INFLUENCE OF MI GENES ON GRAFTED *SOLANUM LYCOPERSICUM* L. CULTIVARS FOR CONTROL OF TRIALEURODES VAPORARIORUM (INSECTA) IN THE PRESENCE AND ABSENCE OF *MELOIDOGYNE INCOGNITA* (NEMATODA).

By

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ABSTRACT

INFLUENCE OF MI GENES ON GRAFTED *SOLANUM LYCOPERSICUM* L. CULTIVARS FOR CONTROL OF *TRIALEURODES VAPORARIORUM* (INSECTA) IN THE PRESENCE AND ABSENCE OF *MELOIDOGYNE INCOGNITA* (NEMATODA).

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*Solanum lycopersicum L (tomato) is n*ative to South and Central America. It is grown world-wide on about ten million acres, yielding *circa* 185 million tons of fruit on an annual basis. To make this crop available on a year-round basis, greenhouse tomatoes are commonly grown in countries such as the Netherlands, Canada and Uzbekistan. Tomato is the second staple vegetable in Central Asia after potato. About four million tons were produced in Central Asia in 2013. Tomatoes are eaten raw or cooked and are rich in essential vitamins and minerals. Their color comes from lycopene, which is responsible for the antioxidants reported to alleviate risk to human health issues such as heart disease, hypertension, congestive heart failure, prostate cancer and atherosclerosis.

Tomato can be susceptible to numerous insect pests and infectious disease pathogens. Tomato hornworm (*Manduca quinquemaculata*), cotton bollworm (*Helicoverpa armigera*), corn earworm (*Helicoverpa zea*), potato aphid (*Macrosiphum euphorbiae*), green peach aphid (*Myzus persicae*), silver leaf whitefly (*Bemisia tabaci*), and greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) are key insect pests of tomato. Whitefly is important because of its ability to cause direct feeding damage and vector viruses such as tomato yellow leaf curl virus (TYLCV) of the begomovirus genus.

Numerous tomato cultivars have been developed for desirable, pest, horticultural and food attributes. Some have been adopted for their pest or pathogen resistance characteristics. For example, Anahu is a determinant tomato cv that has the Mi gene which confers resistance to

southern root knot nematode (*Meloidogyne incognita (Kofoid & White)* Chitwood), and cross-resistance to potato aphid (*Macrosiphum euphorbiae* (Thomas)) and sweet potato whitefly (*Bemisia tabaci* (Gennadius)). Not all resistant tomato varieties, however, are suitable for tomato production, or preferred by farmers and consumers. Grafting has the potential to bring all the essential traits together.

Grafting is a practice used in vegetable production systems to decrease risk to soil-borne diseases, increase salt tolerance, increase tolerance to other abiotic factors, increase drought tolerance, increase plant vigor, and increase yield.

The research presented herein was aimed at determining if the grafted susceptible tomato cv Rutgers on top of resistant tomato cv Anahu will control two important tomato pests. Moreover, we measured plant biomass (fruit, stem and leaf, and root) of these two tomato cultivara with and without grafting in order to evaluate grafting impact on plant biomass allocation. Also, we counted the Type-D trichome density as these structures are thought to be responsible for pest resistance by acting as mechanical and chemical barriers.

The results show that Anahu is not a good tomato cultivar to control *M. incognita* and *T. vaporariorum*. Anahu was not completely resistant to *M. incognita*. Moreover, on Anahu, significant decrease in *T. vaporariorum* population density occurred only when the *M. incognita* was present. Type-D trichome density was not responsible for a reduction of *T. vaporariorum* population density on Anahu. Thus, in order to draw a strong conclusion about Mi gene resistant to *T. vaporariorum* and resistance translocation from the rootstock to scion in grafted plants, there is a need for more advanced research, including molecular studies.

This thesis is dedicated to those who supported and believed in me during the course of my Ph.D. program.
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KEY TO SYMBOLS AND ABBREVIATIONS

cv(s) - cultivar(s) (variety)

g - grams

P_i - nematode initial population density per plant

P_f - female final population density per plant

RH - relative humidity

Stem/leaf - stem and weight measured together

INTRODUCTION

The Michigan State University, Central Asia, Integrated Pest Management, Collaborative Research Support Program (IPM CRSP) was initiated in 2004, with funding from USAID. It included three nations: Kyrgyzstan, Tajikistan, and Uzbekistan. The project had two phases. The first phase consisted of conducting landscape ecology research in Tajikistan, BioLab enhancement in Uzbekistan and farmer training in Kyrgyzstan. The activities were implemented by local partners and three project managers. The second phase of the project consisted of development and implementation of IPM programs for three key-staple Central Asia food crops: potato in Kyrgyzstan, tomato in Uzbekistan and wheat in Tajikistan. Moreover, the Central Asia IPM project initiated capacity building for Central Asia through bringing young scientists from Central Asia to the U.S.A. for a graduate-level education. Thus, as one of these individuals, my Ph.D. dissertation is directly interlinked with the MSU IPM CRSP for Central Asia.

On a global basis, tomato production under both greenhouse and field conditions has numerous pest problems. One of the most difficult to control in greenhouse production is the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood, 1856*)*. Current, *T. vaporariorum* control in tomato production is based mainly on the use of chemical pesticides. For example, in Uzbekistan and elsewhere, tomatoes grown in greenhouses are sprayed with insecticides weekly in order to maintain the *T. vaporariorum* population density under the damage threshold level. These chemicals are not only dangerous to humans, but their use also can cause long-term environmental damage that is difficult to remediate.

There are several alternates to chemical control, including the use of resistant or tolerant cultivars and hybrids. Nevertheless, these does not always keep *T. vaporariorum* populations under the damage threshold. Moreover, pests can overcome resistance due to their rapid rates of reproduction. Another obstacle is the time and cost of breading resistant varieties and hybrids.

Another option for pest and disease control in tomato production is vegetable grafting. Grafting can be used to incorporate several desired characteristics of different varieties together into a single plant. Vegetable grafting is a surgical procedure. It has been used extensively in East and South Asia for many ears. Currently, vegetable grafting is being introduced into many European and North American countries. Thus, it should be possible to use grafted tomato plants to maintain *T. vaporariorum* population densities below the threshold level. Moreover, chemical insecticides, resistant varieties and grafting, mentioned above, can be combined together for control of *T. vaporariorum* under greenhouse and field tomato production conditions.

My Ph.D. dissertation entitled "Influence of Mi genes on grafted *Solanum lycopersicum* L. cultivars for control of *Trialeurodes vaporariorum* (Insecta) in the presence and absence of *Meloidogyne incognita* (Nematoda)" consists of five chapters. Each chapter was developed from the results of hypotheses tested during my Ph.D. research program. Plans are to have each chapter published individually in preview science journals. The hypotheses related to the impact of tomato grafting, *T. vaporariorum, M. incognita* and trichomes on biomass partitioning. Since each chapter is self-contained with a comprehensive literature review this information is not repeated in this general introduction. The five chapters include:

Chapter 1 "Impact of Grafting and Pruning on Resource Partitioning of Greenhouse *Solanum lycopersicum* L. cvs Rutgers and Anahu: With Special Reference to Central Asia"

Chapter 2 "Biomass allocations associated with homo-grafted and hetero-grafted *Solanum lycopersicum* L. cvs Rutgers and Anahu grown under field conditions."

Chapter 3 "Influence of Grafting *Solanum lycopersicum* L. cvs Anahu and Rutgers on Plant Biomass Partitioning in the Presence and Absence of *Meloidogyne incognita* (Nematoda): With the specific reference to Central Asia."

Chapter 4 “Impact of *Solanum lycopersicum* L. Grafting on *Trialeurodes vaporariorum* Westwood (Insecta) Development Under Growth Chamber Conditions: With Special Reference to the Mi Gene, Type-D Trichomes and Central Asia.”

Chapter 5 “Joint Impact of *Meloidogyne incognita (Kofoid and White*1919*) Chitwood* 1949 and Westwood on Grafted *Solanum lycopersicum* L. Cultivars: With Special Reference to the Mi Gene.”

CHAPTER 1: IMPACT OF GRAFTING AND PRUNING ON RESOURCE PARTITIONING OF GREENHOUSE *SOLANUM LYCOPERSICUM* L. CVS RUTGERS AND ANAHU: WITH SPECIAL REFERENCE TO CENTRAL ASIA

Abstract

Numerous tomato cultivars have been developed with desirable horticultural and food attributes. Some have been adopted due to their pest or pathogen resistance characteristics. However, not all resistant tomato cultivars are favorable for tomato fruit production. Plant grafting allows for combining two or more desired characteristics into one plant. Tomato cultivars, however, vary in how they allocate biomass among their shoot, root and fruit tissues. Understanding biomass partitioning is important for the selection of appropriate cultivars for grafting and greenhouse production systems. Rutgers and Anahu are two important tomato cultivars that have desirable characteristics for tomato production in central Asia. The major objective of this research was to determine the effects of homo-grafting and hetero-grafting of the two cultivars on plant biomass partitioning among stem/leaf, root and fruit tissue under greenhouse conditions. Rutgers partitioned the majority of its biomass to fruit tissue and less to stem/leaf tissue and root tissues. In contrast, Anahu allocated the majority of its biomass to stem/leaf tissue. Homo-grafted Rutgers shifted its biomass from fruit to stem/leaf tissue, while homo-grafted Anahu shifted its biomass to fruit tissue. Hetero-grafted Rutgers shifted its biomass even more towards stem/leaf tissue, whereas hetero-grafted Anahu retained its original biomass partitioning signature. Thus, Anahu appears to be manipulating the scion's biomass partitioning when used as a rootstock. The impact was most pronounced with respect to fruit tissue or stem/leaf tissue. Root tissue resource partitioning was not impacted by grafting or pruning. An additional objective was to determine the impact of light (optimal) or heavy pruning (single stem) on the partitioning of plant biomass of these tomato cultivars under greenhouse conditions. Heavy pruning entailed removing all suckers and leaves up to the first fruit cluster and resulted in Anahu allocating more

of its biomass to fruit tissue compared to stem/leaf tissue. Under heavy pruning, Rutgers' biomass partitioning signature was not impacted by homo-grafting or hetero-grafting. The biomass signature for lightly pruned Rutgers and Anahu was similar to its non-pruned signature. Understanding biomass partitioning at the cultivar level is important before selecting a rootstock for commercial production under greenhouse conditions. In general, Anahu is not a good rootstock for tomato fruit production under greenhouse conditions. Future research with Rutgers and Anahu grafting and pruning needs to be evaluated in regards to biotic and abiotic stress factors.

Introduction

Solanum lycopersicum L (tomato) is native to South and Central America (Peralta et al. 2006). It is grown world-wide on about ten million acres, yielding *circa* 185 million tons of fruit on an annual basis (FAOSTAT, 2011). To make this crop available on a year-round basis, greenhouse tomatoes are commonly grown in countries such as the Netherlands, Canada and Uzbekistan. Tomato is the second staple vegetable in Central Asia after potato. About four million tons were produced in Central Asia in 2013 (FAOSTAT, 2014). Tomatoes are eaten raw or cooked and are rich in essential vitamins and minerals. Their color comes from lycopene, which is responsible for the antioxidants reported to alleviate risk to human health issues such as heart disease, hypertension, congestive heart failure, prostate cancer and atherosclerosis (Marry, Datta, Bedi, & Kaur, 2015).

Tomato can be susceptible to numerous insect pests and infectious disease pathogens. Tomato hornworm (*Manduca quinquemaculata*), cotton bollworm (*Helicoverpa armigera*), corn earworm (*Helicoverpa zea*), potato aphid (*Macrosiphum euphorbiae*), green peach aphid (*Myzus persicae*), silver leaf whitefly (*Bemisia tabaci*), and greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) are key insect pests of tomato. Whitefly is important because of its

ability to cause direct feeding damage and vector viruses such as tomato yellow leaf curl virus (TYLCV) of the begomovirus genus. TYLCV causes substantial tomato yield loss annually on a global basis (Zalom, 2012). Another key greenhouse pathogen is the southern root-knot nematode (*Meloidogyne incognita*). In addition to being a pathogen, it can also pre-dispose plants to soil-borne wilt diseases such as those caused by *Verticillium dahliae* and *Fusarium oxysporium* f. sp. *Lycopersici* (Zalom, 2012).

Numerous tomato cultivars have been developed for desirable horticultural and food attributes. Some have been adopted for their pest or pathogen resistance characteristics. Another way to achieve resistance is through grafting. Plant grafting has been used for many years, including as a means of managing pests and pathogens through resistance (Lee, 1994). Vegetable grafting was first practiced widely early in the 20th century (Lee, 1994), as described in *Grafted of Herbaceous Vegetable and Ornamental Crops* in Scientia Horticulturae Ashita (1927, 1930, 1934). The technique allows combining two or more desired vegetable characteristics into one plant. In addition, grafting is used to control soil-borne diseases, increase slat tolerance, increase nutrient uptake, and increase plant yield.

Tomato cultivars vary in how they allocate biomass among their shoot, root and fruit tissues. Understanding biomass partitioning is important for the selection of appropriate cultivars for greenhouse production systems. Biomass partitioning has been studied in detail on non-grafted tomato plants. It has been shown that a biomass increase in one part of plant effects the biomass allocated to other parts of the plant (Enquist & Niklas, 2002; Heuvelink, 1997; Marcelis, Heuvelink, & Goudriaan, 1998). This phenomenon has been studied as a means of increasing fruit yield, but less extensively for impacts on overall plant biomass partitioning (Dimitrios et al., 2009, Charles and Xin, 2012). The impact of grafting on biomass partitioning has not been

studied under greenhouse conditions (Marcelis, 1993). It has also not been studied in regards to homo-grafted, and hetero-grafted plants. In homo-grafting, the scion and root tissue are from the same cultivar, but different plants are grafted. In hetero-grafting, the scion and root tissue come from two different cultivars.

Rutgers and Anahu are important tomato cultivars that have desirable horticultural and food characteristics. Rutgers is a determinant cultivar that is typically favored by farmers for its good quality and high productivity. Anahu is a determinant cv that has the Mi gene which confers resistant to *Meloidogyne incognita (Kofoid & White) Chitwood,* and cross-resistance to *Macrosiphum euphorbiae* (Thomas) (Goggin, Williamson, & Ullman, 2001; Vos et al., 1998) and sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Nombela, Williamson, & Muñiz, 2003; Goggin et al., 2001). There are several Mi genes in cultivated and wild tomatoes, including Mi-1 to Mi-9. Mi-1 has seven homologues, Mi-1.1 through Mi-1.7 (Seah, Telleen, & Williamson, 2007). Among the Mi-1 homologues, Mi-1.2 is the only functional nematode resistant gene for *M. incognita* (Milligan et al., 1998). Mi-1.2 is the gene present in cv Anahu. Anahu is one of the parents for all other commercial cultivars containing the Mi gene. Anahu is resistant to three different key pests of tomato. It usually grows to a height of 3-4 feet and produces approximately five kg of tomato fruit under field conditions (Bobisud, Martin, & Sekioka, 1996).

Pruning tomato plants can be used to increase air flow between leaves and decrease relative humidity, resulting in less favorable conditions for pathogens (Ferrandino, 1999). Moreover, Kanyomeka and Shivute (2005) (Kanyomeka & Shivute, 2005)(Kanyomeka & Shivute, 2005)(Kanyomeka & Shivute, 2005)(Kanyomeka & Shivute, 2005)(Kanyomeka & Shivute, 2005) suggested that pruning increases fruit quality, plant health and makes plants less prone to pest attack. There are two common methods of pruning, simple pruning and Missouri pruning.

Simple pruning consists of pinching-off suckers when the suckers are young. This does not take much effort and does not reduce the plant's photosynthetic area very much. With the Missouri system, two leaves are left and the apical meristems of suckers are pinched-off (Ferrandino, 1999). Moreover, pruning is used in greenhouse tomato production to increase the number of plants per unit area. There are several degrees of severity of pruning tomato plants. Pruning may be light (optimal) or heavy. In light pruning, 50% of the suckers are removed up to the first fruit cluster. In heavy pruning in a two stem system, all suckers are removed from the ground to the first fork (Olson, 1989). Pruning effects have been studied in non-grafted as well as in grafted tomato plants. On non-grafted tomatoes, heavily-pruned single-stem plants resulted in low total yield and increased fruit size (Olson, 1989). Double stem pruned tomato plants yielded more than 3 or 4-stem pruned plants (Mourão, Teixeira, Brito, Ferreira, & Moura, 2014).

The aim of this research was to evaluate the potential of using grafted cv Rutgers and cv Anahu tomato plants for greenhouse tomato production in Central Asia. The specific objectives of the research were 1) to determine the effects of homo and hetero grafting of tomato cv Rutgers and cv Anahu on partitioning of plant biomass among stem/leaf, root and fruit tissue under greenhouse conditions and 2) to determine the effects of light (optimal) and heavy pruning on tomato plant biomasses partitioning of plant biomass on the tomato cultivars under greenhouse conditions.

Materials and Methods

Plant material

The tomato biomass resource partitioning study was conducted under greenhouse conditions at Michigan State University using the cultivars Anahu and Rutgers. It consisted of five experiments, each with six grafting treatments and five replicates of each treatment. A single tomato cv Anahu (University of Hawaii Seed Program) or cv Rutgers (Sustainable Seed Co.) was

sown in each cell in 50 or 72 cell seedling trays containing plant growth media (SUREMIX professional growing media, Michigan Grower Products Inc.) and held in a growth chamber maintained at 26 C for 16 hours and 20 C for eight hours, day and night, respectively. The seedlings were watered every other day. Anahu seeds were planted two days before Rutgers seeds to ensure the same seedling diameter for grafting. This time-interval was based on the results of preliminary research. After 14 days in the growth chamber, the seedlings were appropriate for grafting.

Grafting

Rootstocks and scions were cut below the cotyledon at a 45-65 degree angle using a Miter-Cut Grafting Knife (Johnny's Selected Seeds). Scions and rootstocks were clipped together with silicon tubes to hold the rootstock and scion together until the grafting wound healed. Silicon clipper (Hydro-Gardens, Colorado Springs, CO) size varied from 1.5-2.5 mm, depending on seedling diameter. During graft wound healing, a humidifier (Air Innovations Model # HUMID06 1.37-gal. Ultrasonic Digital Humidifier) was used to control the relative humidity (RH). The grafted plants were maintained in a growth chamber for seven days at 24-26 C. The RH was decreased gradually from 90% after three days, to 85% two days later, to 75% one day later and then to 65% for the final day. After 7 days, the grafted seedlings were moved to the laboratory for three days of acclimatization. The six grafting treatments were: 1) non-grafted Anahu, 2) non-grafted Rutgers, 3) homo-grafted Anahu, 4) homo-grafted Rutgers, 5) hetero-grafted Anahu (Anahu scion and Rutgers rootstock) and 6) hetero-grafted Rutgers (Rutgers scion and Anahu rootstock).

Greenhouse

Following acclimatization, the 21-day-old tomato plants were transplanted into five-gallon plastic pots filled with sterilized sandy soil and placed in a greenhouse maintained at 26-28 C.

They were organized in a randomized block design and watered as necessary for 125 to 135 days (Figure 1-Figure 3).

Pruning

Experiments were conducted using both pruned and non-pruned tomato plants. The grafted and non-grafted plants in the first experiment were not pruned. Those in the second experiment were lightly pruned and those in the third experiment were heavily pruned. The lightly pruned experiment was conducted three times. Simple pruning methods were used to remove suckers from the node. Each sucker was pinched of by hand when it was small, or with garden clippers when suckers were larger. Under the lightly pruned system, all the suckers and leaves were removed up to first fruit cluster. On heavily pruned plants, all of the suckers were removed from the entire plant and leaves were removed to a 3-4-foot height. The greenhouse space for the research accommodated 60 non-pruned plants; whereas, it accommodated 90 lightly and 90 heavily pruned tomato plants.

Data collection

Throughout the experiments, ripe fruit (90% or more of the skin red in color) were harvested weekly. Each fruit was weighed individually on a 200g scale on the day of harvest. After 125 days, all remaining fruit were harvested and included in the yield as fruit fresh weight. The stem/leaf tissues also were collected and weighed right after collection on a five kg scale. Fresh weights were recorded and the tissue placed in a drying oven at 30-35 C the same day for dry weight determination. The dry mass was weighed with the same scale after seven days of oven drying. Tomato plant roots were collected, washed and dried in a salad leaf spin dryer for 2-3 minutes. Fresh weight of root tissue was determined on a 200 g scale. The roots were placed in a drying oven at the same temperature used for the stem/leaf tissue. Root dry weights were measured after seven days of drying with the same scale.

Data analysis

The data were analyzed using SPSS 24 Grad Primmum Pack. Data distribution normality was checked with a histogram plot for skewness of the distribution. If the data were positively skewed, they were transformed using a natural Log or Log_{10} prior to analysis. If the data were negatively skewed, the Square Root Method was applied. If data were not normalized, the non-parametric Kruskal-Wallis Test was used as the substitute for a One-Way ANOVA. If the One-Way ANOVA showed a significant difference at the $\alpha=0.05$, means were separated using the Tukey's Test. If the non-parametric Kruskal-Wallis Test was significant at the $\alpha=0.05$, means were separated using the Dunn's Test.

Results

Tomato Plant Fresh Weight Biomass Partitioning of Rutgers and Anahu and Impact of Grafting

There were significant differences among the six grafted treatments for stem/leaf fresh weight in the non-pruned experiment (Appendix A. Statistical Tables
Table 1). The hetero-grafted Rutgers stem/leaf tissue was significantly greater than that of hetero-grafted Anahu. Non-grafted Rutgers and Anahu, however, had different biomass partitioning signatures and allocated their biomass inversely. Rutgers allocated the majority of its biomass to fruit tissue (Figure 4). On the other hand, Anahu allocated the greatest amount of biomass to stem/leaf tissue. In both tomato cultivars, root tissue comprised the least amount of biomass compared to other parts of the plant. Biomass partitioning in Rutgers changed when it was homo-grafted, with a gain in stem/leaf tissue and a loss of fruit tissue. Stem/leaf tissue biomass comprised 52% of the total biomass, while 10% of fruit biomass was lost. Root tissue biomass allocation, however, did not change on homo-grafted Rutgers, compared to non-grafted Rutgers. Conversely, Anahu shifted its biomass towards fruit after homo-grafting. An average of

9% of the biomass shifted to fruit tissue from stem/leaf tissue, compared to non-grafted Anahu. Root tissue biomass was essentially unchanged, decreasing by only 1%. . Hetero-grafting Rutgers shifted 13% of its biomass to stem/leaf tissue, compared to non-grafted Rutgers. Root tissue biomass did not change compared to non-grafted Rutgers. With hetero-grafted Anahu, biomass partitioning shifted towards stem/leaf tissue compared to non-grafted Anahu. Stem/leaf tissue comprised 3% more of the biomass compared to non-grafted Anahu. Root biomass partitioning was not altered by hetero-grafting.

Lightly Pruning Impact on Plant Fresh Weight Biomass Partitioning

There were no significant difference between the six grafted treatments after light pruning on fruit, stem/leaf, and root tissue (Table 2). Moreover, with light pruning, there was very little change in biomass partitioning of Rutgers and Anahu, compare to the non-pruned experiment (Figure 4Figure 5). Rutgers retained it biomass signature after light pruning, allocating the highest proportion of biomass to fruit tissue. Light pruning, however, decreased fruit biomass by 3% and 10%, compared to non-pruned and heavy pruned treatments. Root tissue biomass did not change after light pruning. Anahu also retained its biomass signature after light pruning, allocating the highest proportion of biomass to stem/leaf tissue (Figure 4Figure 5). However, there was a slight 4% increase in fruit biomass after lightly pruning. Root tissue biomass allocation did not change. The biomass of homo-grafted Rutgers that was light pruned consisted of 55% stem/leaf tissue and 35% fruit tissue (Figure 4Figure 5). This represented a 9% decrease in fruit biomass compared to non-pruned plants where 3% shifted to stem/leaf tissue and 6% shifted to root tissue after light pruning. After light pruning, the biomass of homo-grafted Anahu consisted of 64% stem/leaf tissue and 31% fruit tissue (Figure 4Figure 5). This represented a 9% shift in biomass partitioning from fruit to stem/leaf tissue after homo-grafting Anahu. In hetero-

grafted Rutgers after light pruning, biomass was almost equally allocated between stem/leaf tissue and fruit tissue, 49% and 47%, respectively (Figure 4Figure 5). This represented a 6% shift in biomass partioning from leaf/stem tissue to fruits after light pruning. Root tissue biomass did not change. Light pruning produced a similar change in biomass partitioning of hetero-grafted Anahu, with about a 7% increase in fruit biomass (Figure 4Figure 6). Root tissue biomass did not change.

Heavy Pruning Impact on Plant Fresh Weight Biomass Partitioning

There were no significant differences among fruit, stem/leaf, and root tissue on the six grafted treatments after heavy pruning (Table 3). Heavy pruning, however, drastically change biomass partitioning among fruit, stem/leaf and root tissues. The heavily pruned Rutgers biomass signature was similar to the non-pruned Rutgers biomass partitioning signature (Figure 4Figure 6). The greatest amount of biomass was allocated to fruit tissue; there was 7% more fruit biomass following heavy pruning. Stem/leaf tissue biomass was second greatest and root tissue was the least. Anahu did not retain its fresh biomass partitioning signature following heavy pruning, losing 15% of stem/leaf tissue and allocating 16% more biomass to fruit (Figure 4Figure 6). Heavy pruning of homo-grafting Rutgers resulted in a substantial shift in biomass from fruit towards stem/leaf tissue, with 19% more biomass allocated to stem/leaf tissue (Figure 4Figure 6). Root tissue biomass was essentially unchanged, decreasing by only 1%. Heavily pruned homo-grafted Anahu plants allocated 41% of their biomass to stem/leaf tissue and 55% to fruit tissue. This represented a 9% increase in allocation of biomass to fruit (Figure 4-Figure 6). Root tissue of homo-grafting Anahu was not impacted by heavy pruning. Heavy pruning of hetero-grafted Rutgers plants significantly altered the partitioning of resources, shifting 23% of their biomass from leaf/stem to fruit (Figure 4Figure 6). Root tissue biomass was essentially

unchanged, decreasing by only 1%. Biomass partitioning of hetero-grafted Anahu also was substantially shifted by heavy pruning, with 24% more biomass allocated to fruit (Figure 4,Figure 6).

Dry Weights of Rutgers and Anahu Among Grafting Treatments

Dry weight among the six grafting treatments was significantly different (F=4.332, P=0.006) in the non-pruned experiment. The greatest dry weight of stem/leaf tissue occurred in Hetero-grafted Anahu. The least amount of dry weight was recorded on hetero-grafted Rutgers (Table 4). Hetero-grafted Anahu produced about twice as much and significantly more (P=0.015) stem/leaf tissue than hetero-grafted Rutgers. Root tissue dry weight biomass was not significantly different among the six grafted treatments (Table 5). The least amount of root tissue dry weight was observed on hetero-grafted Rutgers. The greatest amount of root tissue dry weight biomass was observed on non-grafted Anahu.

There were no significant differences in stem/leaf tissue dry biomass among the six treatments when the plants were lightly pruned (Table 4). The greatest amount of stem/leaf tissue was observed on homo-grafted Anahu after light pruning. The lowest amount of stem/leaf tissue biomass occurred on non-grafted Rutgers. Root tissue dry weight biomass following light pruning also was not significant different among the six treatments (Table 5). The greatest amount of root tissue dry weight was observed on hetero-grafted Anahu. The lowest amount of root dry weight was recorded on non-grafted Anahu.

Heavy pruning of Rutgers and Anahu did not significantly impact stem/leaf tissue biomass among the six treatments (Table 4). The greatest amount of stem/leaf tissue biomass was observed on non-grated Anahu after heavy pruning. The lowest tissue dry weight was recorded on homo-grafted Anahu. Root tissue dry weight also was not significantly different among the six grafted treatments following light pruning. The greatest amount of root dry tissue was

associated with homo-grafted Anahu. The lowest root tissue dry weight was recorded on non-grafted Anahu.

Discussion

Rutgers and Anahu are important tomato cultivars that have desirable characteristics for tomato production in Central Asia. Rutgers is favored by farmers for its good quality and high productivity, while Anahu has the Mi gene which confers resistant to some important nematode and insect pests (Nombela et al., 2003; Goggin et al., 2001). Plant grafting allows for combining the desired characteristics of each into one plant. A crucial first step in the grafting process is to ensure that fruit production is not negatively impacted when the cultivars are combined. Understanding the biomass partitioning signature of each cultivar involved should be a component of plant grafting systems.

Homo-grafting and hetero-grafting of Rutgers and Anahu impacted the partitioning of plant biomass among stem/leaf, root and fruit tissue under greenhouse conditions. It is well documented that non-grafted tomato plants vary in how they allocate biomass (Brian and Karl, 2002; Heuvelink, 1996, 1999; Marcelis et al., 1998). Higashide et al. (2014) did detailed comparison between two scions and two rootstocks after grafting where hetero-grafting and homo-grafting impacted scion biomass partitioning differently. Comparison and shift of biomass partitioning between non-grafted tomato cultivars, homo-grafted, and hetero-grafted plants, however, is a novel approach to understanding the biomass allocation of the grafted plant.

