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Population Density and Spatial Distribution Pattern of *Tuta absoluta* (Lepidoptera: Gelechiidae) on Different Tomato Cultivars

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ABSTRACT

One of the most important factors in a sampling and integrated pest management program is to know the population density and spatial pattern of the insects, especially the invasive ones such as *Tuta absoluta* (Meyrick) that cause a serious problem in tomato cultivation. Therefore, population density and spatial distribution pattern of *T. absoluta* was determined in Varamin region (Tehran, Iran) during two growing seasons of 2015 and 2016 on seven tomato cultivars ('Primo Early', 'Rio Grande', 'Cal JN3', 'Petomech', 'Early Urbana Y', 'Super Strain B', and 'Super 2270'). The *T. absoluta* density was estimated as active mines (with live larvae) and inactive mines (without live larvae) per plant, which, summed together, resulted in the total infestation (total mines per plant). In 2015, the highest mean number of total mines made by *T. absoluta* was on 'Cal JN3' (21.82 mines plant^{-1}) and the lowest mean was on 'Early Urbana Y' (11.08 mines plant^{-1}). In 2016, the highest and lowest mean population density of *T. absoluta* was observed on 'Cal JN3' (14.44 larvae plant^{-1}) and 'Super Strain B' (4.60 larvae plant^{-1}), respectively. The spatial distribution pattern of *T. absoluta* was determined by using Morisita's coefficient, Taylor's power law, and Iwao's patchiness regression method as well as dispersion index of variance-to-mean-ratio. The dispersion index indicated the aggregated pattern of spatial distribution in all tomato cultivars during both years. Taylor's and Iwao's models showed aggregated pattern of distribution on Primo Early' and 'Early Urbana Y', respectively, in 2015 and on 'Super 2270' in 2016. But, on the rest of cultivars, the pattern was determined random. Also, Morisita's coefficient revealed a random distribution pattern for *T. absoluta* in all of the sampling dates. The smallest optimum sample sizes were estimated with Taylors' coefficients. These results revealed that tomato cultivars affected the population density and spatial distribution pattern of *T. absoluta*. The coefficients of the spatial pattern can be used for improving the sampling program to estimate the population density of *T. absoluta* accurately.

Keywords: Population fluctuation, Sampling program, Spatial pattern, Tomato leaf miner.

INTRODUCTION

The invasive pest, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) that is known by the common names of tomato leaf miner and South American tomato pinworm, is a oligophagous pest that attacks plants of the family Solanaceae, especially tomato, and is considered to be a serious threat to tomato production in Mediterranean region. This pest causes 50-100% yield reduction on tomato crops and its other host plants are *Solanum tuberosum* L. *S. melogena* L. *Nicotiana tabacum* L. *S. nigrum* L. and *Datura ferox* L. (Desneux *et al.*, 2010, 2011; Harizanova *et al.,* 2009). It is originated from South America but is rapidly spreading across in many areas including North Africa,

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Europe, and Mediterranean countries such as Iran (Desneux *et al*., 2010, 2011; Baniameri and Cheraghian, 2012). *T. absoluta* has a high reproductive potential, i.e. each female may deposit up to 250-300 eggs during its life, and it has 10–12 generations per year in which each life cycle lasts 24-38 days in different areas depending on temperature (Harizanova *et al.,* 2009; Korycinska and Moran, 2009). Tomato leaf miner has four larval instars and they feed on mesophyll tissues and make irregular mines on leaf surface in which their damage can reach up to 100% (Hassan and Alzaidi, 2009). Tomato fruit may be attacked only when the infestation is heavy, but even small amount of damage means that the fruits will not be suitable for market (Korycinska and Moran, 2009). Both quantity and quality of tomato fruits can be significantly reduced by direct feeding of the pest as well as the secondary pathogens which may then enter through the wounds made by the pest, so, severely attacked tomato fruits lose their commercial value (Cristina *et al.,* 2008). In designing a pest management program, the methods for estimating population densities as well as sampling program including sampling unit, identification of the appropriate sampling time, determination of sampling pattern, and sample size have crucial role (Southwood and Henderson, 2000). By a comprehensive sampling program, a lot of information can be obtained which are used in ecological investigations such as study of population dynamics, detecting pest levels that lead to a justification of control measures and assessing crop loss (Haughes, 1996; Jarosik *et al.,* 2003).

The spatial distribution pattern of arthropods provides an informative description of a population and influences on the sampling program and the method of data analysis (Iwao, 1968; Southwood and Henderson, 2000). There are different methods to determine spatial distribution pattern including the index of dispersion (e.g., Variance-to-mean ratio, Lloyd's mean crowding, Morisita's coefficient, Cole's index of dispersion, David and Moore's index, Green's index, Coefficient of 'K'), Iwao's patchiness regression, Taylor's power law, (Sedaratian *et al.,* 2010; Darbemamieh *et al.,* 2011). Among the various methods, Iwao's patchiness regression and Taylor's power law were more accurate than the others to estimate the spatial pattern of the insects (Khodayari *et al.,* 2010; Rahmani *et al.*, 2010).

Although diagnosing all interactions among individuals of a pest population in field conditions is difficult, it is possible to identify the pattern of pest distribution as well as the changes made in biological traits of a species as the result of the changes in population density. It can be considered as one of the most important biological aspects of a pest population since the distribution of a population is the result of the interactions between species and their environment. The estimation of a pest population density and spatial distribution is necessary to perform the basic research for integrated pest management program. Therefore, the precise monitoring is needed in IPM tactics by conducting proper sampling programs and estimating population density which are necessary for effective pest control (Pedigo and Buntin, 1994; Castle and Naranjo, 2009). There is no detailed information on the population density and spatial distribution of tomato leaf miner in tomato cultivation. Therefore, the present study aimed to develop a sampling procedure suitable for population dynamics studies of *T. absoluta* on different tomato cultivars, and to determine the differences in spatial distribution and abundance of the pest on different tomato cultivars in Varamin (Tehran Province, Iran) during two growing seasons. The results would optimize the monitoring methods for establishing integrated management strategies against the pest.

