Running title: Sequential sampling plans for *T. absoluta* in protected tomato crops

Spatial distribution and sequential sampling plans for *Tuta absoluta* (Lepidoptera: Gelechiidae) in greenhouse tomato crops

Arturo Cocco,[a*] Giuseppe Serra,[b] Andrea Lentini,[a] Salvatore Deliperi[a] and Gavino Delrio[a]

* Correspondence to: Arturo Cocco, Department of Agriculture, University of Sassari, Viale Italia 39, 07100 Sassari, Italy.
E-mail: acocco@uniss.it

[a] Department of Agriculture, University of Sassari, Sassari, Italy

[b] Institute of Ecosystem Study, National Research Council, Sassari, Italy

Abstract

BACKGROUND: This work investigated the within- and between-plant distribution of the tomato leafminer, *Tuta absoluta* (Meyrick), in order to define action thresholds based on leaf infestation and propose enumerative and binomial sequential sampling plans for pest management applications in protected crops.

RESULTS: The pest spatial distribution was aggregated between plants, and median leaves were the most suitable sample to evaluate its density. Action thresholds of 36 and 48%, 43 and 56%, 60 and 73% of infested leaves, corresponding to economic thresholds of 1 and 3% of damaged fruits, were defined for tomato cultivars with big, medium and small fruits, respectively. Green's method was a more suitable enumerative sampling plan as it required a lower sampling effort. Binomial sampling plans needed lower average sample sizes than enumerative plans to make a treatment decision, with probabilities of error < 0.10.

CONCLUSIONS: Enumerative sampling plan required 87 or 343 leaves to estimate the population density in extensive or intensive ecological studies, respectively. Binomial plans would be more practical and efficient for control purposes, needing average sample sizes of 17, 20 and 14 leaves to take a pest management decision in order to avoid fruit damage higher than 1% in cultivars with big, medium and small fruits, respectively.

Keywords: tomato leafminer; Taylor's power law; Iwao's patchiness regression; enumerative sampling; binomial sampling; resampling validation

1 INTRODUCTION

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), has been considered a key pest of tomato crops since its establishment in the Mediterranean Basin in 2006.[1] *Tuta absoluta* has a tropical origin (South America) and causes severe damages especially to protected tomato crops, with fruit loss up to 80-100%.[2] Females oviposit mainly on leaves and larvae produce expanding mines on mesophyll, thereby reducing the photosynthetic activity. Moreover, young and mature larvae bore into green and ripe tomato fruits causing yield loss and rendering the fruit unmarketable.[1] In addition, fruit infestations increase production costs, due to sorting of damaged tomatoes, and affect international trade flows, in view of the quarantine regulations designed by pest-free countries.[3] Studies on *T. absoluta* infestation dynamics demonstrated that larvae were mainly phyllophagous, infesting fruits when pest densities exceeded a certain magnitude.[4] A preliminary research about the biology of *T. absoluta* in Spain further pointed out a positive correlation between the proportion of infested fruits and the infestation on leaves.[5]

In South America, repeated applications of broad-spectrum pesticides (up to 36 in a single tomato growing season) to control the tomato leafminer led to the development of resistance to a number of active ingredients.[6,7] Accordingly, *T. absoluta* was resistant to most insecticides registered for use on tomato when it established in Europe,[8] compelling the adaptation of pest control tactics that have been tested with promising results (e.g. biological and pheromone-mediated control strategies).[9,10] Nevertheless, chemical control is the conventional strategy applied against this pest[11] and novel effective active ingredients, among which emamectin benzoate, flubendiamide and chlorantraniliprole, have been registered and effectively used in many European countries.[12] The implementation of rational and sustainable management strategies against the tomato leafminer is strongly required to avoid developing resistance to the newly-registered insecticides, reduce unnecessary pesticide applications, and then to preserve indigenous natural enemies[13,14] and pollinators from the unwanted exposure to these insecticides.[15–17] The spread of IPM practices is also supported by the European Union through the Directive 2009/128/EC,[18] which promotes the sustainable use of pesticides and the development of practical tools to help growers in making pest control decisions. Solid and generalized sampling plans require a deep knowledge of within-crop and within-plant distribution, aggregation patterns of the pest and the establishment of action thresholds that prevent fruit damage and crop loss.

