



# Pest density influences on tomato pigment contents: the South American tomato pinworm scenario

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With 2 figures and 6 tables

**Abstract:** It has long been hypothesized that there is a relationship between pigment content of host plant and pest densities. This study aimed to assess the amount of tomato carotenoid, chlorophyll content and leaf area damage in response to different levels of damage caused by the South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). The results confirmed the effect of pest density on chlorophyll a, b, a+b, and carotenoids contents were all significant in the first year of study. During the second year, the effect of *T. absoluta* treatments on chlorophyll “a”, “b”, “a+b” and on carotenoids was significant. The highest and lowest values of pigment content were recorded in control and plant treatments of eight *T. absoluta* eggs in the first year and plant treatments of sixteen eggs in the second year, respectively. The results of leaf loss assessment demonstrated a significantly different consumed leaf area across the different densities of *T. absoluta* eggs in both years. The results are useful in gaining an oversight of pest density influences on tomato pigment content. By estimating the reduction of the pigments content and or reducing the green tissue of the plant and its relationship with the pest density, damage can be prevented before it reaches the economic injury level.

**Keywords:** pest density, tomato pinworm, plant-insect interactions, crop loss, chlorophyll, carotenoids

## 1 Introduction

The tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest of tomato originally reported from South America (in 1964 from Chile) (García & Espul 1982). Soon after, it rapidly spread to other neighboring South American countries. In Europe, its presence was first reported in Spain in 2006 (Urbaneja et al. 2009) and then it was found in other European, as well as African and Asian countries (Desneux et al. 2010, Campos et al. 2017, Biondi et al. 2018, Mansour et al. 2018). In Iran (South-West Asia), *T. absoluta* was reported in more than twenty provinces (Baniameri & Cheraghian 2012). Several factors, including, among others, the absence of effective biological control agents, a high reproductive rate and a short generation time of *T. absoluta*, have contributed to making this species a key economic pest quickly after its invasion in a new area (Desneux et al. 2010, Biondi et al. 2018, Mansour et al. 2018). It is a harmful moth with a strong preference for

tomato but it can also attack other solanaceous crop plants including aubergine, potato, pepper and tobacco (Pereyra & Sanchez 2006, Desneux et al. 2010, Cuthbertson et al. 2013, Sylla et al. 2019, Satishchandra et al. 2019). The control strategy primarily used against this pest is the application of chemicals, however the widespread use of chemicals can have adverse non-target effects (Desneux et al. 2007, Biondi et al. 2013). Thus, other management strategies (e.g. biological control, host plant resistance, and pheromones) have been employed recently in an IPM framework (Biondi et al. 2018).

The major pigments of plants which influence photosynthesis rate are the chlorophylls. They are categorized as chlorophyll “a” and “b”, and can absorb different wavelengths of sunlight, especially blue and red and so contribute to photosynthesis (Solomon et al. 2011). Additionally, there are yellow/orange pigments present within the chloroplast called carotenoids which indirectly contribute to photosynthesis.

Carotenoids are mostly C<sub>40</sub> terpenoids. These pigments are a class of hydrocarbons that contribute to many biological processes within plants including photomorphogenesis and photosynthesis (Nisar et al. 2015). They also serve as precursors for two plant hormones and a diverse set of apocarotenoids. Carotenoids are also colourants and indeed in the form of antioxidants and provitamin “A” are an essential part of human diets (Nisar et al. 2015). These pigments are mainly synthesized in differentiated plastids found in the roots, flowers, fruits, and seeds of plants. Following their production, they then accumulate within chloroplasts and chromoplasts, but can also be found in amyloplasts, leucoplasts, etioplasts and elaioplasts (Cazzonelli & Pogson 2010). Overall, carotenoids represent an essential means by which energy is captured from sunlight (Vesk & Jeffrey 1977).