In comparing non-grafted, homo-grafted and hetero-grafted tomato plants, I found that Anahu and Rutgers allocated their biomass differently among fruit, stem/leaf, and root fresh tissue. Non-grafted Rutgers partitioned the majority of its biomass to fruit tissue and less to stem/leaf and root tissues. In contrast, non-grafted Anahu allocated the majority of its biomass to stem/leaf tissue. I considered these as the biomass partitioning signatures of tomato cultivars

Rutgers and Anahu. The effect of grafting and pruning on the two cultivars was compared to these characteristic biomass partitioning signatures. Homo-grafting and hetero-grafting shifted the allocation of plant biomass among the various plant parts. In homo-grafted Rutgers, biomass shifted from fruit to stem/leaf tissue, while in homo-grafted Anahu fruit tissue comprised more of the biomass. Hetero-grafting of Rutgers shifted biomass even more towards stem/leaf tissue; whereas, hetero-grafted Anahu retained its biomass partitioning signature. There was little change in root biomass allocation following grafting. With the exception of root crop vegetables, the root system is only a small portion of the vegetable plant's biomass (Marcelis, 1991). Overall, Anahu appears to be manipulating the scion's biomass partitioning when it is used as the rootstock.

Pruning also had a substantial impact on biomass partitioning of grafted and non-grafted Anahu and Rutgers tomato plants. Furthermore, the effects varied depending on the extensiveness of pruning. This is consistent with previous findings that plant biomass allocation varies depending on factors such as the age of the plant and environmental conditions (Marcelis et al., 1998). Heavy pruning generally had a beneficial effect on Anahu by shifting the allocation of biomass to fruit production. There was a 16-24% gain in fruit biomass for non-grafted Anahu and homo-grafted or hetero-grafted Anahu. Light pruning of hetero-grafted Rutgers also shifted a high percentage of biomass to fruit. However, 16% more biomass was allocated to stem/leaf tissue following heavy pruning of homo-grafted Rutgers. Light pruning had less of an effect on biomass partitioning. In our studies, the greatest increase in fruit biomass was associated after heavy pruning.

Conclusion

Overall, grafting and pruning impacted the biomass allocation of homo-grafted and hetero-grafted Rutgers and Anahu plants in different ways. Homo-grafted Anahu appears to not be a

good option for greenhouse tomato production due to grafting incompatibility and adverse effect on fruit yield. In contrast, hetero-grafted Rutgers may by a good choice as it improves yield under greenhouse conditions after heavy pruning. We propose that hetero-grafting can be useful in developing tomato plants for commercial greenhouse production systems. Light pruning can also benefit greenhouse tomato production by increasing yield per plant or total production by allowing space for more plants per unit area. We were able to place three times more plants in the greenhouse after pruning, resulting in an increase in total crop yield. Additionally, pruning of tomato plants can provide more favorable growing conditions and possibly make tomato plants less susceptible to risk of disease and yield loss associated with various pathogens and pests (Ferrandino, 1999).

APPENDICES

Appendix A. Statistical Tables

Table 1. Impact of homo-grafting and hetero-grafting on biomass partitioning of tomato cvs Rutgers and Anahu.

Non-pruned						
Treatments	**Fruit (g)**		**Stem/leaf (g)**		**Root ()**	
Non-grafting	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Rutgers	**2161.00**	466.85	**1685.22 ab[1]**	21.95	**159.6**	14.82
Anahu	**1441.51**	340.20	**2350.62 ab**	186.19	**217.6**	31.62
Homo-grafting						
Anahu/Anahu	**1760.13**	463.11	**1838.62 ab**	216.77	**142**	31.11
Rutgers/Rutgers	**1678.12**	603.80	**1975.02 ab**	194.81	**158.4**	14.12
Hetero-grafting						
Rutgers/Anahu	**1007.96**	538.93	**1629.82 a**	154.76	**120**	17.65
Anahu/Rutgers	**1345.29**	520.74	**2483.42 b**	283.29	**195.2**	18.62

[1]Column means followed by different letters are significantly (P = 0.05) different.

Table 2. Impact of homo-grafting and hetero-grafting on biomass partitioning of tomato cvs Rutgers and Anahu with light pruning.

Lightly pruning						
Treatments	**Fruit**		**Stem/leaf**		**Root**	
Non-grafting	**Mean (g)**	**SE**	**Mean (g)**	**SE**	**Mean (g)**	**SE**
Rutgers	**1690.82**	320.82	**1537.82**	107.44	**151.8**	8.9
Anahu	**1262.04**	305.43	**1676.02**	188.01	**156.75**	56.87
Homo-grafting						
Anahu/Anahu	**841.85**	157.65	**1686.22**	240.63	**128.2**	31.74
Rutgers/Rutgers	**1076.28**	214.68	**1660.22**	53.77	**285.8**	89.92
Hetero-grafting						
Rutgers/Anahu	**1686.38**	299.15	**1620.42**	211.92	**136**	28.61
Anahu/Rutgers	**1219.74**	122.51	**1621.42**	104.67	**214**	57.55

Table 3. Impact of homo-grafting and hetero-grafting on biomass partitioning of tomato cvs Rutgers and Anahu with heavy pruning.

Heavy pruning						
Treatments	**Fruit**		**Stem/leaf**		**Root**	
Non-grafting	**Mean (g)**	**SE**	**Mean (g)**	**SE**	**Mean (g)**	**SE**
Rutgers	**1269.38**	222.17	**861.82**	55.29	**82.8**	11.43
Anahu	**1433.93**	214.56	**1024.02**	129.11	**90.8**	28.45
Homo-grafting						
Anahu/Anahu	**1180.17**	271.63	**839.42**	75.57	**88**	6.58
Rutgers/Rutgers	**1738.76**	317.39	**923.42**	111.87	**93.6**	22.79
Hetero-grafting						
Rutgers/Anahu	**1810.15**	148.61	**873.82**	87.75	**73**	14.49
Anahu/Rutgers	**1222.26**	240.73	**827.62**	72.52	**76.4**	11.17

Table 4. Influence of tomato grafting on stem/leaf dry weight biomass partitioning associated with three pruning regimes, including: non-pruned, lightly pruned, and heavily pruned.

Stem/leaf	Non-pruned		Lightly pruned		Heavy pruned	
Grafting	**Mean (g)**	**SE**	**Mean (g)**	**SE**	**Mean (g)**	**SE**
Anahu	**460.62 ab[1]**	38.58	**213.82**	33.77	**143.02**	16.61
Anahu/Anahu	**317.02 ab**	54.76	**248.62**	35.60	**103.62**	7.84
Rutgers/Anahu	**283.42 a**	28.30	**229.62**	32.65	**121.42**	6.62
Anahu/Rutgers	**508.62 b**	60.35	**227.22**	19.52	**116.82**	7.80
Rutgers/Rutgers	**333.82 ab**	50.87	**209.82**	15.08	**127.62**	13.84
Rutgers	**319.02 ab**	4.03	**202.42**	10.58	**118.22**	9.78

[1]Column means followed by different letters are significantly (P = 0.05) different.

Table 5. Influence of tomato grafting on root dry weight biomass partitioning with three pruning regimes, including: non-pruned, lightly pruned, and heavily pruned.

Root	Non-pruned		Lightly pruned		Heavy pruned	
Grafting	**Mean (g)**	**SE**	**Mean (g)**	**SE**	**Mean (g)**	**SE**
Anahu	**25.75**	3.42	**13.38**	2.40	**5.66**	0.62
Anahu/Anahu	**18.31**	4.09	**28.25**	13.03	**10.71**	1.84
Rutgers/Anahu	**16.80**	1.89	**30.30**	10.96	**7.29**	1.26
Anahu/Rutgers	**22.97**	2.92	**50.49**	20.20	**7.39**	1.00
Rutgers/Rutgers	**20.94**	2.92	**49.15**	16.58	**9.67**	2.10
Rutgers	**19.18**	1.39	**27.61**	5.06	**8.15**	1.57

[1]Column means followed by different letters are significantly (P = 0.05) different.

Appendix B. Figures

Figure 1. Young non-pruned tomato plants under greenhouse research conditions.

Figure 2. Maturing and lightly pruned tomato plants under greenhouse research conditions.

Figure 3. Mature fruit bearing and heavily pruned tomato plants under greenhouse research conditions.

Figure 4. Anahu and Rutgers tomato biomass partitioning signatures for non-grafted, homo-grafted and hetero-grafted plants.

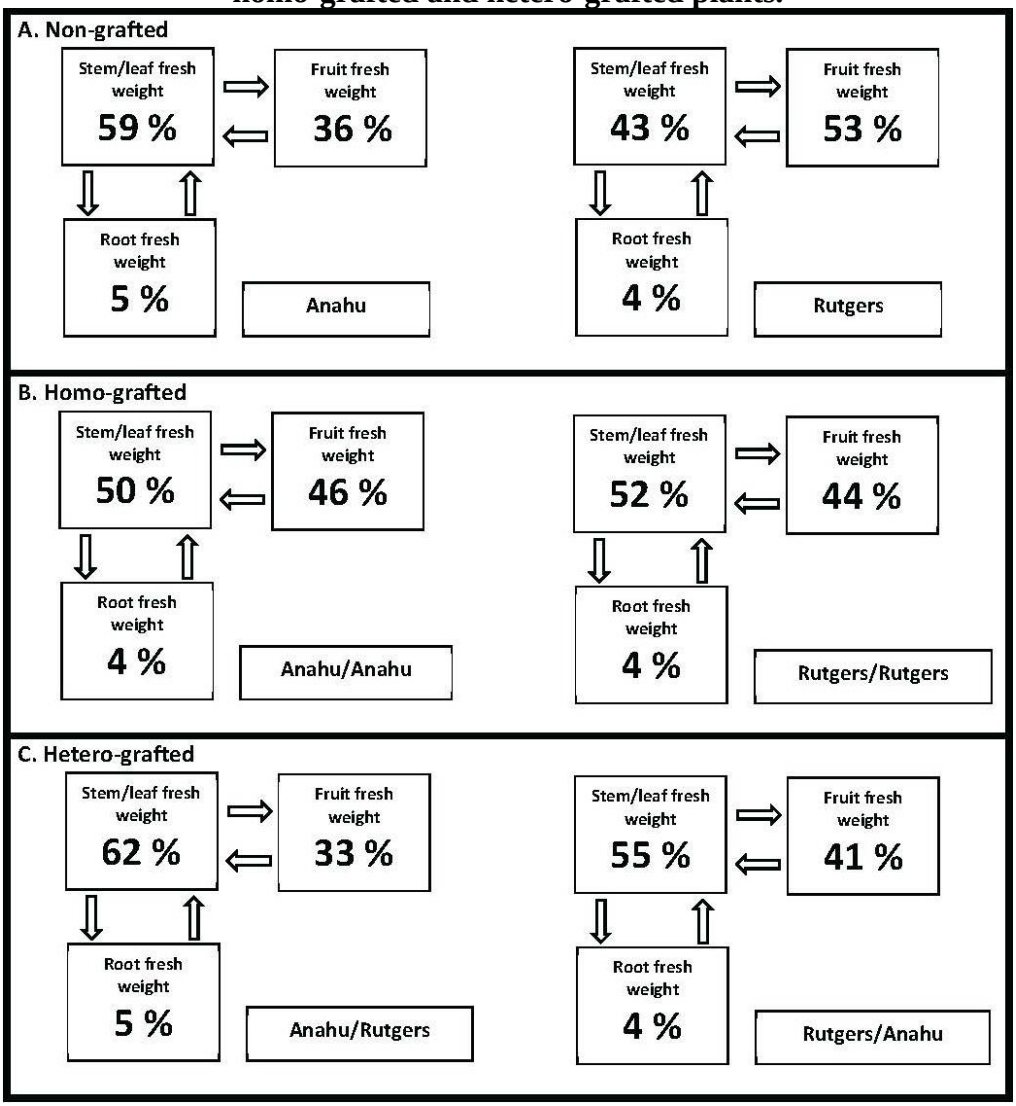


Figure 5. Influence of light pruning on Anahu and Rutgers tomato biomass partitioning signatures for non-grafted, homo-grafted and hetero-grafted plants.

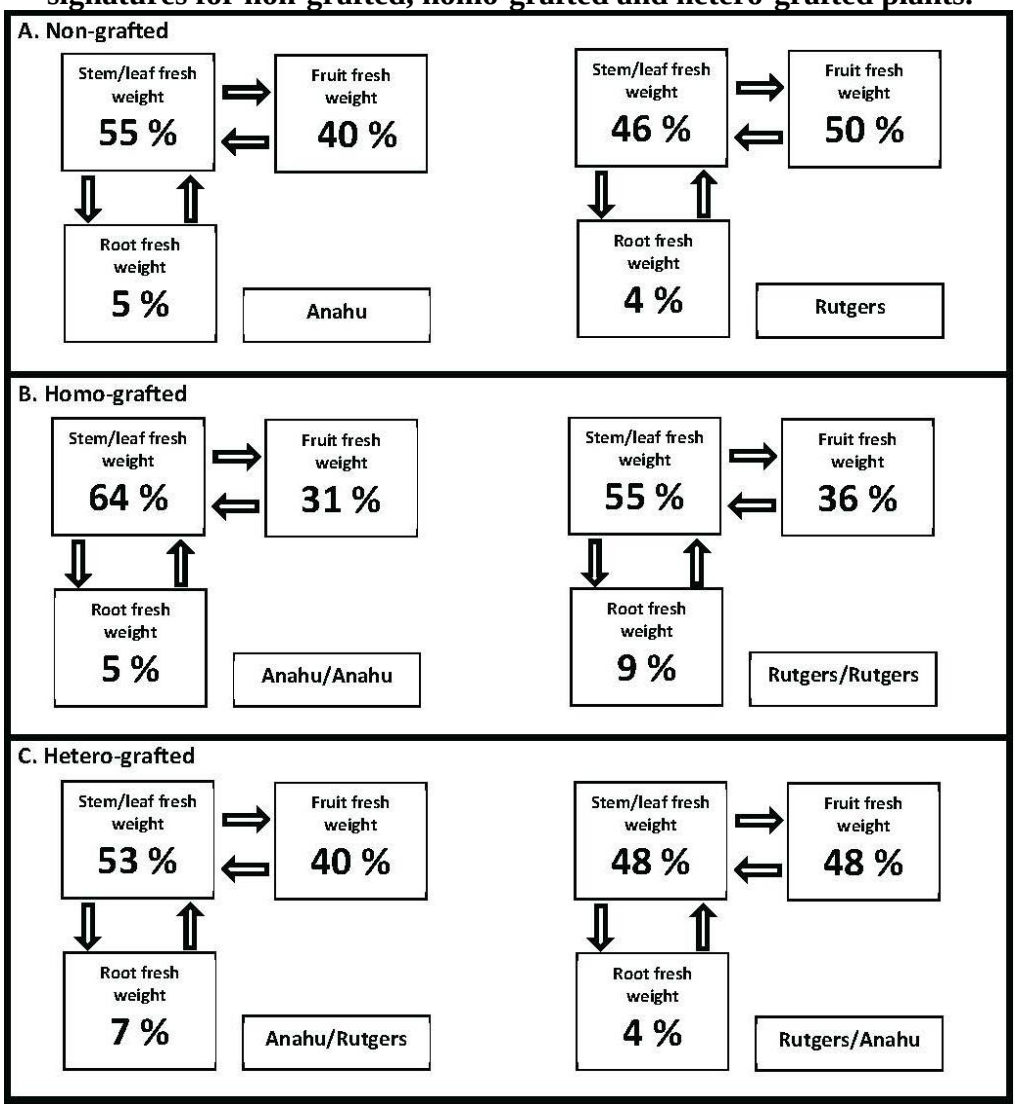
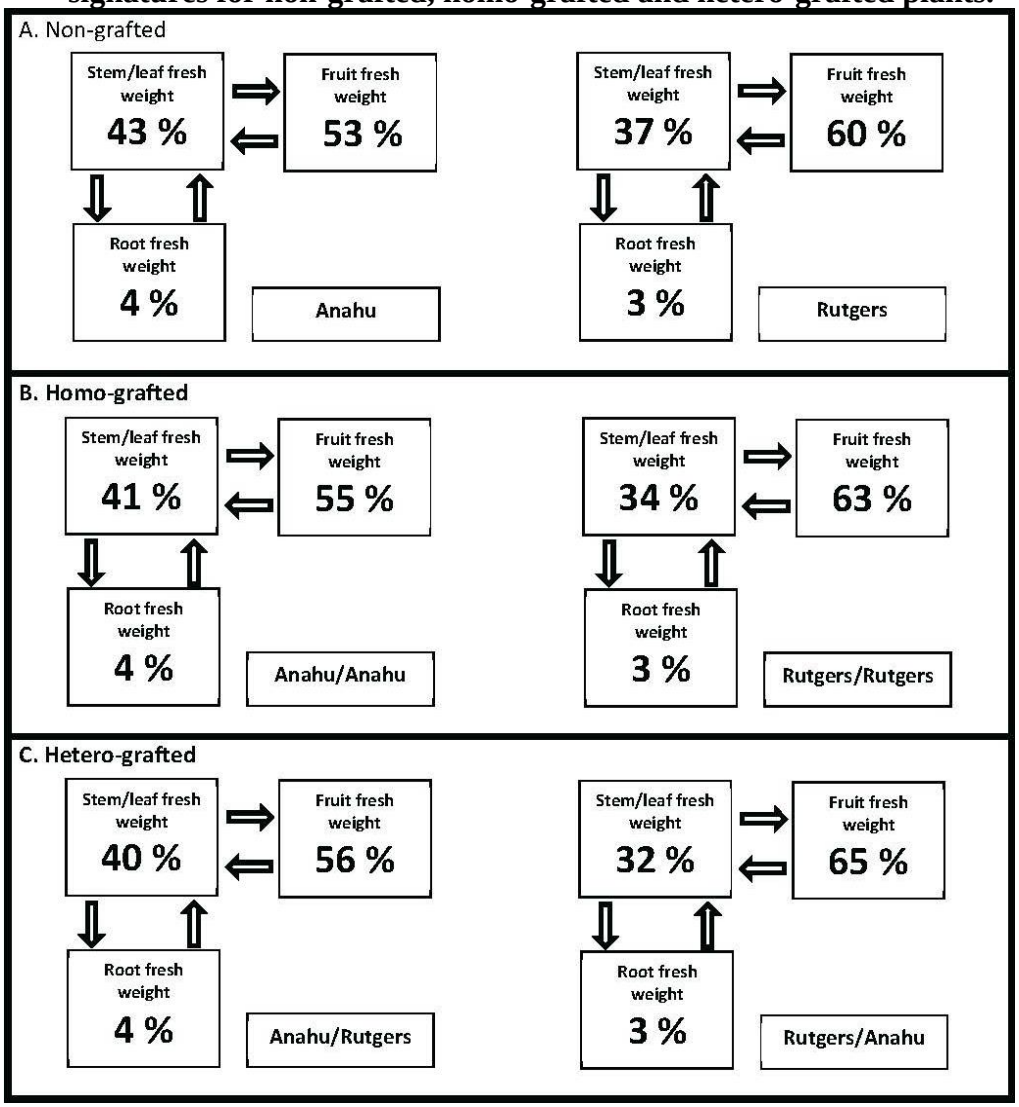


Figure 6. Influence of heavy pruning on Anahu and Rutgers tomato biomass partitioning signatures for non-grafted, homo-grafted and hetero-grafted plants.



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CHAPTER 2: BIOMASS ALLOCATION ASSOCIATED WITH HOMO-GRAFTED AND HETERO-GRAFTED *SOLANUM LYCOPERSICUM* L. CVS RUTGERS AND ANAHU GROWN UNDER FIELD CONDITIONS.

Abstract

Biomass partitioning of tomato (*Solanum lycopersicum* L.) was studied under field and greenhouse conditions. Tomato grafting is of interest because of its potential for disease and pest control through resistance. Grafted tomato resource partitioning into stem, leaf, root, and fruit tissue, however, has not been thoroughly investigated. Understanding allocation of biomass in grafted tomato plants is important for selection of optimal rootstocks for grafting. Two experiments, each with six treatments with cvs Anahu and Rutgers were used to study the impacts of homo-grafting and hetero-grafting of tomato biomass partitioning under field conditions. The research consisted of a partial-season and a full-season experiment. The partial-season experiment was terminated after 50 days. Under these conditions, stem/leaf dry weight, root fresh weight and root dry weight of homo-grafting and hetero-grafted cv Anahu were significantly less than those of non-grafted plants. The same was true for root fresh weight, but not dry weight of cv Rutgers. Homo-grafting, but not hetero-grafting cv Rutgers resulted in less stem/leaf dry weight compared to non-grafted plants. The full-season experiment was terminated after 100 days at the end of fruit harvest. Under these conditions, grafting had no significant impact on the partitioning of stem/leaf dry weight, root fresh weight, root dry weight or fruit fresh weight. Overall, grafting did not adversely impacted plant development and fruit yield. In order to evaluate the complete biological impact of homo-grafting and hetero-grafting, it will be necessary to conduct the research in the presence of key biological (e.g. disease or pest) or physical stress factors.

Introduction

Solanum lycopersicum L. (tomato) and other vegetable crop biomass partitioning has been studied extensively in regards to shoot system dry matter (stem/leaf and fruit tissue) under greenhouse and field conditions (Heuvelink 1999; Heuvelink 1997; Marcelis 1991; Marcelis, Heuvelink, and Goudriaan 1998; Enquist and Niklas 2002). Biomass allocation models are based on dry or fresh weights of tissue to simulate how plants integrate their biomass to different parts the plant. Cultivars have specific biomass partitioning signatures (Chapter 1).

Relatively few biomass partitioning studies have been conducted with grafted vegetables. Rootstocks are usually selected to overcome various biotic and abiotic stress factors. Following grafting, biomass resources can be directed to parts of a plant other than fruit tissue. For instance, rootstock can manipulate scion growth (Kudo and Harada 2007). Thus, biomass partitioning is important for selecting appropriate rootstocks for grafting.

Grafting is a practice used in vegetable production systems to decrease risk to soil-borne diseases (Na Liu et al. 2009), increase salt tolerance (Huang et al. 2009; He et al. 2009; Martinez-Rodriguez et al. 2008), increase tolerance to other abiotic factors (Schwarz et al. 2010), increase drought tolerance (Altunlu and Gul 2011), increase plant vigor (Venema et al. 2008; Estañ et al. 2005), and increase yield (Turhan et al. 2011; Huang et al. 2009; Khah et al. 2006b). Grafting has been used for many years in Japan and Korea to overcome soil-borne diseases (Lee 1994). Interest in grafting vegetable crops is not only increasing in Asian countries but in North America as well. For instance, 40 million seedlings are grafted in North America each year. These are produced mainly in Ontario and British Columbia, Canada (Kubota et al. 2008). Moreover, grafting is environmental friendly and interest/demand is increasing for organic food production. In addition, pesticide regulations have increased in conventional food production systems, making alternatives more attractive. It has been demonstrated that grafting can

decrease the use of chemical pesticides in both organic and conventional crop production (Rivard and Louws 2008), making grafting a very important technique in both types of vegetable production systems.

One of the benefits of vegetable grafting is increased yield and overall plant biomass. There are numerous studies about the impact of grafting on tomato fruit yield and vigor under environmental stress conditions (López-Pérez et al. 2006; Khah et al. 2006a). There is, however, very limited information about the impact of homo- grafting and hetero-grafting on fruit yield and plant vigor in the absence of environmental stress. Despite the benefit of grafting to overcome diseases or increase yield, it is important to understand biomass partitioning of a specific plant cultivar before selecting it as a rootstock. The objective of this study is to determine the impact of homo-grafted and hetero-grafted tomato cultivars Rutgers and Anahu on the fresh and dry biomass partitioning of under field conditions: with special reference to tomato fruit tissue fresh weight, stem/leaf tissue fresh and dry weight, root tissue fresh, and dry weight of both young plants and plants grown through harvest. An additional objective is to evaluate Anahu as a compatible rootstock for hetero-grafting for use under field conditions.

Materials and methods

This research was conducted under field conditions at the Michigan State University, Department of Entomology, Collins Road Research Farm in East Lansing, MI, 42.690396, -84.496250 (Figure 7). It consisted of a partial-season and a full-season experiment, each with six grafting treatments. The partial season experiment had 15 replicates of each treatment and was terminated after 50 days. The full-season trial had 10 replicates of each treatment and was terminated 100 days after transplanting. The six grafting treatments were: 1) non-grafted Anahu, 2) non-grafted Rutgers, 3) homo-grafted Anahu, 4) homo-grafted Rutgers, 5) hetero-grafted

Anahu (Anahu scion and Rutgers rootstock) and 6) hetero-grafted Rutgers (Rutgers scion and Anahu rootstock).

Plant material

The production of grafted tomato plants for the research consisted of planting a single tomato cv Anahu (University of Hawaii Seed Program) or cv Rutgers (Sustainable Seed Co.) seed in each cell in 50 or 72 cell seedling trays containing plant growth media (SUREMIX professional growing media, Michigan Grower Products Inc.) and placed in an environmental chamber maintained at 26 C for 16 hours and 20 C for eight hours, day and night, respectively. The seedlings were watered every other day. Anahu seeds were planted two days before Rutgers seeds to ensure the same seedling diameter at the time of grafting. This time-interval was based on preliminary research results. After 14 days in the environmental chamber, Anahu and Rutgers seedlings were 1.5-2.5 mm in diameter, an appropriate size for grafting.

Grafting

Rootstocks and scions were cut below the cotyledon at a 45-65 degree angle using a Miter-Cut Grafting Knife (Johnny's Selected Seeds). Scions and rootstocks were clipped together with silicon tubes (Silicon clipper, Hydro-Gardens, Colorado Springs, CO) until the grafting wound healed. Silicon clipper size varied from 1.5-2.5 mm depending on seedling diameter. A humidifier (Air Innovations Model # HUMID06 1.37-gal) was used to control the relative humidity during graft wound healing. The grafted plants were held in an environmental chamber for seven days at 24-26 C temperature. The relative humidity was decreased gradually from 90% after three days, to 85% two days later, to 75% one day later and then to 65% for the final day. After seven days, the grafted seedlings were moved to the laboratory for three days of acclimatization

Field

The young tomato plants were transplanted into three raised beds: 60 centimeter wide, 30 centimeter high (Figure 8). The distance from the center of the row was 120 centimeters and each row was 10 meters in length. There were 40 centimeters between each plant within the row. Each treatment was replicated fifteen times in the 50-day experiment and ten times in the 100-day. The plants were maintained in a completely randomized design under field conditions.

Data colocation

The plants were watered as necessary using a drip irrigation system and fertilized every other week. Tomato harvest in the full-season experiment began on September 19th, 2015. Soon after the first harvest, the plants were infected with late blight and the remaining tomatoes were harvested on October 8, 2015. The stem/leaf fresh weights were not recorded. Immediately after collection, stem/leaf tissue was placed in a drying oven at 30-35 C. Tomato plant roots were washed and fresh weight recorded. The roots were immediately placed in a drying oven at the same temperature used for the stem/leaf tissue. After seven days, the stem/leaf and root dry weights were recorded.

Data analysis

The data were analyzed using SPSS 24 Grad Primmum Pack. The data distribution normality was checked with a histogram plot for skewness. If the data were positively skewed, a natural Log or Log_{10} transformation was used. If the data were negatively skewed, the Square Root Method was applied. If the data were not normalized, the non-parametric Kruskal-Wallis Test was used as the substitute for One-Way ANOVA. If the One-Way ANOVA was significant at $\alpha=0.05$, it was followed by Tukey's Test. When the non-parametric Kruskal-Wallis Test was significant at the $\alpha=0.05$, it was followed with Dunn's Test.

Results

Impact of Grafting on Biomass Partitioning After 50 Days Under Field Conditions

There were significant differences in stem/leaf dry, root fresh, and root dry weights among the six treatments of 50-day-old tomato plants maintained under field conditions (Table 6). The stem/leaf dry weight was significantly different among the six treatments ($F_{5,14}$=9.286, p=0.001). Non-grafted Rutgers stem/leaf dry weight was similar to non-grafted Anahu. Non-grafted Anahu had the greatest amount of stem/leaf dry weight among the six treatments. There was, however, no significant difference between the non-grafted treatments. Homo-grafted Anahu and homo-grafted Rutgers also produced similar stem/leaf dry weights. However, stem leaf dry weights for both homo-grafted Rutgers and Anahu were significantly less than non-grafted Rutgers and Anahu (Table 6). Hetero-grafted Rutgers and hetero-grafted Anahu stem/leaf dry weight was similar. Stem/leaf dry weight was significantly less for hetero-grafted Rutgers than either non-grafted Rutgers or Anahu. On the other hand, hetero-grafted Anahu stem/leaf dry weight was not significantly different than non-grafted Rutgers, but it was significantly less than non-grafted Anahu (Table 6).

The root fresh weight was significantly different among the six treatments ($F_{5,14}$=7.263, p=0.001). Non-grafted Rutgers and non-grafted Anahu had a similar amount of root fresh weight (Table 6). Both non-grafted treatments, however, had significantly greater amounts of root fresh weight than all other grafted treatments. Homo-grafted and hetero-grafted treatments had a similar amount of root fresh weight.

The root dry weight was significantly different among the six treatments ($F_{5,15}$=4.014, p=0.003). Non-grafted Rutgers produced similar amount of root dry weight compare to non-grafted Anahu. Homo-grafted Anahu had the lowest root dry weight among the six treatments and it was not significantly different than homo-grafted Rutgers. However, homo-grafted Anahu produced less

root dry weight then non-grafted Anahu, but similar to non-grafted Rutgers. On the other hand, homo-grafted Rutgers was not significantly different than non-grafted treatments. Hetero-grafted Rutgers and hetero-grafted Anahu had similar amount of root dry weight and both were significantly less than non-grafted Anahu but similar to non-grafted Rutgers.

Impact of Grafting on Tomato Plant Biomass Partitioning at After 100 Days Under Field Conditions

Non-grafted Rutgers fruit fresh weight was the lowest among all six treatments (Table 7). Non-grafted Anahu produced a similar amount of fruit fresh weight as non-grafted Rutgers. Homo-grafted Anahu produced the greatest amount of fruit fresh weight among all six treatments. Homo-grafted Rutgers produced a similar amount of fresh fruit weight as home-grafted Anahu. Hetero-grafted Rutgers produced the second greatest amount of fruit fresh weight. Hetero-grafted Anahu produced much lower fruit fresh weight than hetero-grafted Rutgers. However, there was no significant difference between hetero-grafted Rutgers and hetero-grafted Anahu.

