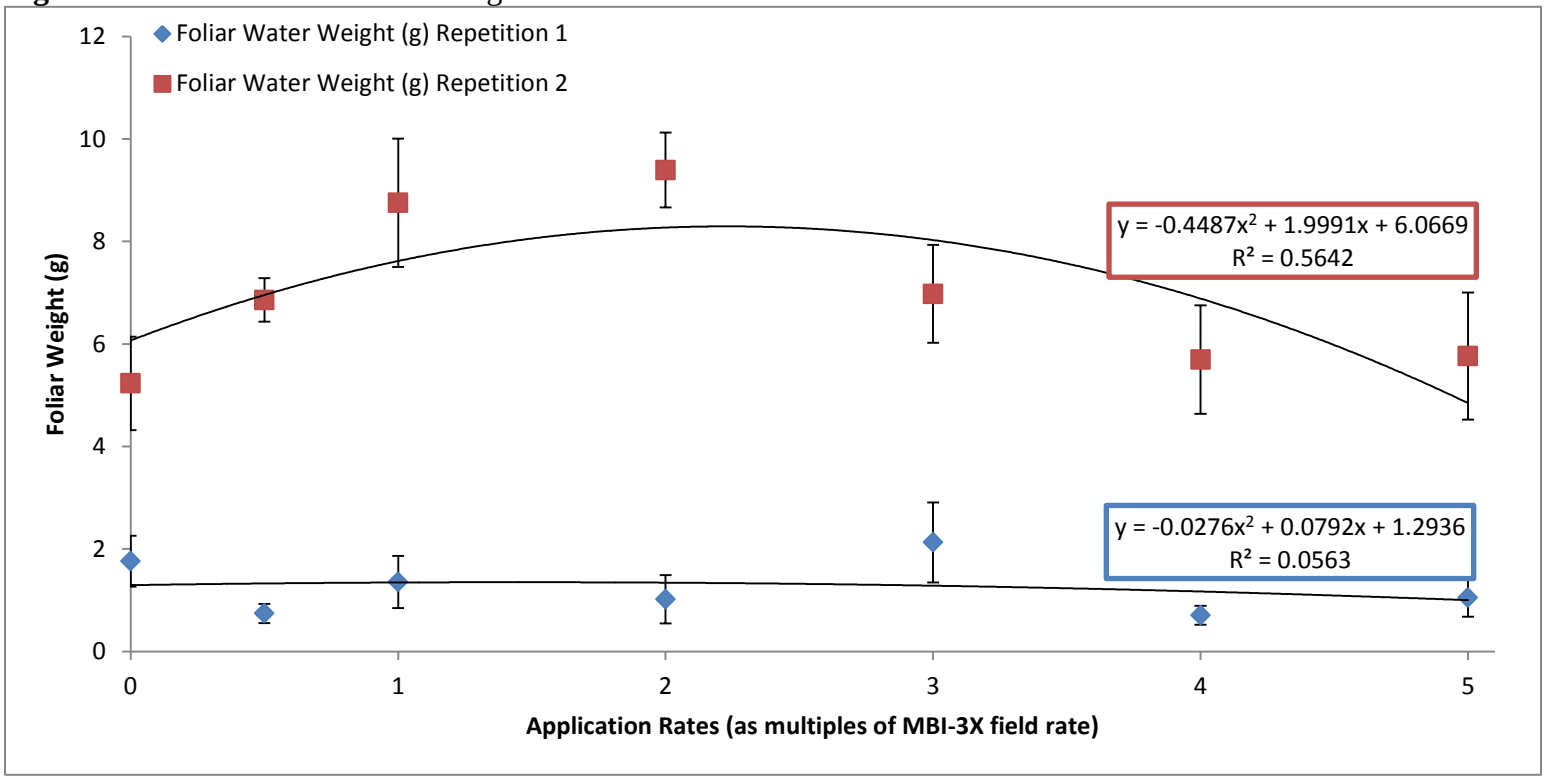


**Figure 12.** Walla Walla Onion fine root lengths in 0.0 to 0.5 mm range.

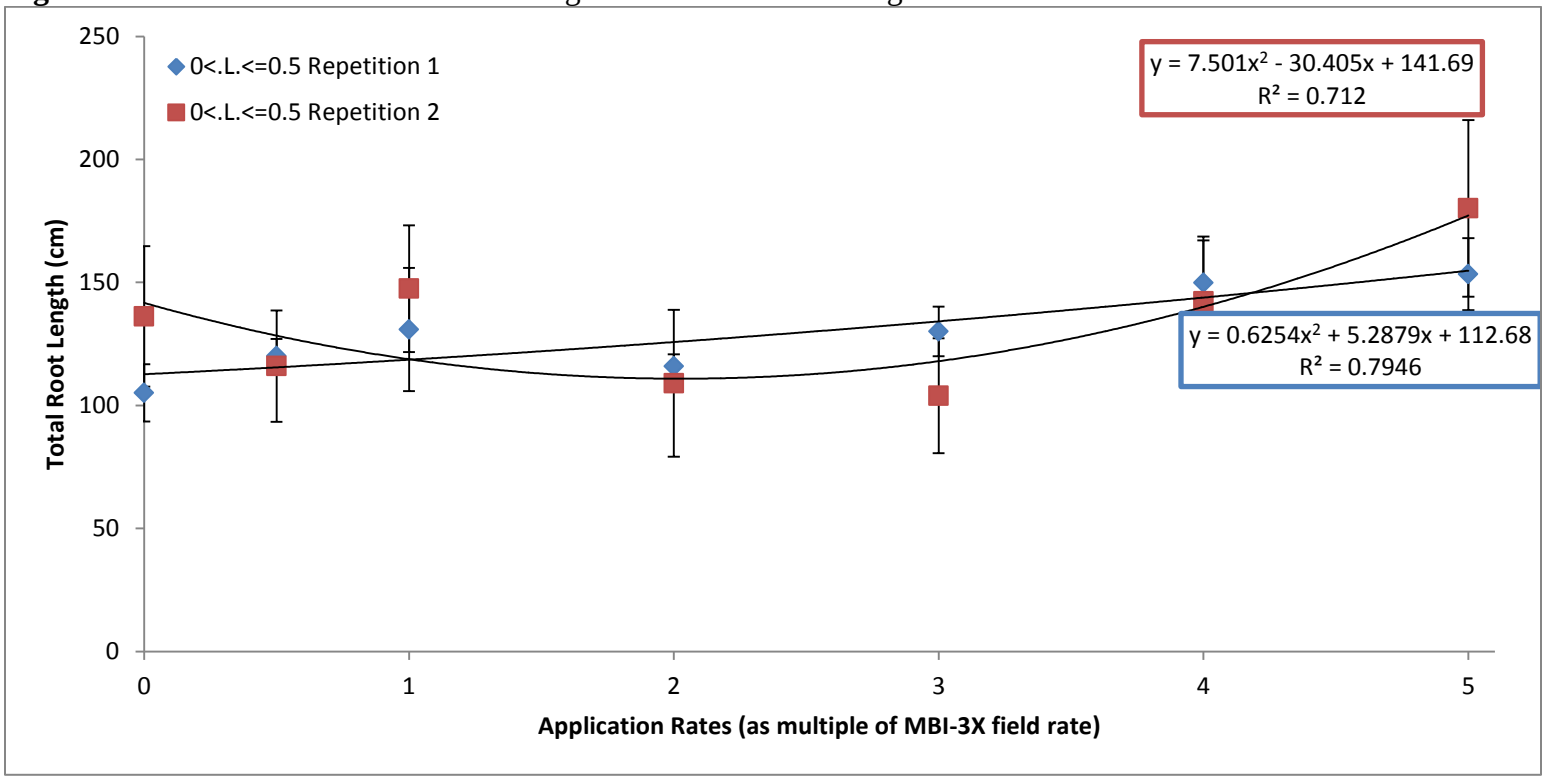