MATERIALS AND METHODS

The experiment was carried out in Varamin region, Southeast of Tehran, Iran

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(35° 19' 31" North, 51° 38' 44" East) during two growing seasons of 2015 (19.2°C, 44% RH and 93.40 mm annual rainfall) and 2016 (18.4°C, 46% RH and 173.3 mm annual rainfall). Seeds of seven extensively cultivated tomato cultivars including 'Primo Early', 'Rio Grande', 'Cal JN3', 'Petomech', 'Early Urbana Y', 'Super Strain B', and 'Super 2270' were provided and planted in a field using a randomized complete block design. The farmland area was about 1,200 m^2 in 2015 and 2016, divided into four blocks. No pesticides were applied during the experiments. The population density and spatial distributions of tomato leaf miner as well as total leaf mines (active and inactive) were determined for the seven tomato cultivars during two growing years.

Sampling Procedure

Sampling Unit

The whole plant was considered as a sampling unit. Randomly selected plants were visually inspected to record the number of *T. absoluta* larvae (active mines) and total mines including active and inactive mines (without larvae) for each tomato cultivar during two growing seasons of 2015 and 2016.

Pattern and Timing of Sampling

Samplings of tomato plants as well as the movement among plants were performed randomly. In both years, the samples were taken weekly in the morning. In 2015 and 2016 sampling started on, respectively, 2 and 19 June and continued until late July and August.

Sample Size

In order to determine the sample size, primary sampling was performed in an equal number on different tomato cultivars on 26 May in 2015 and 12 June in 2016. To know if the number of initial samples is adequate, the Relative Variation (RV) was calculated as follow:

$$RV = (SE/m)\times 100 \quad (1)$$

Where, *SE* is the Standard Error of the mean and *m* is the mean of initial sampling data. The reliable sample size was estimated by using the following equation:

$$N = [t\times s/d\times m]^2 \quad (2)$$

Where, *N*= Sample size, *t*= *T*-student, *s*= Standard deviation, *d*= Desired fixed proportion of the mean (or range of accuracy), and *m*= The mean of initial sampling data (Pedigo and Buntin, 1994).

Population Density

The larval population density of *T. absoluta* on different tomato cultivars was determined from 2nd June to 7th July in 2015 and from 19th June to 7th August in 2016 by counting the number of mines per plant. The *T. absoluta* density was estimated as mines with live larvae plant^{-1} (active mines) and the mines without live larvae plant^{-1} (inactive mines), which, summed together, resulted in the total infestation (total mines plant^{-1}).

Spatial Distribution Pattern

By using the index of dispersion, Taylor's power law, Iwao's patchiness regression, and Morisita's coefficient of dispersion, the spatial distribution of *T. absoluta* larvae was determined.

Index of Dispersion

By calculating the variance to mean ratio, the dispersion of a population can be classified; namely, S^2/m= 1 random, < 1 regular, and > 1 aggregated. By using the following equation, departure from a random distribution can be tested by calculating the Index of Dispersion (I_D) where *n* denotes the number of samples:

I_D= (n-1)×(S^2/m) (3)

I_D is approximately distributed as χ^2 with *n*-1 degrees of freedom. Values of I_D can be tested by *Z* coefficient as follow:

$$Z = \sqrt{2\, I_D} - \sqrt{(2v-1)} \quad (4)$$

v= *n*-1

The spatial distribution would be random if 1.96≥ Z≥ -1.96, uniform if Z< -1.96, and aggregated if Z> 1.96 (Patil and Stiteler, 1974).

Taylor's Power Law and Iwao's Patchiness Regression

Taylor's power law states that the variance (S^2) of a population is proportional to a fractional power of the arithmetic mean (*m*):

Log S^2= Log a+b Log m (5)

Where, *a* is a scaling factor related to sample size and *b* measures the species aggregation. The distribution is random, regular, and aggregated when *b*= 1, < 1, and > 1, respectively (Taylor, 1961).

To quantify the relationship between mean crowding index (m*) and mean (m) Iwao's patchiness, regression method was employed by using the following equation:

$$m^* = m + (\frac{s^2}{m} - 1) \quad (6)$$

m*= α+βm

Where, α indicates the tendency to crowding (positive) or repulsion (negative) and β reflects the distribution of the population in space and is interpreted in the same manner as *b* of Taylor's power law (Iwao and Kuno, 1968). If the colonies are randomly dispersed, student *t*-test can be used to determine it.

Test *b= 1, t= (b-1)/*SE_b (7)

β= 1, t= (β-1)/SE_β (8)

Where, SE_b and SE_β are the Standard Errors of the slope for the mean crowding regression. Calculated values are compared with tabulated *t*-values with *n*-2 degrees of freedom. If the calculated *t* (t_c)< *t*-table (t_t), the null hypothesis (*b*=1) would be accepted and spatial distribution would be random. If $t_c \geq t_t$, the null hypothesis would be rejected and if *b*> 1 or < 1, the spatial distribution would be aggregated or uniform, respectively.