The root fresh weight was not significantly different among all six treatments. Non-grafted Rutgers produced the lowest amount of root fresh weight. Non-grafted Anahu root fresh weight was not significantly different than non-grafted Rutgers. Homo-grafted Anahu produced the greatest amount of root fresh weight. Homo-grafted Rutgers produced a similar amount of root fresh weight to homo-grafted Anahu and it was the second greatest among the six treatments. Hetero-grafted Rutgers root fresh weight was similar to hetero-grafted Anahu root fresh weight (Table 7).

The stem/leaf dry weight was not significantly different among the six treatments (Table 8). Non-grafted Rutgers stem/leaf dry weight was similar to non-grafted Anahu's stem/leaf dry weight. There was no significant difference between non-grafted Rutgers and non-grafted Anahu. Homo-grafted Anahu stem/leaf dry weight was the greatest among the six treatments

and homo-grafted Rutgers stem/leaf dry weight was the lowest. There was, however, no significant difference in stem/leaf dry weight between the two homo-grafted treatments. Hetero-grafted Rutgers's stem/leaf dry weight was the second greatest among the six treatments. Hetero-grafted Anahu stem/leaf dry weight was not significantly different than hetero-grafted Rutgers's stem/leaf dry weight.

The root dry weight was not significantly different among the six treatments. Non-grafted Rutgers root dry weight was numerically higher than non-grafted Anahu's root dry weight. Non-grafted Anahu's root dry weight was lowest amount all six treatments. The root dry weights of the two non-grafted treatments were not significantly different. Homo-grafted Anahu produced the greatest amount of root dry weight among the six treatments fallowed by homo-grafted Rutgers. There was, however, no significant difference in root dry weight between the two homo-grafted treatments. Hetero-grafted Rutgers and hetero-grafted Anahu also produced a similar amount of root dry weight (Table 8).

Discussion

The impact of grafting tomato cultivars on fruit tissue yield has been studied in detail under various abiotic and biotic stress conditions (Turhan et al. 2011; Schwarz et al. 2010; Martinez-Rodriguez et al. 2008; Venema et al. 2008). Grafting of tomato cultivars Anahu and Rutgers, however, have not been studied under field condition. In addition, most grafting studies have not included both partial and full-season biomass partitioning data. In our research, the stem/leaf dry, root fresh, and root dry weights of 50-day-old homo-grafted and hetero-grafted tomato plants were significantly less than those of non-grated plants. This is most likely due to the wound healing process that took time and slowed plant growth in the early days of vegetative growth. With mature plants, however, there were no significant differences for stem/leaf dry, root fresh, and root dry among the grafted or non-grafted treatments. This result agrees with

Khah (2006b) findings where grafted plants dry and fresh weights were not significantly different than those of non-grafted plants. In other studies, grafted plants have better water and nutrient uptake compared to non-grafted plants (Di Gioia et al. 2013; Lee 1994). In this study, homo-grafted Anahu biomass was slightly larger than other treatments due to water and nutrient uptake by the plants at harvest. Homo-grafted Anahu, however, produced the lowest among the six treatments amounts of fresh and dry weight of stem/leaf and root as well as fruit yield under greenhouse conditions (Chapter 1). Even though other studies have found significant increases in tomato fruit yield on grafted plants compared to non-grafted plants (Rivard and Louws 2008), this was not the case in our research. Moreover, there was no negative effect on grafted plants compared to non-grafted plants. We suspect that a lack of difference in biomass of plants at harvest was due to a lack of biotic or abiotic environmental stress factors. Other studies, however, have demonstrated grafting impacts on the fresh and dry weight and as well as increased the yield under conditions of stress (Kumar et al. 2015; N. Liu et al. 2009; Di Gioia et al. 2013; Rivard and Louws 2008; Savvas et al. 2009).

Conclusion

Grafting has excellent potential for use in integrated pest management due to increased concern about environment quality and human safety associated with the use of pesticides. In this study, there was no significant difference in mature tomato plant dry weight and yield between grafted and non-grafted treatments. This was likely due to the fact there were insufficient biotic or abiotic stress factors associated with the site until the arrival of Late Blight. Thus, future work is needed for investigation of the benefits of homo and hetero-grafting of tomato cvs Anahu and Rutgers under adverse conditions. Albeit, there were no significant effects in this study, grafting has potentially great benefits, including: increase tomato fruit yield, increase plant vigor, overcomes soil borne diseases. For instance, root-knot nematode species are soil-borne

pathogens that cause major economic losses in tomato production. Anahu is a root-knot nematode (*Meloidogyne incognita*) resistant variety (Hu et al. 2015; Williamson et al. 1994; Reddy, Tikoo, and Anand 1987) that can be used as a rootstock grafted to susceptible tomato cultivars to overcome root-knot nematode damage under both field and greenhouse growing conditions. Anahu also is resistant to both potato aphids and sweet potato whitefly (*Bemisia tabaci*) (Nombela, Williamson, and Muñiz 2003; Goggin, Williamson, and Ullman 2001). Thus, future research is required to evaluate use of tomato cv Anahu as a rootstock to confer resistance to the grafted plant.

APPENDICES

Appendix A. Statistical Tables

Table 6. Influence of *Solanum lycopersicum* L. grafting on stem/leaf dry, root fresh, and root dry tissue biomass partitioning after 50 days under field conditions.

Grafting	Stem/leaf dry weight (g)		Root fresh weight (g)		Root dry weight (g)	
	Mean	SE	Mean	SE	Mean	SE
Anahu	**19.33 c**[1]	1.70	**14.90 b**	1.04	**3.14 b**	0.09
Anahu/Anahu	**10.30 a**	1.56	**11.46 a**	0.87	**2.69 a**	0.07
Rutgers/Anahu	**9.94 a**	0.82	**11.22 a**	0.75	**2.74 a**	0.07
Anahu/Rutgers	**12.12 ab**	1.06	**11.34 a**	0.87	**2.77 a**	0.07
Rutgers/Rutgers	**11.63 a**	0.90	**11.53 a**	0.61	**2.81 ab**	0.06
Rutgers	**17.25 bc**	1.43	**16.19 b**	0.70	**3.01 ab**	0.14

[1] Date analyzed with One Way ANOVA. Significantly different means at p-value ≤ 0.05 followed by Tukey's test. Same letter in the column are not different at p-value › 0.05.

Table 7. Impact of grafting on biomass partitioning of *Solanum lycopersicum* cvs Rutgers and Anahu after 100 days under field conditions.

	Fruit tissue weight		Root tissue weight	
Non-grafted	**Mean (kg)**	**SE**	**Mean (g)**	**SE**
Rutgers	**4.4a**[1]	0.60	**38.6a**	6.13
Anahu	**4.8a**	0.75	**57.6a**	6.54
Homo-grafted				
Anahu/Anahu	**5.7a**	0.61	**77.7a**	7.34
Rutgers/Rutgers	**5.2a**	0.67	**65.6a**	11.576
Hetero-grafted				
Rutgers/Anahu	**5.7a**	0.33	**58.3a**	4.21
Anahu/Rutgers	**4.5a**	0.4.8	**59.9a**	11.33

[1] Date analyzed with One Way ANOVA. Significantly different means at p-value ≤ 0.05 followed by Tukey's test. Same letter in the column are not different at p-value › 0.05.

Table 8. Influence of *Solanum lycopersicum* L. grafting on stem/leaf and root dry weight biomass partitioning after 100 days under field conditions.

	Stem/leaf tissue weight		Root tissue weight	
Grafting	**Mean (g)**	**SE**	**Mean (g)**	**SE**
Anahu	**140.10a**[1]	23.03	**4.09a**	0.64
Anahu/Anahu	**211.32a**	22.16	**7.81a**	0.67
Rutgers/Anahu	**162.89a**	14.55	**6.37a**	0.60
Anahu/Rutgers	**145.11a**	14.18	**6.29a**	1.38
Rutgers/Rutgers	**136.89a**	23.93	**6.49a**	1.15
Rutgers	**143.72a**	19.94	**5.69a**	0.74

[1] Date analyzed with One Way ANOVA. Significantly different means at p-value ≤ 0.05 followed by Tukey's test. Same letter in the column are not different at p-value › 0.05.

Appendix B. Figures

Figure 7. Aerial view of the 2015 tomato grafting field research site at the Department of Entomology Research Farm at Collins Road, Michigan State University.

Figure 8. Tomato grafting research site at the Michigan State University, Department of Entomology Collins Road Research Farm.

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CHAPTER 3: INFLUENCE OF GRAFTING *SOLANUM LYCOPERSICUM* L. CVS ANAHU AND RUTGERS ON PLANT BIOMASS PARTITIONING IN THE PRESENCE AND ABSENCE OF *MELOIDOGYNE INCOGNITA* (NEMATODA): WITH THE SPECIFIC REFERENCE TO CENTRAL ASIA.

Abstract

Understanding plant biomass partitioning is important for selection of appropriate rootstocks and scions for grafting vegetable crops. Biomass allocation has not been studied in regards to grafting *Solanum lycopersicum* L. The primary objective of this research was to determine the impact of self-grafting (homo-grafting) and rootstock grafting (hetero-grafting) on tomato plant fresh weight partitioning of stem and leaf, fruit, and root tissue following three types of pruning (none, light, and heavy), in the presence and absence of *Meloidogyne incognita* at initial population densities of $P_{i=1000}$, $P_{i=5000}$ and $P_{i=0}$. Two additional objectives were to evaluate grafting *M. incognita* resistant tomato cv Anahu to the susceptible tomato cv. Rutgers for control of *M. incognita* under greenhouse conditions, and determine if the function of the *M. incognita* resistance gene (Mi) is retained in grafted tomato rootstocks. In addition to biomass partitioning, homo-grafting and hetero-grafting were evaluated in regards to their impact on the final female population density (Pf) of *M. incognita*. Non-grafted and homo-grafted *S. lycopersicum* were used as controls. In the presence of *M. incognita* ($P_{i=1000}$) and no pruning, non-grafted Anahu and non-grafted Rutgers possessed the same biomass partitioning signatures as in the absence of *M. incognita*. With extensive pruning and *M. incognita* at $P_{i=0}$, $P_{i=1000}$, $P_{i=5000}$, all grafted treatments possessed biomass partitioning signatures similar to non-grafted Rutgers. Most of the biomass was partitioned to fruit tissue, compared to stem and leaf or root tissue. Resistant tomato cv Anahu had a significant effect on the Pf of *M. incognita* in root tissue. Over all, Anahu was not completely resistant to *M. incognita*. In the non-pruned experiment, there was an 11-fold difference in Pf between Anahu and Rutgers. Homo-grafted Anahu followed the same

trend and produced 19-fold fewer *M. incognita* at Pf than Rutgers. Nevertheless, hetero-grafted Rutgers produced only 1-fold less Pf compare to Anahu. In the case of heavy and light pruning, Pf was high on both non-grafted Anahu and homo-grafted Anahu, 10-fold and 15-20-fold, respectively. When the plants were not pruned in the presence of *M. incognita* ($P_{i=1000}$), fruit yield was 31 % less, compared to plants maintained in the absence of *M. incognita*. In conclusion, Anahu rootstock grafting may not be beneficial to commercial tomato growers due to its biomass partitioning signature where Anahu produced more leaves and stem under greenhouse conditions. Moreover, Anahu resistance to *M. incognita* is not complete according to these research results. Thus, Anahu should not be used as a rootstock to manage *M. incognita* under greenhouse and possibly field conditions.

Introduction

Tomato (*Solanum lycopersicum* L.) is the second most valuable fruit/vegetable crop on a global basis. Uzbekistan produces 80% of the tomatoes grown in Central Asia (FAO.STAT, 2010). More than three million tons are produced annually. This is about four-fold less than tomato production in the USA, yet Uzbekistan exports most of the tomato fruit to neighboring countries and Russia. Soil-borne diseases represent key limiting factors for tomato production. Sometimes farmers lose nearly 100% of expected yield due to soil-borne pests/pathogens. One of these is *Meloidogyne* spp. (Nematoda: Heteroderidae), a sedentary-endoparasite of numerous key food crops worldwide and one of the most damaging nematode pests in agriculture (Dong et al, 2013, Davis et al, 2014). *Meloidogyne incognita* (Kofoid and White1919) Chitwood 1949 is a common pest under both field and greenhouse tomato production conditions. During the past 60 years, control of *Meloidogyne* spp. has been based mainly on the use of soil fumigants, non-fumigant nematicides and rotation with non-host crops.

Due to environmental and human health risks, Integrated Pest Management (IPM) is recommended for *M. incognita* control. IPM is designed to keep pest damage under threshold levels using good monitoring plus all available control tactics, including, biocontrol agents, cultural controls, host plant resistance, and when it is needed or required, chemical pesticides (Bird, 2003). Not all IPM strategies work for *M. incognita* management under greenhouse conditions. Use of pest resistant tomato varieties and hybrids can be an important aspect of IPM. Some tomato varieties are resistant to *M. incognita*. These varieties carry the Mi gene. Mi stands for resistance to *M. incognita*. Not all resistant tomato varieties are suitable for tomato production, or preferred by farmers and consumers. Grafting has the potential to bring all the essential traits together.

Vegetable grafting began early in the 20th century in Japan and Korea (J.-M. Lee & Oda, 2002) and is now practiced on a global basis (J. Lee, 1994). Vegetable grafting is used to reduce risk to detrimental abiotic and biotic factors (Pradeep et al., 2015; Charles et al., 2012; Theodore McAvoy et al, 2012; Na Liu et al., 2009; Alfonso et al., 2008). Grafting is a key practice for control of soil-borne diseases in East and South Asia (J.-M. Lee & Oda, 2002). In some cases, both grafting and use of a resistant plant will enhance the crop's overall vigor and protect it from pests (Khah et al, 2006b). In several countries, vegetables are grafted to enhance overall plant vigor (Estañ, 2005, Venema 2008). Grafting, however, can increase plant vigor, yet at the same time, reduce yield potential.

It is important to understand biomass allocation in grafted plants in order to select optimal rootstock and scion combinations. In tomato biomass resource allocation, it is well known that increasing one type of tissue impacts other parts of the plant (Brian and Karl, 2002; Heuvelink, 1999). Plant resource partitioning has been extensively researched on non-grafted tomato plants

under greenhouse conditions (Albacete et al., 2008; Heuvelink, 1999). The impact of grafting on resource partitioning is not well understood under severe or sub-optimal biotic stress conditions (Marcelis, 1991). Tomato biomass partitioning has been studied extensively in regards to fruit yield (Marcelis et al., 1998; Heuvelink, 1996), but much less is known about overall biomass partitioning (Dimitrios et al., 2009, Charles and Xin, 2012). In particular, tomato has not been studied in detail in regards to biomass partitioning after grafting and when grafted plants are under stress, especially under greenhouse conditions. In addition, plant biomass partitioning has not been studied in relation to homo-grafted (self-grafted), and hetero-grafted (rootstock grafted) plants.

The specific objective of this research was to determine the impact of homo and hetero-grafting on tomato plant fresh weight partitioning to stem and leaf, fruit and root tissues in the presence and absence of the biological stress factor, *M. incognita*. The second objective was to evaluate *M. incognita* resistant tomato cv Anahu grafted with susceptible tomato cv. Rutgers for future use in greenhouse and field condition; including identification of the extent the Mi gene mechanism is retained in grafted tomato plants in the case of Anahu resistant and Rutgers susceptible grafted plants.

Materials and Methods

The research was conducted under greenhouse conditions at Michigan State University to evaluate the impact of grafting *S. lycopersicum* on biomass partitioning. The Materials and Methods Section describes the procedures used to determine tomato plant biomass partitioning and nematode resistance evaluation of Anahu and Rutgers plants before and after grafting. It includes the experimental design, plant materials, grafting procedures, greenhouse conditions, final *M. incognita* population density and data analysis.

Experimental Design

The research included three initial population densities of *M. incognita* ($P_{i=0}$, $P_{i=1000}$, and $P_{i=5000}$ eggs per experimental unit) and grafting. There were six grafting treatments: 1) non-grafted Anahu, 2) non-grafted Rutgers, 3) homo-grafted Anahu, 4) homo-grafted Rutgers, 5) hetero-grafted Anahu (comprised of Anahu scion, Rutgers rootstock) and 6) hetero-grafted Rutgers (comprised of Rutgers scion, Anahu rootstock). Each treatment was replicated five times. The plants were maintained in a randomized complete block design under greenhouse conditions for 125 to 135 days after being transplanted into 5-gallon plastic pots.

Plant materials

A single tomato cv Anahu (University of Hawaii Seed Program) or cv Rutgers (Sustainable Seed Co.) was sown in each cell in 50 or 72 cell seedling trays containing plant growth media (SUREMIX professional growing media, Michigan Grower Products Inc.) and held in a growth chamber maintained at 26 C for 16 hours and 20 C for eight hours, day and night, respectively. The seedlings were watered every other day. Anahu seeds were planted two days before Rutgers seeds to ensure the same seedling diameter for grafting. This time-interval was based on preliminary research results. Approximately 30-day-old tomato plants were transplanted into five-gallon plastic pots filled with sterilized sandy soil and placed in a greenhouse maintained at 26-28 C. Plants were watered as necessary and the ripe fruit (90% or more of the skin red in color) harvested weekly. At the end of each experiment, all remaining tomato fruit were harvested. Combined stem and leaf fresh weights were recorded and the tissue placed in a drying oven at 30-35 C the same day. The tomato plant intact root system was removed from the soil by washing with pistol nozzles in tap water and fresh root tissue weight recorded. The root tissue was placed in a drying oven at the same temperature used for drying the stem and leaf tissue. After seven days, stem and leaf and root dry weights were recorded.

Pruning

Pruning treatments consisted of three pruning levels including: non-pruned, lightly pruned and heavily pruned. Simple pruning methods were used to remove suckers from the nodes. Each sucker was pinched off entirely by hand when the sucker was small or with garden clippers when suckers were larger. In heavy pruning, all of the suckers were removed from the entire plant and leaves were removed up to a 3-4-foot height. Under the lightly pruned conditions, all the suckers and leaves were removed up to first fruit clusters. The reason for pruning tomato plants was to accommodate potential limited space in the greenhouse. In the non-pruned experiment (Figure 9), there were 60 tomato plants, five plants per greenhouse bench (60 in widths x 66 in length). In Experiment 3, the plants were lightly pruned and there were 90 tomato plants, 15 tomato plants per bench. The lightly pruned experiment was conducted three times. Heavily pruned experiment was conducted only once and the numbers of plants were identical to the lightly pruned experiments.

Grafting

After 14 days in the growth chamber, the seedlings were of appropriate size for grafting. Rootstocks and scions were cut below the cotyledon, at a 45-65 degree angle using a Miter-Cut Grafting Knife (Johnny's Selected Seeds). Scions and rootstocks were clipped together with silicon tubes used to hold the rootstock and scion together until the grafting wound healed. Silicon clipper (Hydro-Gardens, Colorado Springs, CO) size varied from 1.5-2.5 mm depending on seedling diameter. During the graft wound healing process, a humidifier (Air Innovations Model # HUMID06 1.37-gallons Ultrasonic Digital Humidifier) was used to control relative humidity (RH). The grafted plants were maintained in a growth chamber for seven days at 24-26 C. The RH was decreased gradually from 90% at three days, to 85% at two days, and then to 75% one day and 65% the final day. After seven days, the grafted seedlings were moved to the

laboratory for three days of acclimatization. The six treatments included: 1) Non-grafted Anahu, 2) Non-grafted Rutgers, 3) Homo-grafted Anahu, 4) Homo-grafted Rutgers, 5) Hetero-grafted Anahu (Anahu scion and Rutgers rootstock) and 6) Hetero-grafted Rutgers (Rutgers scion and Anahu rootstock).

***Meloidogyne. incognita* Population Density**

Only the root systems of plants inoculated with *M. incognita* and expressing root galls, were used for estimating final female population densities of *M. incognita* (Figure 11). From each experimental unit, five grams of root tissue were used for estimation of the final female population density. The root tissue for analysis was cut at random from different parts of each root system. The root staining procedure of Daykin and Hussey (1985) was used. The staining helps to see female nematode in root system under microscope. Five grams of root tissue were wrapped with a fine mashed nylon cloth tagged with corresponding treatment label. In this procedure, nylon cloth was used for the first time in root staining. This prevented loss of root tissue during the staining procedure and allowed for the staining of 20-40 roots at a time (Figure 12). Staining several roots together shortens the time spent for staining and increases staining efficacy. Moreover, staining several roots together increases the staining quality by accurately timing the staining procedure. Fixed roots were placed in 600 ml beakers containing 1:1 ratio of 8.25% NaOCl (Clorox Regular Bleach 3.78qt) and tap water for four minutes, stirring several times. The roots were rinsed with tap water and soaked for 15 minutes in tap water. They were then drained and washed under tap water several times in order to remove the rest of the bleach from roots. After washing, the roots were soaked in water for 15 minutes. Next, the roots were placed into a 600-ml beaker and filled with water until all the fixed roots were submerged. Stock acid-fuchsine-stain solution was added at a concentration of 1.0 ml for each 30-50 ml water required to fill the beaker. The beaker was placed on a hot plate, brought to a boil for one-two

minutes and cooled at room temperature. The cooled roots were drained, rinsed with tap water and dried with a paper towel. The fixed roots were then filled with acidified glycerin with a few drops of 5N HCl and boiled on a hot plate for 30-60 seconds. After the roots cooled, the P_f of M. incognia were determined under a binocular microscope.

Data Analysis

The data were analyzed using SPSS 24 Grad Primmum Pack. First, data distribution normality was checked with a histogram plot for skewness of the distribution. If the data were positively skewed, natural Log or Log_{10} was used to transform the data. If the data were negatively skewed, the Square Root Method was applied. If data were not normalized, a non-parametric Kruskal-Wallis Test was used as the substitute for One-Way ANOVA. If the One-Way ANOVA showed a significant difference at the α=0.05, means were separated using the Tukey's Test. If the non-parametric Kruskal-Wallis Test was significant at the α=0.05, means were separated using the Dunn's Test.

Results

Grafting Impact on Fruit Fresh Tissue Weight

When left unpruned, grafting did not increase the fruit tissue biomass (p=0.128), compared to non-grafted tomato cultivars under *M. incognita* densities of $P_{i=0}$ and $P_{i=1000}$. Non-grafted Rutgers produced the greatest amount of fruit tissue in both $P_{i=0}$ and $P_{i=1000}$ (Table 9A). Non-grafted Anahu, homo-grafted Anahu and hetero-grafted Rutgers produced least fruit weight at $P_{i=1000}$. Overall fruit tissue was significantly less in $P_{i=1000}$, compared to $P_{i=0}$ (t_{49}=2.105 P=0.04). With light pruning, fruit tissue biomass was not significantly different among any treatments at the P_i (Table 9B). This also was the case for tomato fruit tissue compared among P_i of *M. incognita* ($F_{2,6}$= 3.018, P=0.055).

With heavy pruning, grafting did not alter tomato fruit tissue biomass in any treatments with $P_{i=0}$. There are significant differences among the six treatments for $P_{i=1000}$ ($F_{5,24}$=5.075, P=0.003) when the plants were heavily pruned (Table 9C). Rutgers produced the greatest amount of fruit tissue, while the lowest amount of fruit tissue was observed on homo-grafted Anahu. There also were significant differences among the six treatments for $P_{i=5000}$ ($F_{5,24}$=3.544, P=0.015) when the plants were heavily pruned (Table 9C). The greatest amount of fruit tissue was produced by homo-grafted Rutgers, while homo-grafted Anahu produced the least amount of fruit tissue. There was no significant difference on overall fruit yield among any P_is.

Impact of Pruning on Plant Population Density Under Greenhouse Conditions

In the non-pruned experiment, there were only two initial population densities of *M. incognita* ($P_{i=0}$ and $P_{i=1000}$), due to greenhouse space. Sixty tomato plants were accommodated. With light or heavy pruning, we were able to place 180 tomato plants in the same amount of space required for 60 tomato plants in the non-pruned experiment.

Lightly and heavily pruned experiments had three level of *M. incognita* initial population densities ($P_{i=0}$, $P_{i=1000}$, and $P_{i=5000}$). With heavy pruning, 90 tomato plants were placed in half of the space required for the non-pruned experiment. Moreover, there were significant differences in total fruit weight among non-pruned and lightly or heavily pruned experiments (H_2=7.041, P=0.03). Lightly pruned plants had 100 kg of fruit tissue, which was significantly more than plants in the non-pruned experiment (P=0.043). With heavy pruning, tomato plants yielded 120 kg of fruit, which was significantly more compared to non-pruned plants which produced 68 kg (P=0.012). In the heavily pruned experiment, however, total fruit tissue per plant was significantly less compared to non-pruned plants (P=0.038) with $P_{i=0}$ and $P_{i=1000}$ of *M. incognita*.

Overall, tomato fruit production was increased by heavy pruning due to accommodating three times more plants per unit area in the greenhouse.

Grafting Impact on Stem/Leaf Fresh Weight

Tomato stem/leaf fresh tissue weight was significantly different among treatments of non-pruned plants in the absence of the *M. incognita* (H_5=19.13, P=0.02) (Table 10). The greatest amount of stem/leaf fresh tissue weight was produced on hetero-grafted Anahu and it was significantly different than hetero-grafted Rutgers without pruning and in the absence of *M. incognita*. In the absence of pruning and *M. incognita*, stem/leaf fresh weight was not significantly different on non-grafted Anahu compared to non-grafted Rutgers. Similarly, homo-grafted Anahu and homo-grafted Rutgers produced a similar amount of stem/leaf fresh tissue weight without pruning and in the absence of *M. incognita*. Tomato stem/leaf fresh tissue weight was not impacted by any grafting treatments of lightly pruned or heavily pruned plants (Table 10).

Grafting Impact on Root Fresh Weight

The root fresh weight of the six treatments was significantly different on lightly pruned plants with a nematode initial population density of 1000 ($F_{5,24}$=2.692, P=0.046). The greatest amount of root fresh tissue weight was observed on homo-grafted Rutgers and it was significantly different than the root fresh tissue weight on homo-grafted Anahu (P=0.023). Hetero-grafted Anahu root fresh tissue weight was not significantly different than hetero-grafted Rutgers. Non-grafted Anahu produced a similar amount of root fresh tissue to non-grafted Rutgers (Table 11B).

***Meloidogyne incognita* Population Density**

In the absence of pruning, resistant tomato cv Anahu had a significant negative effect on *M. incognita* female population density in root tissue. There was an 11-fold reduction (P=0.014) in female density in non-grafted Anahu compared to non-grafted Rutgers (Table 12A). Homo-

grafted Anahu followed a similar trend and produced 19-fold fewer females than homo-grafted Rutgers (P=0.014). Hetero-grafted Rutgers had only a 1-fold reduction in female density compared to hetero-grafted Anahu.

Under light pruning and a *M. incognita* density of $P_{i=1000}$, resistant tomato cv Anahu P_f increased; however, the change was not significantly different than susceptible tomato cv Rutgers (Table 12B). Non-grafted Anahu produced 8% more P_f than non-grafted Rutgers. Homo-grafted Anahu produced 30% less P_f compared to homo-grafted Rutgers. Hetero-grafted Rutgers, however, increased P_f by 6% compared to hetero-grafted Anahu.

Under a higher density of $P_{i=5000}$ and with light pruning, non-grafted Anahu produced 2-fold more P_f compared to non-grafted Rutgers. Homo-grafted Anahu produced 15-fold more P_f compared to homo-grafted Rutgers and it was the highest P_f among all six treatments. Hetero-grafted Rutgers produced 30% more P_f compared to hetero-grafted Anahu (Table 12B).

The lightly pruned experiment was conducted three times. In the first experiment, there were significantly more tomato fruit than in the second experiment (stem/leaf tissue: P=0.140, fruit tissue: P=0.02, root tissue: P=0.176). In the third experiment, all measurements were significantly less than in the first lightly pruned experiment (stem/leaf tissue: P=0.049, fruit tissue: P=0.031, root tissue: P=0.003). The data for the second and third experiments were not analyzed due to plant growth issues that made it difficult to accurately record the results and abnormal tomato fruit yield production. In particular, foliar necrosis took place during the growth period, resulting in a lot of outlier data (Data is not presented).

With heavy pruning and a *M. incognita* density of $P_{i=1000}$, resistant cv. Anahu did not have a significant impact on the population density of females in root tissue, compared to susceptible cv Rutgers under the same conditions (Table 12C). Non-grafted Anahu produced one-fold fewer

females compare to non-grafted Rutgers. Homo-grafted Anahu reduced P_f by 44 %. Nematode density did not differ on hetero-grafted Rutgers compared to hetero-grafted Anahu, supporting 2101 and 2487 *M. incognita* females, respectively. Overall, with heavy pruning there was no significant difference in P_f among the six treatments at $P_{i=1000}$. There was no statistical significance among six grafted treatments after heavy pruning at $P_{i=5000}$ (Table 12C). The greatest P_f was on non-grafted Rutgers. It was 3.5-fold more, compared to non-grafted Anahu. Hetero-grafted Rutgers produced the lowest P_f, with close to eight-fold fewer *M. incognita* females compared to non-grafted Rutgers (P=0.032).

