Spatial patterns of *Tuta absoluta* and heterorhabditid nematodes

Mahfouz M.M. Abd-Elgawad

Phytopathology Department, National Research Center, EI-Tahrir St., Dokki 12622, Giza, Egypt
e-mail: mahfouzian2000@yahoo.com

Accepted for publication 31 May 2014

Summary. The tomato leafminer, *Tuta absoluta*, is a serious pest that can spread rapidly and damage tomato production substantially. Understanding the spatial distribution of the pest population and its natural enemies under specific conditions will aid in the formation and application of integrated control strategies against the pest. The dispersion parameter of *T. absoluta* larval population derived from Taylor's Power Law was studied at plant, leaf and leaflet scales in three tomato fields in Egypt. Values of *b*, the distribution parameter of the Power Law, increased from plant to leaflet, indicating a more aggregated distribution as the size scale diminished. Data transformations were calculated from parameters of the Power Law fitted to the data obtained across the three fields and compared with log and square root transformations. Using baiting technique with *Galleria mellonella*, entomopathogenic nematodes (EPN) were not detected from the three fields but were found in 29 out of 30 soil samples obtained from a nearby mango orchard. Mortality of *T. absoluta* larvae caused by *Heterorhabditis* species isolated from the orchard ranged from 90.0 to 96.7% suggesting that its inundative release for pest control may be feasible. Horizontal spatial patterns of the nematodes were aggregated within the orchard, with individual soil samples being much more likely to contain sufficient nematodes to infect multiple *G. mellonella* larvae than was predicted based on the total number of positive soil samples. The distribution of EPN-infected *G. mellonella* larvae within a series of extractions was random in 27 out of the 29 samples; the larvae were aggregated in the two remaining positive samples. The possibility of finding infected *G. mellonella* larvae in the positive samples was not more than that expected by chance.

Key words: distribution, entomopathogens, *Galleria mellonella*, insects, mango, tomato.

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a very injurious pest affecting tomato plants from seedlings to mature stage, both in glasshouse and open field cultivations (Zappala *et al*., 2012). Now, it is considered as a serious threat to tomato production worldwide (*e.g*., Desneux *et al*., 2010, 2011). The insect has a high reproductive potential and a short life cycle (Pereyra & Sanchez, 2006). The insect larvae bore into and develop inside the plant, continuously searching for new feeding locations. The larvae usually mine in the leaves producing large galleries, penetrate into the stems causing tunnels leading to fragility of the stem and collapse of the plant, and burrow into the fruits causing a substantial losses of tomato production (Ibrahim & Hasan, 2012). The larval stage (caterpillars) does not enter diapause as long as food is available. There are four larval instars. In between moulting, caterpillars can be found temporarily outside the leaf mines or fruits. The larval period is most damaging period, which usually lasts 12-15 days. *Tuta absoluta* infests other solanaceous crops (eggplant, sweet pepper, potato and tobacco) as well as spontaneous plants, such as the black nightshade, *Solanum nigrum* L.; occasional damage has been reported on green bean (Desneux *et al*., 2010). Moreover, Abolmaaty *et al*. (2010) predicted peaks in *T. absoluta* annual generation numbers under current and expected climate change in Egypt based on future degree days.

Control strategies for *T. absoluta* consist mainly of early detection through sex pheromone traps, the use of insecticides and cultural methods (Batalla-Carrera *et al*., 2010). Biocontrol of the pest by predators, parasitoids, entomopathogens, resistant plants, and botanical insecticides has met with variable success (Miranda *et al*., 1998; Marchiori *et al*., 2004; Desneux *et al*., 2010; Jacobson & Martin, 2011). Batalla-Carrera *et al*. (2010) stressed that effective biocontrol agents against *T. absoluta* are urgently needed to replace broad spectrum

insecticides to avoid hazards to humans and development of resistance in the pest (Siqueira *et al*., 2000).

Entomopathogenic nematodes (EPN) are lethal to a broad range of economically important insect pests (Gaugler & Kaya, 1990; Grewal *et al*., 2005). The only function of a nematode-infective juvenile (IJ) is to seek out and infect insect hosts. After entering an insect, IJ release their associated mutualistic bacterium. The nematodes and bacteria feed on the liquefying host, and reproduce for several generations inside the cadaver. When food resources in the host become scarce, the adult EPN produce new IJ adapted to withstand the outside environment and so they leave the host cadaver in search of new hosts. The nematodes *Heterorhabditis bacteriophora* (Poinar), *Steinernema carpocapsae* (Weiser) and *S. feltiae* (Filipjev) showed high efficacy against *T. absoluta* larvae, but limited success against pupae (Batalla-Carrera *et al*., 2010; Jacobson & Martin, 2011).

The aggregated distribution of nematodes in soil usually confounds parametric statistical analyses necessary for their study and developing management strategy (Goodell & Ferris, 1980; Abd-Elgawad, 1992; Salama & Abd-Elgawad, 2010). Approaches based on estimate of nematode dispersion indices have been used to solve these problems (Barker & Campbell, 1981). Taylor's power law (Taylor, 1961) provides such an index. It states that: the variance (S^2) of a population is proportional to a fractional power (b) of the arithmetic mean (X): $S^2 = aX^b$ or $\log S^2 = \log a + b \log X$ (i), where a and b are population parameters, a depends chiefly on the sample size and b is an index of nematode dispersion (McSorley *et al.*, 1985). The law is useful in determining transformations (Taylor, 1970) and developing nematode sampling plans (Ferris, 1984).

In this paper, the spatial distribution patterns of *T. absoluta* larvae, as the most EPN-susceptible and tomato-damaging insect stage, at three separate tomato fields were studied. The patterns may serve as a basis for management-decision making (Bechinski & Pedigo, 1981; Faleiro *et al*., 2002) and as important information on ecological characteristics of insect and EPN species (Taylor, 1984; Salama & Abd-Elgawad, 2010). On the other hand, most surveys of natural populations have merely assessed the occurrence of EPN in single soil samples at different Egyptian sites and provide no information on the nematode distribution within sites (*e.g*., Abd-Elgawad & Nguyen, 2007; Abd-Elgawad *et al*., 2009; Abu-Shady *et al*., 2011). Hence, EPN populations in the three tomato fields were examined to know the possibility of their use in augmentation or conservation biological control in Egypt. Also, EPN in a nearby area were surveyed to assess their spatial patterns as a solid basis of information for effective use in biocontrol programmes (Ferris, 1985; Cabanillas & Raulston, 1994; Stuart & Gaugler, 1994; Salama & Abd-Elgawad, 2010).

MATERIALS AND METHODS

The study area, the Sugar Beet village, is located near the Alexandria-Cairo desert road running from the southern edge of the Maryut company farm south towards El Nasr canal, 55 to 66 km from Alexandria, Egypt. It represents one of the relatively newly reclaimed areas in the west of the Nile delta. Its climate is semi arid with an annual rainfall varying from 136 mm at the north to 35 mm at the south. No rain falls between June and September and is sporadic during November to February, the main rainy season, when the rainfall is localised and unpredictable (Erian & Yacoub, 2000). The mean monthly maximum temperature rises to 32.2°C at west Nubariya. The mean monthly minimum temperature drops to 7.5°C.