**Figure 13.** Walla Walla Onion fine root tip counts in 0.0 to 0.5 mm range.

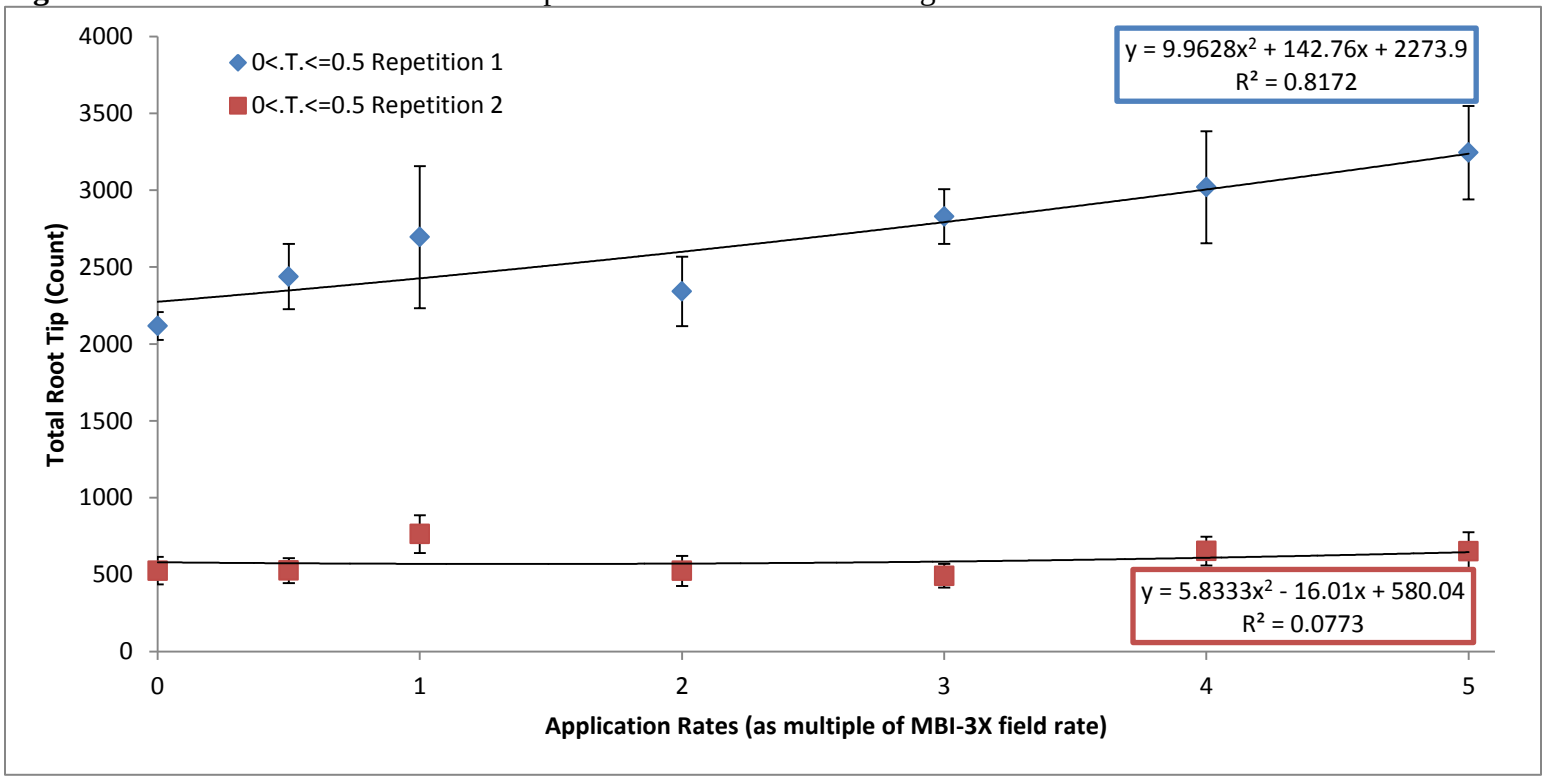


**Figure 14.** Walla Walla Onion fine tip counts in 0.5 to 1.0 mm range.