Biomass Partitioning

Non-pruned Experiment

In the absence of *M. incognita* ($P_{i=0}$), non-grafted Anahu and non-grafted Rutgers possessed unique biomass partitioning signatures (Figure 13A). Non-grafted Anahu plants allocated more of their fresh weight to stem and leaf tissue, compared to non-grafted Rutgers (P=0.029). Non-grafted Rutgers, however, allocated more biomass to fruit tissue, compared to non-grafted Anahu (p=0.038). Root tissue was not statistically significant among the non-grafted tomato cvs Anahu and Rutgers (p=0.226). Homo-grafting Anahu did not change the biomass partitioning signature compared to non-grafted Anahu. Homo-grafted Anahu, however, slightly shifted (9%) biomass towards to fruit tissue, compared to non-grafted Anahu (Figure 13A, B). Biomass partitioning for homo-grafted Rutgers, was not significantly different compared to non-grafted Rutgers, although it shifted slightly (9%) towards to stem/leaf tissue. Hetero-grafted Anahu did not change the biomass partitioning signature, compared to non-grafted Anahu (Figure 13A, C). Hetero-grafted Rutgers biomass shifted towards stem and leaf tissue (12%), compared to non-grafted Rutgers.

In the presence of a moderate density of *M. incognita* ($P_{i=1000}$), non-grafted Anahu and non-grafted Rutgers possessed the same unique biomass partitioning signatures as in the absence of *M. incognita* (Figure 13AFigure 14A). Non-grafted Anahu allocated more of its fresh weight to stem and leaf tissue, compared to non-grafted Rutgers (P=0.012). Non-grafted Rutgers, however, allocated more biomass to fruit tissue, compared to non-grafted Anahu (P=0.043). Root tissue fresh weights were not statistically different among the non-grafted tomato cvs Anahu and Rutgers (P=0.760). Homo-grafting Anahu did not change the biomass partitioning signature of fruit, stem/leaf, and root tissue, compared to non-grafted Anahu (Figure 14A, B). Homo-grafted Rutgers biomass partitioning was not significantly different from that of non-grafted Rutgers, shifting slightly (4 %) from fruit tissue to stem and leaf tissue in comparison to non-grafted Rutgers. The biomass partitioning signature of hetero-grafted Anahu was the same as that of non-grafted Anahu (Figure 14A, C). Non-grafted Anahu, however, shifted its biomass signature to fruit tissue (12%) in comparison to non-grafted Anahu. Hetero-grafted Rutgers s allocated it biomass differently than non-grafted Rutgers, with hetero-grafted Rutgers shifting more of its fresh weight to stem and leaf tissue than fruit tissue (P=0.05). Fruit tissue was significantly less compared to non-grafted Rutgers. In all of the experimental treatments, the amount of fresh weight allocated to root tissue was less than that allocated to fruit or stem/leaf tissues.

Lightly-pruned Experiment

Under light pruning and in the absence of *M. incognita* ($P_{i=0}$), non-grafted Anahu and non-grafted Rutgers possessed unique biomass partitioning signatures (Figure 15A). There were no significant differences among the grafting treatments. Non-grafted Anahu allocated more fresh weight to stem and leaf tissue, compared to non-grafted Rutgers. Root tissue was not significantly different in the non-grafted tomato cvs Anahu and Rutgers. Non-grafted Rutgers,

however, allocated more biomass to fruit tissue, compared to non-grafted Anahu. Homo-grafting Anahu did not change the biomass partitioning signature compare to non-grafted Anahu. Homo-grafted Anahu biomass shifted more to stem and leaf tissue after grafting, compared to non-grafted Anahu (Figure 15A, B). Homo-grafted Rutgers biomass partitioning, however, was not significantly different than non-grafted Rutgers, although there were slight shifts toward stem and leaf tissue (9%) and root tissue (5%), compared to non-grafted Rutgers. Hetero-grafted Anahu did not change the partitioning signature, compared to non-grafted Anahu (Figure 15A, C). Hetero-grafted Rutgers biomass did not change the biomass partitioning signature, compared to non-grafted Rutgers (Figure 15A, C).

Under light pruning and in the presence of a moderate density of *M. incognita* ($P_{i=1000}$), non-grafted Anahu and non-grafted Rutgers, again, possessed the same unique biomass partitioning signatures (Figure 16A). Non-grafted Anahu allocated more of its fresh weight to stem and leaf tissue, compared to non-grafted Rutgers. Non-grafted Rutgers, however, allocated more biomass to fruit tissue, compared to non-grafted Anahu. Root tissue was not significantly different for the non-grafted tomato cvs Anahu and Rutgers. Homo-grafted Anahu biomass partitioning signature was the same as for non-grafted Anahu (Figure 16A, B). Homo-grafted Rutgers biomass partitioning was also not significantly different from non-grafted Rutgers, but did shift towards stem and leaf tissue (11%) and root tissue (9%), in comparison to non-grafted Rutgers. Hetero-grafted Anahu did not alter the partitioning signature, compared to non-grafted Anahu (Figure 16A, C). Hetero-grafted Rutgers allocated biomass differently than non-grafted Rutgers. Hetero-grafted Rutgers allocated most of its fresh weight to fruit tissue.

Under light pruning and in the presence of a high density of *M. incognita* ($P_{i=5000}$), non-grafted Anahu and non-grafted Rutgers possessed similar unique biomass partitioning signatures as in

the absence of *M. incognita* (Figure 17A). Non-grafted Anahu allocated more of its fresh weight to stem and leaf tissue, compared to non-grafted Rutgers. Non-grafted Rutgers, however, allocated more biomass to fruit tissue compared to non-grafted Anahu. Root tissues were not significantly different for the non-grafted tomato cvs Anahu and Rutgers (Figure 17A). Homo-grafting Anahu did not change its biomass partitioning signature in comparison to non-grafted Anahu (Figure 17A, B). Homo-grafted Rutgers did not change its biomass partitioning signature compared to non-grafted Rutgers. Likewise, hetero-grafted Anahu did not change the partitioning signature compared to non-grafted Anahu, but shifted slightly toward root tissue (5%) (Figure 17A, C). Hetero-grafted Rutgers did not change the biomass partitioning compare non-grafted Rutgers.

Heavily-pruned Experiment

After heavy pruning and in the absence of *M. incognita* ($P_{i=0}$), non-grafted Anahu and non-grafted Rutgers possessed similar biomass partitioning signatures (Figure 18A). All six treatments allocated most of their biomass to fruit tissue, compared to stem and leaf tissue. Root tissue had the least fresh weight in all six treatments. In the presence of *M. incognita* ($P_{i=1000}$), non-grafted Anahu and non-grafted Rutgers possessed similar biomass partitioning signatures (Figure 19 A). All six treatments allocated more of the biomass to fruit tissue than to stem and leaf tissue. Root tissue had the least weight in all six treatments. In the presence of *M. incognita* ($P_{i=5000}$), non-grafted Anahu and non-grafted Rutgers possessed similar biomass partitioning signatures (Figure 20A). All treatments allocated more of the biomass to fruit tissue than to stem and leaf tissue. Root tissue had the least weight in all treatments.

Discussion

Understanding how a plant partitions its biomass is critical to choosing root-stocks for commercial tomato grafting (Khah, Kakava, Mavromatis, Chachalis, & Goulas, 2006a). Tomato

cultivars differ in how they allocate biomass and often very little is known about the effect of grafting on biomass. Rootstocks can allocate plant biomass differently and allocation of biomass on the scion can be manipulated by rootstocks (Kudo & Harada, 2007; Yang et al., 2015). Plant biomass allocation has been studied in the past by a number of researchers (Marcelis, 1991; Heuvelink, 1997; Marcelis, Heuvelink, & Goudriaan, 1998; Heuvelink, 1999; Enquist & Niklas, 2002). However, none of studies investigated biomass partitioning of grafted vegetable plants. This is the first paper describing biomass partitioning of grafted tomato plants with comparison to non-grafted plants. Moreover, this research includes biomass allocation with three types of pruning in the presence and absence of the southern root-knot nematode, *M. incognita*.

In the absence of *M. incognita* and pruning, non-grafted Anahu and non-grafted Rutgers, possessed unique biomass partitioning signatures. Non-grafted Anahu allocated more of its fresh weight to stem and leaf tissue; whereas, non-grafted Rutgers allocated more of its fresh weight to fruit tissue. Homo-grafted Rutgers, shifted its biomass from fruit to stem and leaf tissue. This is consistent with other studies demonstrating that grafting makes plants more vigorous (Estañ, Martinez-Rodriguez, Perez-Alfocea, Flowers, & Bolarin, 2005; Venema, Dijk, Bax, van Hasselt, & Elzenga, 2008), but does not increase fruit tissue (Romano & Paratore, 2000). In contrast, hetero-grafted Anahu and hetero-grafted Rutgers biomass signatures were the same as the non-grafted Anahu biomass allocation signature. One possible explanation for the increased vigor is that the Anahu scion is not influenced by the Rutgers biomass partitioning signature. In contrast, the Rutgers biomass signature is manipulated by the Anahu biomass partitioning signature in hetero-grafted Rutgers. A second possibility is that wounding during grafting triggers plant defensive mechanisms. As a result, the grafted tomato cultivar scion becomes more vigorous without producing more fruit. Consistently, the literature indicates that plants become more

vigorous after grafting (Khah et al., 2006b). Contradictory to this, we found that non-grafted Anahu allocated more biomass towards to stem and leaf tissue than fruit tissue. Thus, the Anahu biomass allocation signature appears to be unique, along with its ability to control scion biomass partitioning. Authors who reported plant vigor or fruit increase after grafting have neglected to compare and contrast the biomass partitioning with the non-grated cultivar's biomass partitioning signature.

In the presence of a moderate density of *M. incognita* ($P_{i=1000}$) both non-grafted Anahu and non-grafted Rutgers retained their biomass partitioning signatures. Nematode infection resulted in less fruit tissue (Table 9), rather less than stem and leaf tissue (Table 10). Thus, biomass partitioning shifted towards stem/leaf tissue (Figure 13AFigure 14A) and there was a significant decrease (P=0.027) in yield between $P_{i=0}$ and $P_{i=1000}$. Root galling increased root tissue weight slightly in some treatments with $P_{i=1000}$ (Table 11A-C). In the heavy pruned experiment with all P_i, biomasses partitioning shifted from stem and leaf tissue to fruit tissue due to the heavy pruning. Despite the nematode infection resulting in a low amount of fruit tissue, fruit tissue biomass remained high compared to other parts of the plant biomass.

In this research, the Anahu Mi gene for resistance to *M. incognita* (Williamson, Ho, Wu, Miller, & Kaloshian, 1994) did not provide complete resistance to this nematode. While Anahu produced females in the root systems, the Pf was reduced up to 19-fold, compared to Rutgers when plants were not pruned. Reddy, Tikoo and Anand (1987) found Anahu to be susceptible to *M. incognita* at a $P_{i=20}$ per 100 grams of soil, supporting my findings. Another possibility for the susceptibility of Anahu is that a resistance breaking *M. incognita* population was present in our culture (Eddaoudi, Ammati, & Rammah, 1997). An additional possible explanation for Anahu supporting *M. incognita* is that the Mi gene losses resistance above 28 C to *M. incognita*

(Dropkin, 1969; Cap, Roberts, & Thomason, 1993; Veremis, Van Heusden, & Roberts, 1999; Hu et al., 2015). Greenhouse temperature in our study, however, was kept at 26 C during the winter when tomato plants were inoculated with *M. incognita* eggs. Thus, Mi gene segregation due to high temperature was unlikely. With heavy and light pruning, *M. incognita* Pf increased compared to that in the non-pruned experiments, up to 10-fold and 20-fold on non-grafted Anahu and homo-grafted Anahu, respectively. This is evidence that pruning can have a negative impact on resistance.

Root galling was different in $P_{i=1000}$, compared to $P_{i=5000}$. Roots were more heavily galled with $P_{i=1000}$ than $P_{i=5000}$ (Table 12B, C). Moreover, the root system was healthier and produced larger galls on the root systems of susceptible tomato cultivars with $P_{i=1000}$. Also, inoculating tomato seedling with large numbers of eggs resulted in secondary disease infection with symptoms of root necrosis. High P_f in Anahu was observed in the both heavily pruned and lightly pruned experiments. There were no significant differences on P_f between resistant and susceptible cultivars following inoculation with *M. incognita* egg $P_{i=1000}$ or $P_{i=5000}$, per experimental unit. Increase in P_f of *M. incognita* after pruning has not been reported in previous research.

Heavily and lightly pruned tomato plants fit into a smaller space in the greenhouse than non-pruned plants, resulting in a higher yield of fruit per unit area. Heavily pruned tomato plants (90 experimental units) and lightly pruned plants (90 experimental units) were placed in the same area that accommodated only 60 non-pruned plants. The total yield was 68kg of fruit for the 60 non-pruned plants, while a combined tomato yield 220 kg was recorded for the 180 heavily pruned and lightly pruned plants. The findings suggest that pruning can be used as a means of increasing production of greenhouse-grown tomatoes. Pruning plants, however, decreased tomato fruit tissue per plant due to removing most of the leaves, the energy and matter syntheses

factory of the plants (Ferrandino, 1999). This finding was similar to that of Olson (1989) and Kanyomeka and Shivute (2005), where heavily pruned tomato fruit production was less than non-pruned and removal of leaves was negatively correlated with fruit yield.

The results of this study indicate that it is important to understand resource partitioning before selecting a rootstock for commercial tomato production. While resistant rootstocks can suppress soil-borne disease, choosing a resistant rootstock might not be in the best interest of a grower if the plant allocates biomass towards stem and leaf tissue, instead of fruit tissue. A grower must have access to rootstocks that allocate biomass towards fruit tissue and not stem and leaf tissue. This is consistent with the conclusions of Lopez-Perez et al (2006) where homo-grafted plants decreased yield compare to non-grafted or hetero-grafted plants.

Grafting has been reported to result in scion control over the rootstock in fruit trees, including: apricot, peach, apple, pear etc. To eliminate this phenomenon Lopez-Perez et al (2006) compared susceptible scion grafted to resistant rootstock with the same resistant non-grafted tomato cultivar where the number of galls and final egg population density of *M. incognita* was not statistically significant. In contrast, we found in the non-pruned experiment that non-grafted Anahu had a five-fold greater P_f when resistant tomato cv. Anahu was used as the rootstock. In this case, the resistant rootstock was not manipulated by the susceptible scion.

Conclusion

Grafting is known to improve plant salt tolerance, yield, and plant vigor. These have been very well studied. Increased biomass in one part of the plant, however, can lead to decreases in other parts of the plant. Different tomato cultivars allocate biomass differently. Thus, biomass partitioning in tomato is cultivar specific. Based on the biomass partitioning signature results I conclude that Anahu rootstock can controlled biomass partitioning signature of Rutgers when the Rutgers was used as a scion (Figure 13C). My findings provide an understanding of tomato

biomass partitioning that will be useful in the selection of rootstocks favorable for commercial tomato fruit production under greenhouse conditions.

Anahu does not appear to be a good rootstock for grafting for several reasons. First, Anahu is not completely resistant to *M. incognita*. Second, biomass partitioning in Anahu does not favor tomato fruit production. Third, heavily infested Anahu roots are susceptible to secondary diseases that will cause early mortality of tomato plants.

The results demonstrate that pruning tomato plants provides more space to grow additional tomato plants under greenhouse conditions, resulting in greater fruit yields.

APPENDICES

Appendix A. Statistical Tables

Table 9. Influence of *Solanum lycopersicum* L. grafting on fruit tissue in the presence and absence of *Meloidogyne incognita*.at initial nematode population densities of $P_{i=0}$, $P_{i=1000}$, and $P_{i=5,000}$ eggs per experimental unit. A. Non-pruned, B. Lightly pruned, C. Heavily pruned.

A	Non-pruned				
	$P_{i=0}$		$P_{i=1000}$		Difference (g)
Grafting	Mean (g)	SE	Mean (g)	SE	0 vs 1000
Anahu	1441.5	760.7	623.2	488.2	818.3
Anahu/Anahu	1760.1	802.1	675.5	619.5	1084.6
Rutgers/Anahu	1216.7	1282.9	540.6	243.5	676.1
Anahu/Rutgers	1345.3	1041.5	1100.2	833.9	245.0
Rutgers/Rutgers	1678.1	1207.6	1565.2	696.9	113.0
Rutgers	2161.0	1043.9	1691.2	767.5	469.8

B	Lightly pruned							
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference (g)	
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	0 vs 1000	0 vs 5000
Anahu	1262.0	683.0	988.4	460.1	719.5	415.9	273.7	542.5
Anahu/Anahu	841.9	352.5	1026.7	358.1	1008.9	446.4	-184.8	-167.0
Rutgers/Anahu	1686.4	668.9	1141.5	275.2	1330.4	972.3	544.8	356.0
Anahu/Rutgers	1219.7	273.9	969.3	490.4	722.1	449.3	250.4	497.6
Rutgers/Rutgers	1076.3	480.0	879.9	281.6	1179.6	471.7	196.4	-103.3
Rutgers	1690.8	717.4	1168.2	504.0	1058.4	345.1	522.7	632.4

C	Heavily pruned							
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference (g)	
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	0 vs 1000	0 vs 5000
Anahu	1269.4	496.8	1117.2 a	679.6	1134.8 ab[1]	367.5	152.2	134.6
Anahu/Anahu	1180.2	607.4	947.4 a	400.4	768.8 a	216.4	232.8	411.4
Rutgers/Anahu	1810.2	332.3	1306.8 ab	192.7	1297.9 ab	506.5	503.4	512.3
Anahu/Rutgers	1222.3	538.3	932.2 a	239.7	927.7 ab	234.3	290.1	294.5
Rutgers/Rutgers	1738.8	709.7	1740.0 ab	668.7	1641.4 b	560.4	-1.2	97.4
Rutgers	1433.9	479.8	2052.3 b	252.9	1410.3 ab	246.9	-618.3	23.7

[1]Column means followed by different letters are significantly (P = 0.05) different.

Table 10. Influence of *Solanum lycopersicum* L. grafting on stem/leaf tissue in the presence and absence of *Meloidogyne incognita* at initial nematode population densities of $P_{i=0}$, $P_{i=1000}$, and $P_{i=5000}$ eggs per experimental unit. A. Non-pruned, B. Lightly pruned, C. Heavily pruned.

A	Non-pruned				
	$P_{i=0}$		$P_{i=1000}$		Difference (g)
Grafting	Mean (g)	SE	Mean (g)	SE	0 vs 1000
Anahu	2382.4 ab	416.3	2299.8	255.8	82.6
Anahu/Anahu	1870.4 ab	484.7	2257.6	486.6	-387.2
Rutgers/Anahu	1661.6 a	346.1	1752.4	341.4	-90.8
Anahu/Rutgers	2515.2 b	633.5	2082.8	600.6	432.4
Rutgers/Rutgers	2006.8 ab	435.6	1885.2	170.4	121.6
Rutgers	1717.0 ab	49.1	1696.0	245.0	21.0

B	Lightly pruned							
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference (g)	
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	0 vs 1000	0 vs 5000
Anahu	1707.8	420.4	1128.2	452.2	865.8	532.3	579.6	842.1
Anahu/Anahu	1718.0	538.1	1230.8	670.4	1093.0	540.3	487.2	625.0
Rutgers/Anahu	1652.2	473.9	1067.4	651.9	849.2	479.7	584.8	803.0
Anahu/Rutgers	1653.2	234.1	1164.6	181.6	802.8	392.4	488.6	850.4
Rutgers/Rutgers	1692.0	120.2	1019.0	571.6	585.4	105.3	673.0	1106.6
Rutgers	1569.6	240.2	694.8	153.9	603.2	227.9	874.8	966.4

C	Heavily pruned							
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference (g)	
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	0 vs 1000	0 vs 5000
Anahu	1055.8	288.7	738.6	217.1	822.2	207.9	317.2	233.6
Anahu/Anahu	871.2	169.0	971.4	350.1	903.0	387.4	-100.2	-31.8
Rutgers/Anahu	905.6	196.2	1077.8	154.8	689.4	261.3	-172.2	216.2
Anahu/Rutgers	859.4	162.2	775.4	341.6	681.4	154.5	84.0	178.0
Rutgers/Rutgers	955.2	250.1	737.8	118.7	646.8	104.0	217.4	308.4
Rutgers	893.6	123.6	749.6	261.8	834.0	167.6	144.0	59.6

Table 11. Influence of *Solanum lycopersicum* L. grafting on root tissue in the presence and absence of *Meloidogyne incognita* at initial nematode population densities of $P_{i=0}$, $P_{i=1000}$, and $P_{i=5000}$ eggs per experimental unit. A. Non-pruned, B. Lightly pruned, C. Heavily pruned.

A	Non-pruned				
	$P_{i=0}$		$P_{i=1000}$		Difference (g)
Grafting	Mean (g)	SE	Mean (g)	SE	0 vs 1000
Anahu	217.6	70.7	118.8	33.5	98.8
Anahu/Anahu	142.0	69.6	141.6	80.5	0.4
Rutgers/Anahu	120.0	39.5	179.2	100.8	-59.2
Anahu/Rutgers	195.2	41.6	248.8	124.6	-53.6
Rutgers/Rutgers	158.4	31.6	205.2	69.2	-46.8
Rutgers	159.6	33.1	224.0	119.4	-64.4

B	Lightly pruned							
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference (g)	
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	0 vs 1000	0 vs 5000
Anahu	156.8	113.7	181.4 a	75.2	165.1	92.9	-24.7	-8.4
Anahu/Anahu	128.2	71.0	207.8 a	90.7	289.0	105.9	-79.6	-160.8
Rutgers/Anahu	136.0	64.0	158.4 a	73.8	208.4	102.8	-22.4	-72.4
Anahu/Rutgers	214.0	128.7	215.4 a	74.3	241.2	132.5	-1.4	-27.2
Rutgers/Rutgers	285.8	201.1	380.6 b	206.9	168.6	60.7	-94.8	117.2
Rutgers	151.8	19.9	164.4 a	94.0	190.6	83.8	-12.6	-38.8

C	Heavily pruned							
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference (g)	
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	0 vs 1000	0 vs 5000
Anahu	90.8	63.6	105.6	52.8	139.8	67.6	-14.8	-49.0
Anahu/Anahu	88.0	14.7	111.4	60.4	153.2	76.8	-23.4	-65.2
Rutgers/Anahu	73.0	32.4	91.4	54.5	106.2	59.5	-18.4	-33.2
Anahu/Rutgers	76.4	25.0	107.8	67.5	170.8	48.7	-31.4	-94.4
Rutgers/Rutgers	93.6	51.0	175.6	96.1	166.6	48.5	-82.0	-73.0
Rutgers	82.8	25.5	158.8	46.5	210.6	68.7	-76.0	-127.8

Table 12. Influence of *Solanum lycopersicum* L. grafting on final female population densities of *Meloidogyne incognita* associated with initial *M. incognita* egg population densities of $P_{i=1,000}$ and $P_{i=5,000}$ per experimental unit. A. Non-pruned, B. Lightly pruned, C. Heavily pruned.

A	Non-pruned				
	$P_{i=0}$		$P_{i=1000}$		Difference
Grafting	Mean (g)	SE	Mean (g)	SE	0 vs 1000
Anahu	0.0	0.0	297.1	226.9	-297.1
Anahu/Anahu	0.0	0.0	243.0	289.3	-243.0
Rutgers/Anahu	0.0	0.0	1547.4	1665.7	-1547.4
Anahu/Rutgers	0.0	0.0	3370.2	2951.0	-3370.2
Rutgers/Rutgers	0.0	0.0	4618.2	3758.3	-4618.2
Rutgers	0.0	0.0	3329.2	2677.5	-3329.2

B	Lightly pruned						
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	$P_{i=0\ vs}\ P_{i=5000}$
Anahu	0.0	0.0	3042.8	3808.8	5749.4	5635.6	-2706.5
Anahu/Anahu	0.0	0.0	4778.2	4440.5	11590.8	17613.7	-6812.7
Rutgers/Anahu	0.0	0.0	3988.8	2768.7	7754.3	8264.6	-3765.5
Anahu/Rutgers	0.0	0.0	3733.5	4558.6	5469.4	5514.1	-1735.8
Rutgers/Rutgers	0.0	0.0	6732.3	5762.3	778.8	712.6	5953.6
Rutgers	0.0	0.0	2800.8	2948.4	2895.3	4460.0	-94.5

C	Heavily pruned						
	$P_{i=0}$		$P_{i=1000}$		$P_{i=5000}$		Difference
Grafting	Mean (g)	SE	Mean (g)	SE	Mean (g)	SE	$P_{i=0\ vs}\ P_{i=5000}$
Anahu	0.0	0.0	2837.3	3141.4	3769.5	4840.1	-932.2
Anahu/Anahu	0.0	0.0	3748.2	3681.8	6420.3	5692.3	-2672.1
Rutgers/Anahu	0.0	0.0	2100.7	2627.0	1667.4	1163.8	433.3
Anahu/Rutgers	0.0	0.0	2486.8	3238.6	7804.6	6257.2	-5317.8
Rutgers/Rutgers	0.0	0.0	6642.8	3896.5	4495.4	2814.6	2147.4
Rutgers	0.0	0.0	5884.8	1095.1	12886.0	10066.0	-7001.2

Appendix B. Figures

Figure 9. Grafted and non-pruned *Solanum lycopersicum* L. experiment under greenhouse conditions.

Figure 10. Grafted *Solanum lycopersicum* L. heavily pruned and lightly pruned greenhouse trials. Left: heavily pruning experiment, Right: lightly pruned experiment. There were 15 experiment units per bench.

Figure 11. *Solanum lycopersicum* L. root systems: Left. Susceptible tomato cv Rutgers under initial egg population density (P_i) of 1000 per experimental unit. Right. Susceptible tomato cv Rutgers under initial egg population densities (P_i) of 0 per experimental unit.

Figure 12. Root staining method with nylon fine mashed cloth to prevent loss of small pieces of root tissue.

Figure 13. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the absence of *M. incognita* ($P_{i=0}$). A. Non-grafted, B. Homo-grafted, C. Hetero-grafted

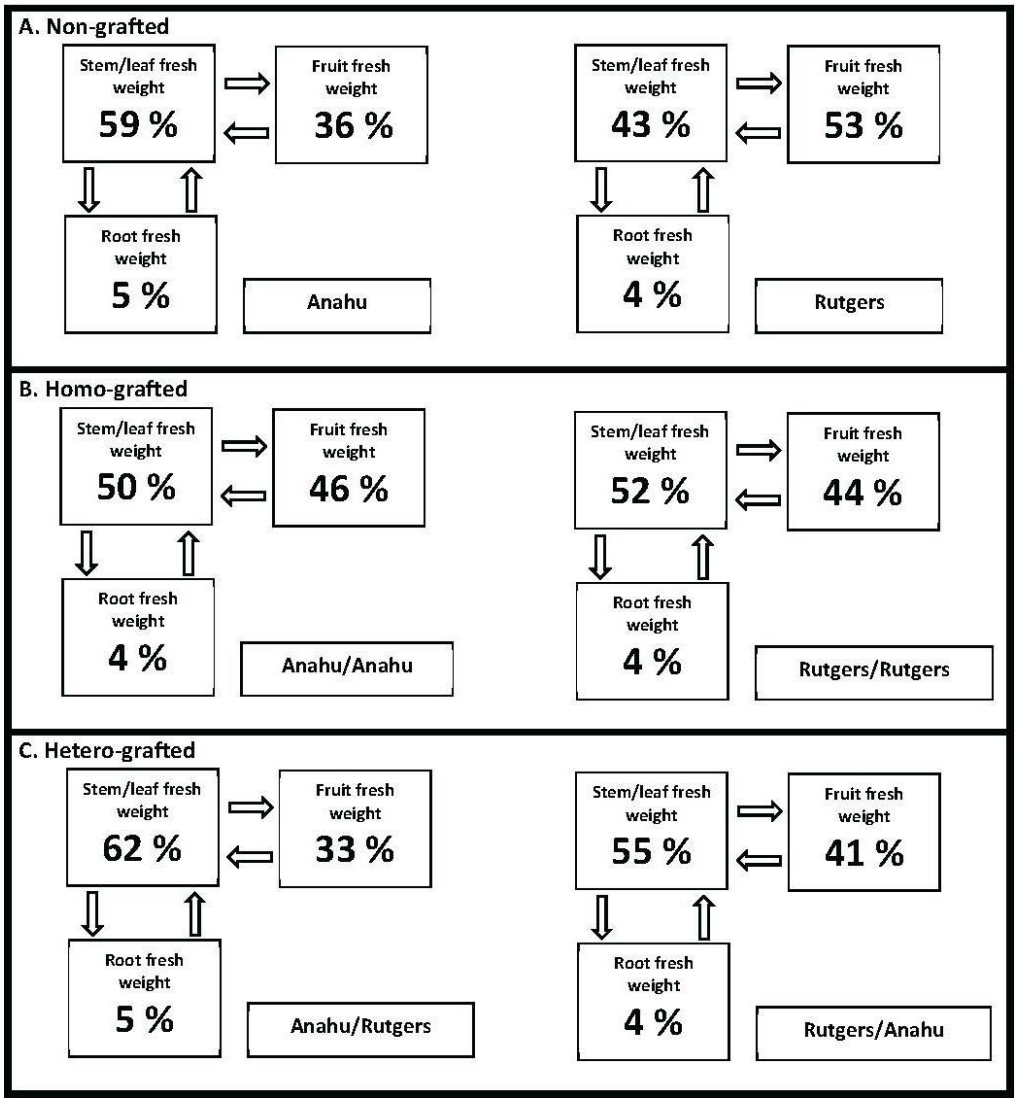


Figure 14. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the presence of *M. incognita* ($P_{i=1000}$). A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.