Insect and EPN sampling and laboratory bioassay. Following cultivation of Egyptian clover (*Trifolium alexandrinum* L.), the sandy soil (sand = 94.7%, silt = 3.2%, clay = 2.1%, pH = 7.88, organic matter = 1.14%) at Sugar beet village was ploughed and prepared for planting seeds of tomato, *Solanum lycopersicum* L. *cvs* Alisa, Hadeer and Hynez, in three separate fields between 3 and 20 March twice in 2011 and 2012. Plants grew on both sides of I m-wide raised beds, and the distance between the plants at each side of a bed was 40-50 cm where seeds were planted, covered and then irrigation took place between beds so that water could spread across to seed level; all agricultural practices were carried out as recommended (Mohamed & Dosuki, 2009). Since *Tuta absoluta* sex pheromone trap is highly attractive to male moths, the pheromone based control system was used according to Ibrahim & Hasan (2012) only in fields with *cvs* Alisa and Hynez. Pheromone traps were placed on the tops of 25 cm wood sticks and randomly scattered, just above the plant. Also, a light trap consisted of bright bulb suspended above blue plastic pan (25 cm diam., 8.5 cm height) filled with 0.3% detergent was randomly scattered in the field with cv. Alisa (six traps/Feddan). Tomato cv. Hadeer served as non-treated control. Thus, the experiment was based on *a priori* reasons: reported efficacy of pheromone and light traps (cv. Alisa), light traps only (cv. Hynez)

and untreated control (cv. Hadeer) for predicting the observed ranks of treatment populations. About 300 m^2 (in 2011) and 400 m^2 (in 2012) of each tomato field was delimited for sampling tomato leafminer larvae according to Sanchez *et al*. (2009) at three different scales: plant, leaf, and leaflet. Only the upper third of the plant was sampled as representative of the distribution of the pest because *T. absoluta* concentrates mainly in the apical parts of the tomato plants (Torres *et al*., 2001). At each field, three plants at three successive beds were randomly sampled from each 100 m^2 of the delimited area; 300 and 400 m^2 of each field were sampled in years 2011 and 2012, respectively. The apical parts of 9 (in 2011) and 12 (in 2012) tomato plants, each plant having seven consecutive expanded leaves, were harvested at random at each field/cultivar. To examine the spatial distribution of *T. absoluta* larvae at the three scales, each apical part of the plant was sorted in all component leaves and each leaf in its component leaflets; seven leaflets were counted per leaf. The leaves were examined under a binocular microscope to count *T. absoluta* larvae at the three spatial scales (Sanchez *et al*., 2009). Insect sampling took place on July 5 (in 2011) and 11 (in 2012) during the tomato harvest season. For EPN sampling, soil samples were simultaneously collected from the rhizosphere of the same insect-sampled plants with a shovel to a depth of *ca* 25 cm beneath the plant shoots. Three subsamples, from three of the insect-sampled plants in 100 m^2 plot, with a total volume of approx. 1500 cm^3 were collected and mixed thoroughly to form a composite soil sample; three (in 2011) and four (in 2012) composited samples were collected from each of the three tomato fields.

On July 5, 2012, soil samples were also collected from 11-year-old mango, *Mangifera indica* L., trees planted at 2.5 m between rows and 5 m between trees of a row in a geographical North/South row direction. The mango orchard, located near to the Sugar Beet village, had sandy soil (sand = 94.8%, silt = 3.5%, clay = 1.7%, pH = 7.83, organic matter = 1.29%). It apparently lacked a tree management programme and seemed as mango semi-jungle. Plastic tube openings for dropping under tree canopies were spaced 70 cm with two parallel longitudinal tubes per a tree row; a tube at each side of the tree stem. The orchard was irrigated at weekly intervals and sampling time corresponded to 3 days after irrigation time. Samples were taken once from 30 trees, ten successive trees in each of three consecutive rows, with a shovel to a depth of ca 25 cm. Under each tree canopy, three subsamples, within a distance of 40-60 cm apart, with a total volume of approx. 1500 cm^3 were randomly collected and mixed to form a composite sample representing the tree canopy. Each sample was mixed thoroughly and a 500-cm^3 aliquot from the soil sample was assayed for EPN using the *Galleria*-bait method (Bedding & Akhurst, 1975) in multiple cycles according to Stuart & Gaugler (1994) with modifications; *i.e*., samples were placed in assay chambers (plastic cups, 10 cm diam., 7 cm height). Each plastic cup was examined twice weekly for the first 2 weeks and once weekly thereafter. Infected juveniles (IJ) emerging from collected insect cadavers in the traps (White, 1927) were used to reinfect additional *Galleria* to confirm pathogenicity and to fulfill Koch's postulates (Pelczar & Reid, 1972).

EPN identification and preparation. Taxonomic identification was based not only on characteristics of cadavers, IJ and males (Poinar, 1990; Stock & Hunt, 2005), but also on morphological and molecular identification of species previously isolated from the same area (Abd-Elgawad *et al*., 2013). Nonetheless, the isolates obtained in the present investigation are receiving further study, and the current analysis remains relevant whether considered at the generic or the species level.

Larval susceptibility in Petri dish assay. Three EPN species/isolates extracted from the mango soil, *i.e*., *H. bacteriophora*, *Heterorhabditis* sp. Mgo2, and *Heterorhabditis* sp. Mgo3, were separately processed through *G. mellonella* last-instar larvae several times to obtain a pure culture of the nematodes. IJ in White traps were harvested then counted (Woodring & Kaya, 1988) and suspended in distilled water within flasks at 12-15°C for 2 days before testing against *T. absoluta* late (third to fourth) larvae instars collected from the tomato fields according to Batalla-Carrera *et al*. (2010) with some modifications; *i.e*., insects were singly exposed to only one nematode dose (50 IJ cm^{-2} or 981 IJ per 5-cm-diameter Petri dish). Insect mortality was checked at 72 h and presence of nematodes inside dead insects was observed to confirm nematode infection. There were 10 replicates/treatment and the experiment was repeated twice.

Statistical analyses. Counts of *T. absoluta* larvae were subjected to ANOVA using a completely randomised design and their arithmetic means were compared using Duncan's New Multiple Range Test. Means of dead insects by the three nematode species/isolates were also subjected to similar analysis. Mean and variance of *T. absoluta* larvae counts were calculated from each seven leaflets

Table 1. Mean (±SE) and ranges of densities of *T. absoluta* larvae at three spatial scales in three Egyptian fields each planted to a tomato cultivar*.