Morisita's Coefficient of Dispersion I_δ

Morisita (1959) reported a hypothesis for testing the uneven distribution coefficient of I_δ which is calculated by the following equation:

$$I_\delta = \frac{n \sum x_i\ (x_i - 1)}{N\ (N-1)} \quad (9)$$

Where, *n*= The number of sample units, x_i= The number of individuals in each sample unit and *N*= Total Number of individuals in *n* samples. To determine if the sampled population significantly differs from random pattern, the following goodness-of-fit equation can be used:

$$Z = \frac{(I_\delta - 1)}{\left(\frac{2}{nm^2}\right)^{\frac{1}{2}}} \quad (10)$$

If 1.96≥ z≥ -1.96 the spatial distribution would be random but if z< -1.96 and z> 1.96 it would be uniform and aggregated, respectively.

Optimum Sample Size

The optimum sample size is the smallest number of sample units that would satisfy the objectives of the sampling program and achieve the desired precision of estimates. Finding out the Taylor's power law and Iwao's patchiness regression coefficients eliminates experimental needs for large sample size (Ifoulis and Savopoulou-Soultani, 2006). The optimum number of sample size was calculated using Taylor's power law coefficients (a and b) as follows:

$$N_{opt} = a \left(\frac{t_{\alpha/2}}{d}\right)^2 (m^{b-2}) \quad (11)$$

and using Iwao's patchiness regression coefficients (α and β) (Wilson, 1985) as follows:

$$N_{opt} = \left(\frac{t_{\alpha/2}}{d}\right)^2 \left(\frac{\alpha+1}{m} + (\beta - 1)\right) \quad (12)$$

Where, N_{opt}= Optimum sample size, $t_{\alpha/2}$= *t*-student of table, *m*= Mean density of primary sampling, and *d*= The range of accuracy, which, in this study, was considered as15% (0.15) level.

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RESULTS

Sampling Procedure

The reliable sample size with a relative variation of 15% from the initial sampling results was 49, 60, 65, 47, 66, 64 and 48 samples in 2015 and 57, 53, 56, 57, 59, 50 and 49 samples in 2016 for Primo Early, Rio Grande, Petomech, Super Strain B, Cal JN3, Super 2270, and Early Urbana Y cultivars, respectively. In both years, the Relative Variation (RV) of the initial sampling data was counted as very appropriate for a sampling program (Table 1).

Population Density

During 2015 and 2016, the pest infestation on seven tomato cultivars was determined from the beginning of the sampling period (Tables 2 and 3). The total mines formed by *T. absoluta* showed a peak at the mid-season and was gradually reduced during the rest of sampling period on all tomato cultivars in both years. In 2015, the highest mean number of total mines was on 'Cal JN3' (21.82 mines/plant) and the lowest mean was on 'Early Urbana Y' (11.08 mines/plant). Pest infestation was moderate at the beginning of June, indicating a slow development of immature insects, then increased gradually at the second week of June (Table 2), in which *T. absoluta* larvae (active mines) peaked at mid-June under favorable climatic conditions. At late June, the active mines decreased to a moderate level. On the other hand, the mines without larvae increased at the end of the tomato growing season, because of plant withering and unsuitable climate conditions. Similarly, almost the same trend was observed in 2016 (Table 3). In 2016, the highest and lowest mean number of total mines was observed on 'Cal JN3' (14.44 mines plant^{-1}) and 'Super Strain B' (4.60 mines plant^{-1}), respectively. *T. absoluta* infestation peaked at the mid of July. Furthermore, gradual reduction in the active mines (with larvae) and increase in the inactive mines (without larvae) happened at the end of the experimental period (Table 3).

Spatial Distribution

The results of the variance to mean ratio

Table 1. Estimated parameters by primary sampling of *Tuta absoluta* on different tomato cultivars during 2015 and 2016.[a]

Year	Cultivars	*n*	*SE*	*SD*	*RV*	*m*	*d*	*N*
2015	Primo Early	25	0.75	3.75	10.52	7.12	0.15	49
	Rio Grande	25	0.73	3.63	11.58	6.28	0.15	60
	Petomech	25	0.83	4.14	12.11	6.84	0.15	65
	Super Strain B	25	0.72	3.59	10.27	7.00	0.15	47
	Cal JN3	25	0.70	3.52	12.15	5.80	0.15	66
	Super 2270	25	0.38	1.92	11.98	3.20	0.15	64
	Early Urbana Y	25	0.58	2.91	10.40	5.60	0.15	48
2016	Primo Early	25	0.72	3.61	11.29	6.40	0.15	57
	Rio Grande	25	0.74	3.70	10.89	6.80	0.15	53
	Petomech	25	0.81	4.04	11.22	7.20	0.15	56
	Super Strain B	25	0.77	3.83	11.28	6.80	0.15	57
	Cal JN3	25	0.74	3.69	11.52	6.40	0.15	59
	Super 2270	25	0.44	2.22	10.59	4.20	0.15	50
	Early Urbana Y	25	0.62	3.09	10.45	5.92	0.15	49

[a] *n*= Number of samples; *SE*= Standard Error of the mean; *SD*= Standard Deviation; *RV*= Relative Variation; *m*= Mean of primary sampling data; *d*= Desired fixed proportion of the mean, *N*= Sample size.