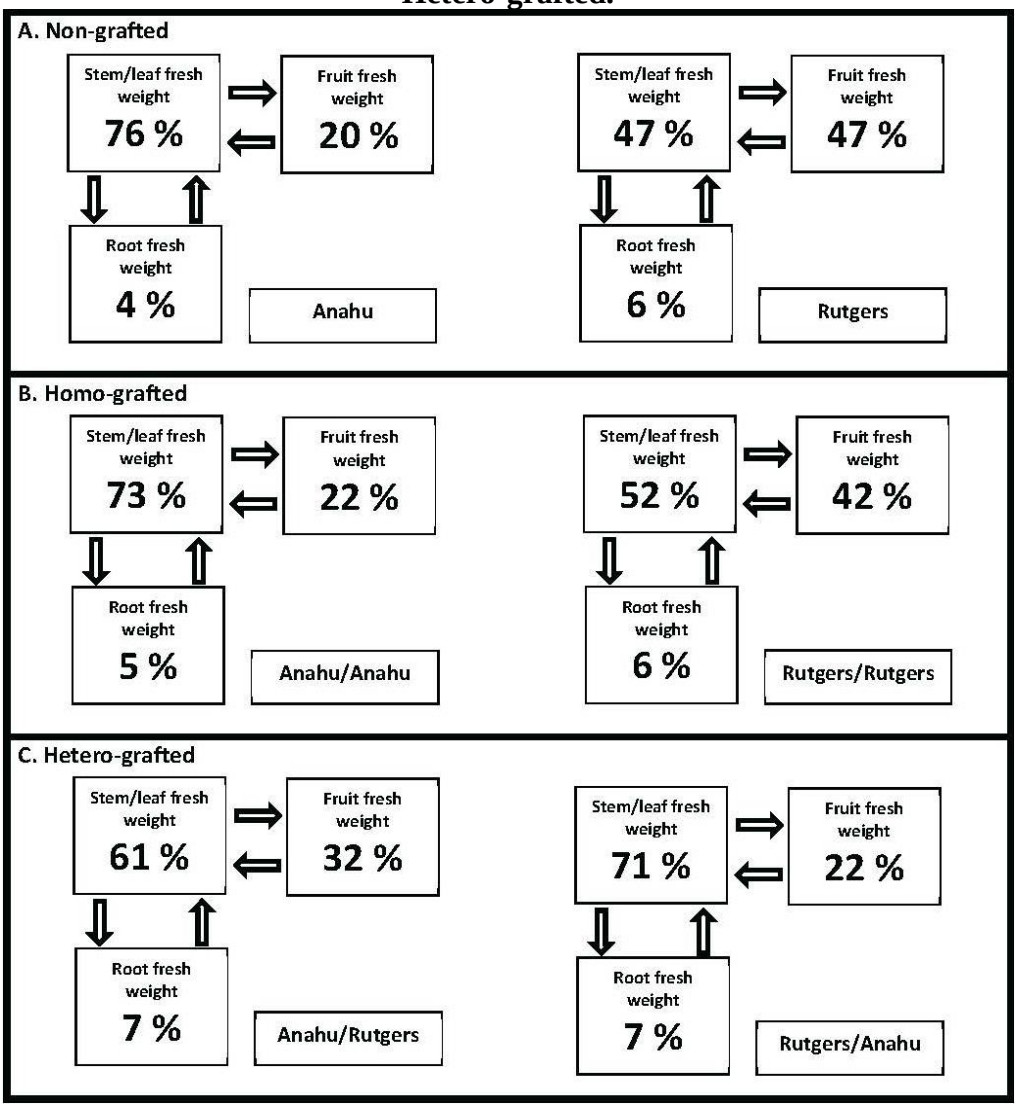


Figure 15. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the absence of *M. incognita* ($P_{i=0}$) and with lightly pruning. A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.

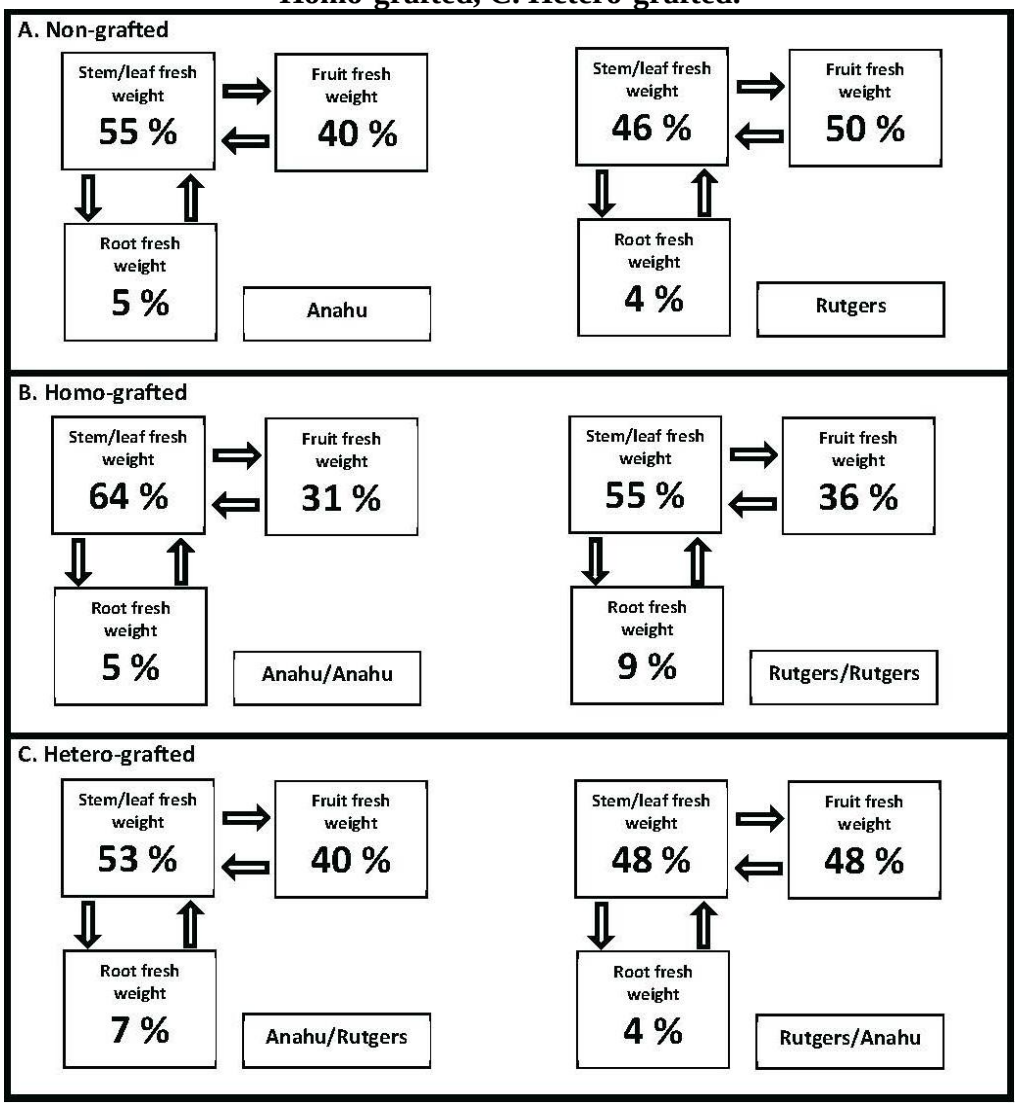


Figure 16. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the presence of *M. incognita* ($P_{i=1000}$) and with lightly pruning. A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.

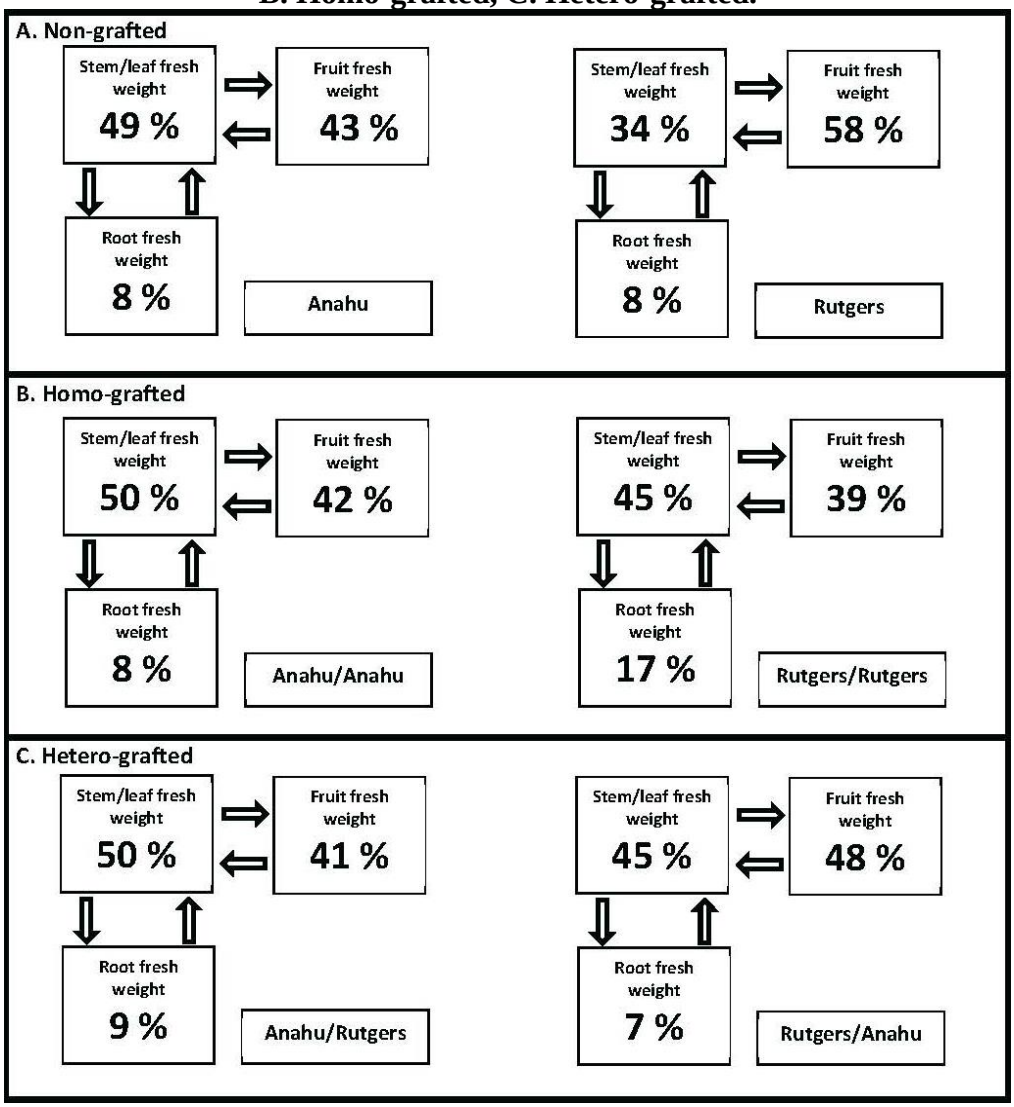


Figure 17. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers with *M. incognita* ($P_{i = 5000}$) and with lightly pruning. A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.

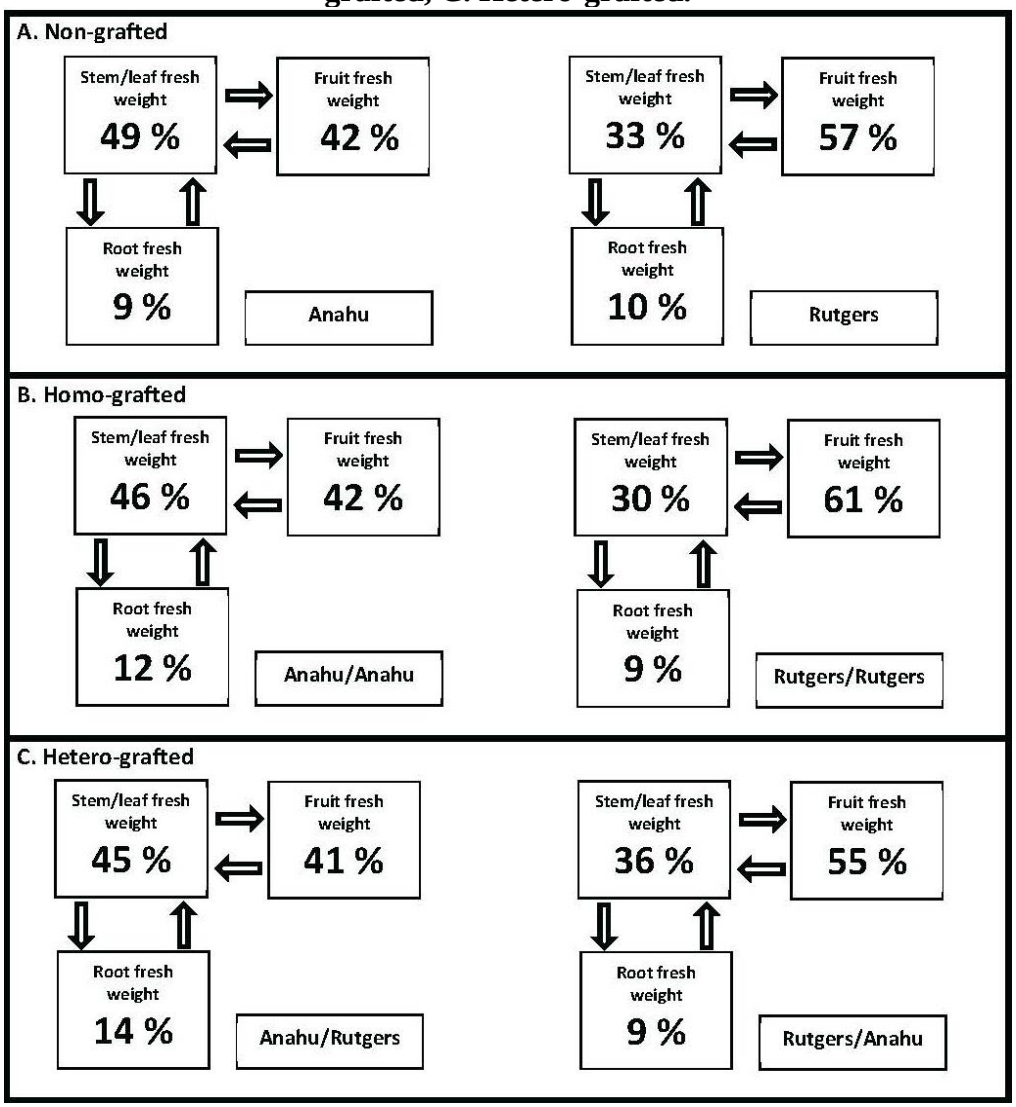


Figure 18. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers with heavily pruning and in the absence of *M. incognita* ($P_{i=0}$). A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.

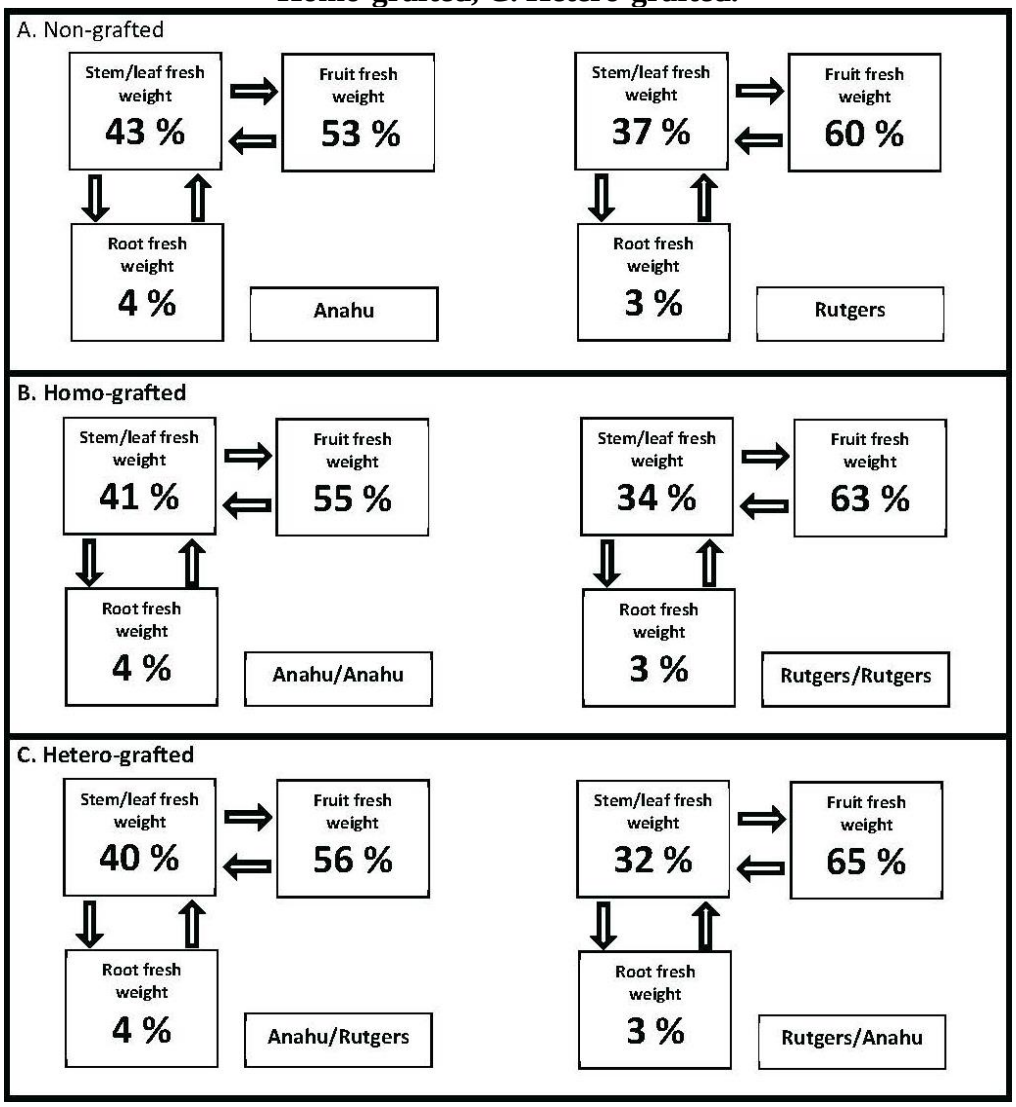


Figure 19. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the presence of *M. incognita* ($P_{i=1000}$) with heavily pruned. A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.

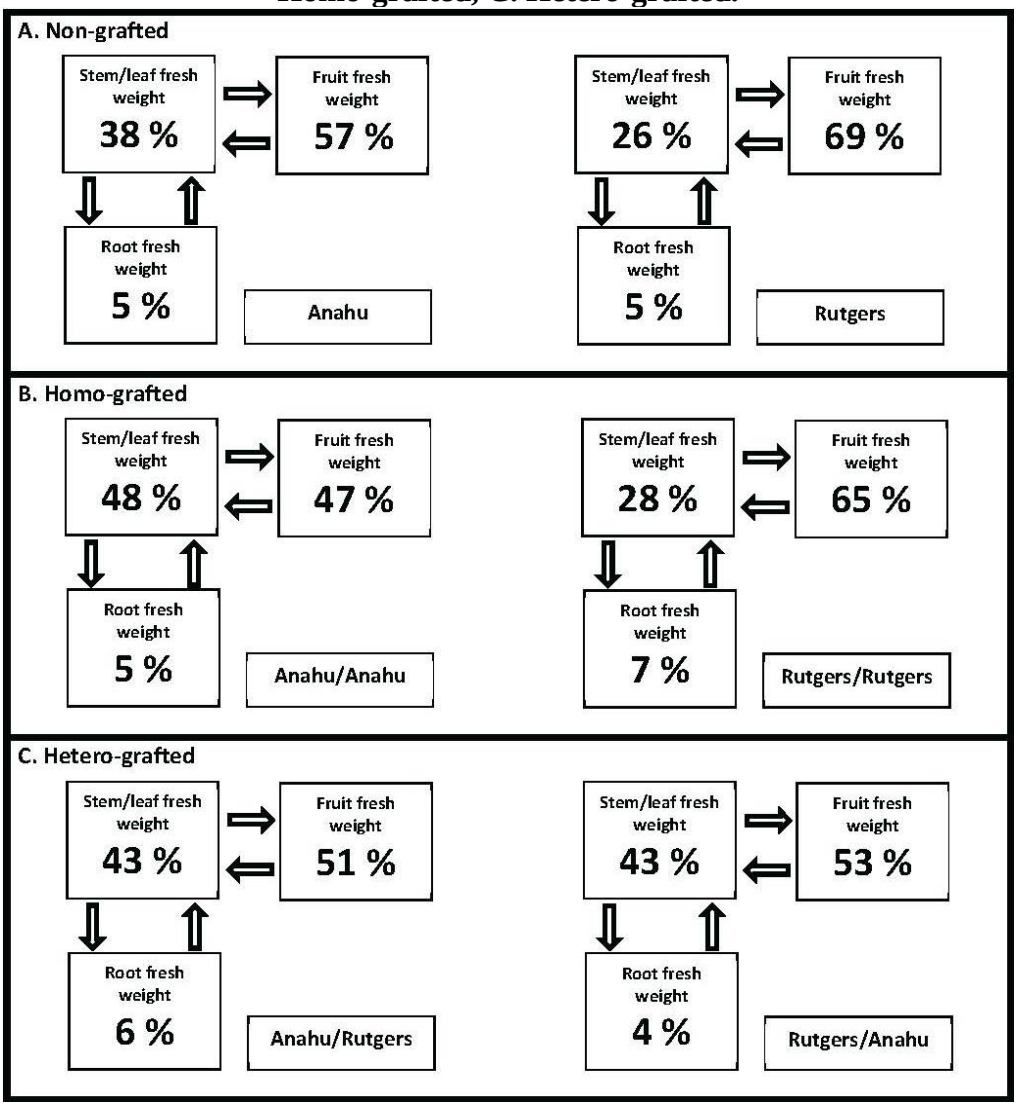
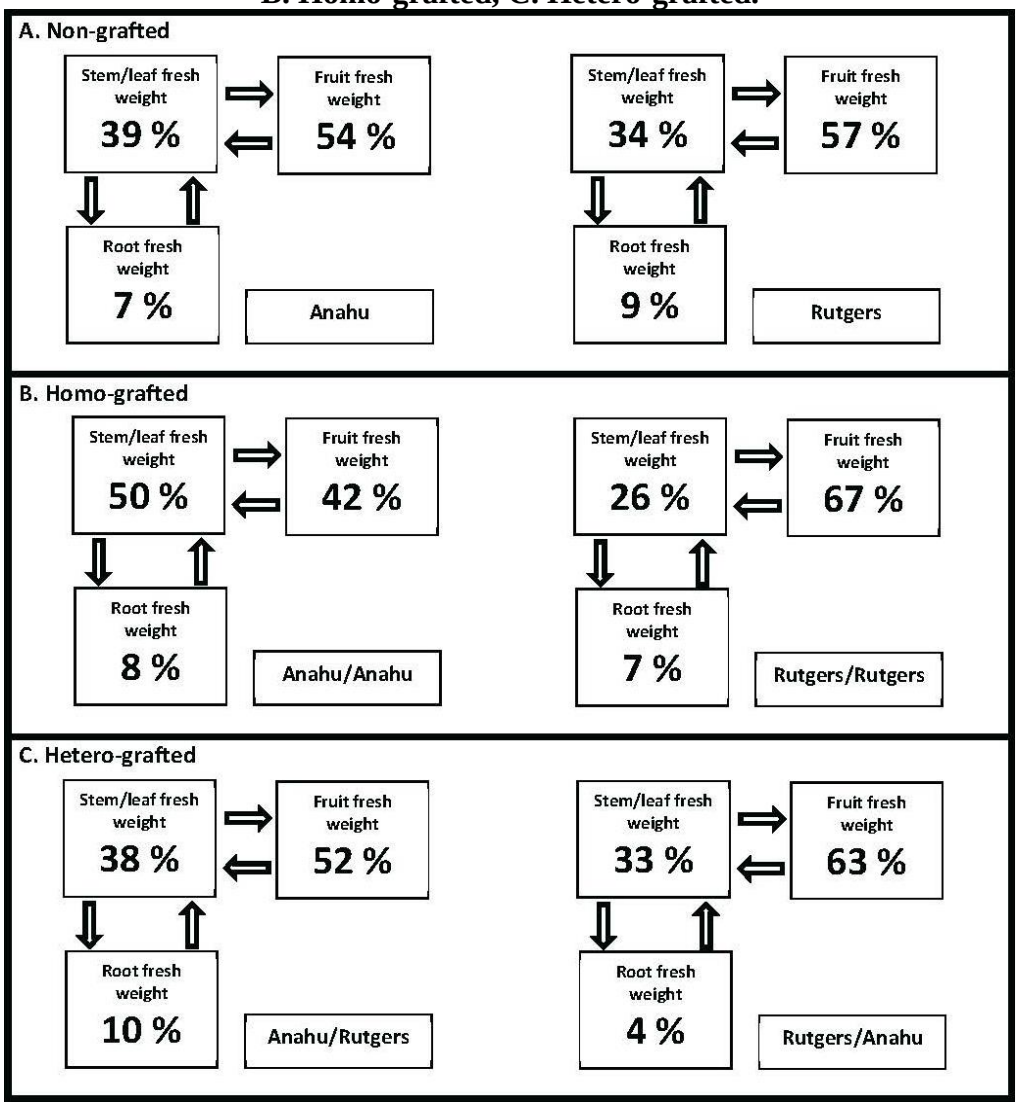


Figure 20. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers with heavily pruning and in the absence of *M. incognita* ($P_{i = 5000}$). A. Non-grafted, B. Homo-grafted, C. Hetero-grafted.



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CHAPTER 4: IMPACT OF *SOLANUM LYCOPERSICUM* L. GRAFTING ON *TRIALEURODES VAPORARIORUM* WESTWOOD (INSECTA) DEVELOPMENT UNDER GROWTH CHAMBER CONDITIONS: WITH SPECIAL REFERENCE TO THE MI GENE, TYPE-D TRICHOMES AND CENTRAL ASIA.

Abstract

Solanum lycopersicum L. resistance to *Trialeurodes vaporariorum* Westwood has not been studied in regards to the Mi gene which confers resistant to *Meloidogyne incognita (Kofoid & White) Chitwood*, *Macrosiphum euphorbiae* (Thomas) and *Bemisia tabaci* (Gennadius). Grafting susceptible cultivar scions to resistant rootstocks with the Mi gene should control, *M. incognita*, but it is not known if the resistance to foliar feeding insects will be conferred to the scion. In this research Anahu with the Mi gene and Rutgers without the Mi gene were homo-grafted, hetero-grafted and evaluated in regards to resistance to *T. vaporariorum*. Glandular trichomes are responsible for mechanical and chemical resistance, thus Type-D Trichome densities differences were evaluated in grafted plants. *T. vaporariorum* population density associated with non-grafted Anahu was significantly less that that associated with homo-grafted Rutgers. Type-D Trichome density was significantly different among three plant heights. The greatest Type D-Trichome density was on leaves immediately basipetal to the apical meristem. Anahu had significantly more Type-D Trichomes than Rutgers. In this research, the Anahu Mi gene carrying tomato cultivar was not resistant to *T. vaporariorum*. Grafting did not impact Type-D Trichome density.

Introduction

Solanum lycopersicum L. is a major vegetable crop in Uzbekistan, where greenhouse whitefly *Trialeurodes vaporariorum* Westwood, 1856 (Hemiptera: Aleyrodidae) is a devastating pest of this plant. In addition to direct damage, *T. vaporariorum* vectors viruses of economic significance (Jones 2003). For instance, *T. vaporariorum* transmits Tomato Infectious Chlorosis Virus (Zalom 2012; Duffus, Liu, and Wisler 1996). In Uzbekistan, whitefly management is

based on insecticidal control in both greenhouse and field production systems. Farmers apply insecticides weekly (personal conversation/observation) under greenhouse conditions. This can be harmful to the environment and result in excessive chemical residues. Moreover, overuse of pesticides increases the selection of populations of *T. vaporariorum* that are resistant to specific active ingredients (Gorman et al. 2007; Gorman et al. 2002). Using resistant varieties is an environmentally friendly management strategy for control of *T. vaporariorum* in tomato production. For instance, the tomato cultivar Anahu has the Mi gene that confers resistant to *Meloidogyne incognita (Kofoid & White) Chitwood* and also cross-resistance to *Macrosiphum euphorbiae* (Thomas) (Goggin, Williamson, and Ullman 2001; Vos et al. 1998) and sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Nombela, Williamson, and Muñiz 2003; Goggin, Williamson, and Ullman 2001). There are several Mi genes in cultivated and wild tomatoes. These include Mi-1 to Mi-9. Mi-1 has seven homologues, Mi-1.1 through Mi-1.7 (Seah, Telleen, and Williamson 2007). Among these, the Mi-1.2 homologue is the only functional nematode resistant gene for *M. incognita* (Milligan et al. 1998). There is no literature indicating that the Anahu Mi gene confers resistant to *T. vaporariorum*. Plants infested with *T. vaporariorum*, however, trigger the same resistance reaction as that of *B. tabaci* infection (Puthoff et al. 2010). Tomato leaves possess trichomes which can provide both mechanical and chemical defenses against insects. Seven types of trichomes exist in tomato plants (Tissier 2012). They are divided in to two groups based on glandular cell presence at the tip of the trichome. Glandular trichomes are responsible for plant resistant and repellency to pests in tomato (Goffreda, Mutschler, and Tingey 1988). Among glandular trichomes, Type-D, otherwise known as Type VI, is responsible for mechanical and chemical resistance (Aina, Rodriguez, and Knavel 1972).

Tomato cultivars preferred in Central Asia do not possess the Mi gene. Grafting local cultivar scions to tomato rootstocks with the Mi gene should control, *M. incognita*, but whether foliar feeding insect resistance can also be conferred to the scion has not been determined. The objective of this research was to determine if the Mi insect resistance trait of cv. Anahu can be transferred to grafted cv. Rutgers in the absence of *M. incognita*. The second objective of this research was to evaluate the relationship of tomato trichomes Type-D to *T. vaporariorum* resistance after grafting.