Tomato cultivar	Treatment	Plant			Leaf			Leaflet		
		Mean (SE)	Min.	Max.	Mean (SE)	Min.	Max.	Mean (SE)	Min.	Max.
Alisa	Phermone and Water traps	18.14b (4.42)	0	67	2.59b (0.36)	0	28	0.37b(0.04)	0	10
Hadeer	Non	35.86a (4.10)	0	75	5.12a (0.45)	0	39	0.73a (0.05)	0	14
Hynez	Phermone	22.67b (4.11)	0	65	3.24b (0.39)	0	31	0.47b(0.04)	0	14

* Means in each column sharing a common letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test.

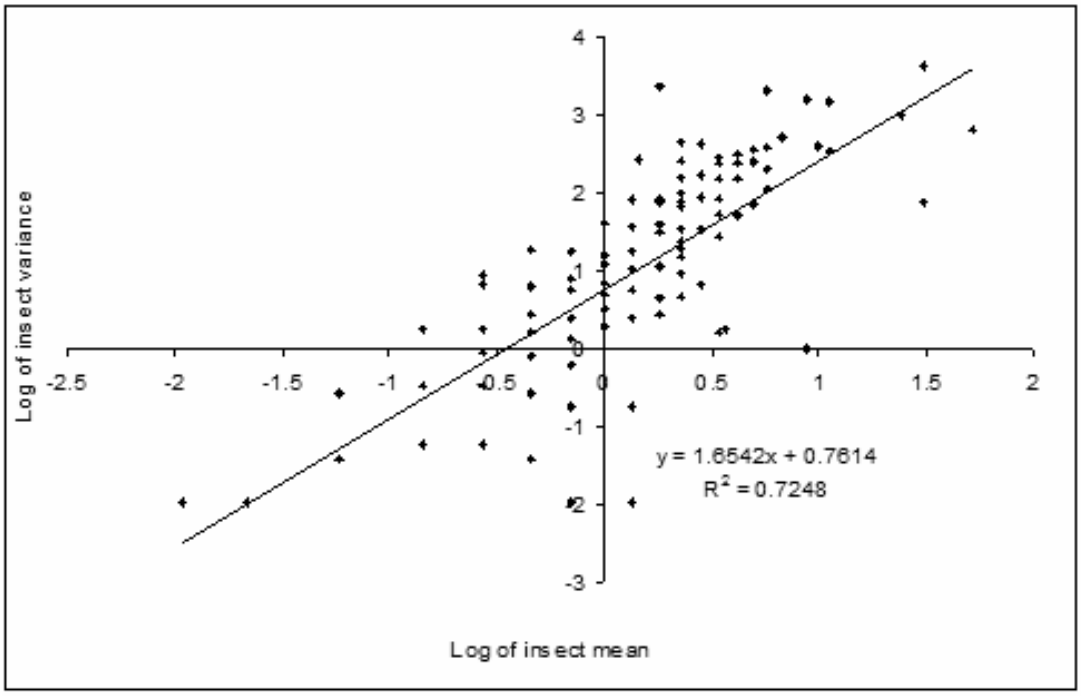

Fig. 1. Relationship between the logarithms of the mean (X) and variance (Y) of *Tuta absoluta* larvae counts on leaflets of tomato plants across three cultivars (n = 229).

of the same plant leaf for the leaflet scale; from each seven leaves of the same plant for the leaf scale; and from 3 plants at 100 m^2 area for the plant scale in each field and pooled for the two years together. These means and variances of larval counts were natural-log transformed. Mean and variance of EPN-infected *Galleria* numbers from the bait method on different observation dates for the positive soil samples were also transformed. Log variance was regressed against log mean to determine coefficients of Taylor's Power Law for each distribution scale of single tomato cultivars, and also for all cultivars pooled together, as well as for distribution of EPN-infected *Galleria* from positive soil samples (Taylor, 1961). For example, log variance was plotted against log mean of each three plants sampled from each 100 m^2 plot in a tomato field for each plant scale (7 plots/field/2 years for plant scale *et seq*). Data of these infected *Galleria* were analysed using the chi-squared, variance-to-mean ratio for agreement with a Poisson series (small samples, n < 30) according to Elliott (1971). The index of dispersion (*I*) or variance-to-mean ratio was calculated: $I = S^2/X'$, where S^2 = sample variance and X' = sample arithmetic mean. The significance of departures from unity was assessed by reference to tables of chi-square values (Pearson & Hartley, 1966).

The chi-squared value was calculated where a scale was not fit to the Power Law. A transformation procedure to normalize data in order to perform parametric statistics was determined (Taylor, 1970) as follows: $Y = X^{(1-0.5b)}$ (ii) and compared.

RESULTS

Insect sampling and spatial distributions. Data of *T. absoluta* larvae for each scale of a tomato cultivar did not differ ($P \leq 0.05$) between the two years; and therefore were pooled together. A total of 3,087 leaflets from 441 leaves of 63 tomato plants and 21 soil samples from the three tomato fields surveyed in 2011 and 2012 as well as 30 soil samples from mango sites in 2012 were examined. Overall mean densities (± standard error) for *T. absoluta* at the three spatial scales were 25.56 (±2.58) leafminer larvae/plant; 3.65 (±0.24) larvae/leaf; and 0.52 (±0.03) larvae/leaflet. Densities of *T. absoluta* larvae in fields planted with *cvs* Alisa or Hynez were lower ($P \leq 0.05$) than on cv. Hadeer at the three scales when calculations were performed on the untransformed insect counts (Table 1). Out of 21 plants, 147 leaves and 1029 leaflets examined, 20, 96, and 284 for cv. Hadeer, 15, 58, and 134 for cv. Alisa, and 20, 75, and 232 for *cv*. Hynez, respectively, were infected by *T. absoluta*.

Taylor's Power Law was fitted to seven of the nine spatial scales related to the three tomato cultivars (Table 2). Generally, slope values of regression lines lie within the range of values which would indicate

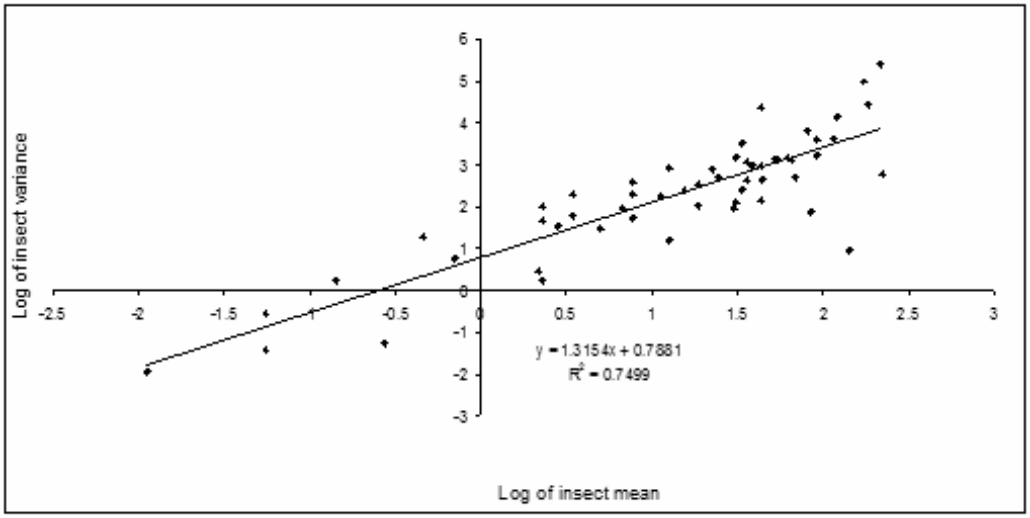

Fig. 2. Relationship between the logarithms of the mean (X) and variance (Y) of *Tuta absoluta* larvae counts on leaves of tomato across three fields (n = 55).