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Table 2. Mean (±SE) number of active mines, inactive mines and total mines of *Tuta absoluta* on different tomato cultivars in 2015.[a]

Infestation	Cultivars	Sampling dates						
		2 June	9 June	16 June	23 June	30 June	7 July	Overall dates
Active mines	Primo Early	1.33±0.188d	3.35±0.281b	3.39±0.311c	1.63±0.201ab	0.86±0.124a	0.33±0.074a	1.81±0.110c
	Rio Grande	1.28±0.119d	3.65±0.219b	5.78±0.349b	1.22±0.137bc	0.88±0.139a	0.25±0.066a	2.18±0.129bc
	Petomech	3.75±0.344b	6.15±0.355a	3.40±0.247c	1.26±0.132bc	0.98±0.141a	0.40±0.084s	2.66±0.141b
	Super Strain B	2.49±0.361c	5.91±0.420a	5.32±0.344b	0.91±0.128c	0.85±0.118a	0.32±0.081a	2.63±0.174b
	Cal JN3	2.23±0.211cd	6.97±0.252a	8.59±0.301a	2.02±0.179a	1.41±0.179a	0.45±0.084a	3.61±0.175a
	Super 2270	5.08±0.328a	6.17±0.304a	7.25±0.379a	2.28±0.207a	1.45±0.151a	0.53±0.083a	3.79±0.167a
	Early Urbana Y	1.31±0.181d	3.27±0.261b	5.85±0.240b	1.25±0.164bc	0.83±0.134a	0.42±0.093a	2.16±0.135bc
		$F_{6, 392}$ = 40.258	$F_{6, 392}$ = 41.870	$F_{6, 392}$ = 30.582	$F_{6, 392}$ = 33.604	$F_{6, 392}$ = 26.691	$F_{6, 392}$ = 38.019	$F_{6,2387}$=25.102
Inactive mines	Primo Early	6.63±0.557b	10.98±0.838c	24.84±1.020a	22.55±1.106b	18.61±0.574b	9.61±0.627ab	15.54±0.518b
	Rio Grande	7.25±0.348b	8.60±0.371de	22.23±1.041a	18.10±0.617c	18.92±0.687b	7.13±0.588cd	13.71±0.420c
	Petomech	5.78±0.324bc	10.52±0.472cd	15.75±0.661b	25.66±0.999ab	19.62±0.593b	8.29±0.607bc	14.27±0.435bc
	Super Strain B	12.66±0.655a	16.51±0.646b	24.45±0.904a	14.74±0.633cd	11.02±0.431d	4.51±0.430e	13.98±0.442bc
	Cal JN3	13.80±0.500a	19.58±0.444a	13.83±0.518b	27.55±0.890a	23.68±0.679a	10.82±0.556a	18.21±0.390a
	Super 2270	12.59±0.415b	11.69±0.674c	24.64±0.972a	24.19±0.663ab	23.59±0.756a	7.47±0.461bcd	17.36±0.451a
	Early Urbana Y	4.31±0.384c	7.63±0.243e	8.96±0.634c	13.54±0.634d	13.85±0.570c	5.27±0.391de	8.93±0.297d
		$F_{6, 392}$ = 42.349	$F_{6, 392}$ = 49.707	$F_{6, 392}$ = 72.902	$F_{6, 392}$ = 56.088	$F_{6, 392}$ = 62.745	$F_{6, 392}$ = 54.008	$F_{6,2387}$=47.271
Total mines	Primo Early	7.96±0.612cd	14.33±0.907de	28.22±1.157ab	24.18±1.121b	19.47±0.584b	9.94±0.648ab	17.35±0.554b
	Rio Grande	8.53±0.361c	12.25±0.451ef	28.02±1.032b	19.32±0.640c	19.80±0.709b	7.38±0.611cd	15.88±0.468b
	Petomech	9.54±0.523c	16.68±0.664cd	19.15±0.683c	26.92±1.037ab	20.60±0.634b	8.69±0.646bc	16.93±0.433b
	Super Strain B	15.15±0.855b	22.43±0.933b	29.77±1.078ab	15.66±0.669d	11.87±0.482c	4.83±0.449e	16.62±0.565b
	Cal JN3	16.03±0.619ab	26.55±0.605a	22.42±0.688c	29.56±0.916a	25.09±0.731a	11.27±0.575a	21.82±0.425a
	Super 2270	17.67±0.553a	17.86±0.903c	31.89±0.962a	26.47±0.706ab	25.05±0.775a	8.00±0.454bcd	21.16±0.496a
	Early Urbana Y	5.63±0.408d	10.90±0.396f	14.81±0.678d	14.79±0.623d	14.69±0.585c	5.69±0.416de	11.08±0.322c
		$F_{6, 392}$ = 45.040	$F_{6, 392}$ = 50.005	$F_{6, 392}$ = 66.494	$F_{6, 392}$ = 65.280	$F_{6, 392}$ = 61.097	$F_{6, 392}$ = 46.024	$F_{6,2387}$=56.212

[a] The means followed by different letters in the same column are significantly different (P< 0.05, Tukey).

Table 3. Mean (±SE) number of active mines, inactive mines and total mines of *Tuta absoluta* on different tomato cultivars in 2016.[a]