Several studies have shown the relationship of plant pigment content and invertebrate pest density. For example, the feeding of sucking pests such as mirid bugs, aphids, scale insects and leafhoppers in particular, reduces photosynthesis substantially resulting in leaf chlorosis (Heng-Moss et al. 2003, Zvereva et al. 2010). Additionally, the amount of chlorophyll “a” and “b” in cereals damaged by the pea aphid, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) was less than that of uninfested crops (Golawska et al. 2010). Dai et al. (2009) stated that chlorophyll pigment in *Hypericum sampsoni* (St. John’s Wort) Hance decreases after feeding of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). Huang et al. (2014) reported that feeding of *Bagrada hilaris* Burmeister (Hemiptera: Pentatomidae) reduced the photosynthesis of brassicaceous plant species (broccoli, green cabbage, red cabbage, cauliflower, kale, and radish). However, the severity of damage caused to the plant depended on the density of mature insects and also the growth stage of the given host plant. It is known that different factors including sap feeder insects alter plant growth and photosynthetic rate by influencing pigment content of the host plant (Golan et al. 2015). The relationship of pest feeding and different injury guilds in regards to photosynthetic rate have been studied in recent years where it has been revealed that there is little or no decrease in photosynthetic rate of the remaining leaves after defoliation in response to herbivory or leaf mining (Peterson et al. 1992). However, in response to sap-feeding, and consequently cellular damage, the photosynthetic rate was reduced as the chlorophyll content decreased in attacked leaves.

Extensive knowledge has been recently generated on several biological and applied aspects of *T. absoluta* and its wide distribution, but little is known about the correlation between density of the moth and pigment content of given host plants. The aim of this study was therefore to investigate the correlation between *T. absoluta* density with chlorophyll and carotenoid contents of tomato leaves. In addition, the impact of initial pest densities on leaf injury was also investigated.

## 2 Materials and methods

### 2.1 Experimental design

This research was carried out over two consecutive years (2014 and 2015) in the research fields of Bu-Ali Sina University, Iran (20 km from Hamedan; latitude 30.2° 01’ 30” North and longitude 42.7° 30’ 48” East; 1,730 m above sea level). A six hundred square meter representative field was selected as an experimental site for both years.

The experiment was undertaken using a complete randomized block design with five treatments including control (i) 0 leafminer eggs, (ii) 1, (iii) 2, (iv) 4, and (v) 8 pest eggs per each host plant (cv. Super Strain B) used in the first year of study (2014) and (i) 0, (ii) 2, (iii) 4, (iv) 8 and (v) 16 *T. absoluta* eggs per each host plant (cv. Super Strain B) used during the second year of study (2015), each with four replicates. Each plot consisted of three rows implanted by six-host seedlings (total 18 seedlings on each plot).

The distance between different tillage rows and between tomato seedlings on each row was set at 75 and 40 cm, respectively. To prevent interplant movement of pest individuals, adjacent rows in each plot were spaced by 1.5 m. All of the tillage practices, e.g. plowing, seeding and weeding were carried out according to common practices of the local farmers. The amount of chemical fertiliser applied was also according to soil fertility tests and local formal recommendations (Pooya Kesht Co®). The soil texture within the field was classed as a medium loam. Soil analysis experiments determined that electrical conductivity, salinity and pH were not limited to tomato cultivation. Soil organic carbon content was also determined. According to the soil analysis results NPK fertilizer (20–20–20) was applied. No additional chemical control against invertebrate pests or diseases was applied.

Transplantation of tomato seedlings (cv. Super Strain B) was carried out on the 21<sup>st</sup> May on both years of study. Following one month, the seedlings were surrounded with ventilated wooden cages (3 × 2 × 1.5 m). Weed control was undertaken mechanically on a weekly basis as necessary.

### 2.2 Artificial plant infestation and sampling

The artificial infestation was carried out on 12<sup>th</sup> of July (50 days after transplanting) for both years. To provide the cohort eggs, *T. absoluta* moths were reared on the same tomato cultivar (cv. Super Strain B) seedlings within the laboratory. Collected eggs were transferred and placed using a fine camel hair brush onto tomato leaves at a given specific rate of infestation. In the first and second year, there were 10 and 7 sampling dates, respectively.

Following egg hatching and the appearance of larval feeding, the number of larval galleries created in each treatment was counted and recorded on each sampling date. Five weeks after initial sampling, total intact and damaged leaf area was accurately measured by using graph paper. For this purpose, from each treatment, three plants in each row were randomly

selected and three infested leaves were cut from the bottom, middle, and top of the plant and transferred to the laboratory. Then, within three weeks of plant growth sampling date, in order to measure the exact leaf area, three plants from each row were randomly selected and destructively sampled. In addition, to determine the leaf pigment content, three plants from each row were also randomly selected from which three leaves of each infested plant (nine leaves for each treatment) were randomly collected from the top, middle, and bottom of the plant and brought back to the laboratory. From these leaves, chlorophyll “a” and “b”, total chlorophyll and carotenoid content were measured using a spectrophotometer. The color absorbance of the solution was estimated by a spectrophotometer using 645 and 663 nm wavelengths against the solvent, because at these wavelengths, maximum absorption of chlorophyll “a” and “b” takes place respectively. Acetone (80%) was used as a blank (APHA, 1989).