Table 2. Taylor's Power Law regression equations for data of *Tuta absoluta* larvae from three tomato cultivars/fields.

Tomato cultivar	Distribution scale	n	R^2	Power law parameters		Effective range	
				a	*b*	x^-	S^2
Alisa	leaflet	58	0.846**	1.08	1.73	0.14-4.0	0.14-20.33
	Leaf	15	0.834**	1.11	1.24	0.29-9.57	0.57-82.60
	Plant	7	0.796**	2.01	1.18	3.33-43.99	25.0-586.4
Hadeer	leaflet	96	0.736**	0.78	1.92	0.14-5.57	0.14-27.48
	Leaf	20	0.294*	0.70	1.22	0.71-10.71	1.57-221.63
	Plant	7	0.022 ns	1.55	1.09	27.33-46.67	56.51-1410.52
Hynez	leaflet	75	0.664**	0.42	1.39	0.14-4.43	0.14-37.94
	Leaf	20	0.921**	0.70	1.50	0.14-9.29	0.14-142.91
	Plant	7	0.459 ns	1.50	0.96	2.0-57.0	4.0-390.33

n = number of non-zero points, R^2 = coefficient of determination for fit to equation: $\log S^2 = \log a + b \log x^-$, where x^- = mean, S^2= variance. Effective range = range of x^- and S^2 data. * and ** = significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns = no significant regression.

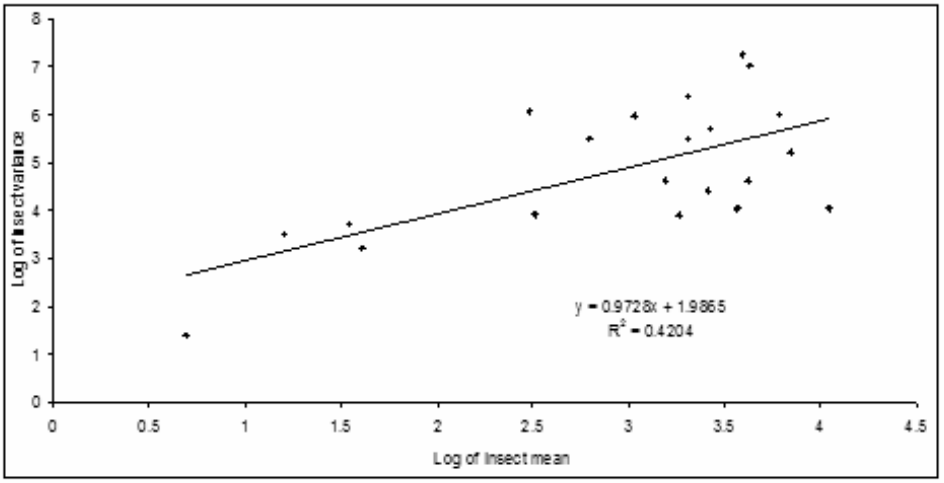

Fig. 3. Relationship between the logarithms of the mean (X) and variance (Y) of *Tuta absoluta* larvae counts on plants of tomato across three fields (n = 21).

Table 3. Comparison of *F* values from Analysis of Variance Tables from *T. absoluta* larvae counts and from transformed counts on three scales of tomato *cvs* Alisa, Hadeer, and Hynez.

Sample scale	Df+	F value			
		Natural log x	Square root	Taylor law*	Untransformed
Leaflet	2&3084	27.61	22.75	26.01	17.16
Leaf	2&144	3.27	3.17	3.15	2.73
Plant	2&18	3.00	3.35	3.35	3.04

Samples were collected from three tomato fields/cultivars, representing three different treatments.

+Degrees of freedom for treatments (the three tomato cultivars) and error of a completely randomized design.

Transformation calculated from $y = x^{(1 - 0.5b)}$ where x is the population count and *b* is the aggregation index from Taylor's Power Law (Table 2; Taylor, 1970).

from equation (ii) that log (slope = 2.0) or square root (slope = 1.0) data transformations are most appropriate to equalise experimental treatment variances (Taylor, 1970). The chi-squared values were well above the upper 5% significance level described by Elliot (1971) in the two plant scales that did not fit to the power law. Therefore, agreement with a Poisson series was rejected at the 95% probability level for cv. Hadeer ($\chi^2 = 197.18$, $P < 0.05$) and cv. Hynez ($\chi^2 = 313.04$, $P < 0.05$) indicating that *T. absoluta* larvae had clumped distribution on their plant scale. When insect counts for each scale of the three cultivars were pooled together, this law was fitted ($P \leq 0.01$) to the three grouped scales, namely leaflets, leaves and plants of the two-year-combined data (Figs 1-3). Consequently, slope values for each of the three grouped scales were used in equation (ii), resulting in the following normalising transformation: $Y = X^{0.17}$, $X^{0.34}$ and $X^{0.51}$ for leaflets, leaves and plants, respectively, across the three tomato fields. The used transformations resulted in different *F* values of ANOVA tables (Table 3) and consequently in various statistical probability (*P*) levels for separation between tomato cultivars concerning the mean counts of insect larvae in each of the three scales. This probability (*P*) for log, square root, Taylor law, and untransformed counts was $P \leq 0.001$ for leaflets, $P = 0.046$, 0.050, 0.051 and 0.075 for leaves, and $P = 0.075$, 0.058, 0.058, and 0.073 for plants, respectively.

For leaflets, results of mean separation techniques were similar regardless of which transformation was used (Table 4). However, in such a scale, use of any of the three transformations (log, square root, Taylor law) permitted separation of treatment population levels, between *cvs* Alisa and Hynez (Table 4), in which differences were

statistically nonsignificant when untransformed data were analysed (Table 1). For leaves, only Taylor law could separate treatment population levels, between *cvs* Alisa and Hadeer (Table 4). Also, for plants, only Taylor law could separate treatment population levels, between *cvs* Hynez and Hadeer (Table 4).

Table 4. Differences in mean separation of tomato leafminer larval population counts according to Duncan's multiple range test on transformed data.