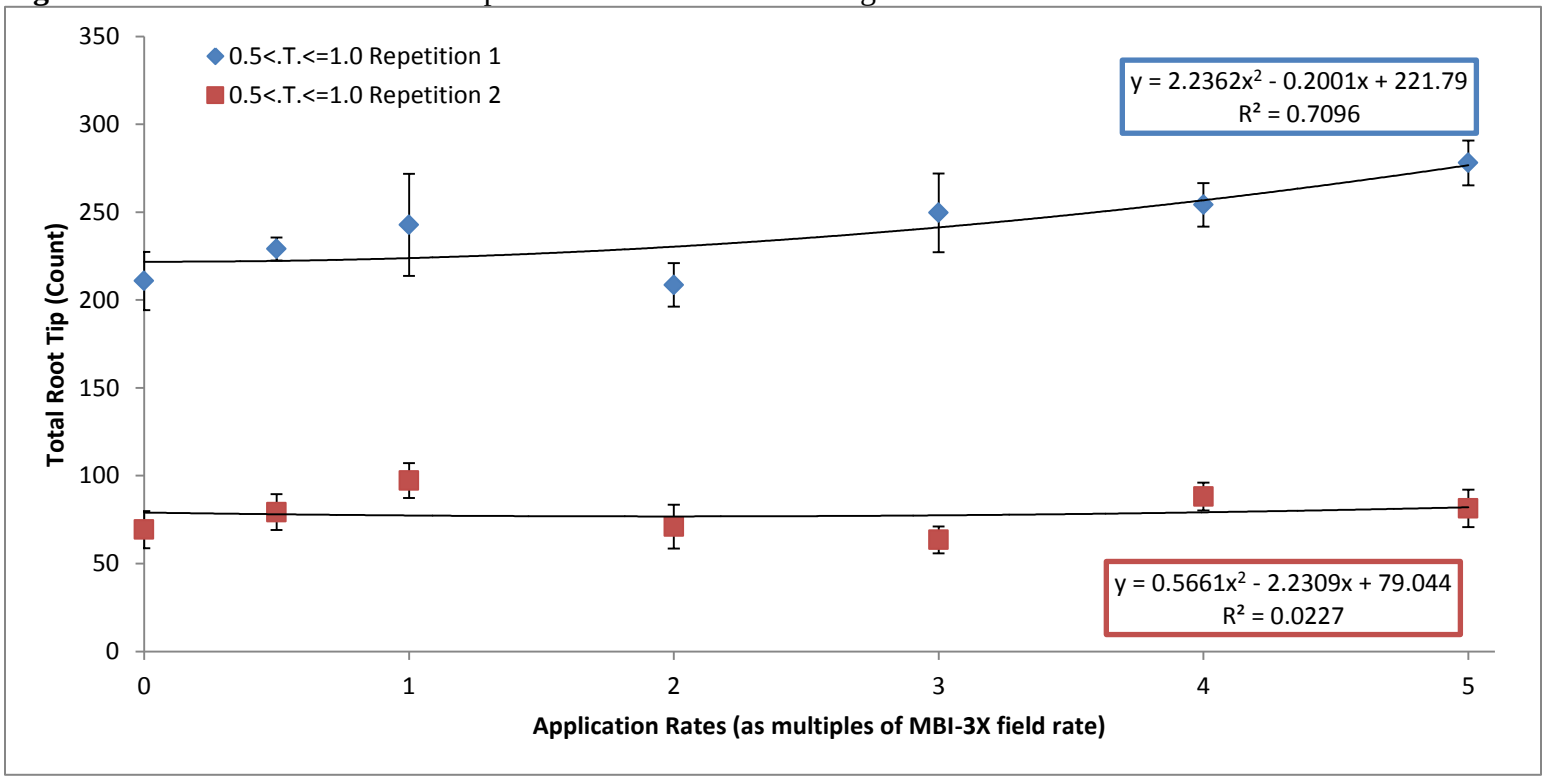


**Figure 15.** Golden potato fine root length counts in 0.0 to 0.5 mm range.

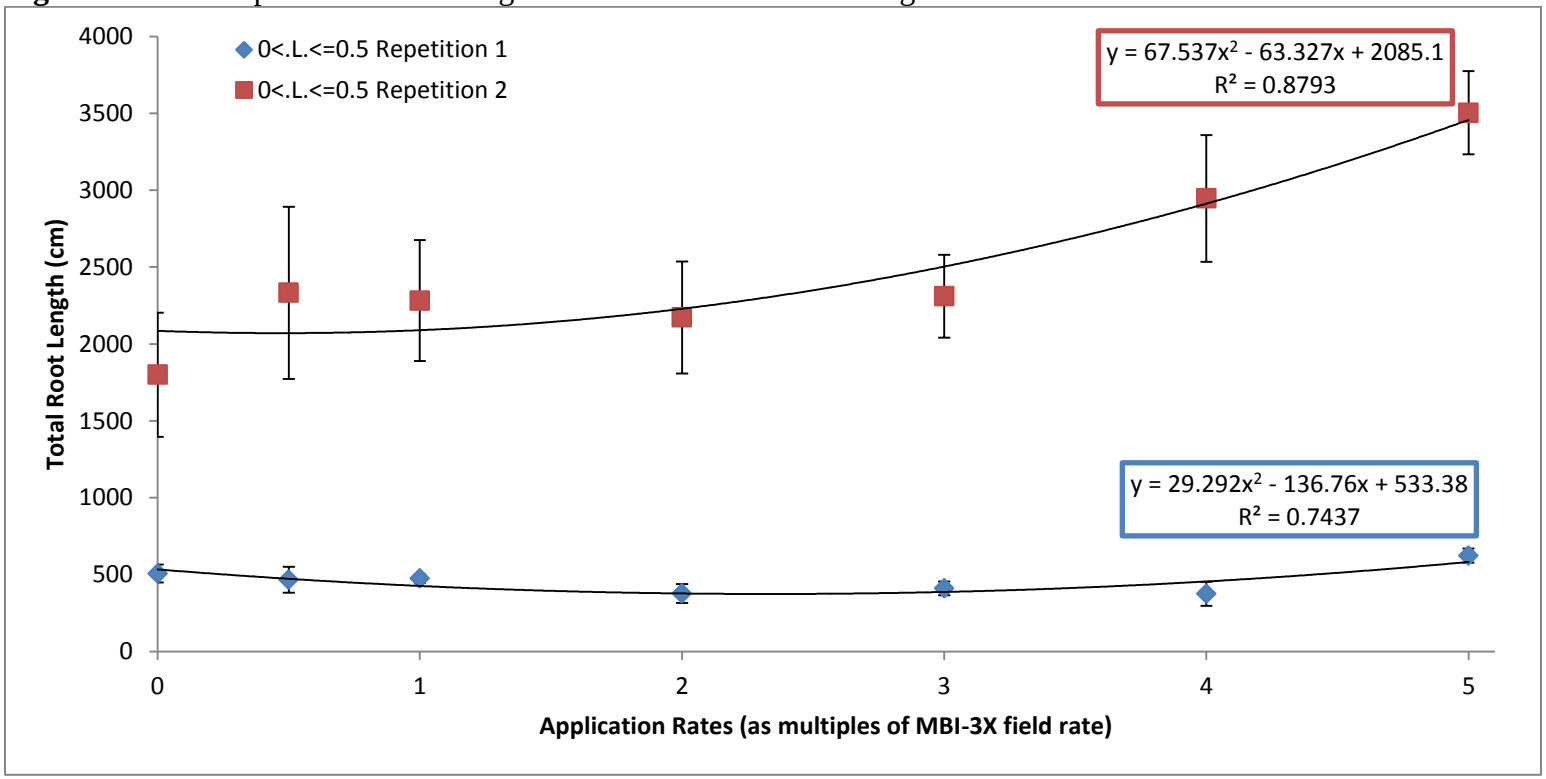