Materials and Methods

The research was conducted under growth chamber conditions at Michigan State University to evaluate the impact of grafted *S. lycopersicum* resistant rootstock on the population development of *T. vaporariorum*. The Materials and Methods Section describes the procedures, including the experimental design, plant materials, grafting procedures, growth chamber conditions, *T. vaporariorum* population density bioassay, trichome Type-D density, and data analysis.

Experimental Design

There were six treatments: 1) non-grafted Anahu, 2) non-grafted Rutgers, 3) homo-grafted Anahu, 4) homo-grafted Rutgers, 5) hetero-grafted Anahu (Anahu scion and Rutgers rootstock) and 6) hetero-grafted Rutgers (Rutgers scion and Anahu rootstock). Each treatment was replicated eight times. The plants were maintained in a completely randomized design under growth chamber conditions for 20 to 25 days after being transplanted into 32 oz styrofoam cups.

Plant materials

A single tomato cv Anahu (University of Hawaii Seed Program) or cv Rutgers (Sustainable Seed Co.) was sown in each cell in 50 cell seedling trays containing plant growth media (SUREMIX professional growing media, Michigan Grower Products Inc.) and maintained under growth chamber conditions. Growth chamber temperature was maintained at 26 C for 16 hours and 20

C for eight hours, day and night, respectively. The seedlings were watered every two days. Anahu seeds were planted two days before Rutgers seeds to ensure the same seedling diameter for grafting. This time-interval was based on preliminary research results.

Grafting

After 14 days under growth-chamber conditions, the seedlings were of appropriate size for grafting. Rootstocks and scions were cut below the cotyledon, at a 45-65 degree angle using a Miter-Cut Grafting Knife (Johnny's Selected Seeds). Scions and rootstocks were clipped together with silicon tubes used to hold the rootstock and scion together until the grafting wound healed. Silicon clipper (Hydro-Gardens, Colorado Springs, CO) size varied from 1.5-2.5 mm depending on seedling diameter. During graft-wound healing, a humidifier (Air Innovations Model # HUMID06 1.37-gallon Ultrasonic Digital Humidifier) was used to control relative humidity. The grafted plants were maintained in a growth chamber for seven days at 24-26 C at 90% RH. The relative humidity was decreased gradually from 90% at three days, to 85% the next two days, 75% the next day and 65% on the final day. After 7 days, the grafted seedlings were moved to the laboratory for three days for acclimatization.

Growth Chamber

Following acclimatization, the tomato plants were transplanted into 32 oz styrofoam cups, filled with steam sterilized sandy soil. Growth chamber temperature was maintained at 26 C for 16 hours and 20 C for eight hours, day and night, respectively. Plants were watered every two days and as necessary.

***T. vaporariorum* Population Density Bioassay**

A colony of *T. vaporariorum* was maintained on Rutgers, a susceptible tomato cultivar, under growth chamber conditions. For each experimental unit, five female and at least one male *T. vaporariorum* were introduced into mini-insectaries in two-dram vials paced under each tomato

plant. The insectaries were made from clear plastic jars with an opening of 3.5 inches designed to fit snugly into the 32 oz cup containing the tomato plant (Figure 21). The insectaries had openings on each side and on the top, covered with fine mashed nylon cloth to prevent white fly escape and allowing for air exchange. The insectaries and tomato plants were removed from the growth chamber after 20-25 days. The abaxial side of leaves of each plant was observed for *T. vaporariorum* eggs, nymphs, pupa, and pupa cases. Pupa and pupa cases were combined and presented as pre-adults. They were counted using a binocular microscope at 60X magnification and recorded.

Type-D trichome density

Glandular trichomes, known as Type-D or Type VI trichomes, were counted under a 60x binocular microscope at three levels of tomato plant height: Type-D trichome density was determined on the abaxial side of the leaves using one leaf from the second leaf branch (lower height), one from top branch (upper height), and one from a mid-branch (mid height) of each plant. The detached leaf adaxial surface was fixed to a glass slide with tape to make the leaf surface flat, making it easy to counting Type-D trichomes under a 60 X binocular (Figure 22).

Data Analysis

The data were analyzed using SPSS 24 Grad Primmum Pack. First, the data distribution normality was checked with a histogram plot for skewness. If the data were positively skewed, a natural Log or Log_{10} transformation was used to normalize the data. If the data were negatively skewed, the Square Root Method was applied. If data were not normalized after transformation, non-parametric Kruskal-Wallis Test was used as the substitute for ANOVA. In the case of the *T. vaporariorum* population density bioassay, two identical bioassay experiments were conducted separately under growth chamber conditions. Growth chamber conditions were as described above. The results were compared using an Independent Sample T Test to identify significance

differences in total population density. Since there were no statistically significant differences in the results of Experiment 1 and 2, the data were combined to increase the number of replicates. A natural square root transformation was used to normalize the data. Since Leaven's Test of Homogeneity was violated for ANOVA, the non-parametric Kruskal-Wallis Test was used to analyze the data. In the trichome density experiment, the data were positively skewed. A natural log transformation was used to normalize the data. For all data, a one-way ANOVA was used to test significant treatment effects. This was followed with Fisher's Least Significance Difference (LSD) test for mean separation.

Results

Nymphs

Nymph population density was significantly different among the six treatments ($F_{5,90}$=2.788, P=0.022). It was significantly less on non-grafted Anahu, compared to non-grafted Rutgers (P=0.047) (Table 13). Homo-grafted Anahu had significantly fewer nymphs compared to homo-grafted Rutgers (P=0.007). Hetero-grafted Anahu nymph density was numerically lower than hetero-grafted Rutgers, but it was not significantly different (Table 13). The highest density of nymphs developed on homo-grafted Rutgers, followed by non-grafted Rutgers. Both had significantly higher densities than all other grafted treatments.

Pre-adults and Hatched Pupa Cases

Pre-adult density was not statistically significant among the six treatments ($F_{5,90}$=1.713, P=0.140). The greatest density of pre-adult life stages were recorded on homo-grafted Rutgers, 70.25 (Table 13). The lowest pre-adult life stage population density was present on non-grafted Anahu, 43.25.

Total Population Density of *T. vaporariorum*

Total greenhouse population density was significantly different among the six treatments ($F_{5,90}$=2.141, P=0.068). The lowest total population density of *T. vaporariorum* was observed on

non-grafted Anahu and it was not significantly different from non-grafted Rutgers (Table 13). The highest total population density of *T. vaporariorum* was observed on homo-grafted Rutgers and it was significantly different than homo-grafted Anahu (P=0.024). Total population density of *T. vaporariorum* on hetero-grafted Anahu and hetero-grafted Rutgers was not significantly different, 61.50 and 76.69, respectively (Table 13)

Type-D Trichome Density after 7 and 25 days of Transplant into Plastic Pots

Leaves from the upper part of the plants had significantly more Type-D trichomes than those at the mid or lower plant heights after seven days (Table 14). There was a significant effect of leaf position on trichome density after seven days ($F_{2,105}$=5.870, P=0.004). Type-D trichome numbers were significantly higher on upper leaves than on lower leaves (P=0.007) or mid-height leaves after seven days (P=0.002) (Table 14). When the data were analyzed between treatments there was not significant difference after seven days (H_5=9.019, P=0.108). Non-grafted Rutgers had the least number of Type-D trichomes among all treatments and it was almost two-fold less than Anahu's Type-D trichome density, 31.89 and 55.50, respectively (Table 15) seven days after transplanting. The highest density of Type-D trichomes was recorded on hetero-grafted Rutgers and it was two-fold greater than homo-grafted Anahu seven days after transplanting. Type-D trichome density was similar between homo-grafted Anahu and homo-grafted Rutgers, 49.94 and 39.17, respectively after seven days of transplant (Table 16).

Type-D trichome density was significantly different at the three heights after 25 day of transplanting ($F_{2, 105}$=9.527, P=0.001), with trichome density increasing with increasing height. Leaves at the low height had the lowest Type-D trichome density and it was significantly different than densities at the mid or upper heights, P=0.02 and P=0.001 respectively. Mid

region Type-D trichome density was significantly less then Type-D trichome density on upper region leaves (P=0.046).

Twenty five days after transplanting, Type-D trichome density did not differ significantly among the six treatments ($F_{5,102}$=1.652, P=0.153). Numerically, the lowest Type-D trichome density was observed on non-grafted Rutgers and it was similar to Type-D trichome density on non-grafted Anahu, 31.11 and 43.72, respectively (Table 17). Type-D trichome density on homo-grafted Anahu and Rutgers was intermediate, 58.44 and 37.72, respectively. The greatest density of Type-D trichomes was recorded on hetero-grafted Rutgers, 70.67; however, Type-D trichome density was not significantly different than hetero-grafted Anahu, 49.39.

Discussion

Solanum lycopersicum has been studied extensively for insect pest resistance. Many resistant wild tomato species and cultivated tomato varieties have been reported to have resistance to tomato pests including: *Tetranychus urticae, Macrosiphum euphorbia, Bemisia tabaci, and Trialeurodes vaporariorum*. Pest resistance in *Solanum lycopersicum* is based on a number of factors including: trichomes, chemical exudes and overall genetics. In this research, we evaluated two *T. vaporariorum* resistance characteristics, the Mi gene and abundance of Type-D trichomes in regards to *S. lycopersicum* cultivars, Anahu and Rutgers. The results indicate that that the Mi gene carrying *S. lycopersicum* cv Anahu was not resistant to *T. vaporariorum*. Moreover, Type-D trichome density did not impact *T. vaporariorum* resistance.

Several Mi gene carrying tomato cultivars have significantly lowered Q-biotype of *B. tabaci* population densities (Nombela, Beitia, and Muñiz 2001). In contrast, our findings document that the Mi gene in cultivar Anahu did not confer resistant to *T. vaporariorum* and there was no difference in population density of *T. vaporariorum* between Anahu and the susceptible cultivar Rutgers. Lucatti et al (2010) found that two different Mi gene carrying tomato cultivars responded

differently to *T. vaporariorum* infestation. Tomato cultivar LC 138 was susceptible and tomato cultivar Uco Plata resistant, producing significantly fewer adult *T. vaporariorum*, 43 and 10, respectively. Kumar et al. (1995), however, found that the tomato cultivar Anahu, with the Mi gene, was significantly less attractive to *T. vaporariorum*, compared to the tomato cultivar Rey de los Tempranos, without the Mi gene. Moreover, Anahu showed moderate resistant to killing or repelling 50% of inoculated *Tetranychus urticae* on a leaf disc experiment (Aina, Rodriguez, and Knavel 1972).

The mini-insectary used in this research to evaluate *T. vaporariorum* population development enabled me to test for resistance on intact tomato plants. This procedure was used because it is difficult and not very accurate to use population density models to estimate whitefly population densities (Rumei 1982). Mini-insectaries, however, can prevent direct light from reaching the plant. As a result, plants in the mini-insectaries might have received lower levels of light, which could impact photosynthesis and impacted plant growth and its overall physiology (Crafts-Brandner and Chu 1999). Based on over observation we do not believe the light intensity had an impact on over research.

In wild tomato *Solanum pennellii* and *Solanum peruvianum* resistance to *Macrosiphum euphorbiae* is attributed to their sticky glandular trichromes and trichome density, respectively (Gentile and Stoner 1968). Gentile et al (1968) later reported that *Solanum pennellii* and *Solanum hirsutum* Humb. and Bonpl. had the same sticky glandular hairs that were death traps to *T. vaporariorum*. However, these two reports did not specify which types of the trichomes are responsible for insect pest resistance. Trichome types are discussed by Tissier (2012) after Luckwill (1943), describing the presence of glandular trichomes in wild species and cultivated tomatoes. According to this article, Type-D trichomes produce sticky exudes that are death traps

to several tomato pests. Rodriguez et al., found that Type-D trichomes were repellent and toxic to mites. De Ponti and Hogenboom (1975) reported that resistance to *T. vaporariorum* in *Solanum hirsutum* and *Solanum hirsutum glabratum* is based on internal factors in addition to glandular sticky trichomes. This factor in *Solanum hirsutum f. glabratum* is reported as 2-tridiconon, a Type-D trichome exude which is toxic to various pests of wild tomato species (Williams et al. 1980). Moreover, Williams et al. (1980) reported that wild tomato species have 72 times more of 2-tridecanon chemical then cultivated tomatoes. Kevin and Frank (1995), however, screened 20 commercial cultivars of processing tomato and seven species of wild tomatoes. They found that *Bemisia tabaci* B-biotype (*Bemisia argentifolii)* resistance was not due to trichome density. This is consistent with the results of my findings where there was a weak relationship between trichome density and *T. vaporariorum* population density (Figure 23).

The numbers of Type-D trichomes increased in density with increasing plant height. I also visually observed that *T. vaporariorum* laid eggs preferentially on the lower region leaves where tomato trichomes Type-D are less prevalent. Hargreaves (1915) mentioned that *T. vaporariorum* lays eggs on younger leaves and trichome density interfered with oviposition rate and fecundity. This characteristic, however, was not consistent with earlier observations on this research. The eggs were distributed randomly among leaves in various positions when the density of ovipositing adults was high. Gentile et al. (1968) failed to find significant difference of eggs and nymphs of *T. vaporariorum* at four plant heights of wild tomato plants, using 16-mm-diam leaf discs. I counted trichome Type-D density in each leaf and concluded that this should not be done where leaf veins are present. Moreover, trichome Type-D density was not equal in each half of the single leaf. Thus, the entire leaf Type-D trichomes were counted and samples were taken from three heights of the plant.

Conclusion

The primary objective for this research was to determine if the Mi gene in Anahu confers resistant to *T. vaporariorum*. This was not supported by the results. This may have been due to the large population density of *T. vaporariorum* on Anahu. Moreover, Anahu was developed through the embryo rescue method by breading *Solanum lycopersicum* cv Michigan State Forcing and *Solanum peruvianum* PI128657 (Liharska and Williamson 1997). Thus, a lack of resistance in tomato cv Anahu can possibly be explained with *S. peruvianum* not being resistant to *T. vaporariorum* (De Ponti, Pet, and Hogenboom 1975; Gentile, Webb, and Stoner 1968). Additionally, plants exhibit high whitefly resistance at temperatures above 28 C under greenhouse conditions. An additional possible explanation for Anahu not being resistant to *T. vaporariorum* is that the Mi gene segregates above 28 C (Dropkin 1969; Cap, Roberts, and Thomason 1993; Veremis, Van Heusden, and Roberts 1999; Hu et al. 2015). This is another clue that the Mi gene is not responsible for *T. vaporariorum* resistance in tomato. Interestingly, Bas et al. (1992) found a larger difference between genotypes in older plants. In this study, all bioassays were conducted using 20-25 days old tomato plants. Bas et al. (1992) and Byrne and Draeger (1989) reported that tomato plant resistance increases with plant age in some wild tomato species. Therefore, it would be of interest to investigate the impact of plant age on the Mi gene carrying tomato cultivar's resistance to *T. vaporariorum*.

APPENDICES

Appendix A. Statistical Tables

Table 13. Influence of *Solanum lycopersicum* L grafting on *T. vaporariorum* life stage population densities.

Grafting Treatment	Nymph	SE	Pre-adult	SE	Total	SE
Anahu	12.50 a[1]*	3.32	43.25	5.34	55.75 a	5.51
Anahu/Anahu	10.50 a	2.44	55.00	10.41	65.50 a	12.48
Rutgers/Anahu	13.75 ab	2.01	62.94	7.61	76.69 ab	8.35
Anahu/Rutgers	12.75 a	2.38	48.75	6.79	61.50 a	8.00
Rutgers/Rutgers	21.06 b	3.24	70.25	8.03	91.31 b	10.17
Rutgers	17.88 b	2.71	58.44	7.10	76.31 ab	7.41

[1] Data were natural log transformed. *T. vaporariorum* population density was recorded at the end of each experiment in entire plant. None of the data were violated Homogeny of variance after natural log transformation. The data analyzed by One Way ANOVA and followed by Fisher's LSD for mean separation. Column means fallowed by the same letter are not significantly different at α=0.10

Table 14. *Solanum lycopersicum* L Type-D trichome density at three plant heights seven days after transplanting into plastic pot. Lower region (leaves from second branch), Mid-region (leaves from a mid-region branch), and Upper region (leaves from last branch one before merry stem).

	N	Type-D Trichomes	SE
Lower region	36	42.72 a[1]	6.81
Mid region	36	43.19 a	7.53
Upper region	36	71.42 b	8.80

[1] Data were natural log transformed. The data did not violated Homogeny of variance after natural log transformation. The data analyzed by One Way ANOVA and followed by Fisher's LSD for mean separation.

* Different letter show significant difference at α=0.05

Table 15. Impact of *Solanum lycopersicum* L grafting on Type-D Trichrome density seven days after transplanting into plastic pot. Means for six replicates of each grafting treatments. Three leaf samples were taken from three leaves of the each tomato plant.

Grafting	N	Mean	SE
Anahu	**18**	**55.50[1]**	**12.71**
Anahu/Anahu	**18**	**49.94**	**11.29**
Rutgers/Anahu	**18**	**85.33**	**17.59**
Anahu/Rutgers	**18**	**52.83**	**5.75**
Rutgers/Rutgers	**18**	**39.17**	**7.61**
Rutgers	**18**	**31.89**	**4.17**

[1]Data violated the Homogeneity of variance. Data natural log transformation for fixing normality of the data. Homogeneity of variance was violated. Thus, the data were analyzed by using Kruskal–Wallis test non parametric test equivalent of One-way analysis of variance.

Table 16. Impact of *Solanum lycopersicum* L grafting on Type-D trichome density at three plant heights 25 days after transplanting into plastic pot. Lower region (leaves from second branch), Mid-region (leaves from a mid-region branch), and Upper region (leaves from last branch one before merry stem).

	N	Type-D Trichomes	SE
Lower region	**36**	**26.44 a[1]**	**3.80**
Mid region	**36**	**42.25 b**	**5.19**
Upper region	**36**	**76.83 c**	**11.08**

[1] Data were natural log transformed. The data did not violated Homogeny of variance after natural log transformation. The data analyzed by One Way ANOVA and followed by Fisher's LSD for mean separation. Different letter show significant difference at $\alpha=0.05$

Table 17. Impact of *Solanum lycopersicum* L grafting on Type-D Trichrome density 25 days after transplanting into plastic pot. Means for six replicates of each grafting treatments. Three leaf samples were taken from three leaves of the each tomato plant.

Grafting	N	Mean	SE
Anahu	**18**	**43.72[1]**	**10.59**
Anahu/Anahu	**18**	**58.44**	**17.95**
Rutgers/Anahu	**18**	**70.67**	**12.81**
Anahu/Rutgers	**18**	**49.39**	**8.03**
Rutgers/Rutgers	**18**	**37.72**	**7.48**
Rutgers	**18**	**31.11**	**7.22**

[1] Data were natural log transformed. The data did not violated Homogeny of variance after natural log transformation. The data analyzed by One Way ANOVA.

Appendix B. Figures

Figure 21. Mini-insectary for *T. vaporariorum* population density bioassay. A: Young grafted tomato seedling in a mini-insectary. B: Experimental units after inoculation with *T. vaporariorum* in a completely randomized design under growth chamber conditions.

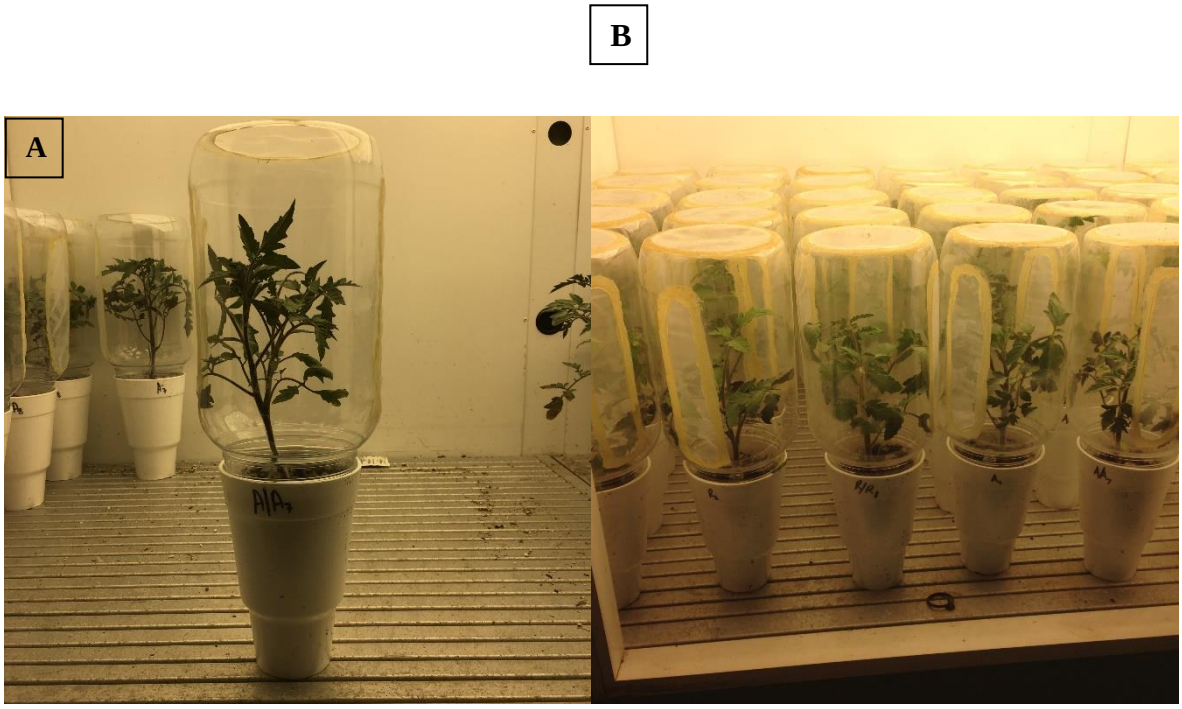


Figure 22. Tomato leaf fixed with tape to make the leaf flat to assist counting whiteflies and tomato trichomes.

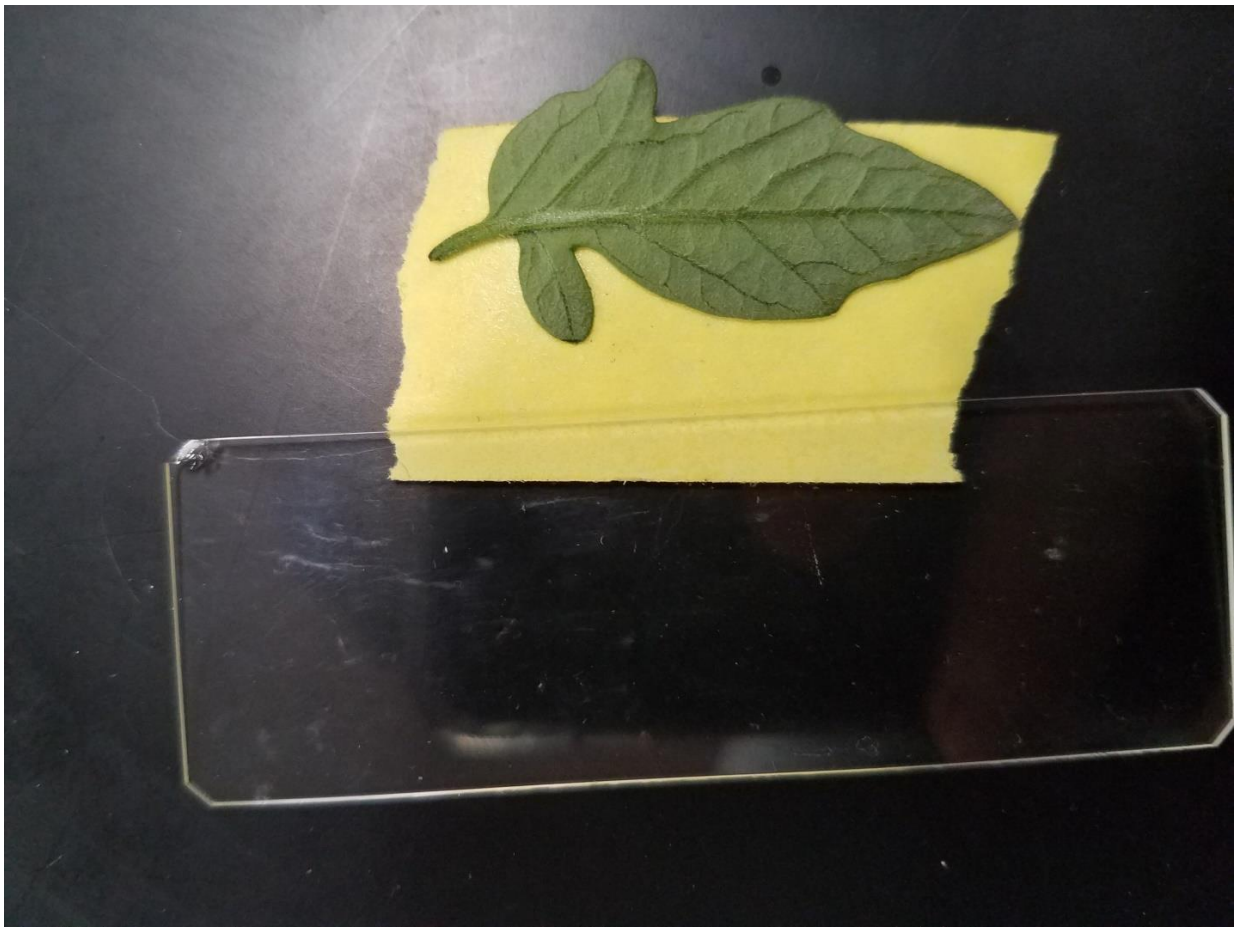
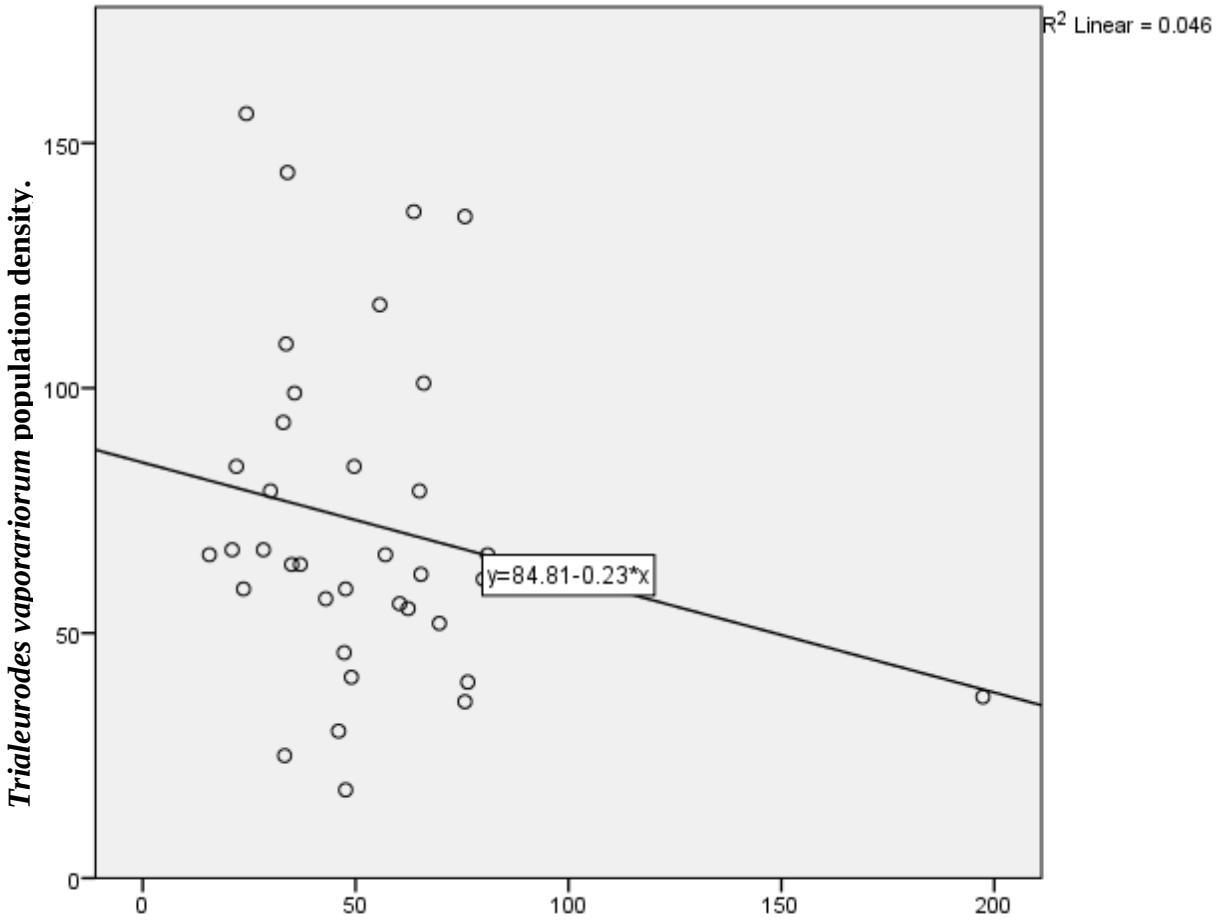


Figure 23. Relationship between lower region Type-D trichome density and total *T. vaporariorum* population density.



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CHAPTER 5: JOINT IMPACT OF *MELOIDOGYNE INCOGNITA (KOFOID AND WHITE*1919*) CHITWOOD* 1949 AND WESTWOOD ON GRAFTED *SOLANUM LYCOPERSICUM* L. CULTIVARS: WITH SPECIAL REFERENCE TO THE MI GENE.