The concentration of chlorophyll “a” and “b” and total chlorophyll content were measured using the following equations (Porra et al. 1989) 1, 2 and 3.

$$\text{Chl}_a = 12.25A^{663.6} - 2.55A^{646.6} \quad (1)$$

$$\text{Chl}_b = 20.31A^{646.6} - 4.91A^{663.6} \quad (2)$$

$$\text{Chl}_{a+b} = 17.76A^{646.6} + 7.34A^{663.6} \quad (3)$$

Where A is light absorption at wavelengths 663.3 and 646.6 nm.

The concentration of carotenoid was measured by reading the absorption at 470 nm wavelength using equation 4:

$$\text{Carotenoid content} = (1000 \times 0.8797 - 1.82 \times \text{Chl}_a - 85.02 \times \text{Chl}_b) / 198 \quad (4)$$

### 2.3 Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the differences among chlorophyll “a” and “b” and carotenoid content of selected leaves in response to *T. absoluta* feeding (SAS Institute Inc 2013). Data residual normality and homogeneity of variance were checked by Shapiro-Wilk (Shapiro & Wilk 1965) and Levene’s (Levene 1960) tests. Whenever, the normality assumption was not met using different transformation methods (logarithmic, square-root conversion, adding constant, Box-Cox, Johnson conversion), a non-parametric test, Wilcoxon test (Upton & Cook 2008) was used. The correlation test was investigated using the number of galleries as the independent variable and pigment content as the dependent variable. The means were separated using Duncan’s multiple range test.

## 3 Results

### 3.1 Chlorophyll and carotenoid content

There was a relatively uniform decreasing trend of chlorophyll content in response to different *T. absoluta* infestation

in the first year of study. Different densities of *T. absoluta* larvae on chlorophyll “a” ( $F = 21.48$ ,  $df = 4, 12$ ,  $P < 0.0001$ ); chlorophyll “a+b” content ( $F = 20.72$ ,  $df = 4, 12$ ,  $P < 0.0001$ ); carotenoid content ( $F = 15.49$ ,  $df = 4, 12$ ,  $P < 0.0001$ ) were highly significant while regarding chlorophyll “b”, it was just slightly significant ( $F = 3.09$ ,  $df = 4, 12$ ,  $P < 0.47$ ). In treatments containing 1, 2, and 4 eggs per plant, there were no significant differences in the amount of chlorophyll “a” or “b”. The highest and lowest amount of pigment content was observed in the control and 8 tomato leafminer eggs per plant treatments, respectively (Fig. 1).

In the second year of study (2015), the effect of pest density treatment on chlorophyll “a” ( $F = 18.26$ ,  $d.f. = 4, 12$ ,  $P < 0.0001$ ); “b” ( $F = 8.06$ ,  $d.f. = 4, 12$ ,  $P = 0.0027$ ); “a+b” ( $F = 25.05$ ,  $d.f. = 4, 12$ ,  $P < 0.0001$ ) and carotenoid ( $F = 4.06$ ,  $d.f. = 4, 12 = 4$ ,  $P < 0.01$ ) pigment content was significant. Similarly, the highest and lowest amounts of chlorophyll “a”, “b”, “a+b” and carotenoid contents were observed in the control treatment and 16 eggs per plant, respectively (Fig. 2).

There were no significant differences in the amount of chlorophyll “a” and “b” and carotenoid content at 2, 4 and 8 eggs per plant.