Spatial scale	Tomato cultivar	Treatment mean*		
		Log x	Square root	Taylor law**
Leaflet	Alisa	0.066c	1.114c	1.032c
	Hadeer	0.137a	1.231a	1.065a
	Hynez	0.098b	1.157b	1.045b
Leaf	Alisa	3.58a	2.28a	1.359b
	Hadeer	3.11ab	1.90ab	1.681a
	Hynez	2.77b	1.68b	1.454b
Plant	Alisa	2.48b	3.87b	3.940b
	Hadeer	3.57a	5.97a	6.246a
	Hynez	2.79ab	4.44ab	4.720b

Data are from experiment in Table 1. The three tomato cultivars were treated differently for control of *Tuta absoluta*.

* Means in each column sharing a common letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test.

** Transformation calculated from $y = x^{(1 - 0.5b)}$ where x is the population count and *b* is the aggregation index from Taylor's Power Law (Table 2; Taylor, 1970).

EPN sampling and spatial distributions. Insect-parasitic nematode isolates of the genus *Heterorhabditis* Poinar were recovered from 29 (56.9%) of 51 soil samples (21 from tomato + 30 from mango) tested. EPN were not detected from any tomato soil samples in 2011 and 2012, while 29 (96.7%) of 30 samples from mango soil in 2012 were positive. Morphological and molecular identification revealed the presence of *H. bacteriophora* (25 samples), and two unidentified species designated as *Heterorhabditis* sp. Mgo2 (in 2 samples), and *Heterorhabditis* sp. Mgo3 (in 2 samples). These three isolates induced 90.0, 93.3 and 96.7% mortality, respectively, to late larvae instars of the tomato leafminer. All dead larvae were dissected to confirm nematode parasitism.

Individual soil samples from the mango orchard often contained sufficient EPN to infect multiple *Galleria*. Overall, 152 *Galleria* of 29 soil samples from the mango orchard were infected with heterorhabditid nematodes with three samples producing one infection, one producing two infections, seven producing three infections, five producing four infections, seven producing six infections, one producing eight infections, two producing nine infections, one producing 12 infections, and two producing 13 infections. Such a large number of heterorhabditid isolates obtained from the mango orchard permits analysis of their distribution in three different levels. Firstly, the distribution of infected *G. mellonella* larvae within each positive sample was mostly random, that is, chi squared (χ^2) value for each of 27 positive samples lie between the appropriate 5% significance levels

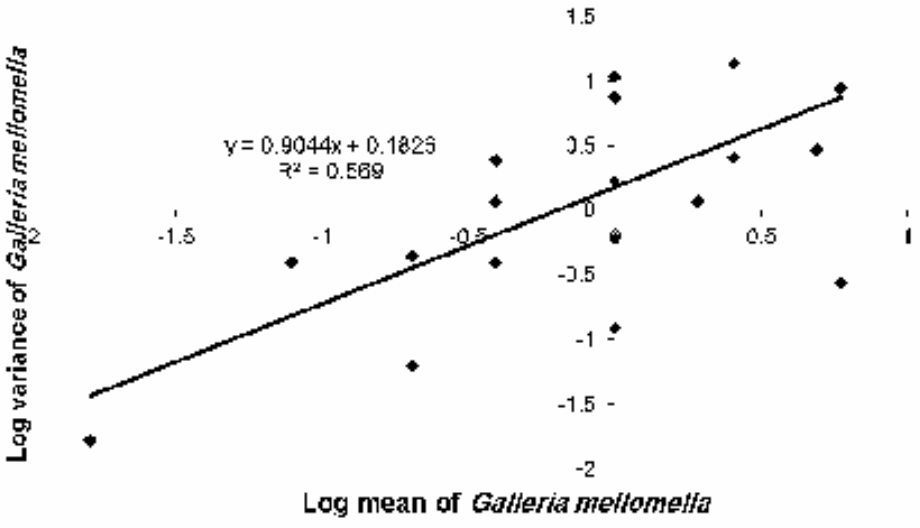

Fig. 4. Relationship between the logarithms of the mean (X) and variance (Y) of *Galleria mellonella* larvae infected by heterorhabditid nematodes (n = 29).

for agreement with a Poisson series (χ^2 = 2-12, P > 0.05). These chi-squared values were well above the upper 5% significance level described by Elliot (1971) in the remaining two positive samples with equal chi-squared value. Therefore, agreement with a Poisson series was rejected at the 95% probability level (χ^2 = 14, P < 0.05). So, in these two samples, infected *G. mellonella* larvae were aggregated in the observation schedule of the multiple *Galleria* bait method. So, the spatial variability of infective EPN-natural populations would mostly change randomly over time. Secondly, when log variance was regressed against log mean for the positive samples (n = 29), Taylor's Power Law fitted (P < 0.01) to the counts of infected *G. mellonella* larvae obtained from multiple cycles of the *Galleria* bait method (Fig. 4). The value of parameter *b* of the Power Law as an index of dispersion was close to one suggesting randomness. This indicated that the possibility of finding larvae infected with *G. mellonella* during any cycle of the multiple *Galleria* bait method is not more than expected by chance. Thirdly, the value for the variance-to-mean ratio in the 29 positive samples was significantly greater than unity (chi-square test). The chi-squared value was well above the upper 5% significance and therefore, agreement with a Poisson series was rejected at the 95% probability level (χ^2 = 59.776, P < 0.05). This high value of the variance-to-mean ratio indicated that the horizontal spatial pattern of heterorhabditid nematodes had aggregated distribution in the mango orchard.

DISCUSSION

Pheromone treatment could significantly affect the abundance of *T. absoluta* larvae on the three scales for the tested tomato cultivars (Table 1) documenting the efficacy of the pheromone (Caparros Megido *et al*., 2013). Also, Batalla-Carrera *et al*. (2010) demonstrated that EPN are capable of infecting larvae, pupae and adults of *T. absoluta* and killing the larvae inside the galleries in tomato plant leaves. They found that all three EPN tested, *i.e*., *Steinernema feltiae*, *S. carpocapsae* and *H. bacteriophora* had high efficacy against larvae irrespective of the dose. The high mortality obtained on *T. absoluta* larvae in the present study is similar to the mortality observed by them (86.6-100%) and by Rodriguez *et al*. (2005) in Petri dish trials using the entomopathogenic fungus *Beauveria bassiana* (96% mortality) but superior to results found by Goncalves-Gervasio & Vendamim (2007) using neem seed extract (52.4-95.4% mortality).