Abstract

The impact of root-scion grafting combinations of the Mi gene containing *Solanum lycopersicum* L cv, Anahu with cv Rutgers (Mi gene absent) on *Trialeurodes vaporariorum* population density was evaluated under growth chamber conditions in no choice assays in the presence of *Meloidogyne incognita*. The treatments included homo-grafted Anahu, homo-grafted Rutgers, hetero-grafted Anahu and hetero-grafted Rutgers. Non-grafted Anahu and Rutgers served as the controls. The hypotheses were that the presence of *M. incognita* triggers *T. vaporariorum* resistance conferred by the Mi gene, or an increase in Type-D trichome density. In this research, there were no significant differences in Type-D trichome density between cvs Anahu and Rutgers. The presence of *M. incognita* triggered resistant to *T. vaporariorum* on non-grafted Anahu and homo-grafted Anahu, compared to non-grafted Rutgers and homo-grafted Rutgers. Moreover, the presence of *M. incognita* delayed the life cycle of *T vaporariorum* in all grafted treatments. In our previous study in the absence of *M. incognita*, the Mi gene in Anahu did not impact *T. vaporariorum* population density. Anahu was partially resistant to *T. vaporariorum* in the presence of the *M. incognita*, while also expressing partial resistance to *M. incognita*. In conclusion, *S. lycopersicum* cv Anahu does not appear to be an optimal cultivar for *T. vaporariorum* or *M. incognita* management, despite being the parent of most Mi gene carrying commercial *S. lycopersicum* cultivars and hybrids.

Introduction

Trialeurodes vaporariorum (Westwood) is a key pest of *Solanum lycopersicum* L, especially under greenhouse conditions. *T. vaporariorum* is difficult to control because of its rapid rate of population growth and the fact that most currently available chemical pesticides are only highly

effective against adults. It's high reproduction increases the risk associated with rapid development of resistance to insecticides. Furthermore, *T. vaporariorum* causes indirect damage by transmitting plant viruses during feeding and probing of a plant (Jones, 2003). According to Duffus et al. (1996) *T. vaporariorum* is a vector of Tomato Infectious Chlorosis Virus (TICV). Therefore, it is of major interest to have plants that are not only resistant to *T. vaporariorum*, but also repellant in order to prevent the spread of TICV during white fly probing.

Tomato leaves possess trichomes which can provide both mechanical and chemical defenses against insects. Seven types of trichomes exist in tomato plants (Tissier 2012). They are divided in to two groups based on glandular cell presence at the tip of the trichome. Glandular trichomes are responsible for plant resistant and repellency to pests in tomato (Goffreda, Mutschler, and Tingey 1988). Among glandular trichomes, Type-D, otherwise known as Type VI, is responsible for mechanical and chemical resistance (Aina, Rodriguez, and Knavel 1972). Tomato breeding programs have identified and extensively studied several wild species of *S. lycopersicumI* that are resistant to *T. vaporariorum*. De Ponti et al. (1975) discovered and evaluated several wild tomato species, including: *Lycopersicon hirsutum, L. hirsutum glabratum* and *S. pennellii* that are resistant to *T. vaporariorum*. Bas et *al.* (1992) mentioned that older *L. hirsutum f. glabratum* plants were highly resistance to *T. vaporariorum*. Moreover, some wild tomato species possess the Mi gene which was introduced into *S. lycopersicum* through the embryo rescue method (Smith 1944). Among Mi-1 homologues, the Mi-1.2 gene has conferred resistance to several organisms including *Meloidogyne incognita (Kofoid & White) Chitwood* (Milligan et al., 1998; Seah, Telleen, & Williamson, 2007), and cross-resistance to both *Macrosiphum euphorbiae* (Thomas) (Goggin, Williamson, & Ullman, 2001; Vos et al., 1998) and sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Nombela, Williamson, & Muñiz, 2003;

Goggin et al., 2001). Utilization of resistant *S. lycopersicum* has not, however, proven to be an achievable way to control *T. vaporariorum*.

Breeding for new hybrids or cultivars is time consuming and not all favored traits of the parents are transferred to the next generation. Grafting provides an alternative approach to integrate both the pest resistant and food quality traits desired for successful *S. lycopersicum* production under greenhouse or field conditions. The objective of this research is to evaluate the impact of a grafted *S. lycopersicum* resistant rootstock on the population development of *T. vaporariorum* in the presence of the nematode, *M. incognita*. Furthermore, to determine if the Mi insect resistance trait of cv. Anahu can be transferred to grafted cv. Rutgers in the presence of *M. incognita*. Additionally, the research involves determination of the relationship between *M. incognita* and *S. lycopersicum* Type-D trichome density at three plant heights: at lower region, mid region and upper region.

Materials and Methods

The research was conducted under growth chamber conditions at Michigan State University. This section describes the procedures used to determine the population density evaluation of *T. vaporariorum* associated with Anahu and Rutgers plants, before and after grafting. It includes the experimental design, plant materials, grafting procedures, growth chamber conditions, *T. vaporariorum* population density bioassay, trichome type density, data analysis and abbreviations.

Experimental Design

The research consists of six treatments: 1) non-grafted Anahu, 2) non-grafted Rutgers, 3) homo-grafted Anahu, 4) homo-grafted Rutgers, 5) hetero-grafted Anahu (Anahu scion and Rutgers rootstock) and 6) hetero-grafted Rutgers (Rutgers scion and Anahu rootstock). Two other treatments were the presence of *T. vaporariorum*, replicated three times, and the absence of *T.*

vaporariorum, replicated five times. The plants were maintained in a completely randomized design under growth chamber conditions for 20 to 25 days after being transplanted into 32 oz styrofoam cups.

Plant Materials

A single tomato cv Anahu (University of Hawaii Seed Program) or cv Rutgers (Sustainable Seed Co.) was sown in each cell of 50 cell seedling trays containing plant growth media (SUREMIX professional growing media, Michigan Grower Products Inc.) and maintained under growth chamber conditions at 26 C for 16 hours and 20 C for eight hours, day and night, respectively. The seedlings were watered every two days. Anahu seeds were planted two days before Rutgers seeds to ensure the same seedling diameter for grafting. This time-interval was based on preliminary research results. At the termination of the experiment, after 20-25 days, the fresh and dry weights of the combined stem and leaf tissues, as well as the fresh and dry weights of the root tissue were determined using a 200 g scale.

Grafting

After 14 days under growth-chamber conditions, the seedlings were grafted. Rootstocks and scions were cut below the cotyledon, at a 45-65 degree angle using a Miter-Cut Grafting Knife (Johnny's Selected Seeds). Scions and rootstocks were clipped together with silicon tubes used to hold the rootstock and scion together until the grafting wound healed. Silicon clipper (Hydro-Gardens, Colorado Springs, CO) size varied from 1.5-2.5 mm, depending on seedling diameter. During graft-wound healing, a humidifier (Air Innovations Model # HUMID06 1.37-gal. Ultrasonic Digital Humidifier) was used to control relative humidity (RH). The grafted plants were maintained in a growth chamber for seven days at 24-26 C. The RH was decreased gradually from 90% for the first three days, 85% the next two days, 75% the next day and 65%

on the final day. After seven days, the grafted seedlings were moved to the laboratory for three days for acclimatization.

Growth Chamber

The tomato plants were transplanted into 32 oz styrofoam cups, filled with steam sterilized sandy soil. Growth chamber temperature was maintained at 26 C for 16 hours and 20 C for eight hours, day and night, respectively. The plants were watered every two days, or as necessary.

***Trialeurodes vaporariorum* Population Density Bioassay**

A colony of *T. vaporariorum* was maintained on Rutgers, a susceptible tomato cultivar, under growth chamber conditions. For each experimental unit, five females and at least one male *T. vaporariorum* were introduced into mini-insectaries in two-dram vials placed under each tomato plant. The insectary was constructed from a clear plastic jar with an opening of 3.5 inches designed to fit snugly into the 32 oz cup containing the tomato plant. The insectary had openings on each side and on the top. The openings were covered with fine mashed nylon cloth to prevent white fly escape and allow for air flow. The insectaries and tomato plants were removed from the growth chamber after 20-25 days. The abaxial sides of leaves on each plant were observed for *T. vaporariorum* eggs, nymphs, pupa, and pupa cases. They were counted using a binocular microscope at 60X magnification and the data recorded.

***Meloidogyne incognita* Population Density**

The root systems of plants inoculated with *M. incognita* and expressing root galls were used for estimating final female population densities of *M. incognita*. From each experimental unit, three grams of root tissue were used to estimate the final female population density. Root tissue for nematode analysis was cut at random from various parts of each root system. A slightly modified root staining procedure of Bybd Jr et al. (1983) was used to determine nematode female final population density. Three grams of root tissue were wrapped with a fine mashed nylon cloth

tagged with corresponding treatment label. This prevented loss of root tissue and allowed for the staining of 20-40 roots at a time. Staining several roots together shortened the time spent for staining and increased staining efficacy. Moreover, staining several roots together increased the staining quality by accurately timing the procedure. Fixed roots were placed in 600 ml beakers containing 1:1 ratio of 8.25% NaOCl (Clorox Regular Bleach 3.78qt) and tap water for four minutes, stirring several times. The roots were rinsed with tap water and soaked for 15 minutes in tap water. They were then drained and washed under tap water several times to remove the rest of the bleach from roots. After washing, the roots were soaked in water for 15 minutes. Next, the roots were placed into a 600-ml beaker and filled with water until all the fixed roots were submerged. Based on how much water was used to fill the beaker, 1.0 ml of stock acid-fuchsine-stain solution was added for each 30-50 ml water. The beaker was placed on a hot plate, brought to a boil for one-two minutes and cooled at room temperature. The room-temperature roots were drained, rinsed with tap water and dried with a paper towel. They were then filled with acidified glycerin with a few drops of 5N HCl and boiled on a hot plate for 30–60 seconds. After cooling, the final population density (P_f) of *M. incognita* infesting the roots was determined under a binocular microscope.

Trichome Types

Glandular trichomes, known as Type-D or Type VI trichomes (Tissier 2012), were counted on the abaxial side of the leaves under a 60X binocular microscope. The determinations were made at three plant height levels: upper, mid and lower. Lower leaves were selected from a position immediately above the first leaf branch. The upper leaves from the upper most leaf branch and mid leaves from a branch in the middle of the plant.

Data Analysis

The data were analyzed using SPSS 24 Grad Primum Pack. First, data normality was checked with a histogram plot for skewness of the distribution. If the data were positively skewed, natural Log or Log_{10} was used to transform the data. If the data were negatively skewed, the Square Root Method was applied. Normalized data after transformation were analyzed with a One Way ANOVA test, followed by a Fisher's LSD pairwise test for mean separation if the ANOVA p value was equal or less than α=0.05. If the data were not normalized after transformation, the non-parametric Kruskal-Wallis Test was used as the substitute for the One Way ANOVA, followed by a Dunn pairwise test for mean separation if the Kruskal-Wallis Test p-value was equal or less than α=0.05.

Results

Greenhouse Whitefly

After 20-25 days under growth chamber conditions at 26 C for 16 hours and 20 C for eight hours, day and night, respectively, *T. vaporariorum* population densities were significantly different ($F_{5,90}$=2.788, P=0.022) among the six treatments. Homo-grafted Anahu had the lowest population density of *T. vaporariorum* and it was significantly lower than all other treatments, except non-grafted Anahu (Table 18). Non-grafted Anahu had a significantly lower population density of *T. vaporariorum* than Non-grafted Rutgers. Hetero-grafted Anahu had the highest population density of *T. vaporariorum*, although it was not significantly different than hetero-grafted Rutgers.

Type-D Trichome Density in the Presence of *M. incognita*

There were no Type-D trichome density differences among the six treatments after seven days of plant growth in the presence of *M. incognita* ($F_{5,102}$=1.286, P=0.276). The highest Type-D trichomes density was observed on hetero-grafted Rutgers (Table 19). The lowest number of Type-D trichomes density was associated with non-grafted Rutgers. There were significant

differences in Type-D trichome densities relative to the vertical location of leaves ($F_{2,102}$=11.235, P=0.001). The greatest density of Type-D trichomes, were found on the most recently formed leaves (Upper region leaves) (Table 20). Leaves at this location had significantly more Type-D Trichomes than those at the mid and lower positions (P=0.004 and P=0.001, respectively). Twenty-five days after inoculation with *M. incognita*, trichome density did not differ significantly among the six treatments ($F_{5,102}$=0.889, P=0.491). Numerically, the greatest number of Type-D trichome was observed on hetero-grafted Anahu plants. The lowest numbers were associated with the homo-grafted Anahu plants (Table 21). There was a significant difference in Type-D trichome density on leaves at different heights ($F_{2,105}$=7.774, P=0.001). Type-D trichome density observed on the upper region leaves was statistically greater compared to those observed on the lower region (P=0.001) and mid region (P=0.007) leaves (Table 22).

Stem/leaf Biomass in the Presence and Absence of *Trialeurodes vaporariorum*

Fresh weight of stem/leaf tissue was not significantly different among the six treatments when *T. vaporariorum* was absent (H5=1.139, P=0.951). Numerically, the greatest fresh weight was observed on non-grafted Anahu. The lowest stem/leaf tissue fresh weight was observed on hetero-grafted Anahu (Table 23).

Dry weight of stem/leaf tissue was not significantly different among six grafted treatments when *T. vaporariorum was absent* (F5.24=1.778, P=0.156). Numerically, the highest amount of stem/leaf biomass was observed on non-grafted Rutgers and the lowest amount on homo-grafted Anahu (Table 24).

When *T. vaporariorum* was present, the fresh weight of stem/leaf tissue was substantially greater than in the absence of this insect (Table 24, Table 25). The fresh weight, however, was not significantly different among the six treatments ($F_{5,12}$=1.152, P=0.387). Numrically, the greatest

amount stem/leaf fresh weight was observed on hetero-grafted Anahu. The lowest amount of stem/leaf tissue fresh weight was observed on homo-grafted Anahu.

Dry weights of stem/leaf tissue also were not significantly different amount the six treatments ($F_{5,12}$=0.518, P=0.758). Numerically, the greatest amount of dry weight of stem/leaf tissue was observed on hetero-grafted Anahu (Table 26). The lowest amount of dry weight of stem/leaf tissue was observed on homo-grafted Anahu. While the fresh weight of stem/leaf tissue was not significantly different in the presence or absence of *T. vaporariorum*, dry weight of stem/leaf tissue was significantly less (P=0.03) in the presence of *T. vaporariorum* compared to when this insect was absent (Table 23 and Table 26).

Root Biomass in the Presence and the Absence of the Trialeurodes vaporariorum

In the absence of *T. vaporariorum*, there were significant differences in root fresh weights among the treatments (F5.24=3.183, P=0.024). Non-grafted Rutgers had the greatest root fresh weight and it was not significantly different than non-grafted Anahu (Table 27). Homo-grafted Anahu had the lowest root tissue fresh weight among the six treatments and it was significantly less than homo-grafted Rutgers (P=0.029). Hetero-grafted Anahu and hetero-grafted Rutgers had similar root tissue fresh weights.

In the presence of *T. vaporariorum*, root tissue fresh weights were not significantly different among the six treatments (H_5=8.196, P=0.146). Numerically, hetero-grafted Anahu produced the greatest amount of root tissue fresh weight in the presence of the *T. vaporariorum* (Table 28). Hetero-grafted Anahu had the lowest amount of root tissue fresh weight.

There were significant differences in dry weights of root tissue among the six treatments in the absence of *T. vaporariorum* ($F_{5,24}$=6.073, P=0.01). The greatest amount of root tissue dry weight was associated with non-grafted Rutgers and it was significantly more than non-grafted Anahu

(P=0.026) (Table 29). Homo-grafted Anahu produced the lowest amount of root tissue dry weight and it was significantly less than homo-grafted Rutgers (P = 0.003). Hetero-grafted Anahu and hetero-grafted Rutgers had similar amounts of root tissue dry weight.

In contrast, In the presence of *T. vaporariorum* there were no significant differences among the six treatments (H_5=9.117, P=0.102). numerically, the greatest amount of root tissue dry weight was associated with non-grafted Rutgers and the lowest root tissue dry weight was observed on hetero-grafted Rutgers (Table 30). Overall, there were no significant differences in fresh and dry root weights when *T. vaporariorum* was present or absent.

Meloidogyne incognita Population Development in the Presence and the Absence of Trialeurodes vaporariorum

In the absence of *T. vaporariorum*, *M. incognita* final female population densities were not significantly different among the six treatments ($F_{2,24}$=2.410, P=0.066) (Table 30). Numerically, the highest number of *M. incognita* females was observed on homo-grafted Rutgers. The lowest number *M. incognita* females were observed on homo-grafted Anahu. When *T. vaporariorum* was present, the *M. incognita* final female population densities also were not significantly different (Table 32). The highest and lowest population densities were observed on non-grafted Rutgers and hetero-grafted Rutgers, respectively.

Biomass Partitioning in the Presence and Absence of *Trialeurodes vaporariorum* and *Meloidogyne incognita*

Plants associated with all six treatments allocated greater biomass towards to stem/leaf compared to root tissue (Figure 24). After grafting, homo-grafted Anahu and Hetero-grafted Anahu shifted their biomass allocation more towards stem/leaf tissue than root tissue, compared to non-grafted Anahu. Grafting also impacted homo-grafted Rutgers and hetero-grafted Rutgers, where

biomass allocation shifted to stem/leaf compared to root tissue associated with non-grafted Rutgers. When *T. vaporariorum* was present, non-grafted Anahu shifted its biomass towards stem/leaf rather than root tissue (Figure 25). The same effect was observed on non-grafted Rutgers where 2% of biomass shifted from root to stem/leaf in the presence of *T. vaporariorum*. Homo-grafted Anahu did not follow this pattern, but rather shifted 1% towards to root fresh weight in the absence compared to the presence of *T. vaporariorum*. On homo-grafted Rutgers, biomass shifted more towards to stem/leaf in the presence of *T. vaporariorum*. Hetero-grafted Anahu biomass partitioning was identical to homo-grafted Anahu, but compared to hetero-grafted Anahu in the absence it partitioned 1% less of its biomass to stem/leaf than root fresh weight. Hetero-grafted Rutgers had the highest biomass partitioned towards stem/leaf among the six treatments. Hetero-grafted Rutgers shifted 4% of the biomass from root fresh to stem/leaf weight in the presence of *T. vaporariorum*.

Relationship between *Solanum lycopersicum* L Root Fresh Weight and Trialeurodes vaporariorum

Fresh root weight was used as a predictor variable and *T. vaporariorum* population density used as response variable. When 18 data points were pooled together from the six treatments, fresh root weight used to predict *T. vaporariorum* population density with the increase of the root fresh weight the *T. vaporariorum* population density increased (Figure 26). When data points were grouped by grafting treatments, the results were very different. Non-grafted Anahu had a negative impact on *T. vaporariorum* population density (Figure 27,Figure 28). An increase in root fresh weight of non-grafted Rutgers had no impact on the *T. vaporariorum* population density (Figure 27Figure 28). Homo-grafted Anahu did not impact the *T. vaporariorum* population density with its increase in root fresh weight. Homo-grafted Rutgers, however, had a positive impact on the *T. vaporariorum* population density associated with the root fresh weight

increase (Figure 28). *T. vaporariorum* population density increases for hetero-grafted Anahu and hetero-grafted Rutgers were associated with the increase in root fresh weight (Figure 29).

Discussion

This study was carried out to test the impact of grafting on *T. vaporariorum* in the presence of *M. incognita*. Not all Mi gene resistant *S. lycopersicum* cultivars possess complete resistant to *T. vaporariorum*. Partial resistance, however, is important in pest control and can be responsible for decreases in chemical pesticide application. Thus, we evaluated the impact of grafting on resistance of two *S. lycopersicum* cultivars, Anahu (Mi gene resistance reported to *Bemisia tabaci*) and Rutgers (Mi gene absent) after 20-25 days under growth chamber conditions.

Mini insectaries were used to prevent *T. vaporariorum* dispersal from the plants. This mini insectary system had both advantages and disadvantages. The advantage is that the mini insectary prevented *T. vaporariorum* dispersal, allowing us to accurately determine the exact population density of *T. vaporariorum.* The disadvantage is that the material used to build the insectaries can prevent direct light and also increase temperature inside the insectary (Crafts-Brandner & Chu, 1999). In our five-female oviposition system, the total number of eggs produced by five female of *T. vaporariorum* ranged from 25-129, with a mean of 72 per female at 26 C. This is consistent with the approximately 30 eggs per female at 27 C and 124 eggs per female at 24 C reported by Burnett (1949). *T. vaporariorum* oviposition rate is known to decrease with temperature above 18 C when reared on tomato plants (Burnett 1949). Thus, the number of eggs laid per female in our study appeared to not be impacted by our caging method.

Our findings suggest that the rootstock Anahu transfers its resistance to the susceptible Rutgers scion. Although *T. vaporariorum* population densities on hetero-grafted Rutgers (Rutgers scion and Anahu rootstock) were not significantly different than those associated with non-grafted Rutgers or homo-grafted Rutgers, there was a pattern indicating a decrease in the *T. vaporariorum*

population density on hetero-grafted Rutgers plants (Table 18). The results of our study are in agreement with findings of Lucatti (2010), where the Mi gene carrying *S. lycopersicum* cv Uco Plata supported a lower *T. vaporariorum* population density in a free choice assay, compared to plants without the Mi gene. In our research, however, all experimental units were inoculated with *M. incognita* $P_{i=5000}$. *T. vaporariorum* developed significantly less on non-grafted Anahu, compared to non-grafted Rutgers. A possible explanation for this is the Mi gene or related gene responsible for resistance to *T. vaporariorum* was dormant or turned-off in the absence of *M. incognita* and functional in the presence of *M. incognita*, making the plants more resistant to *T. vaporariorum*. Interestingly, *T. vaporariorum* population density was less on homo-grafted Anahu and homo-grafted Rutgers compared to their non-grafted peers. A possible explanation for this is that grafting made the plant more vigorous after homo-grafting (Khah, Kakava, Mavromatis, Chachalis, & Goulas, 2006).

Type-D trichomes are partially responsible for resistant to pests in tomato (Aina, Rodriguez, and Knavel 1972). In this study, the trichomes were not evenly distributed on the leaves of *S. lycopersicum* cvs Anahu and Rutgers in the presence of *M. incognita*. The Type-D trichome density was greatest in the upper region of both cultivars. In addition, we did not find significant differences in trichome Type-D density between Anahu and Rutgers. Thus, we propose that Type-D trichome is not responsible for *T. vaporariorum* resistance in Anahu. Nombela et al. (2000), similarly, found no association between Type-D trichome density and resistance to *Bemisia argentifolii* and low Type-D trichome density was associated on Mi gene carrying *S. lycopersicum* cultivars compared to cultivars without the Mi gene. The presence of *M. incognita* did not impact the overall trichome Type-D density.

Stem and leaf tissue fresh and dry weights were not impacted by the presence of *T. vaporariorum*. In contrast, Pofu (2012) found that the presence of *T. vaporariorum* and *M. javanica* decreased the dry weight of shoot and root tissues by 86 % and 60 %, respectively. We found that root tissue fresh and dry weight differed when *T. vaporariorum* was absent, however when *T. vaporariorum* was present, the weights were not significantly different. In addition, the presence of *T. vaporariorum* increased the *M. incognita* final female population density in all grafted treatments except hetero-grafted Rutgers. Similarly, Pofu et al. (2012) found that *M. javanica* population density increased in wild watermelon in the presence of *T. vaporariorum.*

Biomass partitioning of *S. lycopersicum* cvs Anahu and Rutgers was not impacted by the presence of *T. vaporariorum*. When all of the root tissue fresh weight data from the six grafted treatments were pooled, and regressed against the *T. vaporariorum* population density, there was a positive correlation (Table 20). One possible explanation is that homo-grafted Rutgers, hetero-grafted Anahu, and hetero-grafted Rutgers had positive correlations, with the correlation being close to 1.00, thus the pooled date became a positively correlated (Figure 28,Figure 29). The facts that non-grafted Anahu and non-grafted Rutgers had negative correlations and homo-grafted Anahu had a correlation that was close to 0.00 decreased the positive correlation coefficient of the pooled data. This is an excellent example why it is important to evaluate both pooled date and from the individual grafting treatment.

Conclusion

S. lycopersicum cv Anahu is only partially resistant to *T. vaporariorum* in the presence of *M. incognita*. In this research, we proved experimentally that the presence of the *M. incognita* delayed life cycle of the *T. vaporariorum* population density. Nevertheless, partial resistance can be beneficial in the management of *T. vaporariorum* when used with conjunction of chemical insecticides. The action threshold for *T. vaporariorum* is ten females or eight nymphs per tomato

leaf (Polack, 2005). In our research, the initial population density of *T. vaporariorum* did not exceed Polack's threshold in any of our grafting treatments. Thus, we conclude that it is necessary to conduct future research that will involve multiple generations of *T vaporariorum*. Moreover, the presence of *T. vaporariorum* increased the *M. incognita* final female population density.

APPENDICES

Appendix A. Statistical Tables

Table 18. *T. vaporariorum* population density on grafted *Solanum lycopersicum* after 25 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean	SE
Anahu	**3**	**48.00 ab**[1]	**5.03**
Anahu/Anahu	**3**	**26.67 a**	**1.20**
Rutgers/Anahu	**3**	**74.67 b**	**7.22**
Anahu/Rutgers	**3**	**100.00 c**	**19.47**
Rutgers/Rutgers	**3**	**87.67 bc**	**23.25**
Rutgers	**3**	**93.00 c**	**12.42**

[1] Data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD for separation of means. Different letter in a column are statistically significant at α=0.05.

Table 19. Grafted *Solanum lycopersicum* Type-D trichome density after seven days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean	SE
Anahu	**18**	**56.83**[1]	**12.69**
Anahu/Anahu	**18**	**32.89**	**6.67**
Rutgers/Anahu	**18**	**45.50**	**11.06**
Anahu/Rutgers	**18**	**66.61**	**18.42**
Rutgers/Rutgers	**18**	**27.00**	**6.94**
Rutgers	**18**	**39.72**	**7.05**

[1] Data violated the Homogeny of variance. Natural log transforming the data normalized. The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 20. *Solanum lycopersicum* L Type-D Trichome density at three plant heights after seven days in the presence of *M. incognita.*

Leaf location	N	Mean	SE
Lower region	**36**	**22.83 a**[1]	**2.83**
Mid region	**36**	**38.81 a**	**6.37**
Upper region	**36**	**72.64 b**	**10.77**

[1] Data violated the Homogeny of variance. Natural log transforming the data normalized. The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 21. Grafted *Solanum lycopersicum* Type-D trichome density after 25 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean	SE
Anahu	**18**	**61.22**[1]	**13.25**
Anahu/Anahu	**18**	**49.78**	**13.17**
Rutgers/Anahu	**18**	**65.17**	**15.83**
Anahu/Rutgers	**18**	**60.00**	**16.56**
Rutgers/Rutgers	**18**	**51.72**	**12.36**
Rutgers	**18**	**30.61**	**4.78**

[1] Data violated the Homogeny of variance. Natural log transforming the data normalized. The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different latter on a column are statistically significant.

Table 22. *Solanum lycopersicum* L Type-D Trichome density at plant three plant heights after 25 days in the presence of *Meloidogyne. incognita*.

Leaf location	N	Mean	SE
Lower region	**36**	**31.81 a**[1]	**4.72**
Mid region	**36**	**39.00 a**	**4.06**
Upper region	**36**	**88.44 b**	**13.15**

[1] Data violated the Homogeny of variance. Natural log transforming the data normalized. The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 23. Grafted *Solanum lycopersicum* fresh weight of stem and leaf tissue after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**5**	**48.26**[1]	**2.31**
Anahu/Anahu	**5**	**47.39**	**2.44**
Rutgers/Anahu	**5**	**46.37**	**3.25**
Anahu/Rutgers	**5**	**46.06**	**1.84**
Rutgers/Rutgers	**5**	**46.43**	**1.56**
Rutgers	**5**	**46.95**	**0.35**

[1]Data violated the Homogeny of variance. Natural log or log 10 transforming the data did not normalize it. Means of experiment analyzed by Kruskal-Wallis Test. If there was statistical significant at α=0.05 followed by Mann-Whitney U.

Table 24. Grafted *Solanum lycopersicum* dry weights of stem and leaf tissue after 24 days in the presence of with *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**5**	**6.84**[1]	**0.16**
Anahu/Anahu	**5**	**6.37**	**0.43**
Rutgers/Anahu	**5**	**7.60**	**0.65**
Anahu/Rutgers	**5**	**6.46**	**0.56**
Rutgers/Rutgers	**5**	**6.87**	**0.30**
Rutgers	**5**	**7.79**	**0.34**

[1] The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 25. Impact of Trialeurodes vaporariorum to grafted *Solanum lycopersicum* fresh weight of stem/leaf after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**3**	**49.81**[1]	**2.29**
Anahu/Anahu	**3**	**44.78**	**2.69**
Rutgers/Anahu	**3**	**48.83**	**1.13**
Anahu/Rutgers	**3**	**51.49**	**2.32**
Rutgers/Rutgers	**3**	**49.00**	**2.22**
Rutgers	**3**	**48.45**	**1.22**

[1] The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 26. Impact of *Trialeurodes vaporariorum* on grafted *Solanum lycopersicum* dry weight of stem and leaf tissue after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**3**	**6.12**[1]	**0.40**
Anahu/Anahu	**3**	**5.71**	**0.64**
Rutgers/Anahu	**3**	**6.19**	**0.35**
Anahu/Rutgers	**3**	**6.48**	**0.21**
Rutgers/Rutgers	**3**	**6.36**	**0.51**
Rutgers	**3**	**5.83**	**0.19**

[1] Data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 27. Grafted *Solanum lycopersicum* fresh weight of root tissue after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**5**	**14.94 abc**[1]	**0.73**
Anahu/Anahu	**5**	**12.76 a**	**0.67**
Rutgers/Anahu	**5**	**15.16 bc**	**0.62**
Anahu/Rutgers	**5**	**13.22 ab**	**0.61**
Rutgers/Rutgers	**5**	**15.23 bc**	**0.88**
Rutgers	**5**	**16.30 c**	**0.94**

[1] The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 28. Grafted *Solanum lycopersicum* dry weight of root tissue after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**5**	**0.85 bc**[1]	**0.07**
Anahu/Anahu	**5**	**0.65 a**	**0.06**
Rutgers/Anahu	**5**	**0.90 cd**	**0.06**
Anahu/Rutgers	**5**	**0.67 ab**	**0.05**
Rutgers/Rutgers	**5**	**0.95 cd**	**0.06**
Rutgers	**5**	**1.07 d**	**0.08**

[1] The data analyzed by One Way ANOVA. If the ANOVA is statistically significant in α=0.05 followed by Fisher's LSD. Different letter in a column are statistically significant.

