

The Influence of Soil Nitrogen Level on Tomato Defence against Whiteflies

BY

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Abstract

Whiteflies are one of the leading causes of tomato yield loss worldwide. Phloem feeders, including whiteflies, prefer foliage with high nitrogen (N) content because organic N is a limiting factor for herbivores. Since soil N is commonly insufficient for adequate crop growth, farmers supplement soils with N-containing fertilizers. While an adequate supply of N and other nutrients is necessary for growth and fruit production, by increasing the N content of the plant tissues, soil N supplementation could cause the plants to be less resistant to whiteflies. Therefore, there must be a level of fertilizer addition beyond which the losses in fruit production caused by greater attraction and feeding of pests exceed the gains brought about by N availability. In this study, we grew four commercial tomato varieties under three levels of soil N fertilization and infested half of these plants with whiteflies after eight weeks of growth. After 20 weeks of growth, we compared the yield and resource allocation in whitefly infected and uninfected plants to find whether soil N supplementation results in greater susceptibility of tomato plants to whitefly infestation and if so, to what extent; and to find whether some varieties of tomato are more resistant or tolerant to whiteflies. We observed no significant reduction in fruit production whereas the resistance increased significantly when the amount of N addition was reduced to half of the commercially recommended amount. The varieties differed in resistance and tolerance to whiteflies and a tradeoff between resistance and tolerance was also noted. Afamia was the most resistant and the least tolerant variety, while Conquistador was the least resistant and the most

tolerant variety. Enhancing the natural defences of tomato varieties by lowering fertilizer application could improve the farmers' economic benefits, thereby balancing the losses in yield due to lower fertilizer application. Decreased use of fertilizers and pesticides can also reduce social and environmental costs of large-scale use of agricultural chemicals.

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Table of Contents

Abstract.....	ii
Acknowledgement.....	iv
Table of contents.....	vi
List of Tables.....	vii
List of Figures.....	x
Introduction.....	1
Chapter I: Selection and propagation of biological materials.....	13
Table 1.1.....	17
Figure 1.1.....	18
Chapter II: Resistance and Tolerance of Tomatoes to Whiteflies at Different Nitrogen Levels	
2.1 Introduction.....	20
2.2 Methods.....	24
2.3 Results.....	31
2.4 Discussion.....	34
Tables.....	41
Figures.....	44
References.....	50
Appendices.....	61

List of Tables

Table 1.1	Flower and fruit production (mean \pm SE) of six tomato varieties grown for five months at the Centennial greenhouse of The University of Winnipeg (N = 5).	19
Table 2.1	Composition of modified Hoagland's nutrient solution.	45
Table 2.2	General linear model for total chlorophyll content of four tomato varieties grown at three N levels in greenhouse.	46
Table 2.3	General linear model for total fruit fresh weight of four tomato varieties grown at three N levels.	46
Table 2.4	General linear model for total fruit dry weight of four tomato varieties grown at three N levels.	47
Table 2.5	General linear model for seed production of four tomato varieties grown at three N levels.	47
Table 2.6	General linear model for total seed weight of four tomato varieties grown at three N levels.	48
Table 2.7	General linear model for vegetative biomass of four tomato varieties grown at three N levels.	48
Table 2.8	General linear model for root to shoot ratio of four tomato varieties grown at three N levels.	49
Table 2.9	General linear model for resistance of four tomato varieties grown at three N levels against whiteflies.	49
Table 2.10	General linear model for germination proportion of four tomato varieties grown at three N levels.	50
Table 2.11	General linear model for compensatory ability of whitefly-infested tomato plants of four varieties grown at three N levels.	50
Table 2.12	Regression analysis showing relation between resistance and compensatory ability of four tomato varieties.	50
Table A1.1	General linear model (full model with interactions) for total seeds per plant of four tomato varieties grown at three N levels.	71

List of Tables (continued)

Table A1.2	General linear model for chlorophyll a content of four tomato varieties grown at three N levels in greenhouse.	71
Table A1.3	General linear model for chlorophyll b content of four tomato varieties grown at three N levels in greenhouse.	72
Table A2.1	Descriptive Statistics for weight gained by five pieces of tomato fruits of the variety Avalantino in 48 hr. The fresh weight of the pieces ranged from 2.88 g to 56.64 g. (n = 7)	78
Table A2.2.	Descriptive Statistics for weight gained by five pieces of tomato fruits of the variety Roma in 48 hr. The fresh weight of the pieces ranged from 2.77 g to 101.42 g.(n = 7)	78
Table A2.3	Descriptive Statistics for the total dry weight of fruits from the tomato variety, Afamia. Twenty plants were grown under three N levels.	78
Table A2.4	Descriptive Statistics for dry weight of fruits from the tomato variety, Patrón. Twenty plants were grown under three N levels.	79
Table A2.5	Descriptive Statistics for dry weight of fruits from the tomato variety, Conquistador. Twenty plants were grown under three N levels.	79

List of Figures

Figure 1.1	Cumulative number of flowers on plants of six tomato varieties grown at Centennial greenhouse in Winter 2016.	20
Figure 2.1	Effect of N level on total chlorophyll content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	51
Figure 2.2	Effect of variety on total chlorophyll content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	51
Figure 2.3	Effect of N on total fruit fresh weight produced per plant. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	52
Figure 2.4	Effect of N on fruit dry weight. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	52
Figure 2.5	Effect of N on total seeds produced per plant. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	53

List of Figures (continued)

Figure 2.6	Effect of variety on the total seed mass produced per plant. LS means of the values from a GLM, including variety, N level, and flies as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	53
Figure 2.7	Effect of N on vegetative biomass of four tomato varieties grown under three different N levels. LS means of the values from a GLM, including variety, N level, and flies as factors, are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	54
Figure 2.8	Effect of variety on vegetative biomass of four tomato varieties grown under three different N levels. LS means of the values from a GLM, including variety, N level, and flies as factors, are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis at 90% confidence level.	54
Figure 2.9	Effect of N on the root to shoot ratio of four tomato varieties grown at three N levels. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	55
Figure 2.10	Effect of variety on the root to shoot ratio of four tomato varieties grown at three N levels. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.	55

List of Figures (continued)

Figure 2.11	Effect of N and variety on resistance to whiteflies (24 hr). LS means from a GLM with N level, variety, and the interaction of the two are plotted. Data were log transformed for analysis.	56
Figure 2.12	The relationship between the total number of seeds produced per plant and their germination proportion obtained by germinating 10 seeds from a randomly-selected fruit from each plant.	56
Figure 2.13	Trade-off between resistance and compensatory ability among four varieties studied. LS means of resistance from a GLM for the choice test (with variety, N level, and the interactions of the two factors as factors) is plotted against LS means of compensatory ability from the GLM (with variety and N level as factors) for compensatory ability. Data for resistance were log transformed and data for compensatory ability were square root transformed and 1 is added to all the data points.	57
Figure A1.1	Chlorophyll _a content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering.	73
Figure A1.2	Chlorophyll _b content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering	74

List of Figures (continued)

Figure A1.3	Effect of N and variety on resistance to whiteflies (6 hr). LS means from a GLM with N level, variety, and the interaction of the two are plotted. Data were log transformed for analysis	75
Figure A1.4	Boxplot of number of nymphs on a leaflet of the second mature leaf from the apex of the plants inside the full cage with whiteflies.	76

INTRODUCTION

For the past several decades, research on plant defence has been a highly active area in plant biology, ecology, and evolution (Grime *et al.*, 1968; Herms and Mattson, 1992; Lucas-Barbosa, 2016). As the majority of energy supporting organisms in food webs comes from the autotrophic action of plants, one of the most prominent sets of adaptations in the history of life is plant defence against natural enemies. Herbivores remove >20% of annual net primary productivity by folivory, sap feeding, and root herbivory (Agrawal, 2011).

Plant defensive strategies against herbivory typically include a broad set of traits that either reduce the probability of being attacked by herbivores (resistance), or diminish the negative fitness effects of the loss of tissue to herbivores (tolerance) (Agrawal and Fishbein 2006; Karban and Baldwin, 1997).

Resistance traits include physical and chemical traits. Physical traits include trichomes (hairs), thicker or tougher cuticles and cell walls, and thorns whereas chemical traits include several secondary metabolites with toxic or anti-digestive properties and volatiles that attract the natural enemies of herbivores (Karbon and Baldwin, 1997; Gong and Zhang, 2014). Examples of plant secondary metabolites used as defensive traits are cyanogenic glucosides, glucosinolates, non-protein amino acids, alkaloids, plant phenolics, phytoalexins, salicylic acid, and methyl jasmonate (Bennett and Wallsgrave, 1994). Similar resistance traits are used by distantly related plants to defend a common assemblage of herbivores. For example, plant species attacked primarily by vertebrate grazers employ spines, leaf toughness etc. while plants primarily attacked by caterpillars employ trichomes, toxins, and parasitoid-attracting volatiles.

Traits that contribute to tolerance are less well understood compared to resistance. These include enhanced photosynthetic efficiency, meristem availability, and the capacity to store and mobilize resources such as carbohydrates and nitrogenous compounds (Strauss and Agrawal, 1999; Tucker and Avila-Sakar, 2010). Some examples of tolerance mechanisms by plants to herbivory include induced compensatory photosynthesis in soybean (*Glycine max*) and drybean (*Phaseolus vulgaris*), of family Fabaceae, by insect herbivory and induced plant growth and reduced leaf total and essential amino acid concentrations in red raspberry (*Rubus idaeus*), of family Rosaceae, by aphid (*Amphorophora idaei*) herbivory (Peterson *et al.*, 1998; Johnson *et al.*, 2012; Karley *et al.*, 2016). Plants vary in tolerance response to different types of damage such as difference in response to apical meristem damage and folivory in *Ipomea purpurea* and difference in tolerance to natural levels of seed herbivory and folivory in *Brassica rapa* (Tiffin and Rausher, 1999; Pilson, 2000). In some plants, such as *Triadica sebifera*, the difference in tolerance traits depended on herbivore species (Carrillo *et al.*, 2014).

In natural habitats, plants are exposed to diverse species of herbivores which have specific eco-evolutionary dynamic with the host (Johnson and Stinchcombe, 2007). Resistance traits can be species-specific and specialist herbivores can adapt to several resistance traits such as specific plant secondary metabolites. An example for this is, the adaptation of specialist insect herbivores to cardenolides, found in plants of several families including Apocynaceae and Zingiberaceae (Agarwal *et al.*, 2012).

Whenever the benefits of resistance are reduced by the herbivores' adaptation, tolerance, which allow plants to compensate for damage by a wide

variety of herbivores, could play a major role in the plant's defence strategy (Fornoni, 2011). Therefore, resistance and tolerance can be considered redundant because they are both expected to confer greater fitness to plants in the presence of herbivores (Siddappaji *et al.*, 2015; Züst and Agrawal, 2017). Based on the principle of allocation, a tradeoff between resistance and tolerance is expected, especially if these traits are functionally redundant (Fineblum and Rausher, 1995; Strauss and Agrawal, 1999; Tucker and Avila-Sakar, 2010). However, the degree of redundancy may be less than originally proposed, as shown by Carmona and Fornoni (2013), who showed that, tolerance and resistance worked more as complementary rather than redundant defence mechanisms against herbivores with different degrees of specialization on *Datura stramonium*, of family Solanaceae.

Even though several hypotheses such as optimal defence, carbon: nutrient balance, growth rate, and growth-differentiation balance hypotheses are cited by researchers to explain mechanisms of plant defence, the expanded growth-differentiation balance hypothesis was suggested to be tested for achieving further clarity about the mechanisms of defence. The growth-differentiation balance hypothesis recognizes the constant physiological tradeoffs between growth and differentiation at the cellular and tissue levels relative to the selective pressures of resource availability; meanwhile it takes into account, plant tolerance of damage by enemies (Stamp, 2003). A recent study that tested this hypothesis using five nutrient levels in the tree *Sclerocarya birrea* (Anacardiaceae) showed limited allocation of carbon to defence (flavonols) at both low and high nutrient availability. This supports the humped response of resource allocation to plant secondary metabolites to nutrient

availability predicted by the growth-differentiation balance hypothesis (Scogings, 2018). On the contrary, a study on the vine *Combretum apiculatum* (Combretaceae) failed to explain allocation to secondary metabolites, which makes the applicability of this hypothesis to species under different abiotic and biotic conditions questionable, and generalizations problematic (Hattas *et al.*, 2017). More research is needed to understand the dynamic interplay between the ecology and evolution of plant-herbivore interactions.

Deploying crops' natural defence traits for pest management has important implications in sustainable agriculture. Global crop losses from damage caused by arthropod pests can exceed 15% annually (Birch *et al.*, 2011). Crop domestication and selection for improved yield and quality can alter the defensive capability of the crop, increasing reliance on crop protection using agrochemicals (Altieri, 2004). Sustainable agriculture, however, depends on reduced chemical inputs and therefore identifying plant defensive traits depending on the nature of damage inflicted by the pest is of great importance for crop improvement. For example, resistance traits are more desirable for maintaining disease vectors below threshold infestation densities, whereas tolerance traits are likely to be useful against non-vector pests that typically cause damage by removing resources and reducing plant growth (Mitchell *et al.*, 2016). This thesis explores the idea of taking advantage of intrinsic natural defences of crop plants while simultaneously considering exogenous factors that can alter defence against pests. I used the tomato-whitefly system because of the worldwide importance of tomato as a crop, and of whiteflies as one of the prevalent pests of this crop. In addition, I focused on nitrogen availability and its modification via fertilizer

application as one of the most important exogenous factors that can modify plant defence against herbivores.