Infestation	Cultivars	Sampling dates								
		19 June	26 June	3 July	10 July	17 July	24 July	31 July	7 August	Overall dates
Active mines	Primo Early	0.19±0.053c	0.70±0.117b	2.72±0.164bc	8.82±0.347c	2.25±0.191cd	4.51±0.094bc	0.28±0.074c	0.51±0.104c	2.50±0.143cd
	Rio Grande	0.79±0.143a	0.04±0.026c	1.55±0.204d	9.68±0.257c	1.55±0.204d	5.23±0.440b	1.64±0.221a	1.11±0.172ab	2.70±0.168c
	Petomech	0.61±0.127ab	0.00±0.000c	2.82±0.239ab	8.38±0.232c	2.64±0.234bc	2.98±0.200cd	0.41±0.091c	0.46±0.108c	2.29±0.136cd
	Super Strain B	0.09±0.038c	0.89±0.127b	1.77±0.182cd	7.42±0.261cd	1.77±0.182cd	2.61±0.283d	1.65±0.203a	0.46±0.100a	2.08±0.121cd
	Cal JN3	0.32±0.070bc	2.76±0.200a	5.64±0.319a	12.32±0.658b	13.07±0.377a	12.95±0.667a	2.12±0.169a	1.51±0.140a	6.34±0.275a
	Super 2270	0.20±0.057c	1.00±0.140b	3.72±0.317ab	23.10±0.658a	3.54±0.327b	4.96±0.367b	1.56±0.244ab	1.26±0.206ab	4.92±0.394b
	Early Urbana Y	0.27±0.064bc	0.69±0.098b	1.33±0.205d	5.94±0.309d	1.31±0.207d	1.90±0.237d	0.88±0.115bc	0.73±0.096bc	1.63±0.107d
		$F_{6,374}$ = 101.544	$F_{6,374}$ = 60.279	$F_{6,374}$ = 17.724	$F_{6,374}$ = 40.802	$F_{6,374}$ =	$F_{6,374}$ = 10.303	$F_{6,374}$ = 97.293	$F_{6,374}$ = 8.473	$F_{6,3041}$ = 68.757
Inactive mines	Primo Early	0.16±0.049c	0.86±0.081cd	2.75±0.142c	9.89±0.467d	2.07±0.212c	5.67±0.202c	10.16±0.351a	5.68±0.529a	4.66±0.200c
	Rio Grande	2.08±0.278a	1.28±0.242b	1.60±0.229d	21.21±0.631b	1.60±0.229c	24.53±1.336a	2.40±0.244cd	3.96±0.438b	7.33±0.484b
	Petomech	0.64±0.131bc	0.13±0.045d	1.66±0.182d	8.13±0.271d	1.63±0.185c	5.11±0.233c	6.98±0.552b	3.25±0.283bc	3.44±0.163cd
	Super Strain B	0.44±0.120c	1.02±0.159b	1.98±0.226cd	9.02±0.279d	1.98±0.226c	2.25±0.295d	1.16±0.137d	2.26±0.236d	2.51±0.141d
	Cal JN3	0.73±0.123bc	2.08±0.200a	5.59±0.308a	9.44±0.466d	13.51±0.474a	21.59±0.464b	8.92±0.349a	3.00±0.224bc	8.11±0.323ab
	Super 2270	1.44±0.466ab	2.40±0.340a	3.72±0.236b	35.78±0.466a	3.58±0.239b	22.04±0.848ab	3.40±0.396c	3.86±0.322b	9.53±0.632a
	Early Urbana Y	0.49±0.124c	0.71±0.101cd	1.76±0.223d	18.12±0.416c	1.61±0.202c	0.92±0.179d	1.27±0.112d	2.43±0.189c	3.41±0.293cd
		$F_{6,374}$ = 271.320	$F_{6,374}$ = 17.766	$F_{6,374}$ = 121.493	$F_{6,374}$ = 43.967	$F_{6,374}$ =	$F_{6,374}$ = 11.945	$F_{6,374}$ = 232.564	$F_{6,374}$ = 9.513	$F_{6,3041}$ = 60.613
Total mines	Primo Early	0.35±0.064c	1.56±0.142c	5.47±0.177c	18.72±0.782de	4.32±0.335c	10.18±0.240c	10.44±0.344a	6.19±0.503a	7.15±0.292c
	Rio Grande	2.87±0.317a	1.32±0.248c	3.15±0.308d	30.89±0.725b	3.15±0.308c	29.75±1.514b	4.04±0.361cd	5.08±0.480ab	10.03±0.617b
	Petomech	1.25±0.168bc	0.13±0.045d	4.48±0.351cd	16.50±0.342e	4.27±0.342c	8.09±0.310cd	7.39±0.555b	3.71±0.329bcd	5.73±0.255cd
	Super Strain B	0.53±0.120c	1.91±0.252c	3.75±0.281d	16.44±0.314e	3.75±0.281c	4.86±0.452de	2.81±0.289de	2.72±0.274de	4.60±0.241d
	Cal JN3	1.05±0.150bc	4.85±0.324a	11.24±0.496a	21.76±0.787cd	26.58±0.643a	34.54±0.819a	11.03±0.369a	4.51±0.281bc	14.44±0.548a
	Super 2270	1.64±0.508b	3.40±0.368b	7.44±0.455b	58.88±0.787a	7.12±0.472b	27.00±1.144b	4.96±0.377c	5.12±0.370ab	14.45±0.980a
	Early Urbana Y	0.76±0.129bc	1.41±0.127c	3.08±0.300d	24.06±0.663c	2.92±0.288c	2.82±0.316e	2.14±0.162e	3.16±0.199cd	5.04±0.382cd
		$F_{6,374}$ = 269.985	$F_{6,374}$ = 43.764	$F_{6,374}$ = 94.009	$F_{6,374}$ = 71.697	$F_{6,374}$ =	$F_{6,374}$ = 11.394	$F_{6,374}$ = 219.867	$F_{6,374}$ = 12.539	$F_{6,3041}$ = 68.057

[a] The means followed by different letters in the same column are significantly different ($P < 0.05$, Tukey)

(S^2/m), Index of Dispersion (I_D) and *Z* test are presented in Table 4. Based on the index of dispersion, the spatial distribution in all tomato cultivars was aggregated during the two study years. For seven tomato cultivars, the regression between log S^2 and log *m* was significant in Taylor's model (P< 0.05) in both years (Table 5). In 2015 and 2016, the

Table 4. Variance to mean ratio (index of dispersion) and the *Z* coefficient for *Tuta absoluta* on different tomato cultivars in 2015 and 2016 (Z test for goodness of fit).