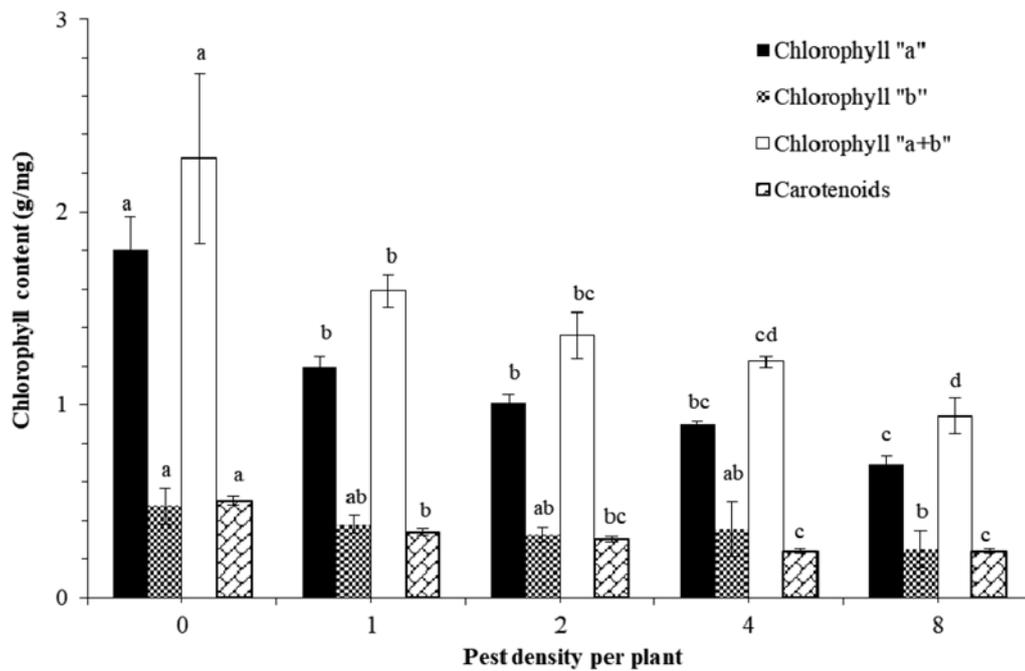
### 3.2 Feed leaf area

The results of statistical analysis demonstrated that the pest densities significantly influenced consumed leaf area of tomato in the first year ( $F = 457.85$ ,  $df = 4, 12$ ,  $P < 0.0001$ ) and second year ( $F = 14.89$ ,  $df = 4, 12$ ,  $P < 0.0001$ ). The control and highest pest density treatments (eight *T. absoluta* eggs per plant) had the greatest and lowest intact leaf surfaces as  $247.25 \pm 6.52$  and  $154.13 \pm 8.14$  cm<sup>2</sup>, respectively in 2014. The highest damaged leaf surface was measured as  $76.58 \pm 2.1$  cm<sup>2</sup> also in the treatment with the highest pest density (Table 1).

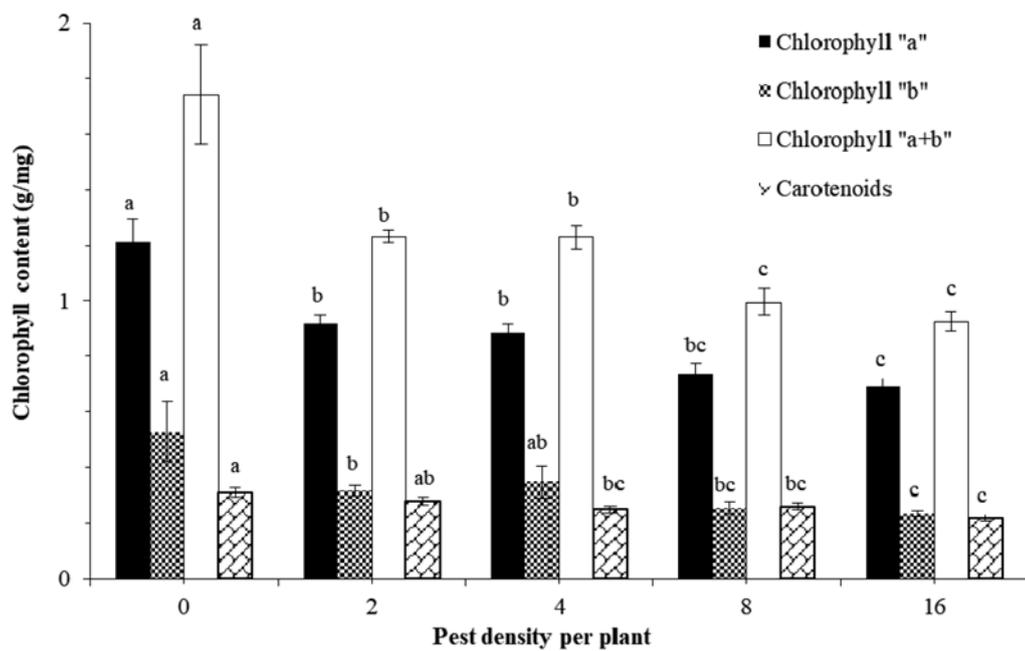
Regarding significant interaction effects of different infestation levels and sampling dates, the results of leaf area index measurements were taken weekly. Accordingly, consumed leaf area was significantly highest on the ninth sampling date (Table 2).