Tomatoes are one of the most important commercial vegetables in the American continent, both in yearly weight consumed and annual production. They are the second most consumed vegetable and thereby the primary source of nutrients among fruits and vegetables. Tomatoes are rich in iron and vitamin A, C, and E (Römer *et al.*, 2000; Halvorsen *et al.*, 2002). Tomatoes provide approximately 0.8 mg of iron, 0.89–3.87 mg of various carotenoids (precursors of Vitamin A), 20 mg of vitamin C and 0.11–1.84 mg of Vitamin E per 100 g of fresh fruit (Craig, 1994; Willcox *et al.*, 2003; Frusciante *et al.*, 2007). Current Recommended Dietary Allowances of these are 11/15 mg, 0.9/0.7 mg, 90/75 mg, and 15/15 mg (men/women) per day, respectively (Health Canada, 2016). Therefore, 100 g of fresh tomato can provide 5–7%, 100–130%, 22–27% and 0.7% of daily needs of iron, carotenoids, Vitamin C and Vitamin E, respectively. Moreover, lycopene, an important antioxidant in tomato, can significantly reduce the risk of developing different types of cancers including colon, rectal, prostate, and stomach cancer (Kucuk *et al.*, 2001; Giovannucci *et al.*, 2002). It is estimated that a major portion of average Canadian daily dietary intake of lycopene (25.2 mg) is from fresh tomatoes and various processed tomato products (Rao *et al.*, 1998).

In Canada, tomatoes are grown on about 8,000 hectares across the country (FAOSTAT, 2017). In the last eight years, Canada has produced, on average, 410,862 metric tons of tomato, which represent a farm gate value of approximately

\$70 million dollars. The greenhouse tomato industry has expanded significantly since the early 1990s and plays an important role in the fresh tomato industry (Statistics Canada, 2011). Canada is the main producer of greenhouse tomatoes in North America, and annually, more than 250,000 tons of tomatoes are produced in greenhouses, with a value of around \$500 million. But since 2008, tomato production has fallen by almost 40% due to several reasons including losses caused by diseases and pests of tomatoes as well as economic decisions of farmers that have resulted in a decrease of 28% in area planted (Agriculture and Agri-Food Canada, 2016). To prevent crop loss due to disease and pests, farmers spend a substantial amount of money on chemical inputs such as pesticides (Horowitz *et al.*, 2005). This increase in the cost of production makes farmers reluctant to cultivate crops with high susceptibility to pests and diseases, thereby decreasing the area of cultivation of those crops. Infestation by virus-bearing whitefly is one of the factors that make farmers reluctant to cultivate tomatoes (Berlinger *et al.*, 1996).

Whiteflies are one of the leading causes of tomato yield loss worldwide, whether in greenhouses or agricultural field operations (Musuna, 1986; Tosh and Brogan, 2015). They are known for their high levels of genetic diversity; the species *Bemisia tabaci* was previously considered as a complex of biotypes but recently, suggested as a complex of separate species (Dinsdale *et al.*, 2010; Parrella *et al.*, 2012). They are phloem feeders, cause general weakening and reduced growth of their host plants because they suck sap, which is rich in sugars and amino acids (Van Lenteren and Noldus, 1990; Byrne and Bellows Jr, 1991). In addition, whiteflies are

vectors that transmit several kinds of viruses, including Gemini viruses, ipomoviruses, closterovirus, and torradoviruses, which cause diseases that limit growth, cause crop loss, and even kill the plants (Cohen and Berlinger, 1986; Morales and Jones, 2004; Tsai *et al.*, 2009). They also mediate virus evolution by host shifts, mixed infections, etc. (Gilbertson *et al.*, 2015). Moreover, both adults and larvae of whiteflies secrete partially digested phloem sap (honeydew) from the apex of their abdomen. This honeydew covers on shoot meristems, leaves, and fruits and acts as a substrate on which dark, sooty mold fungus can grow (Mansour *et al.*, 2012; Nasruddin and Mound, 2016). Fruits covered with this mold require manual cleaning, which increases labor costs of production. In summary, economic losses occur from plant diseases caused by whitefly-transmitted viruses, direct feeding damage, plant physiological disorders, and honeydew contamination and associated fungal growth.

In Canada, whiteflies show widespread yearly occurrence with high pest pressure, which makes the crop loss high (Mansour *et al.*, 2012). So, controls must be implemented even for small populations. Control of whitefly infestations through pesticide application has had limited success (Palumbo *et al.*, 2001; Horowitz *et al.*, 2011). The difficulty in management of whitefly populations is mainly due to their accelerated population growth rates, the rapid evolution of resistance to insecticides, and the relatively protected location of the individuals on the abaxial surfaces of leaves (Sharaf, 1986; Byrne and Bellows Jr, 1991). Monoculture cropping, together with year-round production practices in greenhouses are also factors that favor whitefly and viral disease outbreaks (Brown, 2007). Whiteflies have been found to

adapt rapidly to current environmental or climatic changes especially in subtropical and tropical agroecosystems (Castle *et al.*, 2009).

The pesticides registered for insect and mite management in greenhouse tomato production in Canada include pyriproxyfen, tetrone, and tetramic acid derivatives (Agriculture and Agri-Food Canada, 2014). Whiteflies evolve resistance to insecticides after their long-term application and farmers are forced to apply high doses and later switch to new insecticides, all leading to environmental and economic costs (Georghiou, 1972; Horowitz *et al.*, 2005; Gauthier *et al.*, 2014). Such costs could be minimized through alternative approaches to agriculture that focus on the reduction or elimination of pesticide application and growing tomato varieties with enhanced natural defences against pests and diseases so that the need to apply pesticides during their cultivation is reduced or eliminated (Clark *et al.*, 1999).

Production and maintenance of the structures and compounds for resistance and tolerance represents an important cost to plants and may come at the expense of growth or reproduction when resources are limited (Herms and Mattson, 1992; Cipollini *et al.*, 2014; Züst and Agrawal, 2017). Plants obtain the materials and energy needed for metabolism, growth, and defence through photosynthesis and nutrient uptake (Chapin III *et al.*, 1990; Bazzaz, 1997). Nitrogen (N) is one of the most important essential elements for plants as it is an important component of many compounds including amino acids, structural proteins and enzymes. It is also a constituent of the chlorophyll molecule and a structural constituent of cell walls

(hydroxyproline-rich glycoproteins, glycine-rich proteins, and proline-rich proteins) (Needham, 1973; Bao *et al.*, 1992; Taiz and Zeiger, 2010; Fageria, 2016).

The need for N in plants is high, especially during the vegetative growth period. Soil N availability is crucial for a good yield of crops (Grime, 1977; Sausserde and Adamovics, 2013). Indeed, a doubling of agricultural food production recorded worldwide over the past four decades can be associated with an increase in N fertilizer application (Cassman, 1999; Hirel *et al.*, 2007). China is one of the major consumers of the N fertilizer in the world (about 30%), and this can be considered as one of the reasons for the Chinese success to feed 21.8% of the world's population with only 6.8% of the world's cultivable land.

Nitrogen deficient plants show decreased growth rate, decreased leaf area index, and low radiation use efficiency. The low leaf area index and low radiation use efficiency will result in low photosynthetic activity in plants (Fageria and Baligar, 2005). Nitrogen deficiency can result in stunted and spindly growth of tomato plants. The main symptom of N deficiency is yellowing of mature leaves (chlorosis) while younger leaves remain small and pale green (Nonnecke, 1989; Guidi *et al.*, 1997). The amount and type of nutrients supplied to plants can influence their yield as well as the nutrient content, taste, and post-harvest storage quality of fruits. In tomatoes, decreased soil N level generally results in decreased number and size of fruits, as well as a diminished storage quality, color, and taste of tomatoes (Sainju *et al.*, 2003; Fageria, 2016). Soil N is commonly insufficient for adequate crop growth (Mattson, 1980; Fageria and Baligar, 2005; Fageria *et al.*, 2010). For this reason, farmers supplement soils with N-containing fertilizers.

Although applying N-based fertilizers has proven very effective in increasing crop yields, if overused, these fertilizers may be detrimental to the goal of sustainable agriculture (Naylor 1996). Tilman (1998) estimated that crops absorb only one-third to one-half of the N applied to farmland and the rest may raise the amount of N in groundwater and surface water downstream of the farmland, enters non-agricultural ecosystems, contributing to the degradation of aquatic ecosystems (Rockström *et al.*, 2009).

A frequently overlooked negative effect of soil N supplementation is a direct consequence of the enhanced nutrition experienced by plants with greater access to N. As they build tissues with greater N content, they become a preferred dietary item of a variety of herbivores including whiteflies, for whom N is also a limiting resource (Mattson, 1980; Jauset, 2000). In fact, outbreaks of insect pests have been related to the increase in the amount of readily available and assimilable N in the tissues of the host plants (White, 1984; Minkenberg and Ottenheim, 1990).

Considering that N fertilization enhances plant growth and increases fruit production, but at the same time increases susceptibility of the plants to herbivores, I propose that there must be a level of fertilizer addition beyond which the losses in fruit production caused by greater attraction and feeding of pests (Herms, 2002), exceed the gains brought about by N availability (Žanić *et al.*, 2011). Optimizing the soil N level at which tomato plants show maximum tolerance to whitefly with minimum reduction in yield will be significant in sustainable agriculture. Therefore, to recommend an optimum level of fertilizer addition, the actual decrease in fruit

production and the capacity of the plants to recover from whitefly attack (tolerance) at different levels of reduced fertilization has to be tested. Also, tomato varieties differ in the density and kind of trichomes and the secondary metabolites produced, which are their primary resistance traits (Simmons and Gurr, 2005; Firdaus *et al.*, 2012; Lucatti *et al.*, 2013). Therefore, they should differ in their levels of natural resistance to whiteflies. As the resistance and tolerance traits pose a cost to the plants, there is a possibility of a tradeoff between the resistance and/or tolerance traits and fruit production.

In this study, I was looking for resistant varieties as well as tolerant varieties (less studied), instead of just resistant varieties. I focused on direct effects of whiteflies, i.e., losses in fruit production rather than indirect effects (virus). As a first step to recommend an optimum level of fertilizer addition, I tested the differences in fruit production, resistance, and tolerance to whiteflies at three different levels on soil N in four commercial tomato varieties.

This thesis consists of two chapters. In Chapter I, I present information on the varieties of tomato chosen for this study because they represented a range of resistance to whiteflies. Plants of six varieties of tomato were grown so as to diminish maternal environmental effects, understand more about the flowering pattern and fruit production of the tomato varieties, and obtaining enough seeds for my experiment. Chapter II presents my study on effect of nitrogen availability on resistance and tolerance of tomato plants of these varieties to whiteflies. This study is

presented in article format, as I intend to submit it shortly for publication in an agroecological journal.

Chapter I

Selection and Propagation of Study Material

2.1 Tomatoes (*Solanum lycopersicum* L.)

In this study, I was interested in using tomato varieties which differ in their resistance to whiteflies. The variation in resistance would give me the opportunity to examine possible tradeoffs of resistance with fruit production and tolerance and see the responses to N supplementation. I got the seeds of six commercial tomato varieties from colleagues at the Universidad de Talca (Chile), who were studying the resistance of different tomato varieties to whiteflies under drought conditions. They had some preliminary data about the resistance of a few varieties against whiteflies under different levels of water availability. I propagated the six varieties for my study, from September 2015 to March 2016, in the greenhouse located in Centennial Hall at the University of Winnipeg. They were Afamia, Conquistador, Luciana, Mistral, Patrón, and 7714, which I renamed as Seven. From their study, Verdugo Leal *et al.* found that the varieties, Afamia, Patrón, and Luciana showed more resistance to whiteflies compared to Conquistador, Mistral, and Seven under drought conditions (pers. comm. J. Verdugo Leal, Universidad de Talca).

Patrón is a variety which produces high quality, intense red color fruit with a fresh weight between 180 and 240 g. Luciana is also a variety with high productivity; it produces red fruits with a fresh weight of 240–280 g. The plants have a high capacity of root regeneration. Afamia produces uniform fruits with fresh weight 180–200 g. High resistance to virus and medium resistance to nematodes were noted. In the variety Conquistador, fruits are of excellent color and quality with a fresh weight of 250–260 g. The plants are resistant to viruses (ToMV and ToSRV), nematodes,

Fusarium, and Verticillium. Seven is a variety which produces fruits with intense red color and uniform maturation (fruit weight varies from 180 to 240 g). This variety shows resistance to viruses (ToMV, TSWV) nematodes, Fusarium, and Verticillium.

A total of 10 plants from each variety were grown for obtaining seeds for my major experiment. Two seeds from each variety were sowed per pot in Pro-Mix All-Purpose Soil Mix (Premier Tech Ltd., Quebec) and sand at the ratio of 4:1. They were germinated in a plant growth chamber (Controlled Environments Ltd., Manitoba). The temperature and humidity inside the chamber were set to 28°C, and 66%, respectively. The lights were on a 16-hour light/8-hour dark cycle.

The seeds started germinating on the fourth day after sowing. They were thinned to one seedling per pot on September 18, 2015. On the 29th day after germination, they were moved to the greenhouse. The temperature and relative humidity at the greenhouse ranged from 21 to 28°C, and 66%, respectively. A general purpose water-soluble fertilizer, Plant-Prod 20-20-20 Classic (0.75 g/L), was used for fertilizing the plants.