**Figure 16.** Golden potato fine tip counts in 0.0 to 0.5 mm range.

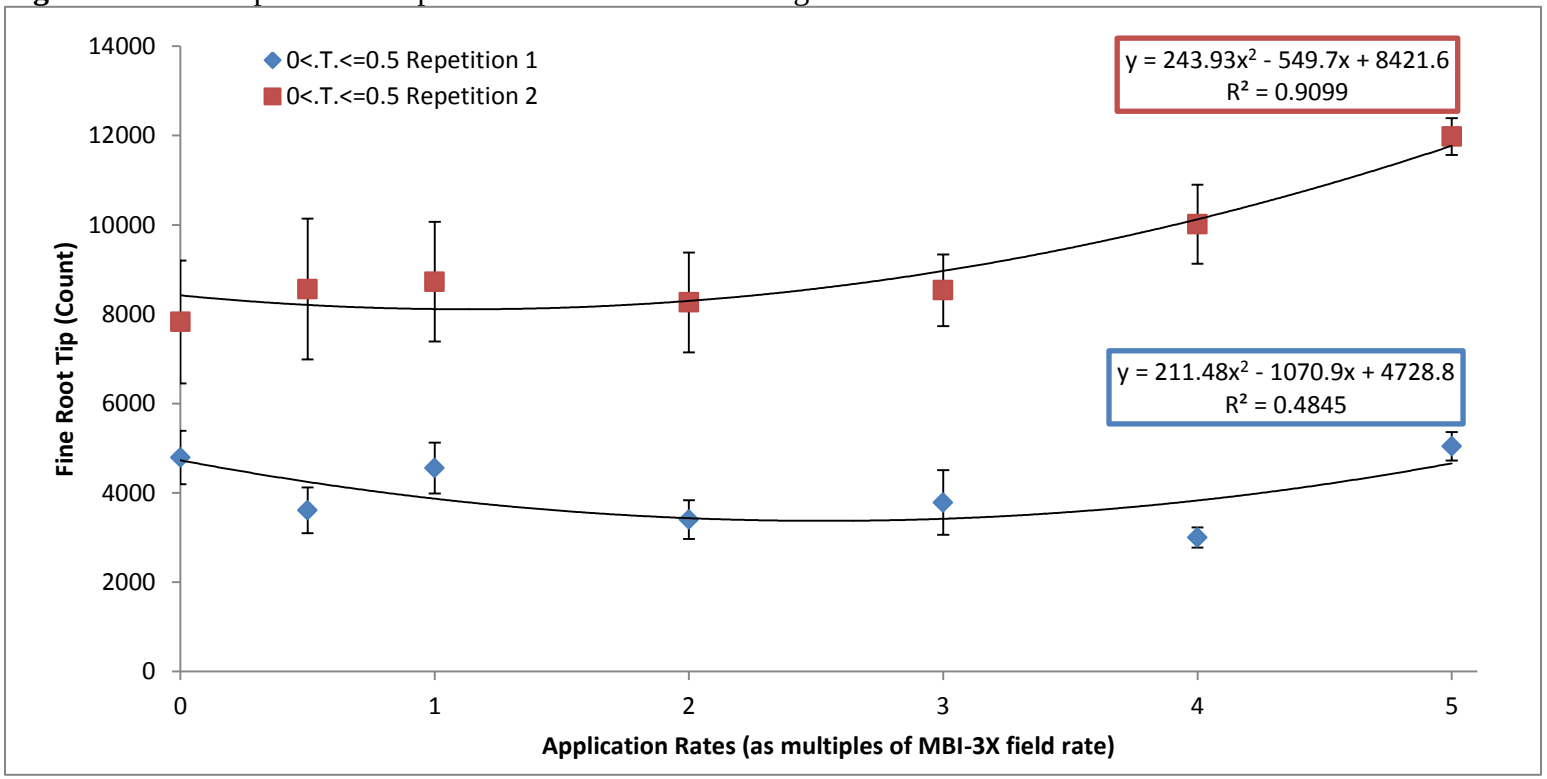


**Figure 17.** Golden potato fine root tip counts in 0.5 to 1.0 mm range.