During the second year of study, the leaf area was measured on the fifth and sixth sampling dates. Similar to the previous year, there was a significant effect of *T. absoluta* infestation levels on consumed leaf area ( $F = 292.26$ ,  $df = 4, 15$ ,  $P < 0.0001$ ). In addition, the least undamaged leaf area surface was recorded at the highest pest densities (16 eggs per plant) (Table 3).

In the second year of study, at the final sampling date, the whole plants were dislodged and leaf area surface measured. The total leaf surface in harvested tomato plants showed a significant difference ( $F = 14.89$ ,  $df = 4, 12$ ,  $P < 0.0001$ ) across different pest densities. Considering the different canopy size and growth of the host plants, there were some variations in total and undamaged leaf area in terms of *T. absoluta* initial densities, however, in the treatment with the highest pest density, the maximum consumed leaf area was recorded (Table 4).



**Fig. 1.** Mean ( $\pm$ SE) of chlorophyll "a", "b", and "a+b" (g/mg of leaf) of tomato leaves in respect to *Tuta absoluta* pest egg density per plant in 2014.



**Fig. 2.** Mean ( $\pm$ SE) of chlorophyll "a", "b", and "a+b" content (g/mg of leaf) of tomato leaves in respect to *Tuta absoluta* pest egg density per plant in 2015.

**Table 1.** Mean values ( $\pm$ SE) of leaf area consumption ( $\text{cm}^2$ ) following three weeks of plant growth in response to *Tuta absoluta* feeding (year 2014).

Pest density	Intact leaf area	Consumed leaf area
Control	247.25 $\pm$ 6.52 a	0.0 e
1	202.38 $\pm$ 5.57 b	26.79 $\pm$ 1.93 d
2	178.92 $\pm$ 5.84 bc	41.91 $\pm$ 2.49 c
4	181.75 $\pm$ 7.54 bc	52.41 $\pm$ 3.54 b
8	154.13 $\pm$ 8.14 d	76.58 $\pm$ 2.1 a

Means with different letters in each column are significantly different (Duncan's multiple range test,  $P < 0.05$ ).

**Table 3.** Mean values ( $\pm$ SE) of leaf area ( $\text{cm}^2$ ) following five and six weeks of plant growth in response to *Tuta absoluta* feeding (year 2015).

Pest density	Intact leaf area	Consumed leaf area
Control	290.00 $\pm$ 6.56a	0.0 d
2	246.13 $\pm$ 16.69a	36.36 $\pm$ 3.1c
4	235.25 $\pm$ 12.06a	32.25 $\pm$ 2.53c
8	229.88 $\pm$ 18.92ab	53.87 $\pm$ 6.52b
16	165.25 $\pm$ 12.99b	81.00 $\pm$ 9.34a

Means with different letters in each column are significantly different (Duncan's multiple range test,  $P < 0.05$ ).

### 3.3 Estimation of *Tuta absoluta* mines

In both years of study (2014 and 2015), after hatching, the feeding activity of larvae indicated as galleries mined inside the leaves were recorded weekly. As it is very difficult to count the number of active and inactive larvae, the number of all galleries created was pooled.

In the first year, there was no difference in the number of galleries among treatments in terms of different infestation levels after three weeks. This period would seem necessary for *T. absoluta* egg densities to hatch and for first instar larvae to begin feeding (Cuthbertson et al. 2013). However, 4 weeks following hatching, there was an increasing trend in the number of galleries recorded which could be assigned to adult emergence and the start of oviposition. Following this date, regarding the biology of the pest within the cages, both adults and larvae were observed simultaneously. From this period, the number of galleries followed an increasing steady trend due to the gradual increase of the pest population until the eighth week of sampling (13<sup>th</sup> September 2014). Here, the sudden increase may be due to egg-laying of the first generation females or early leading females of the second generation. After this peak, there was a slight population increase until the end of sampling time (27<sup>th</sup> October 2014). To further assess the population, we observed the number of corridors produced. As new eggs were laid by adult insects,

**Table 2.** Mean values ( $\pm$ SE) of the effect of pest densities on intact, consumed, and total leaf area sampled following specific weeks of plant growth in response to *Tuta absoluta* feeding (year 2014).