The plants started flowering two months after germination. Variety Seven started flowering first, on the 60th day after germination. Afamia, Luciana, Patrón, and Mistral had their first flower on 67th, 68th, 71st, and the 83rd day after germination, respectively. Conquistador was the last variety to flower and started flowering on the 91st day after germination. There was variation in both flower and fruit production among varieties (Table 1.1). In all varieties, flower production decreased around 150

days after germination (Figure 1.1). I also noted that it took 20–28 days for a fruit to mature or ripen.

Ripe fruits were collected when they attained a dark red color. Fruits were cut open, and the pulp with seeds from each fruit was transferred to a sieve. The seeds were washed under running tap water until they were clean, without any pulp attached to them. They were then transferred to paper towels and air dried. The dried seeds were stored in envelopes for the later use.

2.2 Whiteflies

Whiteflies belong to family Aleyrodidae. They damage crops by extracting large quantities of phloem sap. Two species of whiteflies, *Bemisia tabaci* and *Trialeurodes vaporariorum*, are the major pest of tomato. An adult white fly has a body length of >2 mm and a wing expanse of >3.5–4.0 mm. Females lay eggs on the abaxial surface of leaves. Following an incubation period of 4-12 days, the eggs hatch into nymphs. The first nymphal stage is a crawler stage and is followed by three, sessile, immature instars. The last instar or pupa stage undergoes metamorphosis to become an adult (Gill, 1990; Byrne and Bellows Jr, 1991).

Whiteflies for this study were collected at the Buller greenhouse at the University of Manitoba. Whiteflies were collected from tobacco plants in the greenhouse using a hand-held aspirator and transferred to tobacco plants placed in cages made of anti-aphid screens in a plant growth chamber at The University of Winnipeg. The temperature, humidity, and light were the same as that set for seed germination. Whiteflies were allowed to multiply on the tobacco plants in the growth chamber.

Table 1.1. Flower and fruit production (mean \pm SE) of six tomato varieties grown for five months at the Centennial greenhouse of The University of Winnipeg (N = 5).

Variety	Days to flower	Days for fruiting	Flowers produced	Fruits produced
Afamia	71 \pm 1	91 \pm 3	32 \pm 3	11 \pm 0.3
Conquistador	95 \pm 0	106 \pm 3	25 \pm 0.4	5 \pm 0.3
Luciana	72 \pm 0.5	91 \pm 2	28 \pm 0.2	5 \pm 0.4
Mistral	88 \pm 1	102 \pm 3	18 \pm 0.2	3 \pm 0.2
Patrón	79 \pm 3	93 \pm 3	26 \pm 1	12 \pm 0.2
Seven	69 \pm 1	79 \pm 5	24 \pm 0.7	6 \pm 0.3

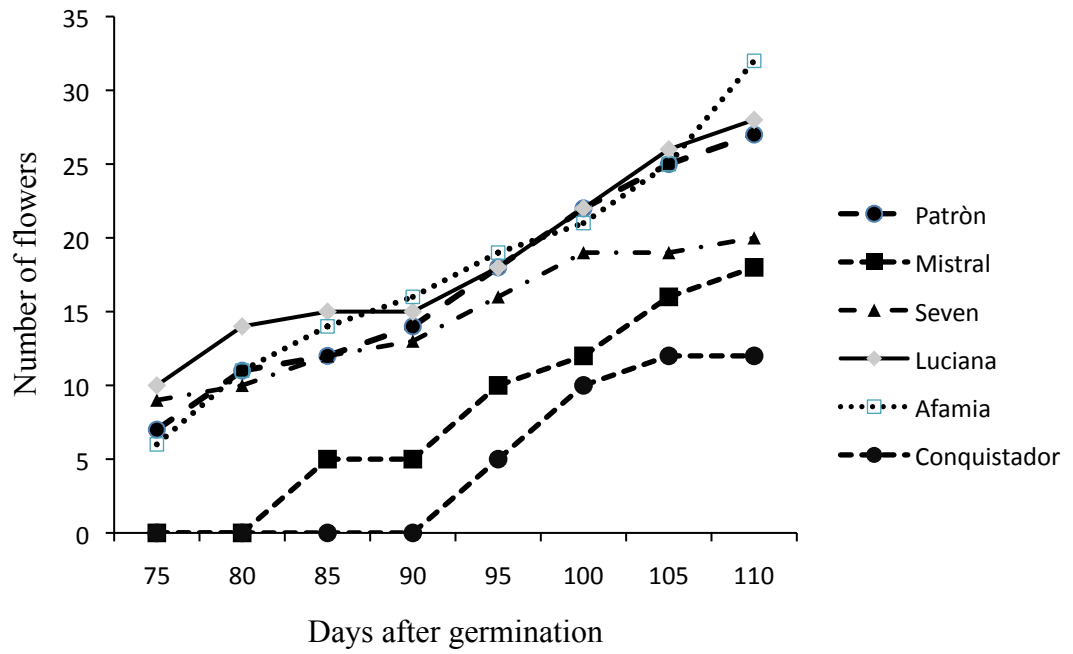


Figure 1.1. Cumulative number of flowers on plants of six tomato varieties grown at Centennial greenhouse in Winter 2016.

CHAPTER II

Resistance and Tolerance of Tomato to Whiteflies at Different Soil Nitrogen Levels

2.1 Introduction

Tomato, *Solanum lycopersicum* L, belongs to family Solanaceae. It is one of the most important commercial vegetables in the American continent. Canada is the topmost producer of greenhouse tomatoes in North America. However, tomato production in Canada has fallen by around 40% during the last seven years (Agriculture and Agri-Food Canada, 2011). Many factors including diseases, pests, weeds, and a decrease of 28% in area planted resulted in the drastic fall in tomato production.

Whiteflies are one of the leading causes of tomato yield loss worldwide, whether in greenhouses or agricultural field operations (Musuna, 1986; Tosh and Brogan, 2015). They are phloem feeders, cause general weakening and reduced growth of their host plants because they suck the phloem sap, which is rich in sugars and amino acids (Van Lenteren and Noldus, 1990; Byrne and Bellows Jr, 1991). In addition, whiteflies are vectors that transmit several kinds of viruses, including Gemini viruses, ipomoviruses, closterovirus, and torradoviruses, which cause diseases that limit growth, cause crop loss, and even kill the plants (Cohen and Berlinger, 1986; Morales and Jones, 2004; Tsai *et al.*, 2009). Losses due to whitefly infestation occur from plant diseases caused by whitefly-transmitted viruses, direct feeding damage, plant physiological disorders, and honeydew contamination and associated fungal growth.

Control of whitefly infestations through pesticide application has had limited success (Palumbo *et al.*, 2001; Horowitz *et al.*, 2011). The difficulty in management of whitefly populations is mainly due to their accelerated population growth rates, the rapid evolution of resistance to insecticides, and their preference for the abaxial surface of leaves, where they are relatively protected from insecticides and most enemies (Sharaf, 1986; Byrne and Bellows Jr, 1991). Soil-applied insecticides are less effective for controlling virus transmission compared to foliar application of insecticides. A study by Castle *et al.* (2017) on eight active ingredients formulated as foliar and soil-applied insecticides showed that foliar formulations had greater knockdown activity than their soil-applied analogs and resulted in lower virus transmission of Cucurbit yellow stunting disorder virus by *B. tabaci* in cucurbit crops. As resistance to insecticides evolves in whitefly populations, farmers are forced to apply higher doses and eventually switch to new insecticides, all leading to environmental and economic costs (Georghiou, 1972; Horowitz *et al.*, 2005; Gauthier *et al.*, 2014). Given the high ecological and social costs of pesticide application, recent approaches to agriculture focus on reduction or elimination of synthetic chemical inputs including chemical fertilizers and pesticides (Clark *et al.*, 1999). Growing tomato varieties with enhanced natural defences against pests would contribute towards that goal.

Plants may defend themselves from herbivores by means of resistance traits (traits that reduce the amount of tissue removed by herbivores), or tolerance traits (mechanisms that reduce the detrimental effects of the loss of tissue on plant fitness)

(Karban and Baldwin, 1997). Resistance traits include trichomes (hairs), thicker or tougher cuticles and cell walls, thorns, and a variety of chemical compounds with toxic or anti-digestive properties (Karban and Baldwin, 1997; Gong and Zhang, 2014). Traits that contribute to tolerance include enhanced photosynthetic efficiency, meristem availability, and the capacity to store and mobilize resources such as carbohydrates and nitrogenous compounds (Karban and Baldwin, 1997; Gong and Zhang, 2014; Mitchell *et al.*, 2016).

Nitrogen is one of the most important essential elements for plants as it is an important component of many compounds including enzymes, amino acids, proteins, and nitrogenous bases among other components. The need for N in plants is high, especially during the vegetative growth period. It should be available in the soil in sufficient quantity for adequate crop growth and good yield (Grime, 1977; Sausserde and Adamovics, 2013). Nitrogen deficient plants show decreased growth rate, decreased leaf area index, and low radiation use efficiency, thereby lowering photosynthetic activity in plants (Fageria and Baligar, 2005). Decreased N level in the soil generally results in the production of fewer and smaller fruits with impoverished storage quality in tomatoes (Sainju *et al.*, 2003; Fageria, 2016). Soil N is commonly insufficient for adequate crop growth (Mattson, 1980; Fageria and Baligar, 2005; Fageria *et al.*, 2010). For this reason, farmers supplement soils with N-containing fertilizers.

Nitrogen-based fertilizers have proven very effective in increasing crop yields (Naylor, 1996). However, if overused, these fertilizers may be detrimental to the goal of sustainable agriculture. Increased N level in the soil will lead to high N content in

plant tissues, thereby increasing the susceptibility of the plants to herbivores (Mattson, 1980; Jauset, 2000). Outbreaks of insect pests have been related to the increase in the amount of readily available and assimilable N in the tissues of the host plants (White, 1984; Minkenberg and Ottenheim, 1990). Therefore, I propose, there must be a level of fertilizer addition beyond which the losses in fruit production caused by greater attraction and feeding of pests, exceed the gains brought about by N availability (Herms, 2002; Žanić *et al.*, 2011). In this scenario, optimizing a nitrogen level at which tomato plants shows maximum tolerance to whitefly with minimum reduction in yield will be significant in sustainable agriculture.

However, the actual decrease in fruit production due to reduced fertilization has not been tested for different varieties of tomatoes with different levels of natural resistance to whitefly. Moreover, the capacity to recover from whitefly attack (tolerance) under different levels of fertilization in these varieties has not been assessed. The main objective of this study is to test three hypotheses. First, that while N addition may increase yield (Cassman, 1999; Hirel *et al.*, 2007), it reduces resistance to whiteflies (Mattson, 1980; Jauset, 2000). Second, that given the differences among varieties in density and kind of trichomes produced on leaves (Luckwill, 1943; Simmons and Gurr, 2005) and the number of flowers produced per inflorescence (Lewis, 1953; Park *et al.*, 2014), varieties will differ in their resistance and tolerance to whiteflies. Lastly, given the cost of resistance and tolerance traits and the possibility that they have some functional redundancy (Siddappaji *et al.*, 2015; Züst and Agrawal, 2017), there may be a tradeoff between them.

To test these hypotheses, four commercial tomato varieties were grown under three levels of soil N fertilization and two levels of whitefly infestation. After 20 weeks of growth, we assessed the effects of N supplementation and whitefly infestation on yield and resistance and tolerance to whiteflies.

2.2 Methods

From the six varieties of tomatoes propagated during Winter 2016, four varieties were used in this study: Afamia, Conquistador, Patrón, and Seven. I chose these varieties based on the preliminary results on their resistance to whiteflies from the study by Verdugo Leal *et al.* (pers. comm. J. Verdugo Leal, Universidad de Talca) on resistance of different tomato varieties to whiteflies under drought conditions and those that produced enough fruits to get sufficient seeds. They observed Afamia and Patrón were more resistant compared to the other two varieties. Pro-Mix All-Purpose Soil Mix (Premier Tech Ltd., Quebec) and sand at a ratio of 4:1 was used for growing the plants. Plants for the study were germinated in a growth chamber in which temperature and humidity were set to 28°C, and 66%, respectively. Two seeds were sowed per pot and 10 days after germination, thinned to one seedling per pot. Plants germinated on the 4th and 5th day after sowing were selected for the experiment. On the 29th day after germination (30 June, 2016), they were transplanted to 1 L pots in the greenhouse at Crop Technology Centre, University of Manitoba, using the same potting mix. The average temperature and relative humidity were $22.5 \pm 1.9^\circ\text{C}$ and $64.1 \pm 6.8\%$, respectively from June to September and $20.3 \pm 0.7^\circ\text{C}$ and $56.3 \pm 3.6\%$, respectively from October to December.

Whiteflies (*Bemisia tabaci*) collected from tobacco plants at the Buller greenhouse, University of Manitoba, were used in this study. They were reared on

tobacco plants placed in anti-aphid screen cages in a growth chamber with same temperature and humidity settings as for germination of tomatoes.