The spatial distribution of *T. absoluta* on tomato plants has been studied in processing and fresh-market tomato crops, showing different results. Some studies reported the preferred oviposition site as the median third of the canopy,[19] whereas others observed a higher proportion of eggs in the apical leaves.[20] Larvae were observed in higher percentages in median leaves than in other parts of the canopy.[20,21] In processing tomato crops, young and mature larvae were mainly detected in the median and upper portions of the canopy and females did not show any oviposition preference between median and apical leaves.[22] Accordingly, comparison of different sampling methods showed the highest correlations between the number of mines and larvae in the median leaves and those in the whole plant, when in fact the number of eggs was more closely related with counts in the upper canopy.[23]

In the early years of establishment in Europe, empirical thresholds used in management programs to control *T. absoluta* infestations were based on male captures in pheromone traps.[24] Male catches were related to plant and fruit damages and were used in South America to adopt action thresholds for open-field crops based on daily captures.[25,5] Adult catches in protected tomato crops were correlated with leaf infestation at low population density (< 25 males per trap per week),[26] in contrast no relationship was observed at high male catches (Cocco A., unpublished data).[27] The definition of reliable economic thresholds based on male captures is difficult to determine considering that they are affected by a number of factors, among which pheromone load of monitoring traps, climatic conditions (temperature and humidity) and control methods employed (e.g. mating disruption, mass trapping). Generalized action thresholds based on direct infestation assessment on leaves and fruits would be more reliable even though time consuming. However, regardless of the several studies performed on the spatial distribution of *T. absoluta*,[19,22,23] it has been noted that no sampling plans for control purposes on tomato crops based on leaf or fruit infestation have been proposed.

The objective of this study was to develop enumerative and binomial sequential sampling plans to estimate the leaf population density of *T. absoluta* and provide suitable and cost-effective tools to assist management decisions in IPM programs. With this aim, the spatial distribution was investigated by means of aggregation indexes and action thresholds were determined by relating the pest population density on leaves to fruit damage.

2 MATERIALS AND METHODS

2.1 Experimental sites

The study was carried out in two areas of an intensive protected tomato-growing district in south-western Sardinia (Italy) (Pula: Lat. 38°58’18” N, Long. 8°57’57” E; Capoterra: Lat. 39°10’22” N, Long. 9°00’20” E) from 2010 to 2012 in 33 commercial plastic and glass greenhouses. Surveyed greenhouses were selected covering a wide range of *T. absoluta* population density and different cultural characteristics, so that the developed sampling plans could be generalized and applicable to a broad range of tomato cultural and management contexts. Experimental plots ranged from 500 to 3,500 m^2 and were cultivated with fresh tomato cultivars with indeterminate growth habit: Cherry (grape tomatoes, 20-35 mm in diameter, 5 greenhouses), Minuetto (oval-shaped tomatoes, 25-35 mm in diameter, 4 greenhouses), Camone (round-shaped fruits, 40-60 mm in diameter, 2 greenhouses), Balente (oval-shaped tomatoes, 40-70 mm in diameter, 2 greenhouses), Belrosso (round-shaped fruits, 60-70 mm in diameter, 2 greenhouses) and Cuore di bue (oxheart-shaped fruits, 60-100 mm in diameter, 18 greenhouses). Tomato plants were grown by twisting the stems to a sisal twine suspended to horizontal support wires above plants. Basal leaves were periodically pruned as they became senescent and lateral shoots were regularly removed. Tomato crops were cultivated in winter-spring and summer-winter growing seasons on coconut fiber or on the soil, which was in some cases covered with plastic mulch, and subjected to conventional cultural practices of drip irrigation and nutrition. Greenhouses were equipped with insect-proof screens and managed by different control methods against *T. absoluta*: chemical and biological control, mating disruption, mass trapping with light or pheromone traps, and untreated greenhouses. The tomato leafminer control was based on 1-3 applications of spinosad, emamectin benzoate, abamectin or *Bacillus thuringiensis* Berliner subsp. *kurstaki*. Control of whiteflies was achieved in the different greenhouses by applying imidacloprid, flonicamid, pyriproxyfen or acetamiprid, whereas oxamil was used against nematodes. Fungicide sprays were targeted against powdery mildew, root rot and late blight and consisted in periodic applications of sulphur, cimoxanil, metalaxil, propamocarb or copper hydroxide. Insecticides and fungicides were applied to runoff by a compressed air sprayer at the recommended label rates.