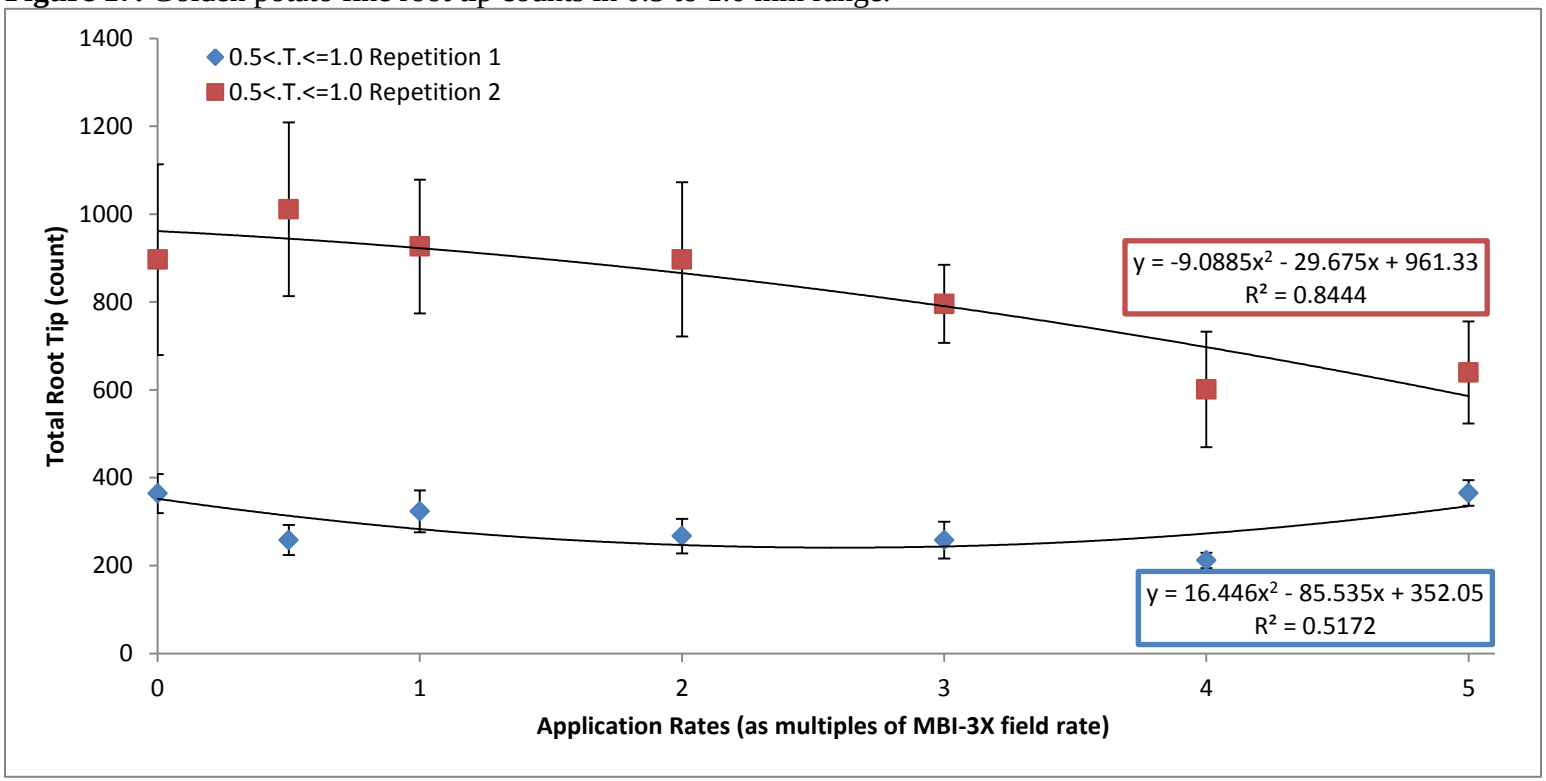


**Figure 18.** Cherry belle radish edible root weight.

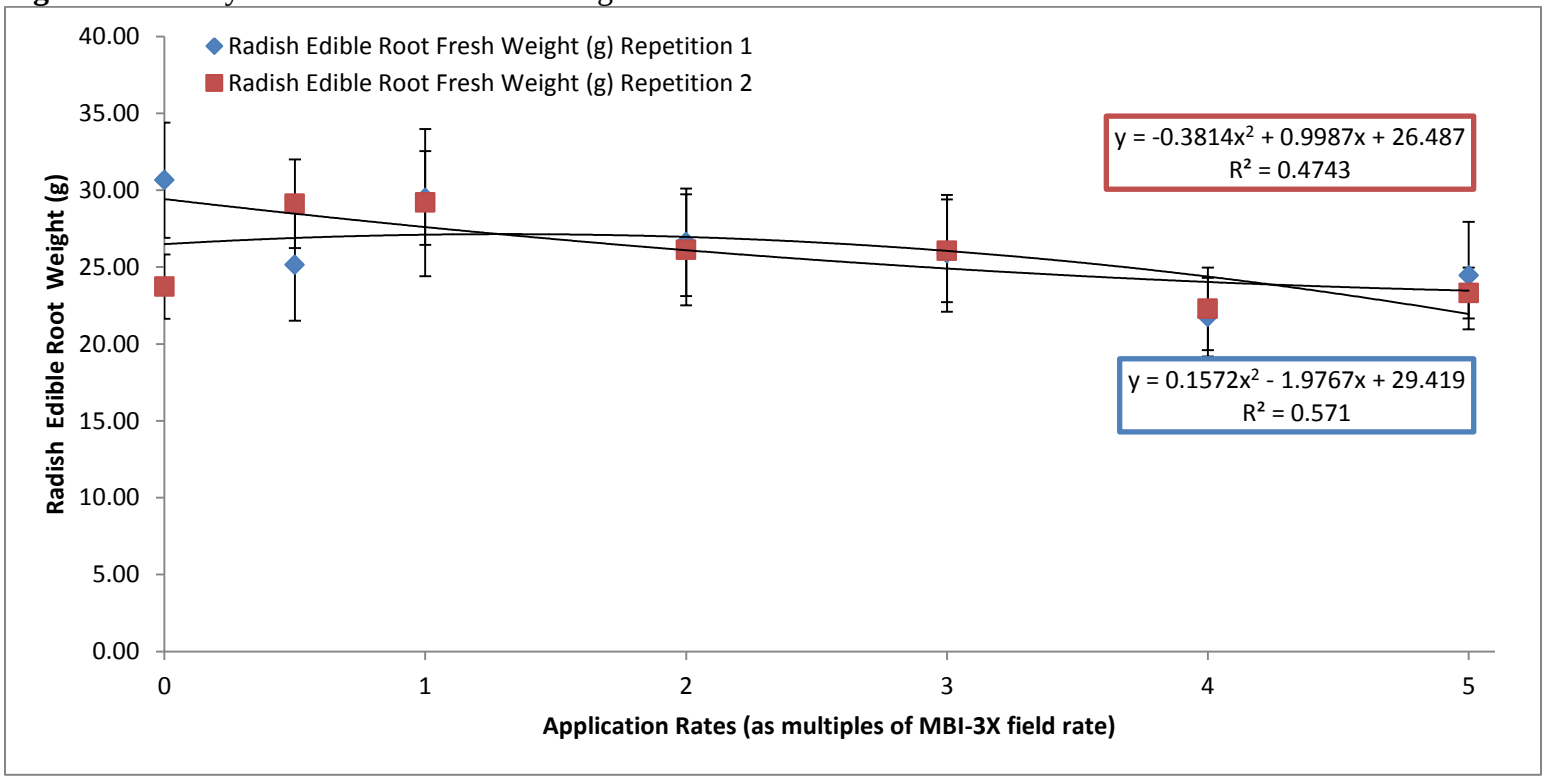
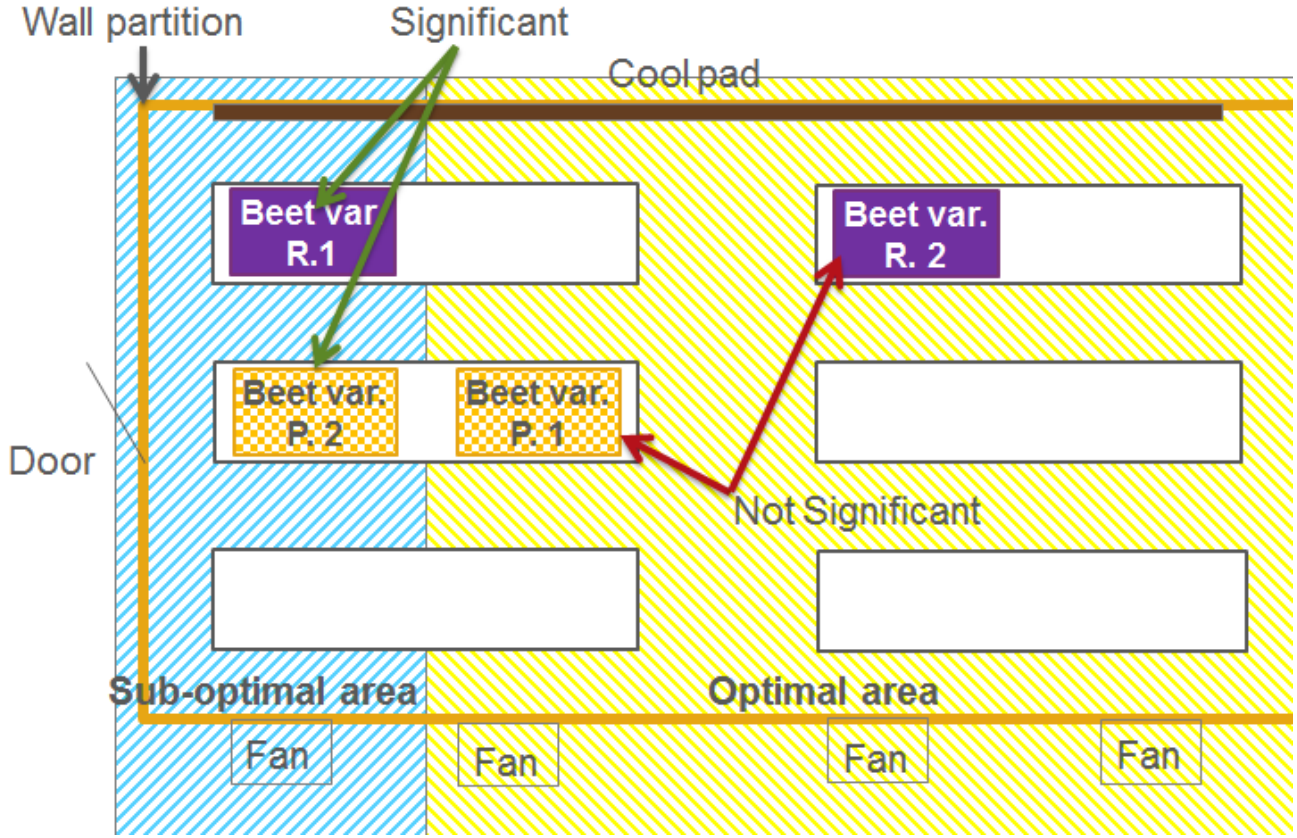


**Figure 19.** Microclimate effects in greenhouse for Pacemaker III and Red Ace beet repetitions.




This is a diagram of beet repetitions placement in the greenhouse. Pacemaker III beet repetition 2 and Red Ace beet repetition 1 are located near a wall partition, which had partial shading. These beet repetitions exhibited significant ANOVA results. Pacemaker III

beet repetition 1 and Red Ace beet repetition 2 were in the middle of the greenhouse with optimal growing conditions. These did not exhibit significant ANOVA results.