2.2.1. Fertilizer application

The recommended amounts of N, phosphorous, and potassium for commercial tomato production are 90 kg/ha, 112 kg/ha, and 135 kg/ha, respectively (Sainju *et al.*, 2003; Heuvelink, 2005). Three different fertilizer treatments were used: High, Medium, and Low N, corresponding to full, half, and 1/6th of the commercially recommended amount of N, respectively. I used modified Hoagland's solution to provide these three levels of N to the plants (Table 2.1). The N source was CaNO₃. For the High N treatment, 89µM CaNO₃ was used. For the Medium N treatment, half the amount of CaNO₃ was replaced with CaCl₂, and for Low N treatment, CaNO₃ was replaced with CaCl₂. Fertilizer was added in five splits based on N requirement for tomato plants at different stages of growth. For High and Medium N plants, the first two splits were given on the 4th and 5th weeks of growth. The other three splits were applied when the plants started flowering (8th and 12th week of growth), the stage at which leaf N content drops rapidly (Locascio *et al.*, 1997; Kelley *et al.*, 2010). Low N plants were not fertilized until they showed N deficiency symptoms. When the plants developed chlorosis on 70% of leaves, they were fertilized with the same solution used for the Medium N plants in two splits (8th and 12th week of growth). Therefore, they got 1/6th of the commercially recommended amount of N.

2.2.2. Experimental design

Plants were arranged randomly for variety and N treatment on two tables (120 plants per table). On the 9th week of growth, half of the plants in each variety-by-fertilizer treatment combination were randomly assigned to a whitefly infestation treatment, and the other half were left to grow without flies. Because of the high cost and logistic difficulties of building separate cages to include (or not) whiteflies, we split the plants into four groups: two with flies and two without flies. Thus, half of the plants on each table were placed inside a cage made of anti-aphid screen while the other half were placed in a mock cage to subject all plants to roughly equal amount of shading (~21%). A total of 1,300 adult whiteflies were introduced into each full cage.

2.2.3. Biomass harvesting

As they ripened, all fruits were collected and their fresh weight was recorded. Fresh weight of a fruit includes the biomass allocated to the fruit and seeds and water. The fruits were cut open and kept in a drying cabinet at 44°C for drying. Dried fruits were weighed again to record dry fruit weight. Seeds were separated from the dried fruits by soaking followed by washing with water. Cleaned seeds were dried, counted, and total seed weight of each fruit was recorded.

After a period of 22 weeks of growth, plants were cut at 2.5 cm above the media and separated into stem and leaves. These were placed in a drying cabinet at 45°C for 72 hr and weighed.

To clean the roots, the whole root with media was placed on two strainers of different mesh size and kept under a continuous flow of tap water for 40 to 45 minutes. The few remaining media particles attached to the roots were washed off the roots by immersing these in water in glass beakers. Roots longer than 2.5 cm were recovered at this time. Root materials were dried and weighed as described above.

2.2.4. Resistance

To estimate the resistance of plants within each variety and N treatment, a choice assay was conducted. Leaf disks were collected from the first mature leaf from the apex of plants before they were exposed to whiteflies. Disks with an area of 1.77 cm² were made using a cork borer. A total of 12 leaf disks (one each from plants of the 12 combinations of variety and N levels) were arranged randomly in a Petri dish. Fifteen whiteflies, reared on tobacco plants, were introduced to the Petri dish and allowed to choose between the disks. The number of flies on each disk was counted after 6, 12, and 24 hr and used as a measure of resistance. Fewer number of whiteflies on a disk reflects greater plant resistance to whiteflies. Twenty replicates of the assay were done to include all the plants in the experiment. Resistance was measured using GLM by estimating the effects of N, variety, and the interaction between the two factors on the mean number of flies on leaf disks in the choice test.

2.2.5. Tolerance

Tolerance can be measured as a slope of function between fitness and damage. However, for the estimate, groups of closely related individuals are needed and statistical power is lost as the number of such groups is usually small (six in this case). Therefore we estimated compensatory ability, a measurement akin to tolerance at the individual level. Compensatory ability was calculated using the equation,

$CA = S_{ij} / \bar{S}_{oj} - 1$, where CA is the compensatory ability, S_{ij} is the number of seeds produced by individual i in variety j , inside the full cage with whiteflies and \bar{S}_{oj} is the mean number of seeds produced by plants of variety j in the mock cage without whiteflies (Tucker and Avila-Sakar, 2010).

The effect of variety, N, and whitefly feeding on biomass of stem, leaves, and roots were used to elucidate the resource allocation patterns that favor tolerance, which will aid in the understanding of the mechanisms of tolerance to whiteflies.

2.2.6. Seed Germination

To test if there is a relationship between the seed size and vigor or viability of the seeds, I did a germination experiment, and the germination proportion of seeds of each plant was calculated. For this, ten seeds from a randomly selected fruit of every plant were used for the experiment. The seeds were soaked in water for 12 hr and then in 90% ethanol for 5 minutes for sterilization. The sterilized seeds were placed on plain agar media (8 g agar/ L) in Petri plates. Seeds from four randomly selected plants were placed in one plate. The plates were sealed and kept in the growth

chamber with the same humidity and temperature set for germination of the seeds for the main experiment. However, they were kept in darkness for germination. The seeds started to germinate from the 5th day onwards. Those seeds which did not produce radicles after 15 days were considered not germinated. The number of seeds germinated per plant was recorded.

2.2.7 Chlorophyll Analysis

Leaf N concentrations could be indirectly determined by measuring the leaf chlorophyll content and can be used as an indicator of leaf N content (Scheppers *et al.*, 1992; Fitzgerald *et al.*, 2010). Thus I determined leaf chlorophyll concentration after the first two fertilizer applications to confirm that the plant N content varied among the three fertilizer treatments. One leaflet from a fully expanded leaf (with length not less than 90% of the longest leaf on the stem) from the apex was collected from the plants on the day on which the first flower opened. The leaflets were placed in plastic bags and kept in a freezer. After collecting samples from all the plants, they were placed in a freeze dryer (Labconco, Missouri) for 24 hrs and stored for chlorophyll analysis.

The leaflets from five plants in each variety-by-fertilizer combination were randomly selected and pooled into one sample. A total of 1 mg of freeze-dried leaf material from each sample was weighed. The leaf samples were transferred to a vial, and 6 ml of methanol was added to each vial and incubated for 24 hr in darkness. The wash in each vial was collected. Absorbance at 650 and 665 nm was recorded for washes from each vial using a spectrophotometer (Ultraspec, Massachusetts).

Chlorophyll concentrations were obtained by the following equations (Sestak *et al.*, 1971; Renault *et al.*, 2001).

$$\text{Chlorophyll}_{a+b} = 4 * A_{665} + 25.5 * A_{650}$$

$$\text{Chlorophyll}_a = 16.5 * A_{665} - 8.3 * A_{650}$$

$$\text{Chlorophyll}_b = 33.8 * A_{650} - 12.5 * A_{665}$$

where A_{665} is the absorbance at 665 nm, A_{650} is the absorbance at 650 nm. The amount of chlorophyll (mg) per gram of leaf tissue was calculated.

2.2.8 Statistical analysis

We had a split-plot experimental design in which each table was divided into two plots, i.e., one with a full cage with flies and the other with a mock cage without whiteflies (four plots in total). A split-plot analysis that included whiteflies, N treatment, variety, and flies nested in table entered as fixed effects and table as a random factor, was used for all analyses. General linear models (GLM) were used to analyze the effects of variety, fertilization treatment, and whitefly feeding on fresh and dry fruit weights, seed production, and biomass allocation to different parts of plants. To analyze the effects of the above-mentioned factors on seed production and compensatory ability, the data were transformed using square root and arcsine transformations, respectively. Tukey's multiple comparison tests were used to determine the difference in means of each factor in all the analyses.

2.3 Results

To estimate the effects of variety, N treatment, and cage (flies) on fruit mass, seed production, seed mass, vegetative biomass, root to shoot ratio, resistance, and compensatory ability, I used models that include the main effects (variety, N treatment, and cage (flies)) and their interactions. For all the explanatory variables except resistance, the two-way and three-way interactions in full models were found not statistically significant.

There was a significant effect of variety and N treatment on chlorophyll content. Total leaf chlorophyll content increased with N addition ($F_{2, 47} = 11.23$, $P < 0.001$). Among the four varieties, Afamia had the highest leaf chlorophyll while Seven had the least ($F_{3, 47} = 10.87$, $P < 0.001$) (Table 2.2 , Figures 2.1 and 2.2). Chlorophyll a and chlorophyll b followed the same trend as total chlorophyll content (Tables A1.1 and A1.12, Figures A1.1 and 1.2).

Fruit fresh and dry fruit weights increased significantly in direct relation to soil N level (fresh weight: $F_{2, 239} = 19.64$, $P < 0.001$; dry weight: $F_{2, 239} = 18.63$, $P < 0.001$). The fruit fresh and dry weights of plants in the High and Medium treatments were significantly greater than those of plants in Low N treatment (Figures 2.3 and 2.4). Variety and the interaction of N level and variety had no significant effect on fresh and dry fruit weights (Tables 2.3 and 2.4).

The total number of seeds produced per plant was significantly affected by N. The variable, variety was found marginally significant in the full model including the

interactions (Table A1.3). Therefore, for greater clarity, I removed the interactions, which were not significant, from the model and here I present the simple model that includes only the main effects: variety, N treatment, and flies (Table 2.5). Plants grown under Medium N produced significantly more of seeds than those in Low N, while the number of seeds produced by the High N was intermediate between that of the Low and Medium N plants ($F_{2, 239} = 3.21, P = 0.042$) (Figure 2.5). Nitrogen treatment had no significant effect on total seed weight per plant. Variety was the only significant factor that affected total seed weight ($F_{3, 239} = 5.11, P = 0.002$). In Afamia, the seed weight was significantly lower than in the other three varieties (Table 2.6 and Figure 2.6).

Variety and N level had significant effects on vegetative biomass produced by the plants (variety: $F_{3, 239} = 2.95, P = 0.034$; N level: $F_{2, 239} = 69.15, P < 0.001$). Total vegetative biomass of the plants increased with soil N level, and the highest vegetative mass was found in High N plants (Table 2.7 and Figure 2.7). Varieties Conquistador and Afamia produced smaller plants compared to the other two (Figure 2.8). Variety and N level had significant effect on root to shoot ratio also as observed in vegetative biomass (variety: $F_{3, 239} = 27.95, P < 0.001$; N level: $F_{2, 239} = 14.59, P < 0.001$). Plants in the Low N treatment had the highest root to shoot ratio while plants in the High N treatment had the lowest root to shoot ratio. Consequently, the root to shoot ratio varied significantly among the four varieties (variety: $F_{3, 239} = 27.52, P < 0.001$; N level: $F_{2, 239} = 14.13, P < 0.001$) (Figures 2.9 and 2.10 and Table 2.8). Seven had the highest root to shoot ratio in all the N treatments while Afamia had the least

in Low and Medium N treatments. In high N treatment, Conquistador had the least root to shoot ratio.

The effects of N, variety, and their interaction on resistance were all significant (variety: $F_{3, 239} = 170.94$, $P < 0.001$; N level: $F_{2, 239} = 309.09$, $P < 0.001$; variety \times N level: $F_{6, 239} = 4.60$, $P < 0.001$). In the choice assay, the number of flies on the leaf disk did not change after 12 hr. I found that the number of flies was the highest on the leaf disks from High N plants and the lowest on the leaf disks from Low N plants. The magnitude of the N effect varied within varieties. Also, some varieties had more flies on them irrespective of N levels compared to others. Afamia had the least and Conquistador had the most number of flies on their leaf disk in all the N treatments (Figure 2.11 and Table 2.9).

Variety and the interaction of variety and N level had significant effect on seed germination, but the germination proportion (viability) was not influenced by the number of seeds produced by the plant (variety: $F_{3, 184} = 9.41$, $P < 0.001$; variety*N level: $F_{6, 184} = 2.76$, $P = 0.014$; total seeds produced : $F_{1, 184} = 2.27$, $P = 0.114$) (Table 2.10 and Figure 2.12). Therefore, I used the number of seeds produced for measuring the compensatory ability of the plants.

Compensatory ability differed significantly among the varieties studied ($F_{3, 77} = 10.27$, $P < 0.001$) (Table 2.11). A regression analysis was done to test if there was a negative relation between resistance and compensatory ability among varieties. The result showed a statistically significant negative association between resistance

and compensatory ability (Regression equation: $Resistance = 15.0 - 0.0434\sqrt{CA}$, where CA is compensatory ability) (Table 2.12, Figure 2.13).

2.4 Discussion

In this study, the effects of N supplementation and whitefly infestation on fruit mass, resource allocation, resistance, and tolerance of four commercial tomato varieties were assessed.

The availability of N has been recognized as a yield-limiting factor of both crops and wild plants, and tomatoes are no exception (Wqeston and Zandstra, 1989; Sainju *et al.*, 2003; Santos *et al.*, 2010). As per my first hypothesis, I observed that fruit production dropped with a decrease in N fertilization. However, the decrease in yield from High to Medium N treatment was not significant while that from Medium to Low N was. This is consistent with the trend observed by Sainju *et al.* (2000) and Heeb *et al.* (2005). In their experiment which compared tomato production under different N levels, Sainju *et al.* (2000) observed that the addition of 90 kg N/ha produced as much tomato yield as 180 kg N/ha. At the same time, the residual soil N accumulation and movement at 180 kg N/ha were high compared to 90 kg N/ha N addition. Although sufficient organically available soil N is needed for adequate plant growth and production of tomato, excess soil N has two major consequences: N leaching from the soil to the groundwater and increased susceptibility of the plants to herbivores (Mattson, 1980; Dinnes *et al.*, 2002).