Source	Total leaf area	Intact leaf area	Consumed leaf area
Eighth week	227.00 $\pm$ 6.23 b	194.05 $\pm$ 5.8 ab	32.95 $\pm$ 2.11 c
Ninth week	223.27 $\pm$ 5.27 b	178.45 $\pm$ 5.2 b	44.82 $\pm$ 2.76 a
Tenth week	247.00 $\pm$ 6.94 a	206.15 $\pm$ 5.73 a	40.85 $\pm$ 2.3 b

Means with different letters in each column are significantly different (Duncan's multiple range test,  $P < 0.05$ ).

**Table 4.** Mean values ( $\pm$ SE) of leaf area ( $\text{cm}^2$ ) on the final sampling date (seventh week), in response to *Tuta absoluta* infestation levels (year 2015).

Pest density	Total leaf area	Intact leaf area	Consumed leaf area
Control	9660 $\pm$ 546.45 <sup>a</sup>	9660 $\pm$ 546.45 <sup>a</sup>	0.0 <sup>c</sup>
2	7348.8 $\pm$ 1218.19 <sup>bc</sup>	5680 $\pm$ 986.27 <sup>b</sup>	1668.8 $\pm$ 249.53 <sup>b</sup>
4	7032.5 $\pm$ 985.59 <sup>bc</sup>	5261 $\pm$ 981.18 <sup>b</sup>	1771.4 $\pm$ 411.94 <sup>b</sup>
8	6471.8 $\pm$ 730 <sup>c</sup>	4852 $\pm$ 668.1 <sup>b</sup>	1620.0 $\pm$ 109.69 <sup>b</sup>
16	8992.8 $\pm$ 385.07 <sup>ab</sup>	5071 $\pm$ 296.15 <sup>b</sup>	3921.8 $\pm$ 678.21 <sup>a</sup>

Means with different letters in each column are significantly different (Duncan's multiple range test,  $P < 0.05$ ).

the number of corridors increased. We did not succeed in examining the next generation due to atmospheric shrinkage and drying of the tomato plants (Table 5).

In both years of study, the number of galleries made and damaged leaf area was reported as being significantly positive ( $P < 0.0001$ ,  $r = 0.943$ ) and ( $P < 0.0055$ ,  $r = 0.365$ ) in 2014 and 2015, respectively. Moreover, the number of galleries and chlorophyll "a" ( $P < 0.0001$ ,  $r = -0.918$ ) and ( $P < 0.0001$ ,  $r = -0.859$ ); "b" ( $P = 0.0012$ ,  $r = -0.672$ ) and ( $P = 0.0011$ ,  $r = -0.674$ ); carotenoid ( $P < 0.0001$ ,  $r = -0.856$ ) and ( $P = 0.0019$ ,  $r = -0.649$ ) in 2014 and 2015, respectively, were reported to be inversely correlated. This means an increasing number of galleries led to a decrease in the amount of chlorophyll "a", "b" and carotenoid content.

In the second year of study, regarding the delay in starting sampling compared with the first year, there was a sharp increase in pest density and number of galleries in the second week of sampling (22<sup>nd</sup> August 2015). This trend increased moderately and reached its peak value on 12<sup>th</sup> September 2015. Then, the number of galleries increased to the end of sampling dates gradually. The increase in population was observed again due to emerging the number of corridors. As new eggs were laid by adult insects, the number of corridors increased. However, as in 2014, we did not succeed in examining the next generation due to atmospheric shrinkage and

**Table 5.** Wilcoxon test effect of initial pest density on the number of galleries in response to *Tuta absoluta* feeding (year 2014).

Source	Df	Mean Squared	F	CV (%)
<b>First week</b>				
Pest density	4	12205.05	2377.61**	4.46
Error	15	5.13	–	–
<b>Second week</b>				
Pest density	4	12205.05	2377.61**	4.46
Error	15	5.13	–	–
<b>Third week</b>				
Pest density	4	12205.05	2377.61**	4.46
Error	15	5.13	–	–
<b>Fourth week</b>				
Pest density	4	2820747.05	1672.59**	5.6
Error	15	1686.45	–	–
<b>Fifth week</b>				
Pest density	4	3083296.68	956.63**	7.14
Error	15	3223.08	–	–
<b>Sixth week</b>				
Pest density	4	3200368.93	832.61**	7.42
Error	15	3843.77	–	–
<b>Seventh week</b>				
Pest density	4	3341254.13	719.51**	7.63
Error	15	4643.82	–	–
<b>Eighth week</b>				
Pest density	4	74617710.9	786.29**	4.86
Error	15	94898	–	–
<b>Ninth week</b>				
Pest density	4	78121957	628.30**	5.32
Error	15	124338.3	–	–
<b>Tenth week</b>				
Pest density	4	78669463.3	625.83**	5.33
Error	15	125704.6	–	–

\*\* indicates significant difference at  $P < 0.01$ .

drying of the tomato plants. It was again determined that the effect of treatment on the number of galleries was significant (Wilcoxon test,  $P < 0.0001$ ) (Table 6).