All the values of the Power Law parameters *a* and *b* (Table 2) lie within the range reported by Taylor (1961) for other biological contexts. Sánchez *et al*. (2009) found that the spatial distribution of *T. absoluta* larvae on the same scales of tomato deviated significantly from three of the most common frequency distributions in three out of 12 studied cases, *i.e*., larval counts on plant scale in one tomato site and leaf as well as leaflet scale in another site did not adjust either to Poisson or to negative or positive binomial distributions. On the other hand, differences in nematode patch size can affect the relationship of the sample variance to the mean (Duncan *et al*., 1989). For EPN, this is particularly evident when variable insect (host) size or environmental factors affect the nematode carrying capacity of the soil. Orchards with low carrying capacities in which many samples are positive, *e.g*., many small-size insects are infected, may have mean EPN population levels similar to orchards with high carrying capacities, but in which fewer samples are positive due to a few infected large-size insects. The EPN variance to mean ratio of the former orchards would be smaller than those of the latter. Therefore, application of the Power Law in a geographic survey using sampling regime similar to the present survey, *i.e*., sampling individual trees, could distinguish between two subsets of data according to the perceived size of patches of the citrus nematode *Tylenchulus semipenetrans* (Duncan *et al*., 1989). This information enables us to select the appropriate EPN application strategy to manage the target insect species, such as EPN augmentation or conservation (Campos-Herrera *et al*., 2009; Salama & Abd-Elgawad, 2010). Therefore, an insect baiting technique was also used herein to give information on the ability of the nematodes to infect insects. A fine scale (*e.g*., 5 × 5 cm) distribution of natural populations may also be studied to visualise where events of EPN emergence from insect cadavers have occurred, and give some indication of how dauer juveniles may have dispersed (Spiridonov *et al*., 2007).

In the present study, F values of ANOVA from transformed counts were generally higher than those of the original counts (Table 3). This resulted in further separation ($P \leq 0.05$) of insect means for each of these scales could be obtained through Taylor's Power Law, natural logarithm, and square root transformations of *T. absoluta* counts (Table 4). Yet, among the three transformation methods used, only the Power Law has the advantage of maintaining the untransformed counts-based separation between the means carried out for both plant and leaf scales (Table 4). This Law covers a wider range compared to the above-mentioned distributions and the transformations derived from

the Law parameter *b* (Table 2; Figs 1-4) are often easier to apply, as index of dispersion, than those of the negative binomial (*e.g.*, Elliott, 1971; McSorley *et al*., 1985; Salama & Abd-Elgawad, 2010). Transformations based on Taylor's Power Law were found by Abd-Elgawad & Hasabo (1995) to be more effective than log or square root transformations in stabilising variances for nematode population data. When the parameter *b* was used as index of citrus nematode dispersion (Duncan *et al*., 1989), it differed ($P \leq 0.05$) from values which would indicate from the Power Law that log (slope = 2.0) or square root (slope = 1.0) data transformations are most appropriate to equalise experimental treatment variances (Taylor, 1970). Yet, the primary thrust of the present study was to compare the spatial patterns of *T. absoluta* and its biocontrol agent, EPN, as a solid basis for drawing up an improved *T. absoluta* management strategy.

The distribution pattern observed often depends upon the scale over which it is measured (Southwood, 1978). Patchy distributions are often an apparent consequence of the distribution of resources upon which a species depends or of interactions among conspecifics or heterospecifics (Stuart & Gaugler, 1994). Admittedly, the very high percentage of positive samples in the mango orchard is an unprecedented case in Egypt (*e.g.*, Shamseldean & Abd-Elgawad, 1994; Abd-Elgawad & Nguyen, 2007; Abu-Shady *et al*., 2011) and elsewhere (*e.g.*, Cabanillas & Raulston, 1994; Stuart & Gaugler, 1994; Zadji *et al*., 2013). This was possibly because numerous small *Ceratitis capitata* larvae, susceptible to EPN (Abd-Elgawad *et al*., 2012), were seen during sampling in and around Mediterranean fruit fly infected-mangoes that have fallen from the trees. Individual positive samples from this orchard often contained sufficient nematodes to infect multiple *Galleria* as reported elsewhere (Stuart & Gaugler, 1994). Also, the number of nematodes infecting a *Galleria* larva under these circumstances is probably highly variable and is probably higher for first infections than later infections, as reported by Stuart & Gaugler (1994), which may have, at least partially, accounted for the randomness in the nematode-infected *G. mellonella* larvae during the series of extractions by the baiting method within and among most of the positive samples. On the other hand, horizontal spatial patterns of the nematodes were aggregated in the orchard, which agreed with EPN distribution in other regions (Cabanillas & Raulston, 1994; Stuart & Gaugler, 1994; Salama & Abd-Elgawad, 2010). Nematode aggregation can be caused by patchy host distribution coupled with high reproductive capabilities and limited mobility (McGraw, 2009). Yet, this latter found that nematode dispersion values at the scale of several square meters fluctuated from aggregated to random in very short time frames (1-2 wk). Such a cycling of dispersion demonstrate that EPN probably exist as complex metapopulations within an area of several square meters, fluctuating from uniform or random to aggregated in response to migration, immigration, death, infection and reproduction (Stuart *et al*., 2006; Spiridonov *et al*., 2007).

The spatial distribution of *T. absoluta* larvae was clumped at plant, leaf and leaflet scales at four sites in Buenos Aires province, Argentina (Sanchez *et al*., 2009). In parallel, eight out of the nine slope values of the insect in the present study (Table 2) had slope > 1.0 indicating different degrees of larval aggregations too. Only plant scale of cv. Hynez had slope (*b*) ≈ 1.0 (Table 2) suggesting random distribution. Moreover, values of *b* generally increased from plant to leaflet at each field, indicating a more aggregate distribution as scale diminished, which corroborated a previous report (Sanchez *et al*., 2009). This same trend was quite obvious at each spatial scale when analyses were also carried out for all fields/cultivars combined (Figs 1-3). Also, when Spiridonov *et al*. (2007) used both Lloyd's index of patchiness and spatial analysis by distance indices (SADIE), they found that EPN species had aggregated distributions. Yet, based on assigning these soil-inhabiting EPN into four groups (I-IV) of increasing physiological age, Lloyds index identified group I as being the most aggregated, whereas SADIE identified group II as the most aggregated; also probably because group I nematodes were aggregated at a scale finer than that used in the sampling regime (Spiridonov *et al*., 2007).

It is likely that the undisturbed habitat of the perennial mango has contributed to such an occurrence of EPN reported herein. On the other hand, because of crop, pest and natural enemy removal at the end of the tomato growing season as well as soil ploughing and preparation for the new crop, and because of usual chemical applications on tomato, no EPN were detected from the three fields. Hence, such factors may hinder the reliability of EPN as conservation biological control agents. Cabanillas & Raulston (1994) attributed low levels of suppression of corn earworm prepupa and pupa by naturally occurring *S. riobravis* to the aggregated distribution of the nematode in soil.

REFERENCES

ABD-ELGAWAD, M.M.M. 1992. Spatial distribution of the phytonematode community in Egyptian citrus groves. *Revue de Nématologie* 15: 367-373.

ABD-ELGAWAD, M.M.M. & HASABO, S.A. 1995. Spatial distribution of the phytonematode community in Egyptian berseem clover fields. *Fundamental and Applied Nematology* 18: 329-334.