Plant uptake of nutrients from manure and fertilizer application averages about 50% of the applied manure or fertilizer for most crops (Allison, 1955; Singandhupe *et al.*, 2003; Jones *et al.*, 2005). In their study, Sweeney *et al.* (1987) reported that N recovered by tomato from N fertilization in Florida ranged from 32 to

53%. Sainju *et al.* (2000) reported only 13 to 30% recovery rate of N by tomato plants in Georgia. As fertilization increases the cost of tomato production, increasing the rate of fertilization without additional yield not only increases the cost of production but also result in environmental damage. Nitrogen leaching from agricultural fields to groundwater has been found to be directly related to N fertilization rate (Owens *et al.*, 1994). The most evident impacts of N leaching to the aquatic ecosystem are eutrophication and direct toxicity to aquatic animals, which impairs their ability to survive, grow, and reproduce (Camargo and Alonso, 2006). Apart from the environmental damage brought about by the excessive application of fertilizer, this practice results in an avoidable economic loss also. To avoid this problem, managing N fertilizer application (rate and time of application) and crop rotations, which limits the N runoff to aquatic ecosystem, are some of the most adoptable strategies in sustainable agriculture (Dinnes *et al.*, 2002; Ding *et al.*, 2016).

The second consequence of high soil N level is the decrease in resistance of the plants to herbivores, which is one part of the first hypothesis I assessed (Mattson, 1980; Jauset, 2000). In my study, the results of choice assay showed that the resistance of tomato plants to whiteflies decreased considerably with increased soil N level. This stems from two factors: the herbivore need for N and a trade-off between growth and defence in plants. For herbivores, N is a limiting resource. Nitrogen is a fundamental component of amino acids, the building blocks of proteins. Many herbivores, in response to selection pressure, have evolved specific behavioral, morphological, physiological, and other adaptations to utilize the available N from

their diet and therefore, plants with high N content in their tissue become a preferred dietary item for herbivores (Mattson, 1980; Jauset, 2000).

In addition, in plants, high nutrient availability can result in decreased resistance due to the shunting of resources towards growth rather than defence (Loomis, 1932; Löyttyniemi, 1981; Bryant *et al.*, 1983). This would apply particularly to plants with resistance based on carbon-rich secondary metabolites that accumulate more when there is not enough N to grow fast (Hoffland *et al.*, 2000). In their review of plant defence, Agrawal and Fishbein (2006) also consider the plant's nutritional quality, as one of the important traits contributing to its defence. Several studies on tomatoes also show that whiteflies, the major pest of tomato, prefer plants with high N content (Tripp *et al.*, 1992; Jauset, 2000; Park *et al.*, 2009). Tripp *et al.* (1992) found a positive correlation between mean whitefly count and increased foliar N content. Jauset (2000) also observed that the number of adult whiteflies was higher on the plants supplied with high N dose. However, these studies did not look for the tolerance of tomatoes to whiteflies.

The expectation that carbohydrates accumulated in leaves of High N plants will be allocated to carbon-rich secondary metabolites as per carbon-nutrient balance hypothesis contradicts the growth rate and growth-differentiation balance hypotheses, which expects higher resistance in lower nutrient availability (Stamp 2003). In my study, plants grown at High N, which had the highest shoot biomass and highest amount of chlorophyll, which could have resulted in increased rate of photosynthesis and increased production of carbohydrates, had the lowest resistance.

This does not support the allocation of accumulated carbon to carbon-based secondary metabolites which could increase resistance of the plants.

Other than resistance traits, tolerance traits provide defence against herbivores to plants that have been damaged by herbivores, via mechanisms that decrease the fitness consequences of damage. Tolerance mechanisms include increases in photosynthetic rate, branching, and storage in below ground tissue (Strauss and Agrawal, 1999; Weinig *et al.*, 2003; Kornelsen and Avila-Sakar, 2015). In my study, I estimated tolerance by measuring the compensatory ability of the plants infested with whiteflies and observed that variety had a significant effect on tolerance. Interestingly, varieties Patrón and Seven, which were the two most tolerant of the four varieties, had relatively high root to shoot ratio (because of high root mass) compared to Afamia, the least tolerant variety, in all the three N treatments. Variety Seven was an exception with high root to shoot ratio but lower tolerance (Figure 2.10). This result is consistent with the conceptual model for resource translocation in plants presented by Orians *et al.* (2011), which propose that upon herbivore attack to their leaves, plants allocate resources to stems and roots for temporarily sequestering the resources. Also, in a previous study in our lab in *Arabidopsis thaliana*, a positive association between tolerance and root to shoot ratio was observed (Hoque and Avila-Sakar, 2014). A study in *Nicotiana attenuata* using ¹⁴C-photosynthate labeling and sugar and enzyme measurements also showed that simulated herbivore attacks increased the allocation of sugars to roots (Schwachtje *et al.*, 2006). In tomatoes, a 20% increase in the relative amount of amino acids allocation to the roots in response to treatment with a defence elicitor was observed

by Gómez *et al.* (2010), but the amount of carbon did not change. Further chemical analysis of the roots would be needed to confirm the type resource allocated to roots in the tomato varieties I studied, which resulted in increased root mass.

As per my third hypothesis, I observed a tradeoff between resistance and tolerance of plants among the four varieties studied (Figure 2.11). A similar tradeoff was observed in *Arabidopsis thaliana* in a previous study in our lab (Kornelsen and Avila-Sakar, 2015). In general, plants acquire defence (resistance and/or tolerance) at the expense of growth and reproduction. In plants, resistance traits can be more species-specific and more than one resistance trait may be present in the same plant to defend from different herbivores (Agarwal *et al.*, 2012). For example, in tomatoes, both chemical (volatile secondary metabolites) and physical (trichomes) resistance traits are present. On the other hand, tolerance traits are more general, i.e., tolerance mechanisms (such as the capacity to allocate resources and high meristem availability) allow plants to compensate for damage by a wide variety of herbivores. So, it is likely that resistance and tolerance are redundant because they can be directed toward the same set of enemies. It is unlikely that plants allocate resources to two redundant traits such as resistance and tolerance (Siddappaji *et al.*, 2015; Züst and Agrawal, 2017). Therefore, a tradeoff between resistance and tolerance is expected in plants (Fineblum and Rausher, 1995; Mauricio *et al.*, 1997; Tucker and Avila-Sakar, 2010). However, plants have both resistance and tolerance traits in them and these traits evolve simultaneously. Therefore they can be two complementary traits (Carmona and Fornoni, 2013).

One limitation of my study is that I could not do the N content analysis of the roots and leaves after whitefly infestation. By comparing the N content of roots and leaves of plants infested and not infested by whiteflies, I would have been able to find whether the plants translocated N, which in higher amounts made them susceptible to whiteflies, from their leaves to roots in response to whitefly infestation. The results of N analysis mentioned above could contribute to understanding the mechanisms of tolerance of tomato to whiteflies. Another factor which I could have tested is the effects of carbon dioxide enrichment on the defensive traits of plants because carbon dioxide enrichment is one of the common practices in greenhouses these days.

In summary, from this study, I found that decreased soil N application decreased fruit production but at the same time increased resistance to whiteflies. When the soil N level was reduced to half of the commercially recommended amount, fruit production dropped only by ~15% and was not significantly different from recommended N, while resistance to whiteflies increased by ~52%. Lower application of fertilizers would help to decrease the cost of production, which is one of the major intentions of farmers. Lower investment on fertilizers and pesticides will improve the farmers' economic benefits, thereby balancing the losses in yield due to lower fertilizer application. Decreased use of fertilizers and pesticides can also reduce environmental costs of large-scale use of agricultural chemicals (Phoenix *et al.*, 2012; Shibata *et al.*, 2015).

My study can be used as a first step in finding an optimum level of fertilizer application, which will increase tomato production while reducing or eliminating the need to control whitefly using pesticides. I used only three N levels and found that the Medium N level (50% of the commercially recommended amount) is the most efficient compared to the other two. Different levels of N between 50% and 100% should be tested to suggest an optimum N fertilization level at which the losses in yield due to reduced N are outweighed by the benefits from the reduction of economic and environmental costs of using chemicals (fertilizers and pesticides) in tomato cultivation. Another anticipated product of this study is a protocol that could be applied for the control of whitefly infestation in greenhouses. The protocol can be extended to agricultural field operations after conducting field studies to discover the effects of many other factors influencing the whitefly infestation in agricultural fields. The findings from the study will also be relevant in development of laws and regulations regarding management of chemicals, such as fertilizers and pesticides, in sustainable or eco-friendly agricultural practices.

Table 2.1. Composition of modified Hoagland's nutrient solution.

	Name	Mol Weight	μM
Macronutrients			
K ₂ HPO ₄	Potassium phosphate (monobasic)	136.6	36
K ₂ SO ₄	Potassium sulfate	174.26	72
Ca(NO ₃) ₂ ·4H ₂ O	Calcium nitrate (tetrahydrate)	236.4	89/44.5/0 (High/Medium/Low)
MgSO ₄ ·7H ₂ O	Magnesium sulfate(heptahydrate)	246.48	1
Reduced N CaCl ₂ ·2H ₂ O	Calcium chloride(dihydrate)	147.01	0/44.5/89 (High/Medium/Low)
Micronutrients			
H ₃ BO ₃	Boric acid	61.83	23
MnCl ₂ ·4H ₂ O	Manganese chloride	197.9	5
ZnSO ₄ ·7H ₂ O	Zinc sulphate	287.5	0.4
CuSO ₄ ·5H ₂ O	Cupric sulphate	249.7	0.2
MoO ₃	Molybdc acid		0.1
FeEDTA	Ethylene Diamine Tetraacetic Acid (ferric-sodium salt)	367.1	7

Table 2.2. General linear model for total chlorophyll content of four tomato varieties grown at three N levels in greenhouse.

Source	df	Adj MS	F	P
Variety	3	12.319	11.22	0.000
N level	2	12.722	11.58	0.000
Variety*N level	6	1.341	1.22	0.319
Error	36	1.098		
Total	47			
R-Sq(adj) = 53.07%				

Adj. MS = Adjusted mean squares; chlorophyll analysis was done at the start of flowering.

Table 2.3 General linear model for total fruit fresh weight of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Flies	1	11	0.01	0.938
Table(Flies)	4	12615	1.90	0.111
Variety	3	3894	0.78	0.504
N level	2	65065	19.64	< 0.001
Variety*N level	6	4735	0.48	0.825
Flies*Variety	3	1109	0.22	0.880
Flies*N level	2	3053	0.92	0.399
Flies*Variety*N level	6	13202	1.33	0.246
Error	212	351130		
Total	239			
R-Sq(adj) = 23.79%				

Adj MS = adjusted mean square; GLM included variety and N level as fixed effects and flies nested in table as a random effect. The plants were arranged in four plots (tables) in four cages, two cages had whiteflies.

Table 2.4 General linear model for total fruit dry weight of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Flies	1	0.540	0.05	0.816
Table (Flies)	4	61.992	1.64	0.166
Variety	3	62.980	2.22	0.087
N level	2	352.473	18.63	< 0.001
Variety*N level	6	88.976	1.57	0.158
Flies*Variety	3	3.022	0.11	0.956
Flies*N level	2	10.516	0.56	0.574
Flies*Variety*N level	6	56.257	0.99	0.432
Error	212	2005.389		
Total	239			

R-Sq(adj) = 25.76%

Adj MS = adjusted mean square; GLM included variety and N level as fixed effects and flies nested in table as a random effect. The plants were arranged in four plots (tables) in four cages, two cages had whiteflies.

Table 2.5. General linear model for seed production of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Cage	1	66.00	1.76	0.316
Table(Flies)	2	37.61	2.58	0.078
Variety	3	34.94	2.40	0.069
N level	2	46.77	3.21	0.042
Error	231	14.55		
Total	239			

R-Sq(adj) = 9.26%

Adj. MS = Adjusted mean squares; Data were square-root transformed. GLM included variety and N level as fixed effects and flies nested in table as a random effect. Two-way and three-way interactions of the factors are avoided for clarity. The plants were arranged in four plots (tables) in four cages, two cages had whiteflies.

Table 2.6. General linear model for total seed weight of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Flies	1	0.00256	0.09	0.770
Table(Flies)	4	0.03663	1.25	0.292
Variety	3	0.14994	5.11	0.002
N level	2	0.04127	1.41	0.247
Variety*N level	6	0.03331	1.14	0.343
Flies*Variety	3	0.00724	0.25	0.864
Flies*N level	2	0.02111	0.72	0.488
Flies*Variety*N level	6	0.02212	0.75	0.607
Error	212	0.02934		
Total	239			

R-Sq(adj) = 14.67%

Adj MS = adjusted mean square; GLM included variety and N level as fixed effects and flies nested in table as a random effect. The plants were arranged in four plots (tables) in four cages, two cages had whiteflies.

Table 2.7. General linear model for vegetative biomass of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Flies	1	78.19	3.08	0.08
Table(Flies)	4	43.08	1.82	0.126
Variety	3	69.93	2.95	0.034
N level	2	1636.72	69.15	< 0.001
Variety*N level	6	33.75	1.43	0.206
Flies*Variety	3	27.70	1.17	0.322
Flies*N level	2	24.98	1.06	0.350
Flies*Variety*N level	6	15.16	0.64	0.698
Error	212	23.67		
Total	239			

R-Sq(adj) = 37.28%

Adj MS = adjusted mean square; GLM included variety and N level as fixed effects and flies nested in table as a random effect. The plants were arranged in four plots (tables) in four cages, two cages had whiteflies.