Year	Cultivar	S^2/m	I_D	*Z*
2015	Primo Early	1.85	660.73	9.63
	Rio Grande	2.73	981.07	17.50
	Petomech	2.90	1129.55	19.64
	Super Strain B	3.22	905.28	18.84
	Cal JN3	3.37	1332.67	23.52
	Super 2270	2.81	1077.73	18.75
	Early Urbana Y	2.42	694.74	13.32
2016	Primo Early	3.72	1693.41	28.03
	Rio Grande	4.42	1868.54	32.04
	Petomech	3.62	1618.81	27.00
	Super Strain B	3.19	14.52.15	23.72
	Cal JN3	5.64	2662.32	42.24
	Super 2270	12.63	50.39.91	72.15
	Early Urbana Y	2.77	1083.05	18.58

Table 5. Estimated parameters by Taylor's power law and Iwao's patchiness regression models for *Tuta absoluta* on different tomato cultivars in 2015 and 2016.

Year		Cultivar	Parameter estimation				Test for slope	
			a±*SE*	*b*±*SE*	r^2	P_{reg}	t_c	t_t
2015	Taylor	Primo Early	0.15±0.026	1.21±0.068	0.987	0.000	3.006	2.776
		Rio Grande	-0.01±0.055	0.99±0.119	0.945	0.001	0.047	2.776
		Petomech	0.08±0.058	1.12±0.119	0.956	0.001	0.956	2.776
		Super Strain B	0.02±0.082	1.18±0.162	0.929	0.002	1.099	2.776
		Cal JN3	0.07±0.069	0.78±0.122	0.910	0.003	1.813	2.776
		Super 2270	-0.02±0.039	1.13±0.067	0.986	0.000	1.891	2.776
		Early Urbana Y	0.006±0.053	0.759±0.129	0.897	0.004	1.871	2.776
	Iwao	Primo Early	-0.11±0.138	1.13±0.064	0.987	0.000	2.042	2.776
		Rio Grande	-0.06±0.173	1.02±0.059	0.987	0.000	0.407	2.776
		Petomech	0.13±0.272	1.071±0.82	0.977	0.000	0.865	2.776
		Super Strain B	0.07±0.434	1.07±0.126	0.947	0.001	0.536	2.776
		Cal JN3	0.33±0.152	0.92±0.032	0.995	0.000	2.555	2.776
		Super 2270	-.0.05±0.141	1.04±0.031	0.996	0.000	1.293	2.776
		Early Urbana Y	0.18±0.089	0.89±0.03	0.995	0.000	3.373	2.776
2016	Taylor	Primo Early	-0.13±0.112	0.70±0.197	0.980	0.001	1.490	2.447
		Rio Grande	0.08±0.086	0.95±0.1280	0.901	0.000	0.407	2.447
		Petomech	-0.004±0.063	0.79±0.119	0.882	0.001	1.717	2.447
		Super Strain B	0.03±0.058	0.95±0.107	0.929	0.000	0.460	2.447
		Cal JN3	0.07±0.095	1.16±0.123	0.937	0.000	1.287	2.447
		Super 2270	0.08±0.048	1.25±0.073	0.980	0.000	3.490	2.447
		Early Urbana Y	0.03±0.064	1.15±0.175	0.877	0.001	0.839	2.447
	Iwao	Primo Early	-0.02±0.160	0.94±0.042	0.988	0.000	1.414	2.447
		Rio Grande	0.50±0.213	0.93±0.053	0.981	0.000	1.295	2.447
		Petomech	0.27±0.124	0.90±0.036	0.990	0.000	2.870	2.447
		Super Strain B	0.28±0.169	0.93±0.056	0.978	0.000	1.273	2.447
		Cal JN3	0.27±0.271	1.06±0.033	0.994	0.000	1.940	2.447
		Super 2270	0.16±0.161	1.10±0.019	0.998	0.000	3.569	2.447
		Early Urbana Y	0.02±0.228	1.01±0.097	0.947	0.000	0.017	2.447

calculated *t* (t_c) was greater than *t*-table (t_t) for Primo Early and Super 2270, respectively, and showed an aggregated spatial distribution of tomato leaf miner. On the other hand, t_c was less than t_t on other cultivars, indicating a random spatial distribution of *T. absoluta* (Table 5). There was a significant relationship between the mean crowding and the density of *T. absoluta* ($P< 0.05$) based on Iwao's model in 2015 and 2016 (Table 5). During these two sampling years, Early Urbana Y in 2015 and Super 2270 in 2016 had an aggregated spatial distribution of tomato leaf miner, while *T. absoluta* had a random pattern on the other tomato cultivars. In Morisita's index, the *Z* value for *T. absoluta* on seven tomato cultivars was between -1.96 and 1.96 at all sampling dates in both growing years, which indicated that spatial distribution pattern of the pest in all sampling dates was random (Table 6). To develop coefficients of regression models based on large amount of data, the exact counts of *T. absoluta* were used. The sample size was re-calculated using Taylor's and Iwao's coefficient (Table 7). Comparison of the two different formulae used for calculating the optimal sample unit size showed that the lowest estimate of the sample size was calculated by using Taylor's formula, for *T. absoluta* on seven tomato cultivars in 2015 and 2016. In order to acquire greater precision, the 15% level was adopted, whereas in IPM programs 25% or 30% level is acceptable.

DISCUSSION

The tomato leaf miner is a noxious pest of tomato cultivation because of its high reproductive potential and serious damage to this economically important crop. Since the direct observation of the plants for the larval stage is an appropriate sampling method, in the present study, a whole plant of tomato was selected as a sampling unit to estimate the number of *T. absoluta* larvae. The population density of tomato leaf miner displayed differences in all tested tomato

Table 6. Morisita's index and Z value for *Tuta absoluta* on seven tomato cultivars in different sampling dates of 2015 and 2016 (Z test for goodness of fit).