#### 4 Discussion

According to Peterson et al. (1992), changes in photosynthetic rate depends on the type of pest species feeding guild. It has been shown that defoliators do not only decrease the photosynthetic rate of the intact leaves but also cause an increase in photosynthetic capacity of the remaining leaves.

However, sucking pests (such as mirid bugs, aphids, scale insects and leafhoppers) and leaf miner feeding also causes cell damage and consequently reduces photosynthetic rate (Haile et al. 1999, Heng-Moss et al. 2003, Desneux et al. 2010, Zvereva et al. 2010, Goławska et al. 2010, Huang et al. 2014, Mansour et al. 2017). Herbivorous insects (leaf consuming and sucking pests) can induce specific molecular or physiological reactions through the leaves or phloem. As a result, these reactions can reduce the photosynthetic capacity of the plants in an increased capacity compared to the direct removal of the leaves. Nability et al. (2009) noted that approximately a 5% reduction in leaf area caused by a chew-

**Table 6.** Effect of initial pest density on the number of galleries in response to *Tuta absoluta* feeding (year 2015).

Source	Df	Mean Squared	F	CV (%)
<b>First week</b>				
Pest density	4	47693	5193.43**	2.99
Error	15	131964.1	–	–
<b>Second week</b>				
Pest density	4	24168069.18	279.18**	12.96
Error	15	86566.68	–	–
<b>Third week</b>				
Pest density	4	25954119.6	196.68**	15.16
Error	15	1319641.1	–	–
<b>Fourth week</b>				
Pest density	4	26661215.7	196.77**	14.69
Error	15	135495.3	–	–
<b>Fifth week</b>				
Pest density	4	150537653.2	120.67**	13.57
Error	15	1247488.2	–	–
<b>Sixth week</b>				
Pest density	4	156426461.1	121.75**	13.16
Error	15	1284795.8	–	–
<b>Seventh week</b>				
Pest density	4	166069204.4	150.10**	11.81
Error	15	1106380	–	–

\*\* indicates significant difference at  $P < 0.01$ .

ing herbivore resulted in approximately 20% reduction in the photosynthetic capacity of the remaining parsnip leaves. In addition, Welter (1989) recorded that interactions between insect pests and plants can lead to a reduction in photosynthetic capacity of up to 50%. However, photosynthetic rate variation depends on several factors including extent of damaged area, the region of damage and physiological state of the host plant (Zangerl et al. 2002). In both years of the current study, the results demonstrated that feeding of *T. absoluta* from the leaves reduced the amount of chlorophyll “a”, “b” and “a+b”. Due to the type of *T. absoluta* feeding from leaf tissue, this conclusion would seem logical. Chlorophyll levels change during plant development, and nutritional deficiencies occur in response to a wide range of stresses including abiotic (e.g. nutrient availability, drought, salinity, and temperature) and biotic stresses such as insect feeding and pathogenic infections (Heng-Moss et al. 2003, Goławska et al. 2010). One of the most crucial parameters in determining photosynthesis rate and plant vigor is the amount of chlorophyll (Mao et al. 2007). It is therefore easy to conclude that low concentrations of chlorophyll directly limit photosynthetic capacity and therefore crop production.

Several studies have shown that insect feeding can influence both chlorophyll “a” and “b” content (Goławska et al. 2010, Hung et al. 2014). The total chlorophyll “a”, “b” and carotenoid contents of plants has been shown to closely depend on pest density, host plant characteristics and the physiological reactions of the host plant in question (Golan et al. 2015).

In the current study there is a very clear relationship between the leafminer feeding and plant photosynthetic rate. Mining on undersides of leaves (Cuthbertson et al. 2013) was found to be more detrimental than mining on the top surface. This could be allocated to the tissue structure of leaves as outlined by Straily (2004) who cited potential damage to stomata and changes to the volume of interstitial space in spongy mesophyll tissue in lower parts of the leaf that may increase photorespiration.