Table 2.8. General linear model for root to shoot ratio of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Flies	1	0.0015284	1.47	0.228
Table(Flies)	4	0.0018389	1.91	0.110
Variety	3	0.0265194	27.52	< 0.001
N level	2	0.0136173	14.13	< 0.001
Variety*N level	6	0.0007636	0.79	0.577
Flies*Variety	3	0.0003880	0.40	0.751
Flies*N level	2	0.0003794	0.39	0.675
Flies*Variety*N level	6	0.0007204	0.75	0.612
Error	212	0.0009635		
Total	239			

R-Sq(adj) = 38.55%

Adj MS = adjusted mean square; GLM included variety and N level as fixed effects and flies nested in table as a random effect. The plants were arranged in four plots (tables) in four cages, two cages had whiteflies.

Table 2.9. General linear model for resistance of four tomato varieties grown at three N levels against whiteflies.

Source	df	Adj MS	F	P
Variety	3	17.523	170.94	< 0.001
N level	2	31.685	309.09	< 0.001
Variety*N Level	6	0.472	4.60	< 0.001
Error	228	0.103		
Total	239			

R-Sq(adj) = 83.56%

Adj. MS = Adjusted mean squares; GLM included variety, N level and interaction of these two as factors.

Table 2.10. General linear model for germination proportion of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Variety	3	0.085732	9.41	< 0.001
N level	2	0.003904	0.43	0.652
Variety*N Level	6	0.025118	2.76	0.014
Total Seeds	1	0.020655	2.27	0.114
Error	172	0.009113		
Total	184			

R-Sq(adj) = 17.06%%

Adj. MS = Adjusted mean squares; Ten seeds from randomly selected fruit were germinated on plain agar. The data were arcsine transformed, and two outliers are removed for clarity of results.

Table 2.11. General linear model for compensatory ability of whitefly-infested tomato plants of four varieties grown at three N levels.

Source	df	Adj MS	F	P
Variety	3	3.5555	10.27	< 0.001
N level	2	0.0503	0.15	0.865
Error	72	0.3462		
Total	77			

R-Sq(adj) = 26.26%

Adj. MS = Adjusted mean squares; GLM included variety and N level as factors and data were square root transformed for analysis.

Table 2.12. Regression analysis showing relation between resistance and compensatory ability of four tomato varieties.

Source	df	MS	F	P
Regression	1	0.068	7.01	0.010
Residual Error	76	0.009		
Total	77			

R-Sq(adj) = 7.2%

MS = mean squares

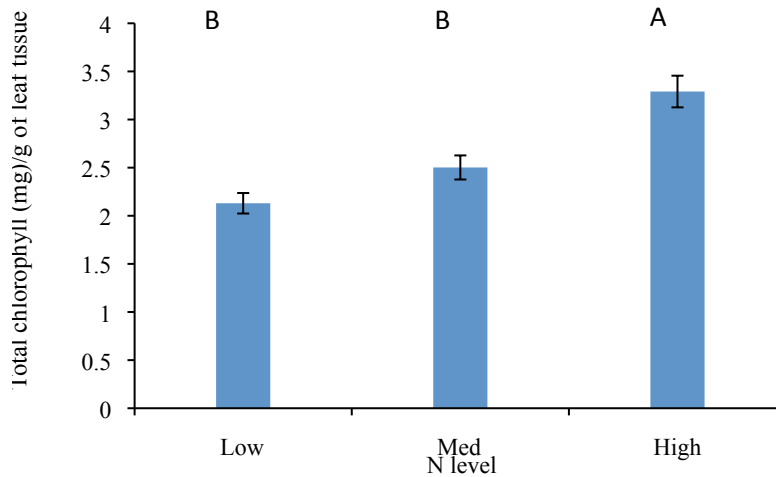


Figure 2.1. Effect of N level on total chlorophyll content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

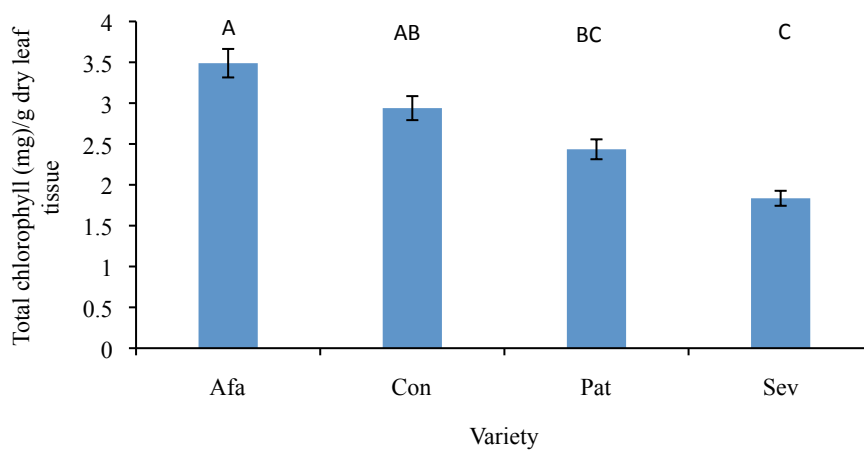


Figure 2.2. Effect of variety on total chlorophyll content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

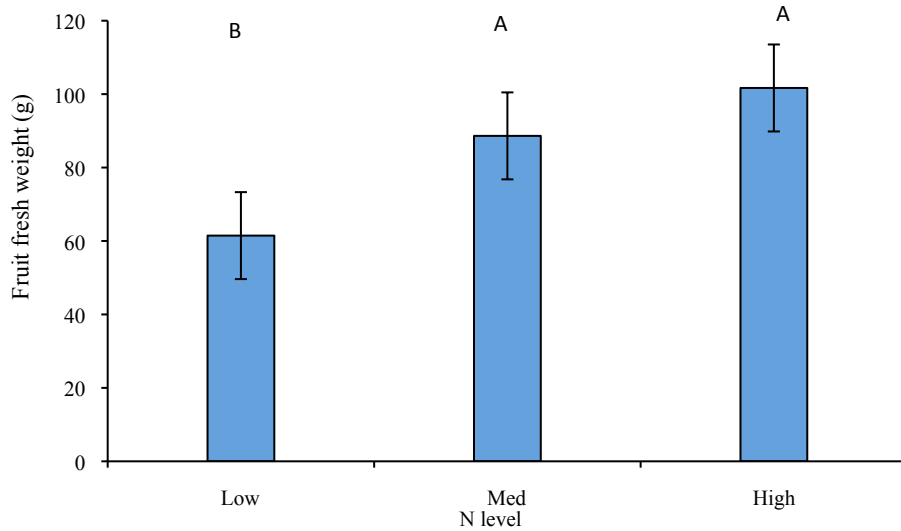


Figure 2.3. Effect of N on total fruit fresh weight produced per plant. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

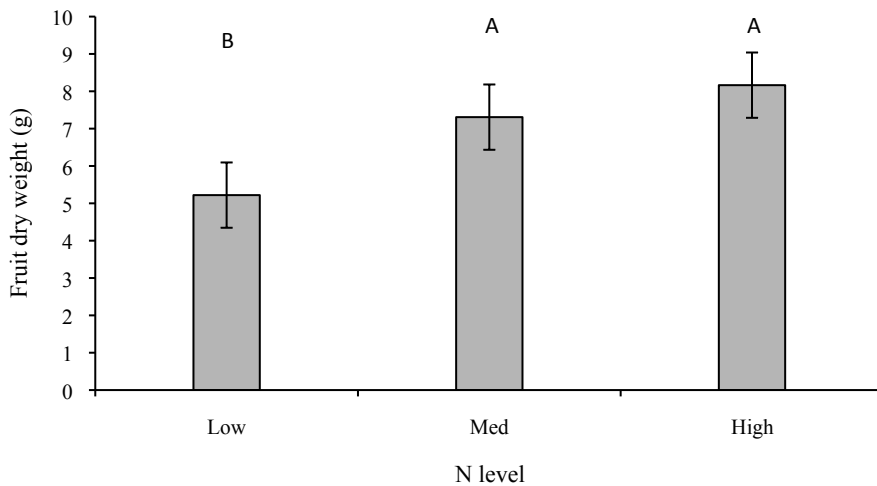


Figure 2.4. Effect of N on fruit dry weight. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

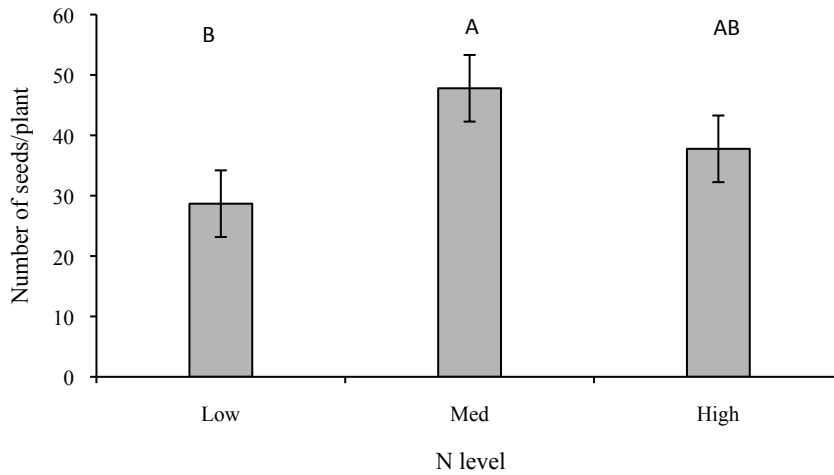


Figure 2.5. Effect of N on total seeds produced per plant. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

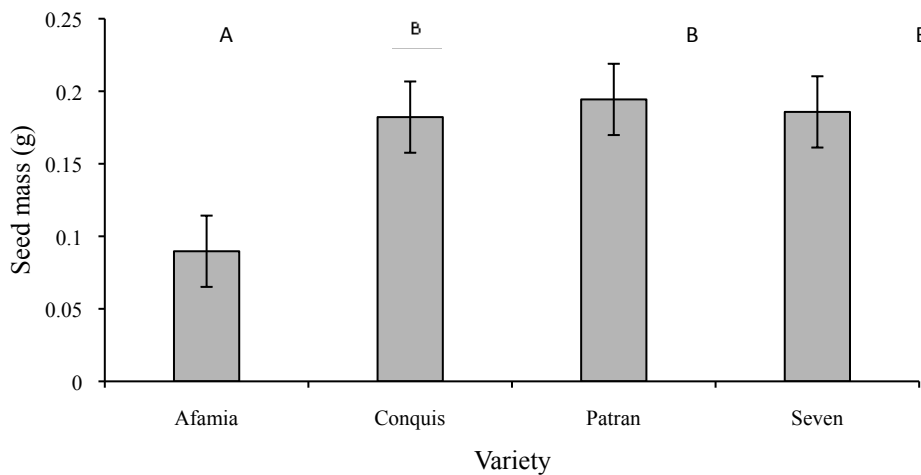


Figure 2.6. Effect of variety on the total seed mass produced per plant. LS means of the values from a GLM, including variety, N level, and flies as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

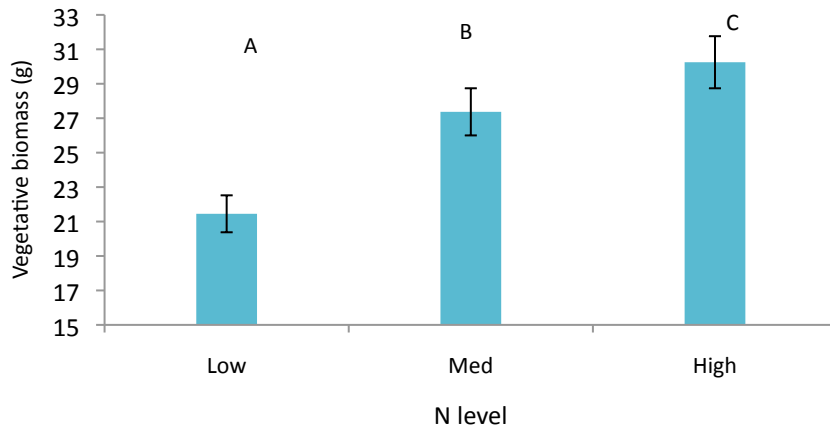


Figure 2.7. Effect of N on vegetative biomass of four tomato varieties grown under three different N levels. LS means of the values from a GLM, including variety, N level, and flies as factors, are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

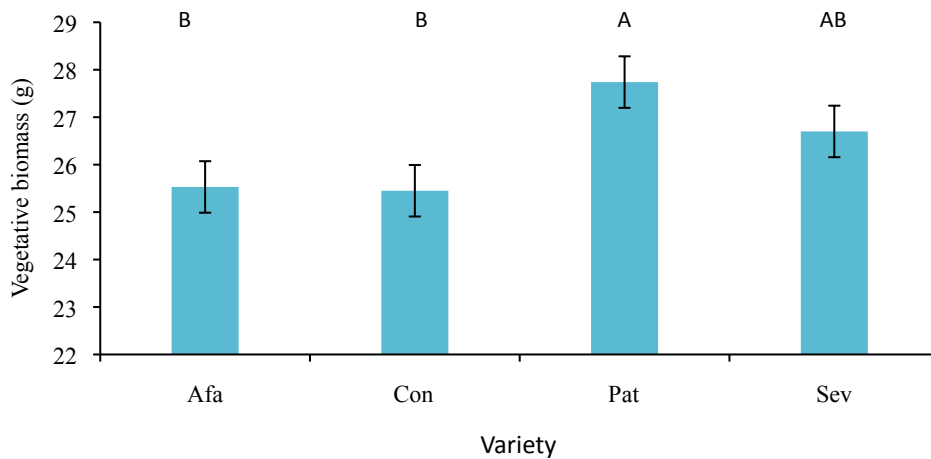


Figure 2.8. Effect of variety on vegetative biomass of four tomato varieties grown under three different N levels. LS means of the values from a GLM, including variety, N level, and flies as factors, are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis at 90% confidence level