Table 29. Impact of *Trialeurodes vaporariorum* on grafted *Solanum lycopersicum* root fresh weight after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean	SE
Anahu	**3**	**14.40**[1]	**0.24**
Anahu/Anahu	**3**	**13.45**	**1.22**
Rutgers/Anahu	**3**	**12.66**	**0.69**
Anahu/Rutgers	**3**	**15.61**	**0.31**
Rutgers/Rutgers	**3**	**14.70**	**1.09**
Rutgers	**3**	**15.39**	**0.39**

[1]Data violated the Homogeny of variance. Natural log or log 10 transforming the data did not normalize it. Means of experiment analyzed by Kruskal-Wallis Test. If there was statistical significant at α=0.05 followed by Mann-Whitney U.

Table 30. Impact of *Trialeurodes vaporariorum* on grafted *Solanum lycopersicum* root dry weight of root tissue after 24 days in the presence of *Meloidogyne incognita*.

Grafting Treatment	N	Mean (g)	SE
Anahu	**3**	**0.73**[1]	**0.04**
Anahu/Anahu	**3**	**0.64**	**0.13**
Rutgers/Anahu	**3**	**0.62**	**0.02**
Anahu/Rutgers	**3**	**0.87**	**0.04**
Rutgers/Rutgers	**3**	**0.87**	**0.08**
Rutgers	**3**	**0.87**	**0.02**

[1]Data violated the Homogeny of variance. Natural log or log 10 transforming the data did not normalize it. Means of experiment analyzed by Kruskal-Wallis Test. If there was statistical significant at α=0.05 followed by Mann-Whitney U.

Table 31. *Meloidogyne incognita* final female population density on six grafted *S. lycopersicum* L. treatments.

Grafting Treatment	N	Mean	SE
Anahu	**5**	**459.00**	**291.42**
Anahu/Anahu	**5**	**347.66**	**204.09**
Rutgers/Anahu	**5**	**898.01**	**254.72**
Anahu/Rutgers	**5**	**1144.21**	**95.84**
Rutgers/Rutgers	**5**	**1149.32**	**137.81**
Rutgers	**5**	**893.52**	**261.33**

Table 32. *Meloidogyne incognita* final female population density on six grafted *S. lycopersicum* L treatments in the presence of *Trialeurodes vaporariorum*.

Grafting Treatment	N	Mean	SE
Anahu	**3**	**1134.40**	**587.04**
Anahu/Anahu	**3**	**688.05**	**648.71**
Rutgers/Anahu	**3**	**493.52**	**474.41**
Anahu/Rutgers	**3**	**1759.56**	**437.62**
Rutgers/Rutgers	**3**	**1522.29**	**539.14**
Rutgers	**3**	**2153.44**	**357.74**

Appendix B. Figures

Figure 24. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the presence of *M. incognita* ($P_{i = 5000}$).

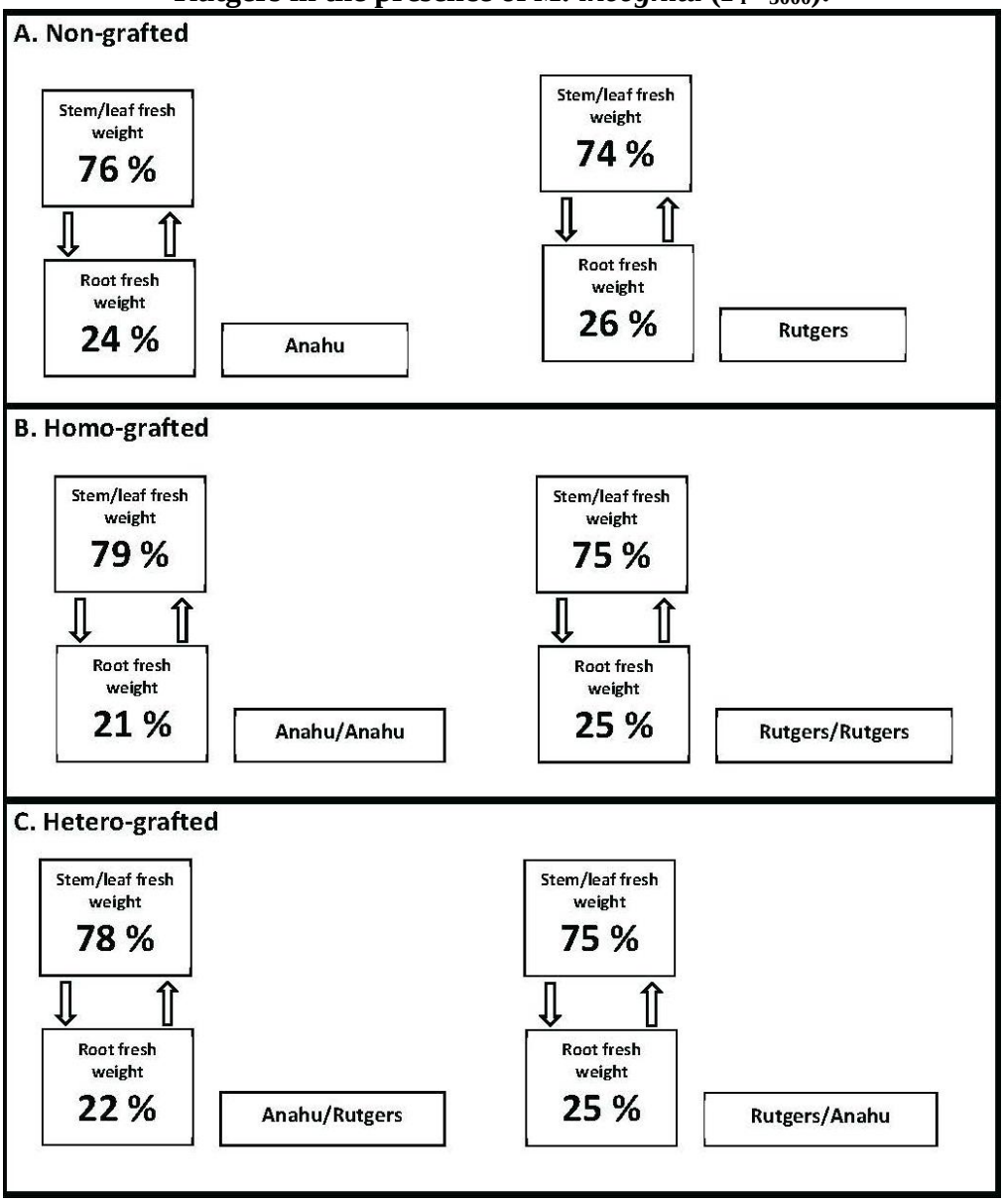


Figure 25. Biomass partitioning signatures of *Solanum lycopersicum* L. cvs Anahu and Rutgers in the presence of *M. incognita* ($P_{i=5000}$) and *T. vaporariorum*.

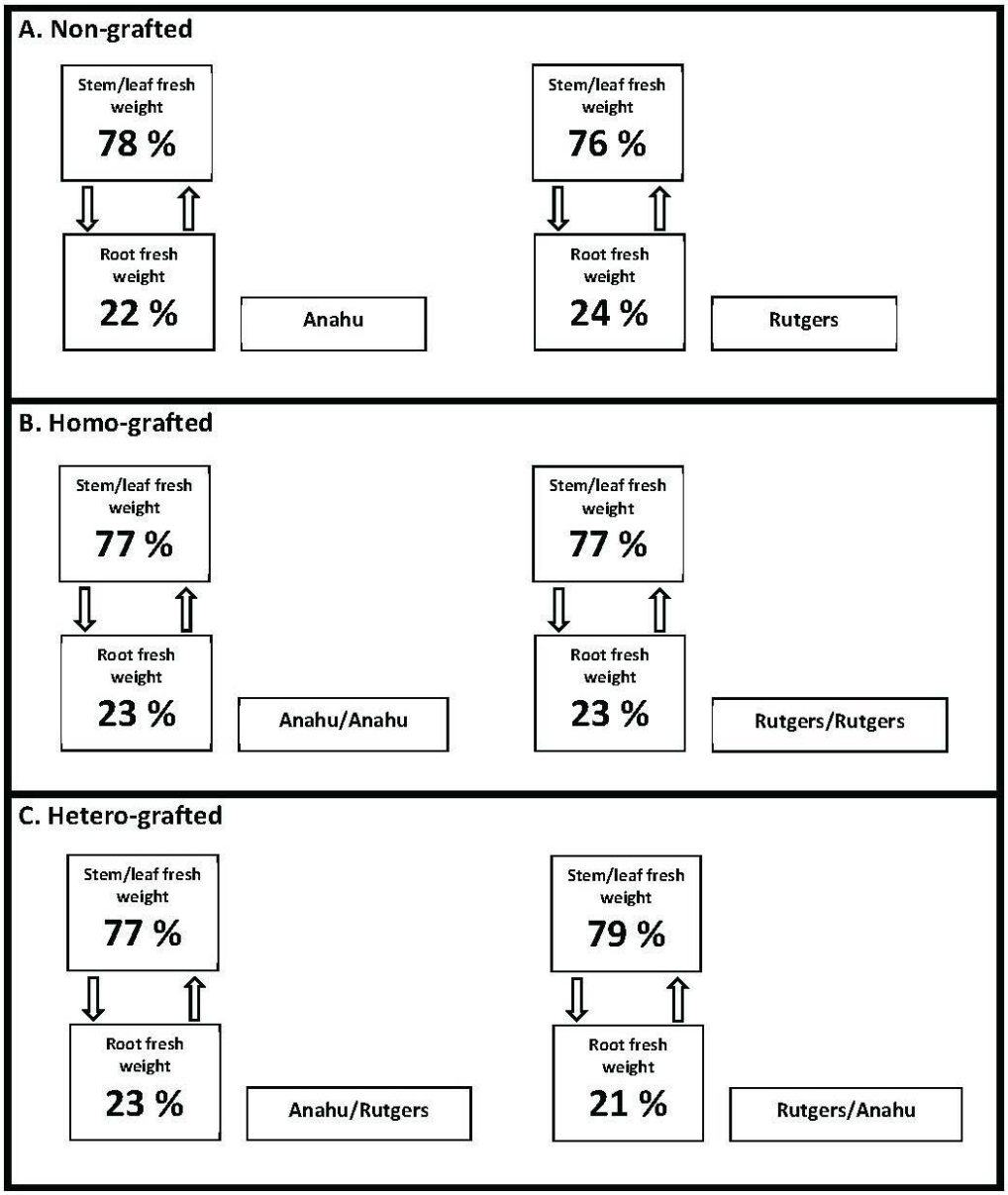


Figure 26. Relationship between *S. lycopersicum* root fresh weight and *T. vaporariorum* population density per plant.

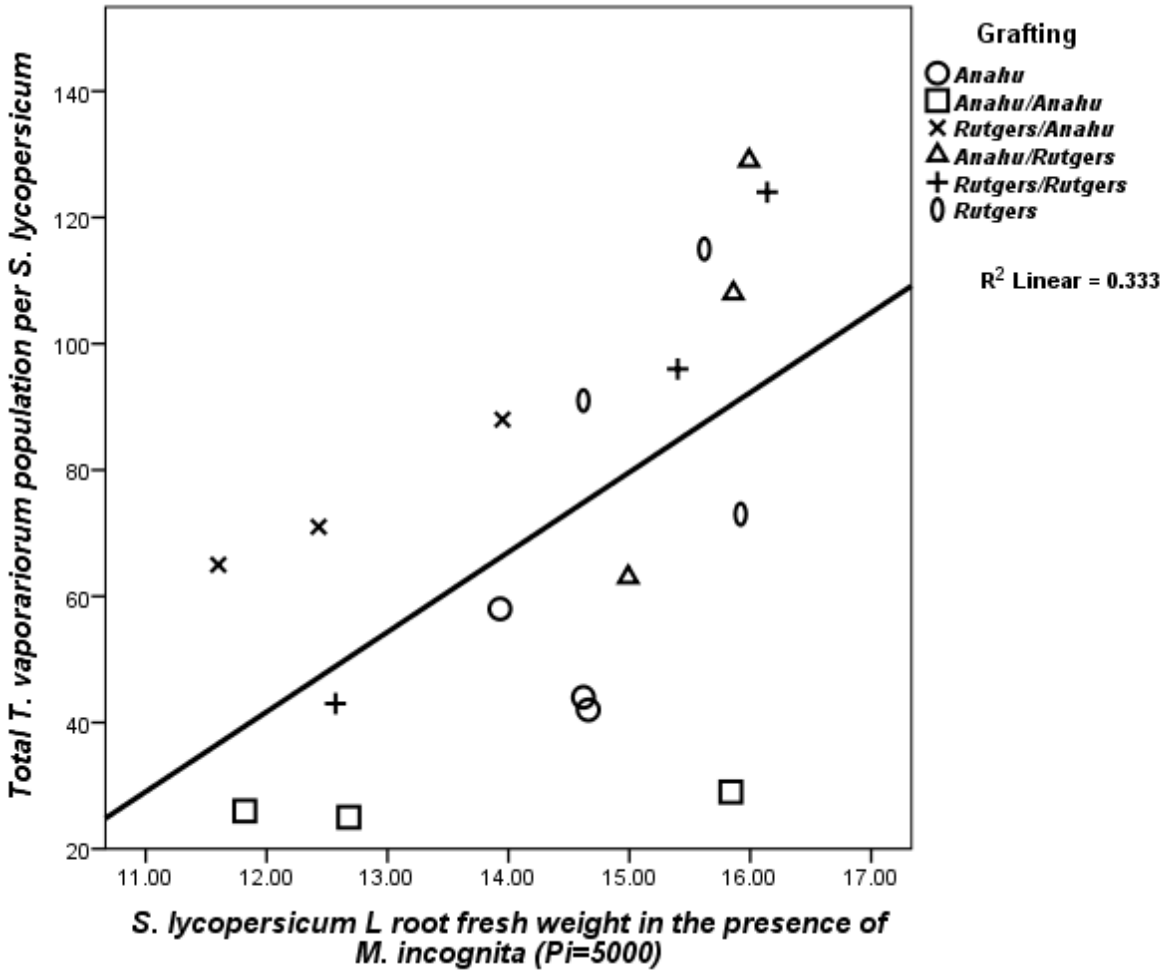


Figure 27. Relationship between *S. lycopersicum* L cv Anahu and cv Rutgers root fresh weight and *T. vaporariorum* population density per plant.

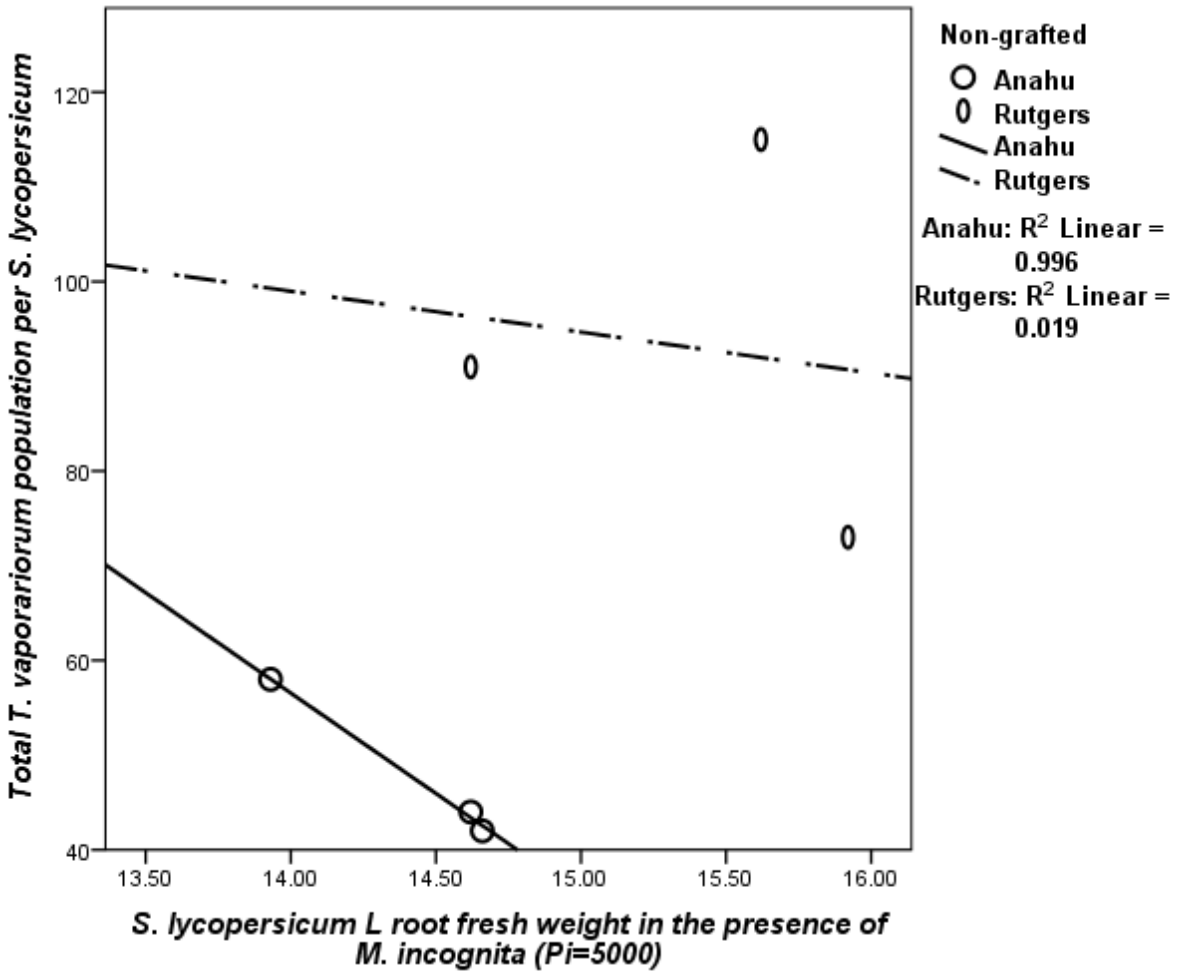


Figure 28. Relationship between *S. lycopersicum* L homo-grafted Anahu and homo-grafted Rutgers root fresh weight and *T. vaporariorum* population density per plant.

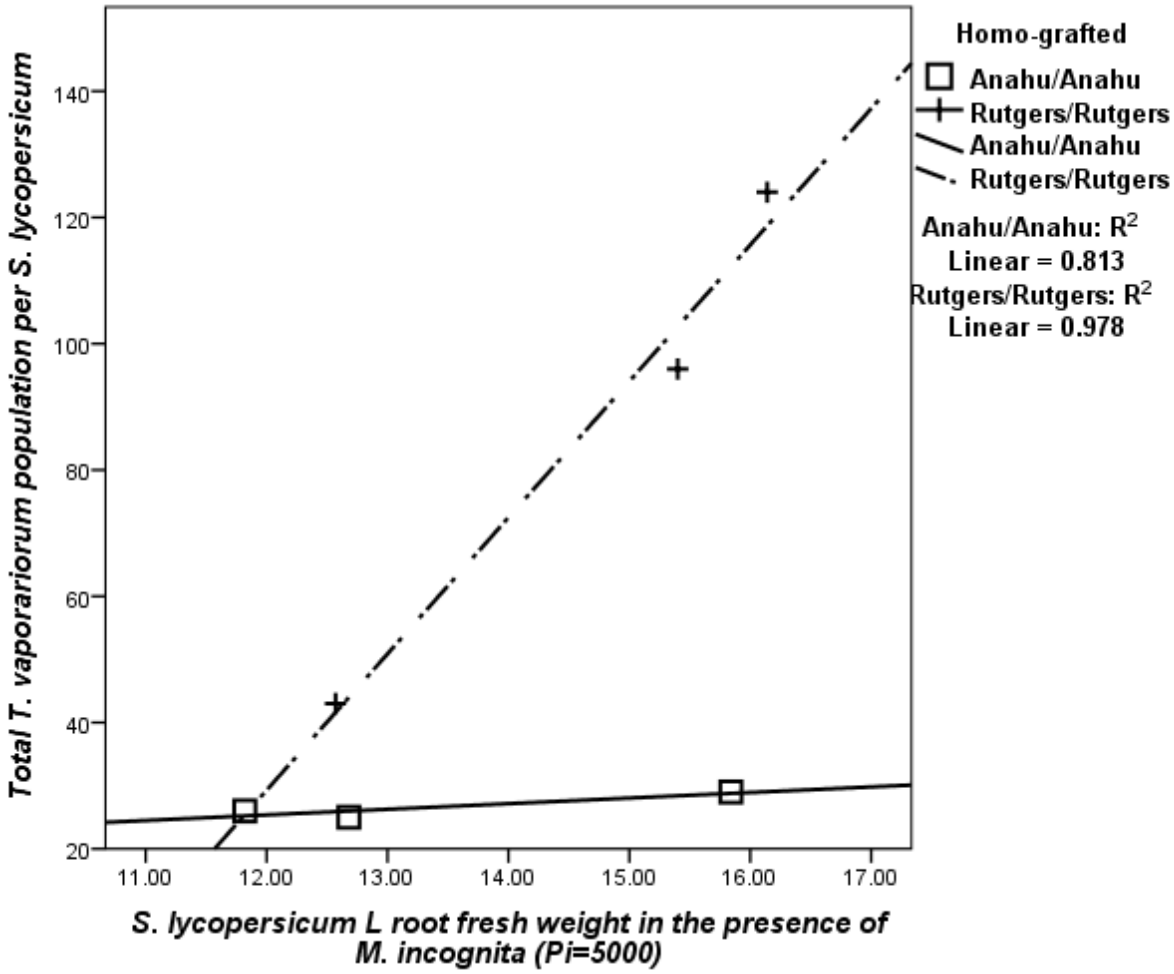
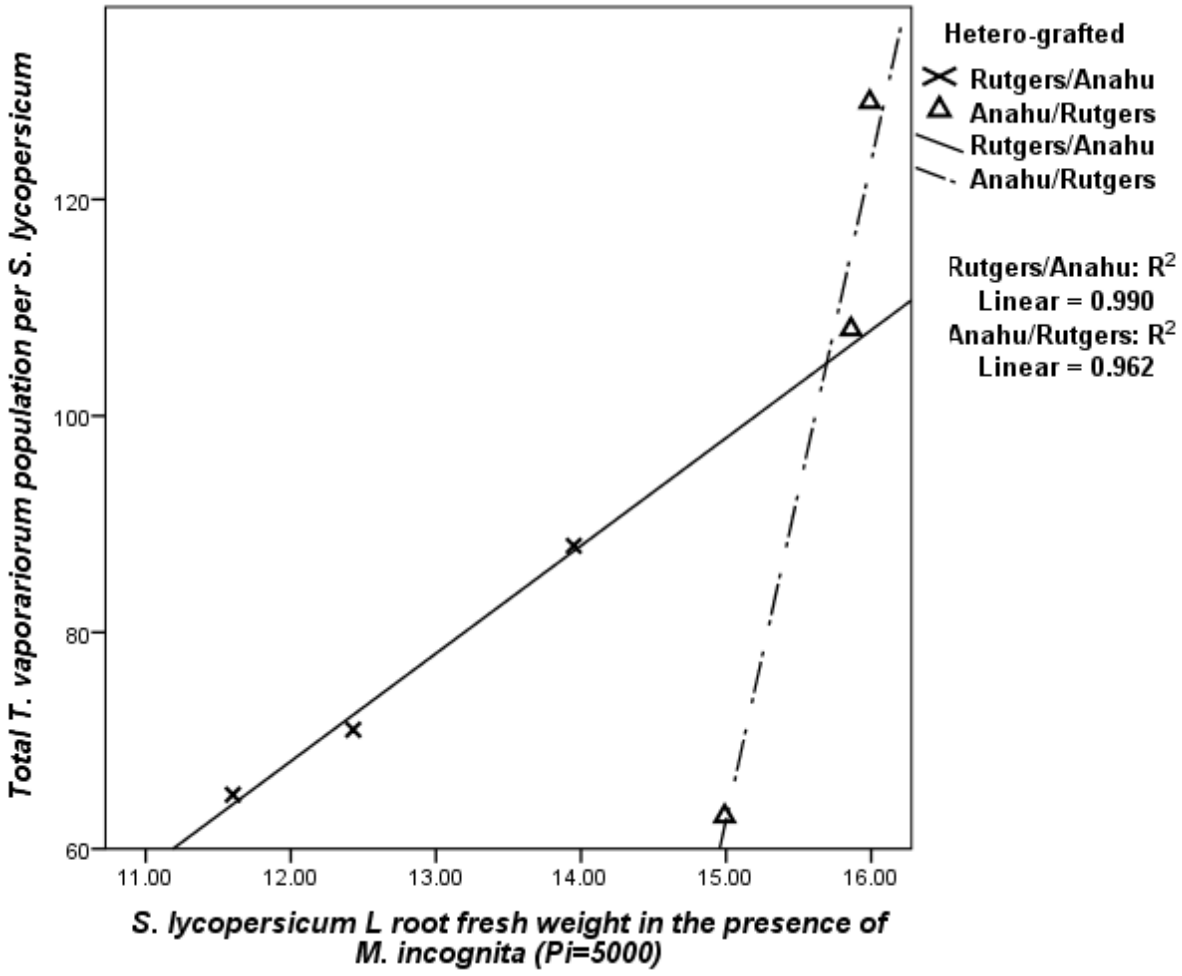


Figure 29. Relationship between *S. lycopersicum* L hetero-grafted Anahu and hetero-grafted Rutgers root fresh weight and *T. vaporariorum* population density per plant.



Appendix C. Specimens

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: _____2017-03_____

Author and Title of thesis:

Bahodir Ruzumboevich Eshchanov

"Influence of Mi Genes on Grafted *Solanum Lycopersicum* L. Cultivars for Control of *Trialeurodes Vaporariorum* (Insecta) in the Presence and Absence of *Meloidogyne Incognita* (Nematoda)."

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Table 33. List of voucher specimens.

Family	Genus/Species	Life Stage	Quantity	Preservation
Aleyrodidae	*Trialeurodes vaporariorum*	Adult	5	Ethanol
Aleyrodidae	*Trialeurodes vaporariorum*	Pupa	5	Ethanol
Aleyrodidae	*Trialeurodes vaporariorum*	Nymph	5	Ethanol

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CONCLUSION

A significant number of important conclusions can be drawn from the results of my Ph.D. dissertation research. Some of these findings are completely new; whereas, others support or contradict the discoveries of other scientists. The major findings of this Ph.D. research program are:

- *Solanum lycopersicum* cultivars Anahu and Rutgers have unique biomass partitioning signatures and allocate their resources differently under greenhouse conditions.

- Anahu biomass allocation is not favorable for greenhouse *S. lycopersicum* production systems.

- Lightly and heavily pruned *S. lycopersicum* allow for more plants per unit area and *S. lycopersicum* yield production is increased by increased plant numbers.

- With the lack of environmental stress, grafted plants produced similar yields under field conditions.

- Fresh and dry biomass of homo-grafted and hetero-grafted plant were significantly less than non-grafted plants at the early stages of the growth period. This difference, however, was not present at the end harvest.

- Biomass allocation is a cultivar specific characteristic and the biomass partitioning signature of a rootstock can manipulate scion biomass allocation.

- Understanding the biomass signature of a cultivar is important before selecting rootstocks for vegetable grafting.

- Anahu, with the Mi resistant gene for *M. incognita*, was not completely resistant to *M. incognita*.

- Pruning significantly increased the final population density of *M. incognita* in both resistant and susceptible plants.

- Young Anahu plants with the Mi gene are not resistant to *T. vaporariorum*.

- Anahu rootstock influenced Rutgers scion resulting in *T. vaporariorum* population density being significantly less on hetero grafted Rutgers than non-grafted Rutgers and hetero-grafted Anahu.

- The presence of *M. incognita* improved the Anahu's resistance to *T. vaporariorum* in 20 to 25 day-old plants.

- *T. vaporariorum* resistance is not related to Type-D Trichome density in Anahu.

- Type-D Trichome density was greater on upper, compared to lower or mid-region leaves. This was not impacted by *M. incognita*.

- Future research recommendations

- Hetero-grafted and homo-grafted *S. lycopersicum* cultivars should be tested in the presence of *M. incognita* under field conditions.

- The entire life cycle of *T. vaporariorum* has to be tested under a distractive experimental design in the presence of *M. incognita* on *S. lycopersicum* cultivar Anahu that caries Mi gene.

- Anahu plants older than 20-25 days need to be tested for resistance to *T. vaporariorum.*

We researched Anahu for biomass allocation and resistance to *M. incognita* and *T. vaporariorum*. The results indicate that Anahu is not completely resistant to *M. incognita* and *T. vaporariorum*. Furthermore, Anahu did not show any significant difference in response to *T. vaporariorum* in the absence of the *M. incognita*. We proved that the Mi gene or other genes that lowered the *T. vaporariorum* population significantly were turned on in the presence of *M. incognita*. The most interesting finding is that the Anahu rootstock significantly impacted *T. vaporariorum* population density on Rutgers scion in the presence of the *M. incognita*.