Cultivar		2015						2016							
		2 June	9 June	16 June	23 June	30 June	7 July	19 June	26 June	3 July	10 July	17 July	24 July	31 July	7 August
Primo Early	I_δ	1.23	1.04	1.12	1.13	0.85	0.41	1.12	1.17	0.84	0.98	0.97	0.81	1.43	1.40
	Z	0.02	0.01	0.01	0.02	0.03	0.09	0.00	0.04	0.01	0.00	0.01	0.01	0.09	0.05
Rio Grande	I_δ	0.74	0.94	1.04	0.94	1.35	1.14	0.98	0.71	1.28	0.94	1.28	1.18	1.35	1.36
	Z	0.02	0.01	0.00	0.02	0.03	0.09	0.03	0.01	0.02	0.00	0.02	0.01	0.02	0.02
Petomech	I_δ	1.28	1.05	1.05	0.92	1.32	1.40	-	-	1.05	0.92	1.06	0.92	1.33	1.90
	Z	0.01	0.00	0.01	0.02	0.02	0.05	-	-	0.01	0.00	0.01	0.01	0.06	0.05
Super Strain B	I_δ	1.58	1.07	1.01	0.83	0.72	0.90	0.98	1.03	1.04	0.94	1.04	1.28	1.25	1.58
	Z	0.01	0.01	0.01	0.03	0.04	0.09	0.03	0.03	0.01	0.00	0.01	0.01	0.02	0.05
Cal JN3	I_δ	1.14	0.94	0.97	1.02	1.36	1.06	0.93	0.95	1.01	1.09	0.97	1.08	0.91	0.84
	Z	0.01	0.00	0.00	0.01	0.02	0.05	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.02
Super 2270	I_δ	1.04	0.97	1.02	1.05	0.99	0.68	0.93	0.98	1.09	1.10	1.14	1.07	1.58	1.54
	Z	0.00	0.00	0.00	0.01	0.02	0.04	0.00	0.03	0.01	0.00	0.01	0.01	0.02	0.02
Early Urbana Y	I_δ	1.16	1.00	0.91	1.03	1.05	1.01	0.74	0.52	1.41	0.97	1.46	1.24	0.71	0.47
	Z	0.02	0.01	0.01	0.02	0.04	0.07	0.07	0.04	0.02	0.00	0.02	0.02	0.03	0.04

Table 7. Calculated sample size of *Tuta absoluta* populations on seven tomato cultivars based on Taylor's power law and Iwao's patchiness coefficients in 2015 and 2016.

Year	Cultivar	N_{opt}		Year	Cultivar	N_{opt}	
		Taylor	Iwao			Taylor	Iwao
2015	Primo Early	25.73	50.83	2016	Primo Early	28.14	47.67
	Rio Grande	11.37	33.56		Rio Grande	16.44	26.77
	Petomech	37.24	41.81		Petomech	37.50	53.58
	Super Strain B	17.53	39.62		Super Strain B	11.47	21.02
	Cal JN3	16.26	26.54		Cal JN3	39.18	45.94
	Super 2270	30.84	65.44		Super 2270	31.50	66.88
	Early Urbana Y	9.03	17.90		Early Urbana Y	20.68	32.41

cultivars. The differences may be due to the presence of trichomes on the leaves, plant age, and Jasmonic acid produced by the plant after caterpillar damages resulting in a decrease in the preference, performance, and abundance of many tomato pests (Constabel *et al*., 1995; Thaler *et al*., 1996; Leite *et al.*, 2001).

Oliveira *et al.* (2012) found that the genotypes of tomato with higher densities of glandular trichomes had greater resistance to *T. absoluta* and, consequently, affected the population density of this pest. More specifically, according to Maluf *et al.* (2007), the length and orientation of leaf hairs, as well as trichoms density could have an effect on the incompatibility of pests on tomato cultivars. Due to lower density of trichoms or amount of Jasmonic acid or other compounds that cause resistant in tomatoes (Maluf *et al.*, 2010), Cal JN3 cultivar could be a suitable host plant for *T. absoluta* (Ghaderi *et al.*, 2017). It leads to an increase in the population density of tomato leaf miner, but further search on this hypothesis is necessary. Because of appropriate climatic conditions for all tomato cultivars, the peak of *T. absoluta* population was observed at mid-season and it gradually decreased during the rest of sampling dates. The activity of larvae which caused young foliage destruction, the plant withering, the nutritional quality of tomato leaves, and unsuitable climatic conditions may lead to decline in *T. absoluta* population towards late growing season (Leite *et al.*, 2001). Cherif *et al.* (2013) reported that tomato leaf miner adults were detected in the Mediterranean basin during the entire year. They also added that the population fluctuation of tomato leaf miner depends on weather conditions and host cues such as leaf volatile compounds. When the temperature and relative humidity were favorable for *T. absoluta*, the population density increased on all tomato cultivars and decreased at the end of growing season, then reached near zero. Our findings in this case are in line with results of other scientists (Abbes and Chermiti, 2011; Balzan and Moonen, 2012; Cherif *et al.*, 2013).