Mating disruption greenhouses were protected with 3-6 g of the natural pheromone blend (ratio 90:10 of [(3E,8Z,11Z)-tetradecatrien-1-yl acetate and (3E,8Z)-tetradecadien-1-yl acetate) per 1000 m^2. The mass trapping was achieved by deploying blacklight traps at densities ranging from 1 trap per 1,000 m^2 to 1 per 350 m^2 or pheromone traps loaded with 0.7 mg of the natural pheromone blend at densities from 1 trap every 350 m^2 to 1 per 100 m^2.

2.2 Within-plant distribution

The study of within-plant distribution of mines and larvae on leaves was conducted in a commercial unheated glasshouse, wide 3000 m^2 and provided with insect-proof screens, with the aim of determining the most suitable portion of the canopy to sample in both intensive and extensive sampling programs. The spatial distribution was investigated in winter-spring during the whole growing season, for a total of 28 sampling dates, starting from when tomato plants were approx 1 m tall (6 weeks after transplant) until the end of the harvest period. Tomato seedlings of cultivar Minuetto were transplanted on the first week of November 2009 and plants were supported with sisal twines, fertilized through drip-irrigation, and pruned monthly by removing senescent leaves and lateral shoots. The tomato crop was sprayed with abamectin and twice with emamectin benzoate at the lowest recommended label rates 4, 7 and 26 weeks after transplant, respectively.

Owing to the difficulty and the time required to detect larvae inside the mines by the naked eye, the spatial distribution was investigated by counting the number of visible mines per leaf (M). On each sampling date, the number of mines was counted on 150 plants in an old, mature and young leaf randomly-chosen from the lower, median, and apical third of the canopy, respectively. In order to evaluate the proportion of mines containing *T. absoluta* larvae (pL), an additional sample of 70 mined leaflets was collected from each plant stratum and examined in the laboratory under a dissecting microscope. Then, the pL values were used to calculate the average number of mines with larvae (ML) and empty mines (ME) on each stratum as follows:

$$ML = M \times pL / 100$$

and

$$ME = M \times (1 - pL) / 100$$

For the purpose of assessing the reliability of field-collected data to estimate the larval density in each canopy stratum, the number of mines with larvae per leaf (ML) was regressed to the total number of mines per leaf (M) ($P < 0.05$) (PROC REG).[28]

The number of mines with larvae on each canopy stratum was normalized as percentage over the total number of mines with larvae, in order to avoid bias effects owing to changes in pest abundance. As the pest abundance along the plant canopy is not independent, significant differences in spatial distribution in the three strata were compared using the Friedman's rank test, a nonparametric procedure that enables to compare dependent samples, followed by rank sum multiple comparison test ($P < 0.05$) (PROC GLM).[29,28] Prior analysis, data within each sampling date were ranked from lowest to highest value (PROC RANK).[29,28] The same procedure was followed to analyze the distribution of empty mines. The variability in the proportions of ML and ME during the tomato growing season was estimated in each stratum by the coefficient of variation (CV = standard deviation mean^{-1}). The stratum of the canopy with higher presence of mines with larvae, lower presence of empty mines and the less variable data (i.e., lowest coefficient of variation values) was accounted as the portion of the plant to sample with the intention of identifying the most reliable assessment of pest population in the field.