ABD-ELGAWAD, M.M.M. & NGUYEN, K.B. 2007. Isolation, identification and environmental tolerance of new heterorhabditid populations from Egypt. *International Journal of Nematology* 17:116-123.

ABD-ELGAWAD, M.M.M., KABEIL, S.S.A. & EL-TAWEEL, M.I.A. 2009. Agricultural features of Borg El-Arab region, Egypt with special reference to nematodes and other ecological factors. *International Journal of Nematology* 19: 33-39.

ABD-ELGAWAD, M.M.M., ABDEL-RAZEK, A.S. & ABD EL-WAHAB, A.E. 2012. Efficacy of kaolin, entomopathogenic nematodes and fungi on the Mediterranean fruit fly infecting citrus. *Egyptian Journal of Agronematology* 11: 178-192.

ABD-ELGAWAD, M.M.M., JIAN, H., QIAO, Y. & HAMMAM, M.M.A. 2013. Entomopathogenic nematodes and their potential against insects and phytonematodes in Egypt. In: *Proceedings of the 10th International Nematological Symposium* (M.V. Pridannikov, Zh.V. Udalova & N.N. Butorina Eds.). pp. 98-101. Moscow (Bolshie Vyazemy), Russia, Russian Research Institute of Phytopathology.

ABOLMAATY, S.M., HASSANEIN, M.K., KHALIL, A.A. & ABOU-HADID, A.F. 2010. Impact of climatic changes in Egypt on degree day's units and generation number for tomato leaf miner moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Nature and Science* 8: 122-129.

ABU-SHADY, N.M., SHAMSELDEAN, M.M., ABD-ELBARY, N.A. & STOCK, S.P. 2011. Diversity and distribution of entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) in Egypt. *Journal of Nematology* 43: 224.

BARKER, K.R. & CAMPBELL, C.L. 1981. Sampling nematode populations. In*: Plant-parasitic nematodes*, V. III (B.M. Zuckerman & R.A. Rohde Eds.). pp. 451-472. New York, USA, Academic Press.

BATALLA-CARRERA, L., MORTON, A. & GARCIA-DEL-PINO, F. 2010. Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions. *BioControl* 55: 523-530.

BECHINSKI, E.J. & PEDIGO, L.P. 1981. Population dispersion and development of sampling plans of *Orius insidiosus* and *Nabis* spp. in soyabeans. *Environmental Entomolo*gy 10: 835-841.

BEDDING, R.A. & AKHURST, R. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica* 21: 109-110.

CABANILLAS, H.E. & RAULSTON, J.R. 1994. Evaluation of the spatial pattern of *Steinernema riobravis* in corn plots. *Journal of Nematology* 26: 25-31.

CAMPOS-HERRERA, R., DUNCAN, L.W., STUART, R.J., EL-BORAI, F. & GUTIERREZ, C. 2009. Entomopathogenic nematode ecology and biological control in Florida citrus orchards. In: *Integrated Management of Arthropod Pests and Insect Borne Diseases* (A. Ciancio & K.G. Mukerji Eds.). pp. 97-126. Dordrecht, the Netherlands, Springer Science + Business Media B.V.

CAPARROS MEGIDO, R., HAUBRUGE, E. & VERHEGGEN, F. 2013. Pheromone-based management strategies to control the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biotechnology, Agronomy, Society and Environment* 17: 475-482.

DESNEUX, N., WAJNBERG, E., WYCKHUYS, K.A.G., BURGIO, G., ARPAIA, S., NARVAEZ-VASQUEZ, C.A., GONZALEZ-CABRERA, J., CATALAN RUESCAS, D., TABONE, E., FRANDON, J., PIZZOL, J., PONCET, C., CABELLO, T. & URBANEJA, A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science* 83: 197-215.

DESNEUX, N., LUNA, M., GUILLEMAUD, T., & URBANEJA, T. 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. *Journal of Pest Science* 84: 403-408.

DUNCAN, L.W., FERGUSON, J.J., DUNN, R.A. & NOLING, J.W. 1989. Application of Taylor's power law to sample statistics of *Tylenchulus semipenetrans* in Florida citrus. *Journal of Nematology* (supplement) 21: 707-711.

ELLIOTT, J.M. 1971. *Some methods for the statistical analysis of samples of benthic invertebrates*. *Freshwater Biological Association* (scientific publication no. 25). UK, the Ferry House, Ambleside. 144 pp.

ERIAN, W.F. & YACOUB, R.K. 2000. The use of the ordinary kriging techniques in measuring the sustainability in sugar beet area, Nubariya, Egypt. *International Archives of Photogrammetry and Remote Sensing.* 33: 403-419.

FALEIRO, J.R., ASHOK KUMAR, J.A. & RANGNEKAR, P.A. 2002. Spatial distribution of red palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae) in coconut plantations. *Crop Protection* 21: 171-176.

FERRIS, H. 1984. Probability range in damage predictions as related to sampling decisions. *Journal of Nematology* 16: 246-251.

FERRIS, H. 1985. Population assessment and management strategies for plant-parasitic nematodes. *Agriculture, Ecosystems and Environment* 12: 285-299.

GAUGLER, R. & KAYA, H. 1990. *Entomopathogenic Nematodes in Biological Control*. USA, CRC Press Inc. 365 pp.

GONCALVES-GERVASIO, R. DE C.R. & VENDAMIM, J.D. 2007. [Bioactivity of aqueous neem seeds extract on the *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) in three ways of application.] *Ciência e Agrotec* 31: 28-34 (in Portuguese).

GOODELL, P. & FERRIS, H. 1980. Plant-parasitic nematode distribution in an alfalfa field. *Journal of Nematology* 12: 136-141.

GREWAL, P.S., EHLERS, R.-U. & SHAPIRO-ILAN, D.I. 2005. *Nematodes as Biocontrol Agents*. UK, CABI Publishing. 528 pp.

IBRAHIM, S.A. & HASAN, E.A. 2012. [*Integrated Control of Tomato Leafminer, Tuta absoluta*]. Egypt, Media Support Center Press of Malawi. 52 pp (in Arabic).

JACOBSON, R.J. & MARTIN, G.A. 2011. A potential role for entomopathogenic nematodes within IPM of *Tuta absoluta* (Meyrick) on organic tomato crops. *IOBC/WPRS Bulletin* 68: 71-74.

MARCHIORI, C.H., SILVA, C.G. & LOBO, A.P. 2004. Parasitoids of *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) collected on tomato plants in larvas, state of Minas Gerais, Brazil. *Brazilian Journal of Biology* 64: 552-554.

MCGRAW, B.A. 2009. The ecology and the biological control of the annual bluegrass weevil, *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae) using entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae). PhD Thesis. 182 pp.

MCSORLEY, R., DANKERS, W.H., PARRADO, J.L. & REYNOLDS, J.S. 1985. Spatial distribution of the nematode community on Perrine marl soil. *Nematropica* 15: 77-92.