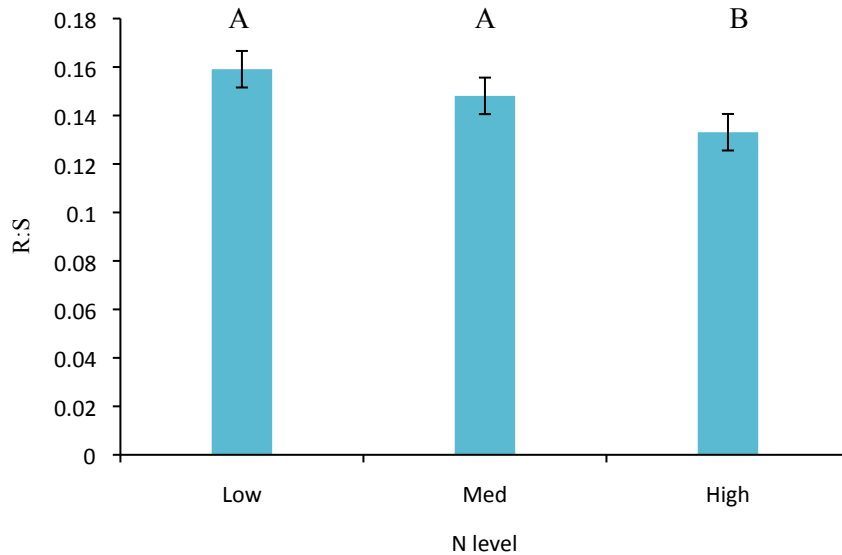


Figure 2.9. Effect of N on the root to shoot ratio of four tomato varieties grown at three N levels. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

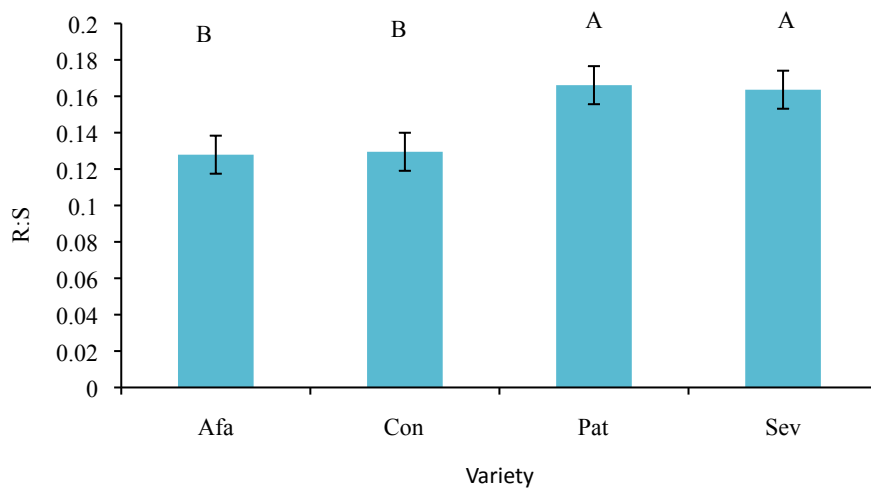


Figure 2.10. Effect of variety on the root to shoot ratio of four tomato varieties grown at three N levels. LS means from a GLM with N level, variety, and flies nested in table as factors are plotted. Letters indicate significant differences among means according to Tukey's multiple comparison analysis.

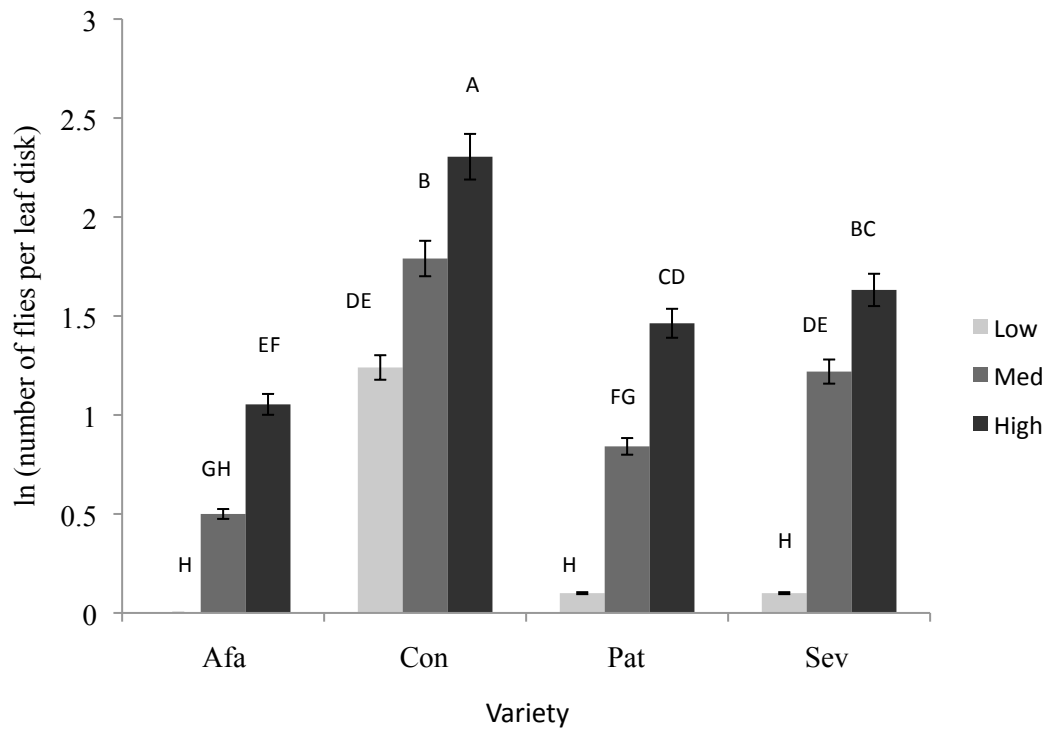


Figure 2.11. Effect of N and variety on resistance to whiteflies (24 hr). LS means from a GLM with N level, variety, and the interaction of the two are plotted. Data were log transformed for analysis.

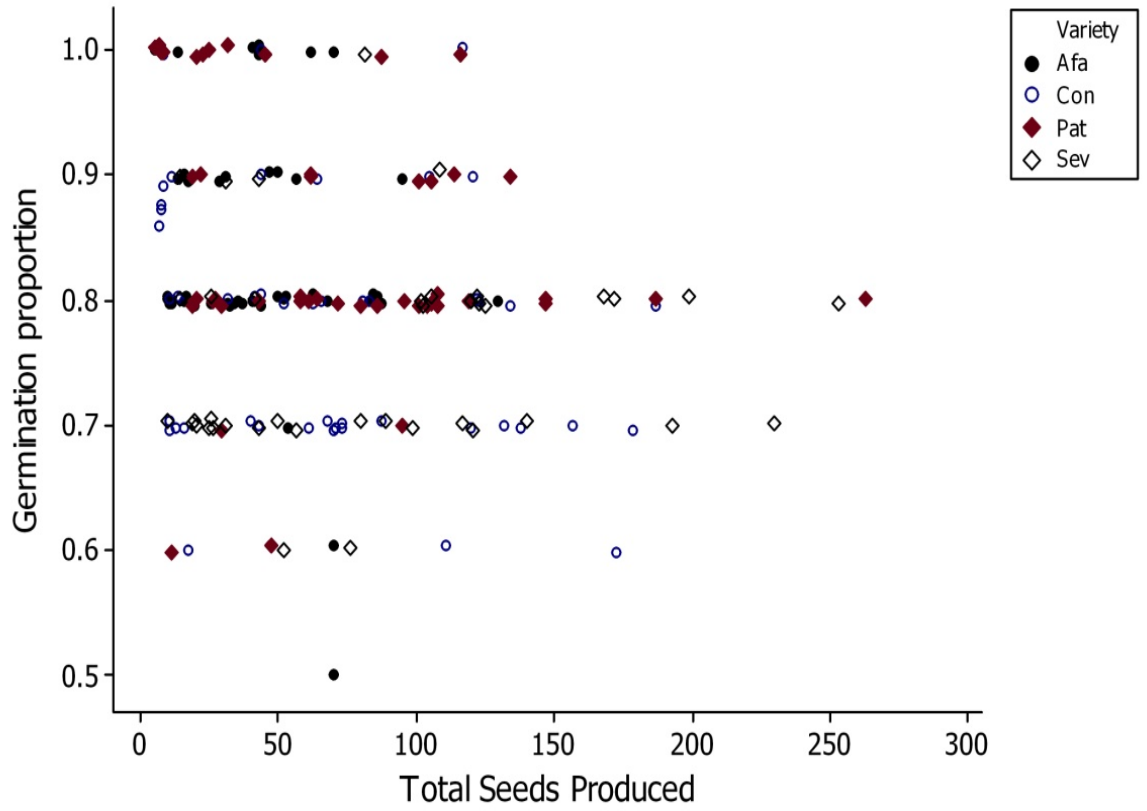


Figure 2.12. The relationship between the total number of seeds produced per plant and their germination proportion obtained by germinating 10 seeds from a randomly-selected fruit from each plant.

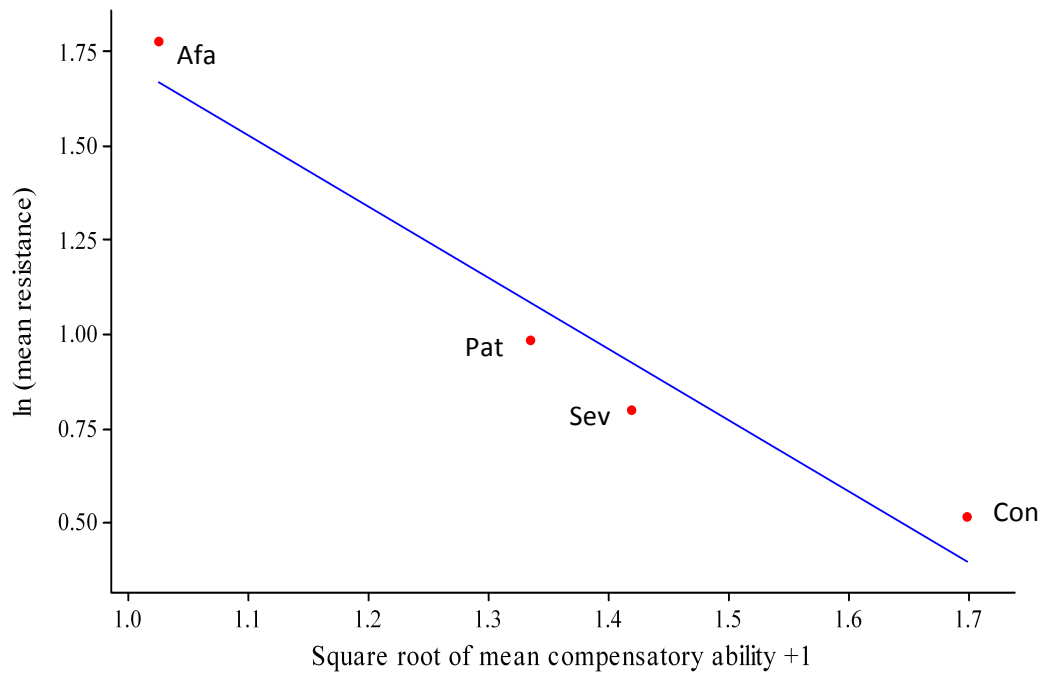


Figure 2.13. Trade-off between resistance and compensatory ability among four varieties studied. LS means of resistance from a GLM for the choice test (with variety, N level, and the interactions of the two factors as factors) is plotted against LS means of compensatory ability from the GLM (with variety and N level as factors) for compensatory ability. Data for resistance were log transformed and data for compensatory ability were square root transformed and 1 is added to all the data points.

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Appendix 1.

Table A1.1. General linear model for chlorophyll_a content of four tomato varieties grown at three N levels in greenhouse.

Source	df	Adj MS	F	P
Variety	3	5.9775	9.90	0.000
N level	2	6.7092	11.12	0.000
Error	42	0.6035		
Total	47			

R-Sq(adj) = 49.97%

Adj. MS = Adjusted mean squares; chlorophyll analysis was done at the start of flowering.

Table A1.2. General linear model for chlorophyll_b content of four tomato varieties grown at three N levels in greenhouse.

Source	df	Adj MS	F	P
Variety	3	1.4107	15.64	0.000
N level	2	0.9688	10.74	0.000
Error	42	0.0902		
Total	47			

R-Sq(adj) = 61.95%

Adj. MS = Adjusted mean squares; chlorophyll analysis was done at the start of flowering.

Table A1.3. General linear model (full model with interactions) for total seeds per plant of four tomato varieties grown at three N levels.

Source	df	Adj MS	F	P
Flies	1	0.22	0.01	0.906
Table(Flies)	4	26.89	1.82	0.127
Variety	3	37.35	2.52	0.059
N level	2	44.85	3.03	0.050
Variety*N level	6	6.10	0.41	0.871
Flies*Variety	3	9.04	0.61	0.609
Flies*N level	2	4.56	0.31	0.735
Flies*Variety*N level	6	21.93	1.48	0.186
Error	212	14.81		
Total	239			

R-Sq(adj) = 23.79%

Adj MS = adjusted mean square; Data is square root transformed for analysis.