Tomato leaf miner had an aggregated distribution pattern based on the results of variance-to-mean ratio on all tomato cultivars tested. Aggregated pattern of different arthropod species are more abundant than other patterns (e.g., Naseri *et al.*, 2009; Kianpour *et al.*, 2010; Darbemamieh *et al.*, 2011). However, Morisita's index and regression models (Taylor's power law and Iwao's patchiness regression methods) indicated the random distribution on tomato cultivars, inferring that in calculating spatial pattern of *T. absoluta*, the different methods might have different results (Khodayari *et al.*, 2010; Rahmani *et al.*, 2010). Random pattern of tomato leaf miner on some tomato cultivars might be due to its lower population density, because Southwood (1978) proved that when a population becomes sparse in an area, the chance of an individual is very low to be in any sample unit, so, the distribution pattern would be random. This result is in

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agreement with our findings of *T. absoluta* on most of the tomato cultivars using Taylor's power law and Iwao's regression model. In this study, the random distribution of *T. absoluta* on some cultivars showed that all leaves had a similar chance of being employed by an individual, and the presence of an individual was not affected by the others. These results suggested that different plant cultivars can affect the spatial distribution of insects as mentioned by other researchers (Sedaratian *et al.*, 2010). Due to high population density of *T. absoluta* on tomato leaves or, perhaps, some behavioral characteristics, Taylor's and Iwao's models showed an aggregated distribution of the pest on 'Primo Early' and 'Early Urbana Y' in 2015, respectively, and on 'Super 2270' in 2016. In both years, the data obtained for seven tomato cultivars fitted better to Iwao's model (r^2= 0.947-0.998) than Taylor's (r^2= 0.877-0.986). However, to estimate the spatial pattern of insects on different plant cultivars, the best-fitted regression model can be different (Naseri *et al.*, 2009). The optimal sample size suggested by Iwao's method was typically higher at low population levels compared with Taylor's method and, also, the results were approximately near the number of samples used in this research. Principally, Taylor's method reduces the necessary sample size compared with Iwao's method (Ifoulis and Savopoulou-Soultani, 2006). In this study, the lower estimate of the sample size was calculated by using Taylor's equation for larval stages. Our findings in this case are in line with results of other researchers including Darbemamieh *et al.* (2011) and Ifoulis and Savopoulou-Soultani, (2006).

This study showed that the population density and spatial distribution of *T. absoluta* is cultivar-dependent. Moreover, estimation of the population density and spatial pattern of tomato leaf miner on different tomato cultivars can be used to develop the sampling plans in integrated management program of this pest.

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انبوهی جمعیت و الگوی پراکنش پهنه ای *Tuta absoluta* (Lepidoptera: Gelechiidae) روی ارقام مختلف گوجه فرنگی

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چکیده

یکی از مهم ترین عوامل در نمونه برداری و برنامه مدیریت تلفیقی آفات دانستن انبوهی جمعیت و الگوی پراکنش پهنه ای حشرات می باشد، به ویژه در مورد آفات مهاجمی همانند مینوز برگ گوجه فرنگی، *Tuta absoluta* (Meyrick) که باعث ایجاد مشکل جدی در کشت گوجه فرنگی شده است. بنابراین، انبوهی جمعیت و الگوی پراکنش پهنه ای *T. absoluta* در طول سال های 1394 و 1395 در منطقه ورامین (استان تهران) روی هفت رقم گوجه فرنگی ('Primo Early', 'Rio Grande', 'Cal JN3', 'Petomech', 'Early Urbana Y', 'Super Strain B' and 'Super 2270') مشخص شد. انبوهی *T. absoluta* با شمارش تعداد دالان های با لارو زنده/بوته (دالان های فعال) و دالان های بدون لارو زنده/بوته (دالان های غیر فعال) محاسبه شد که از مجموع هر دو، انبوهی کل جمعیت (کل دالان ها/بوته) به دست آمد. در سال 1394، بالاترین میانگین تعداد کل دالان های ایجاد شده توسط *T. absoluta* روی رقم 'Cal JN3' (21/82 دالان/بوته) و کمترین میانگین روی رقم 'Early Urbana Y' (11/08 دالان/بوته) بود. در سال 1395، بالاترین و پایین

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ترین میانگین انبوهی جمعیت *T. absoluta* به ترتیب روی رقم 'Cal JN3' (14/44 لارو/ وته) و 'Super Strain B' (4/60 لارو/ وته) مشاهده شد. با استفاده از شاخص موریسیتا، مدل های رگرسیونی تیلور و آیوائو و روش نسبت واریانس به میانگین، الگوی پراکنش پهنه ای *T. absoluta* تعیین شد. در تمام ارقام گوجه فرنگی با استفاده از شاخص پراکندگی، الگوی پراکنش پهنه ای در هر دو سال به صورت تجمعی بود. در مدل های تیلور و آیوائو، پراکنش پهنه ای روی ارقام 'Primo Early' و 'Early Urbana Y' در سال 1394 و روی رقم 'Super 2270' در سال 1395 ار نوع تجمعی بود، اما الگوی پراکنش پهنه ای این آفت روی سایر ارقام از نوع تصادفی بود. همچنین، الگوی پراکنش پهنه ای *T. absoluta* با استفاده از شاخص موریسیتا در همه تاریخ های نمونه برداری از نوع تصادفی بود. کمترین اندازه بهینه نمونه به وسیله ضریب تیلور محاسبه شد. این نتایج نشان داد که ارقام مختلف گوجه فرنگی روی انبوهی جمعیت و الگوی پراکنش پهنه ای *T. absoluta* تاثیر می گذارد. نتایج نشان داد که ارقام گوجه فرنگی روی انبوهی جمعیت و پراکنش پهنه ای *T. absoluta* تاثیر می گذارد. شاخص های پراکنش پهنه ای را می توان در راستای بهبود برنامه های نمونه برداری برای برآورد دقیق انبوهی جمعیت *T. absoluta* استفاده کرد.