MIRANDA, M.M.M., PICANCO, M., ZANUNCIO, J.C. & GUEDES, R.N.C. 1998. Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biocontrol Science and Technology* 8: 597-606.

MOHAMED, M.A. & DOSUKI, S.M. 2009. [*Tomato Production and Trading*]. Egypt, Media Support Center Press of Ismailia. 56 pp (in Arabic).

PEARSON, E.S. & HARTLEY, H.O. 1966. *Biometrika: Tables for Statisticians*. UK, Cambridge University Printing House. 238 pp.

PELCZAR, M.J. & REID, R.D. 1972. *Microbiology*. USA, the McGraw-Hill Companies, 164 pp.

PEREYRA, P.C. & SANCHEZ, N. 2006. Effect of two Solanaceous plants on developmental and population parameters of the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* 35: 671-676.

POINAR, G.O. JR. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. In: *Entomopathogenic Nematodes in Biological Control* (R. Gaugler & H.K. Kaya Eds.). pp. 23-61. Boca Raton, USA, CRC Press Inc.

RODRIGUEZ, S.M., GERDING, P. & FRANCE, A. 2005. [Selection of isolates of entomopathogenic fungi for control of tomato moth *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)]. *Agricultura Técnica* 66: 151-158 (in Spanish).

SALAMA, H.S.A. & ABD-ELGAWAD, M.M.M. 2010. Spatial patterns of the red palm weevil and applied entomopathogenic nematode *Heterorhabditis bacteriophora*. *Archives of Phytopathology and Plant Protection* 43: 689-699.

SÁNCHEZ, N.E., PEREYRA, P.C. & LUNA, M.G. 2009. Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae). *Environmental Entomology* 38: 365-374.

SHAMSELDEAN, M.M. & ABD-ELGAWAD, M.M.M. 1994. Natural occurrence of insect pathogenic nematodes (Rhabditida: Heterorhabditidae) in Egyptian soils. *Afro-Asian Journal of Nematology* 4: 151-154.

SIQUEIRA, H.A., GUEDES, R.N. & PICANCO, M.C. 2000. Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Agricultural and Forest Entomology* 2: 147-153.

SOUTHWOOD, T.R.E. 1978. *Ecological Methods with Particular Reference to the Study of Insect Populations*. UK, Chapman & Hall. 524 pp.

SPIRIDONOV, S.E., MOENS, M. & WILSON, M.J. 2007. Fine scale spatial distributions of two entomopathogenic nematodes in a grassland soil. *Applied Soil Ecology* 7: 192-201.

STOCK, S.P. & HUNT, D.J. 2005. Morphology and systematics of nematodes used in biocontrol. In: *Nematodes as Biocontrol Agents* (P.S. Grewal, R.-U. Ehlers & D.I. Shapiro-Illan Eds). pp. 3-43. Wallingford, UK, CABI Publishing.

STUART, R.J. & GAUGLER, R. 1994. Patchiness in populations of entomopathogenic nematodes. *Journal of Invertebrate Pathology* 64: 39-45.

STUART, R.J., BARBERCHECK, M.E., GREWAL, P.S., TAYLOR, R.A.J. & HOY, C.W. 2006. Population biology of entomopathogenic nematodes: concepts, issues and models. *Biological Control* 38: 80-102.

TAYLOR, L.R. 1961. Aggregation, variance and the mean. *Nature* 189: 732-735.

TAYLOR, L.R. 1970. Aggregation and the transformation of counts of *Aphis fabae* Scop. on beans. *Annals of Applied Biology* 65: 181-189.

TAYLOR, L.R. 1984. Assessing and interpreting the spatial distribution of insect population. *Annual Review of Entomology* 29: 321-358.

TORRES, J.B., FARIA, C.A., EVANGELISTA, W.S. & PRATISSOLI, D. 2001. Within plant distribution of the leafminer *Tuta absoluta* (Meyrick) immature in processing tomatoes, with notes on plant phenology. *International Journal of Pest Management* 47: 173-178.

WHITE, G.F. 1927. A method for obtaining infective nematode larvae from culture. *Science* 66: 302-303.

WOODRING, J.L. & KAYA, H.K. 1988. *Steinernematid and Heterorhabditid Nematodes: a Handbook of Techniques*. USA, Arkansas Agricultural Experiment Station (South Cooperative Bulletin 331). 30 pp.

ZADJI, L., BAIMEY, H., AFOUDA, L., HOUSSOU, F.G., WAEYENBERGE, L., DE SUTTER, N., MOENS, M. & DECRAEMER, W. 2013. First record on the distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Southern Benin. *Russian Journal of Nematology* 21: 117-130.

ZAPPALA, L., BERNARDO, U. BIONDI, A., COCCO, A., DELIPERI, S., DELRIO, G., GIORGINI, M., PEDATA, P., RAPISARDA, C., GARZIA, G.T. & SISCARO, G. 2012. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. *Bulletin of Insectology* 65: 51-61.

M.M.M. Abd-Elgawad. Особенности пространственного распределения *Tuta absoluta* и гетерорабдитидных нематод.

Резюме. Томатная минирующая моль *Tuta absoluta* представляет собой опасного вредителя, который может быстро размножаться и существенно снижать урожай томатов. Понимание пространственного распределения этих вредителей и их естественных врагов несомненно поможет формированию интегрированной борьбы с ними. Параметры дисперсии в личиночной популяции *T. absoluta*, вычисленные с помощью правила степени Тейлора, были изучены на уровне растений, листьев и листовых чешуек на трех плантациях томатов в Египте. Значение коэффициента *b* – параметра, отражающего характер распределения в правиле степени Тейлора, возрастало по мере уменьшения размера исследованного участка (от растения до листовой чешуйки), указывая на возрастание степени аггрегированности распределения при уменьшении размеров участка, для которого оценивается характер распределения. Трансформация данных в соответствии с параметрами правила степени Тейлора давала результаты, сходные с эмпирическими данными, полученными по трем изученным полям. Не удалось выявить энтомопатогенных нематод на трех изученных полях с использованием гусениц вощинной моли *Galleria mellonella* в качестве приманок, однако, нематоды были обнаружены в 29 из 30 почвенных проб, собранных под плодовыми деревьями манго, неподалеку. Смертность личинок *T. absoluta* от нематод из культур варьировала от 90,0 до 96,7%, указывая на имеющийся потенциал этих нематод. Оценка пространственного распределения гетерорабдитид в почве сада показала, что каждая отдельная проба почвы содержит достаточное количество нематод для успешного заражения нескольких гусениц вощинной моли. Распределение числа зараженных нематодами *G. mellonella* в пределах каждой из проб почвы было случайным в 27 из 29 исследованных образцов и аггрегированных в двух оставшихся. В то же время, вероятность обнаружения, зараженных нематодами *G. mellonella*, в положительных пробах не превышала уровня, ожидаемого при случайном заражении.