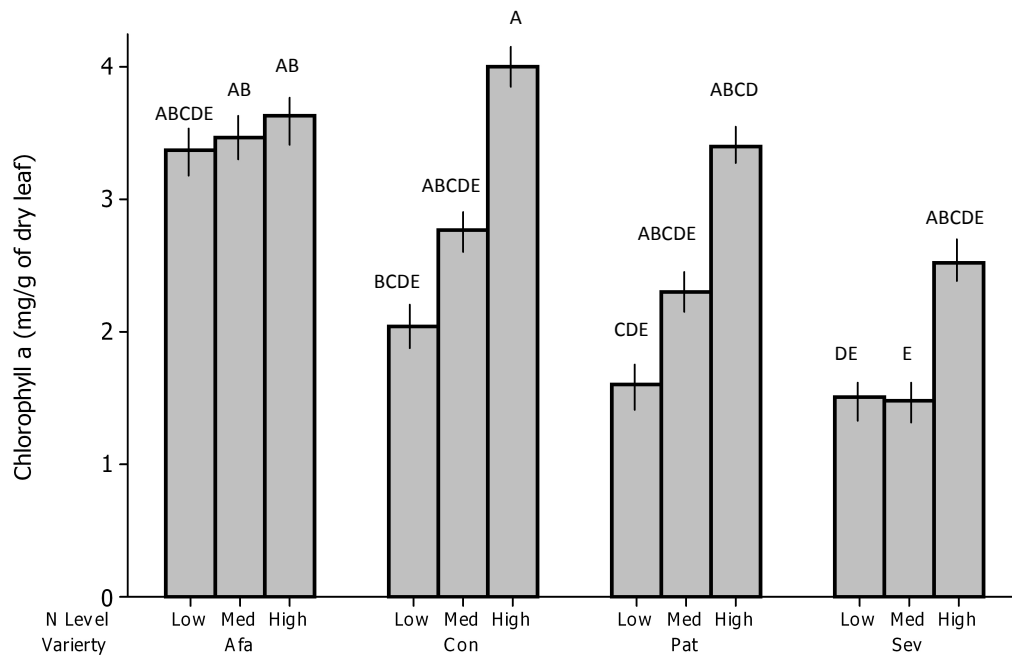


Figure A1.1. Chlorophyll_a content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering.

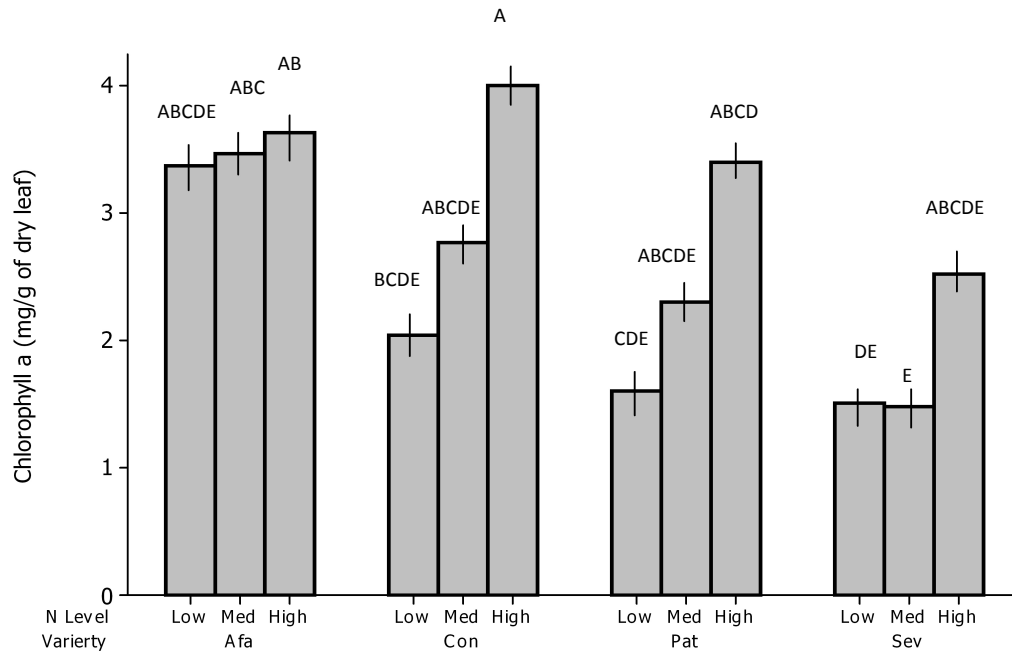


Figure A1.2. Chlorophyll_b content per gram of freeze-dried leaf tissue collected from four tomato varieties grown at three N levels. Fully expanded leaves were used, and analysis was done when the plants started flowering.

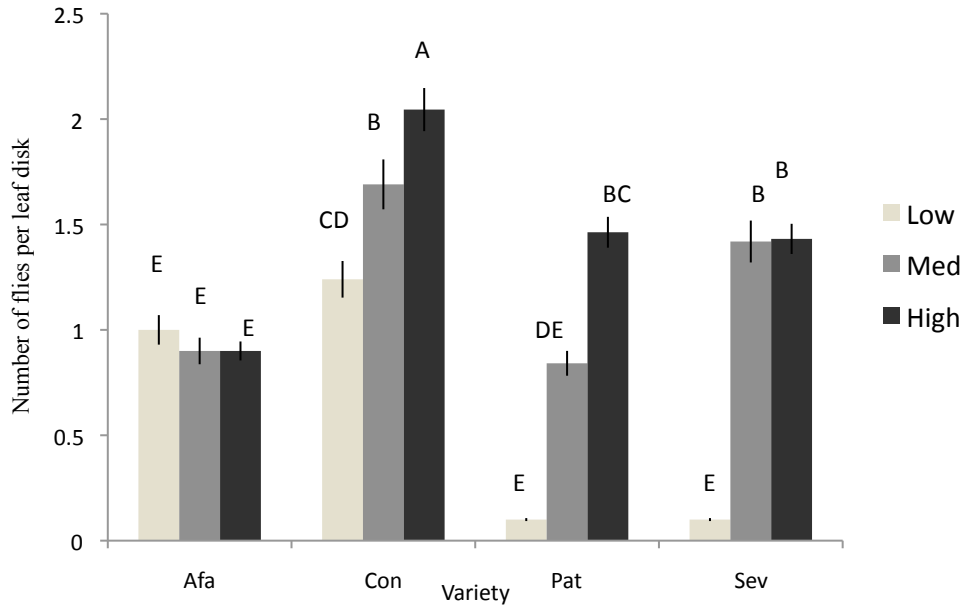


Figure A1.3. Effect of N and variety on resistance to whiteflies (6 hr). LS means from a GLM with N level, variety, and the interaction of the two are plotted. Data were log transformed for analysis.

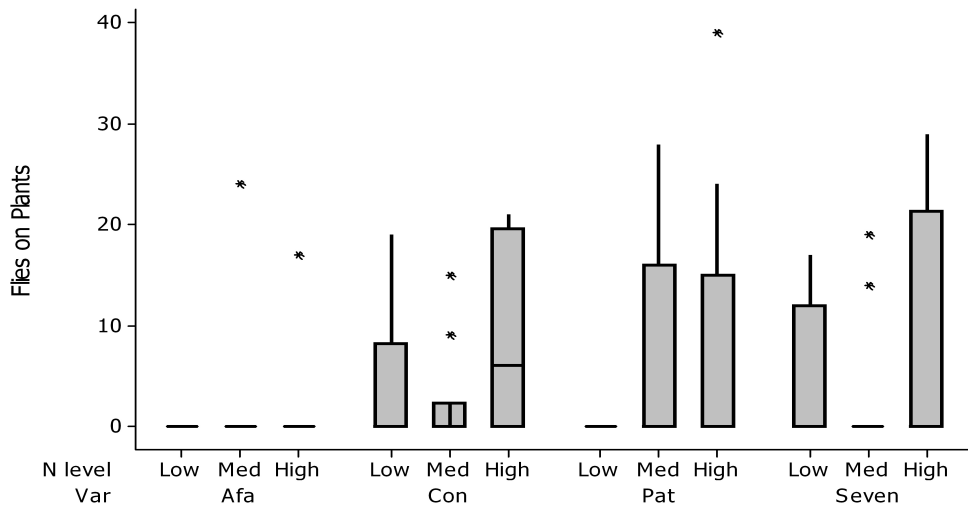


Figure A1.4. Boxplot of number of nymphs on a leaflet of the second mature leaf from the apex of the plants inside the full cage with whiteflies.

Appendix II

To measure the moisture absorbed by dry fruit samples in our lab, I measured the weight gained by dry fruit samples of different sizes from eight fruits from two different varieties. Eight fruits of different sizes from two tomato varieties, Avalantino and Roma, were split into five pieces of different sizes. The fresh weight of the fruits varied from 77.78 to 159.124 and that of the pieces from 4.07 to 92.40 g in Roma. In Avalantino, fruit fresh weight varied from 54.650 to 101.419 g, and fresh weight of the pieces varied from 3.091 to 57.059. The pieces were kept in a drying cabinet at 44°C. The dry weights of the samples were recorded after 48, 72, 96, 120 and 144 hr. The samples were placed in a desiccator during the weighing process, and they were exposed to air only less than 10 sec.

For measuring the amount of moisture they absorbed, the dried samples were weighed after exposing them to air for 2, 4, 8, 10, 12, 24, 48, and 72 hr. During this period, the samples were kept on shelves in the lab for rehydration. The temperature in the lab was 23.5°C and the average humidity varied from 14.5 to 19.2%.

From the descriptive statistics (Table A2.1), I noticed that the maximum weight gained by the samples from Avalantino in 48 hr was 0.025 g by a sample with a dry weight of 1.423, which is only 1.7% of its dry weight. In the variety, Roma, the maximum weight gained by the samples is 0.029 g by a sample with a dry weight of 1.184 g, which is about 2.4% of its dry weight (Table A2.2). Therefore, I found that the weight gained by the samples in 48 hr is negligible.

The maximum dry weight of the samples from my main experiment was 14.37 g (Seven, at High N) and the minimum was 0.635 with a median of 2.461 (Patrón at Low N) (Tables A2.3–A2.6). As observed from the above experiment, the weight gained by the samples will be less than 2.4% of its dry weight, which is negligible.

Table A2.1. Descriptive Statistics for weight gained by five pieces of tomato fruits of the variety Avalantino in 48 hr. The fresh weight of the pieces ranged from 2.88 g to 56.64 g.

(n = 7)

Mean Dry Wt. ± SE	Mean Wt. gained ±SE	Minimum	Median	Maximum
1.667± 0.220	0.006± 0.001	0.002	0.005	0.014
0.653±0.159	0.008± 0.002	0.004	0.006	0.025
0.335±0.110	0.006± 0.001	0.003	0.005	0.016
0.354±0.045	0.004± 0.008	0.002	0.0050	0.007
0.288±0.049	0.004± 0.002	0.003	0.0040	0.005

Table A2.2. Descriptive Statistics for weight gained by five pieces of tomato fruits of the variety Roma in 48 hr. The fresh weight of the pieces ranged from 2.77 g to 101.42 g. (n = 7)

Mean Dry Wt. ± SE	Mean	Minimum	Median	Maximum
2.381± 0.229	0.015 ±0.002	0.009	0.014	0.024
1.348 ± 0.270	0.011 ±0.002	0.008	0.009	0.023
0.647 ± 0.126	0.013±0.002	0.006	0.013	0.029
0.626± 0.131	0.008± 0.003	0.007	0.008	0.012
0.368 ± 0.118	0.009±0.002	0.006	0.007	0.021

Table A2.3. Descriptive Statistics for the total dry weight of fruits from the tomato variety, Afamia. Twenty plants were grown under three N levels.

N Level	n*	Mean Fruit DW ± SE	Minimum	Median	Maximum
Low	16	4.312±0.327	1.786	4.565	6.682
Med	20	3.645±0.327	1.677	3.416	8.143
High	20	3.643±0.305	1.484	3.602	7.183

*n<20 indicates the rest of the plants did not produce fruits.

Table A2.4. Descriptive Statistics for dry weight of fruits from the tomato variety, Patrón.

Twenty plants were grown under three N levels.

N Level	n*	Mean Fruit DW \pm SE	Minimum	Median	Maximum
Low	18	3.377 \pm 0.686	0.635	2.461	11.026
Med	20	5.048 \pm 0.648	1.441	4.483	12.110
High	16	3.848 \pm 0.576	0.897	3.401	8.615

*n<20 indicates the rest of the plants did not produce fruits.

Table A2.5. Descriptive Statistics for dry weight of fruits from the tomato variety,

Conquistador. Twenty plants were grown under three N levels.

N Level	n	Mean	Minimum	Median	Maximum
Low	20	2.839 \pm 0.527	0.000	1.943	9.086
Med	20	4.256 \pm 0.629	0.937	4.147	9.982
High	20	4.726 \pm 0.787	1.078	3.718	14.060

Table A2.6. Descriptive Statistics for dry weight of fruits from the tomato variety, Seven.

Twenty plants were grown under three N levels.

N Level	n	Mean	Minimum	Median	Maximum
Low	20	3.149 \pm 0.461	0.000	3.179	6.746
Med	20	4.723 \pm 0.707	0.000	4.336	10.800
High	19	6.05 \pm 1.03	0.00	4.23	14.37

*n<20 indicates the rest of the plants did not produce fruits.