Few studies have shown the relationship between leaf mining and pigment content of a damaged plant. It has been demonstrated that photosynthesis reduction is related to the reduction of chlorophyll concentration of apple trees in response to the spotted tentiform leafminer, *Phyllocnorycter blancardella* F. (Lep.: Gracillariidae) (Proctor et al. 1982). In addition, Proctor et al. (1982) reported an inverse

relationship between corridor density of citrus leafminer and leaf photosynthesis. Mingdu & Shuxin (1989) also recorded that damage caused by the citrus leafminer reduced photosynthesis rate by approximately 30%. *Liriomyza trifolii* mining significantly reduced photosynthesis on celery (*Apium graveolens* L.) (Trumble et al. 1985), tomato (Johnson et al. 1983), lima beans (Martens & Trumble 1987) and in chrysanthemum plants (Parrella et al. 1985). These studies are all in agreement with that of Johnson et al. (1983), who demonstrated similar reductions in photosynthesis and transpiration on tomatoes in response to *L. sativae* feeding. It has been well proven that the galleries made within leaves and the density of larvae feeding within them has an adverse significant relationship on the resulting photosynthetic rate (Schuster & Everett 1983).

Regarding the reduction of leaf area, and chlorophyll content, especially in the treatment with the highest pest density, a severe reduction in photosynthetic rate was predicted. This however, was not measured in the current study. However, the effect of brown soft scale, *Coccus hesperidum* L. (Hemiptera: Coccidae) feeding on the content of chlorophyll and pigments in lemon needles and leaves showed that with an increase of pest density, the pigment content was decreased (Buntin et al. 1996).

Sap feeders such as *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Stephanitis pyrioides* from tomato and *Azalia* host plants respectively also have been shown to reduce the rate of photosynthesis following feeding by leading to a reduction of leaf chlorophyll content (Buntin et al. 1993, 1996).

Decreasing green leaf area surface of host plants regardless of what factor(s) is involved could be responsible for a reduction in overall plant productivity. According to this claim, leaf area could be considered as one of the most important factors in plant productivity (Eik & Hanway 1966, Williams et al. 1968, Potter & Jones 1977, Huang et al. 2017) and efforts should be made in order to optimize leaf area in order to increase yields. To this end, leaf area index (LAI) is an important variable in climatic and ecological studies (Soltani & Galeshi 2002, Ewert 2004) and precise measurement of LAI is required to understand the reciprocal effects of plant growth and the environment (De Jesus et al. 2001). Peña et al. (2000) demonstrated that damaged leaf area percentage was linearly correlated with the number of citrus leafminer in each leaf and that the percentage of leaf area damaged and cumulative leafminer days was linearly correlated with the number of fruit per tree and total fruit weight per tree.

Bogorni et al. (2003) studied the feeding rate of different instars of larvae on three common tomato cultivars. It was revealed that the fourth instar larvae of *T. absoluta* is the most voracious stage among the larval instars. Accordingly, the fourth instar larvae were reported to be equal to 78.9% of total feeding of all larvae. The feeding rate of the first instar larvae of tomato leafminer from leaf mesophyll has

been reported to be 24.9% (Bogorni et al. 2003). In the present study, increased damage by *T. absoluta* to the plant was related to an increase in larval age. The number of larval galleries of tomato leafminer on different host plants depends on the suitability of the host plant for feeding and oviposition (Ecole et al. 2001). Oliveira et al. (2012) reported that the difference in the number of pest larvae galleries varies in terms of the preference of *T. absoluta* to oviposit on different tomato cultivars. In their assessment, it was shown that the initial infestation level of *T. absoluta* had a negative effect on the growth and development of the host plant.

The effect of tomato pinworm density on leaf area has been reported to be significant (Cely et al. 2010). Additionally, the green biomass of the plant was influenced significantly by different pest densities. The results of the current study are not in line with that of Cely et al. (2010), which can be assumed due to the different growing conditions of plants under field conditions, the number of stems and leaves assessed and the different cultivars of host plants investigated (Heuvelink et al. 2005, Rostami 2017). Damage incurred to crops by arthropods is subject to pest density, the rate of hatching, and the host plant characteristics. In addition to the factors outlined above, environmental changes, host plant age, and presence of other living organisms bring about a complex relationship between pests and plants (Oerke 2006). Therefore, studying the environmental factors and the relationship between plants and herbivores is of great importance in determining control programmes for a given pest. Despite the economic importance of *T. absoluta*, the number of studies undertaken on its management under real conditions are few, leading to ineffective management.

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