2.3 Spatial distribution

Data used to determine the spatial distribution of the pest were obtained by monitoring the leaf infestation and the damage on fruits throughout tomato growing seasons on each of the greenhouses described in the Section 2.1. The tomato leafminer infestation was estimated weekly or bi-weekly by counting in the field the number of mines in one leaf per plant on 30-150 randomly-chosen tomato plants, depending on greenhouse size and pest density. Healthy and fully expanded leaves were sampled from the median third of the canopy, which showed to be the most reliable stratum to sample (see Results, section 3.1, for definition of the sampling unit), adjusting upward the sampling height during the plant growth period so that only mature leaves were examined. Damage on fruits was assessed weekly or bi-weekly by visually inspecting 5-15 randomly-chosen ripe fruits from each sampled plant, for a total of 90-600 fruits per greenhouse, depending on greenhouse surface and fruit size. Fruits were considered damaged when showed larval holes in the tomato peel or under the calyx. Depending on the length of the tomato growing season on each greenhouse, the leaf infestation was monitored 6-29 times, for a total of 524 samplings, whereas the fruit damage was estimated 5-17 times, for a total of 219 samplings.

Spatial patterns of *T. absoluta* infestations were investigated using Taylor's power law (TPL)[30] and Iwao's patchiness regression (IPR).[31,32] Both regression models were tested and compared with the aim of obtaining a more solid sampling plan, as the use of a single dispersion index could lead to unreliable results.[33,34]

Taylor's power law describes the relationship between mean (m) and variance (s^2) as:

$$s^2 = a\, m^b$$

where a is a sampling factor depending on sample and field size and b is the Taylor's aggregation index and indicates a uniform ($b < 1$), random ($b = 1$) or aggregated ($b > 1$) distribution. A linear regression after a natural logarithm (ln) transformation was used to estimate the parameters a and b:

$$\ln(s^2) = \ln(a) + b \ln(m)$$

where $\ln(a)$ is the intercept and b is the slope of the regression line.

Iwao's patchiness regression ($m^* = \alpha + \beta m$) relates Lloyd's mean crowding index (m^*) and mean density (m), where $m^* = m + (s^2 / m - 1)$ and s^2 = variance.[31] Iwao's parameters α and β were obtained by regressing m^* on m. The intercept α is the index of basic contagion and the slope β is an index of spatial dispersion, similarly to b of TPL.

The goodness of fit of TPL and IPR was estimated by coefficient of correlation values (r^2). The coefficients b and β of Taylor's and Iwao's models, respectively, were tested with t-tests ($P < 0.05$) (PROC REG)[28] to determine whether or not the slopes of the regression lines were significantly > 1.

2.4 Correlation between leaf infestation and fruit damage

The direct estimation of pest density by leaf sampling would provide an early response to population increase and would help to prevent fruit damage to a valuable crop such as tomato. Since no economic threshold (ET), expressed as percentage of infested fruits or leaves, has been determined yet for *T. absoluta*, the percentages of 1 and 3% of damaged fruits were proposed as ETs in this study to develop sampling plans.

Surveyed tomato cultivars showed a wide range of fruit sizes (20-100 mm) and a similar tomato yield (6-6.5 kg m^{-2} per growing season). Consequently, *T. absoluta* causes higher yield losses in cultivars with bigger tomatoes (60-100 mm in diameter) than in cherry-like fruits (size = 20-35 mm) as the number of fruits produced is considerably lower. For this reason, cultivars were divided in three product groups according to fruit sizes: big (Cuore di Bue and Belrosso), medium (Balente and Camone) and small fruits (Cherry and Minuetto). In all the fruit groups, relationships between damaged fruits (expressed as the percentage of infested tomatoes) and leaf infestation (expressed as the mean number of mines per leaf or the percentage of infested leaves) were investigated with the aim of defining action thresholds (AT) based on leaf infestation to be used in pest management decisions. The damage on fruits was correlated with the leaf infestation observed on the same sampling date, one and two weeks earlier, in order to determine the most suitable leaf ATs. Correlation equations were calculated for each fruit group using the Statgraphics Centurion XV software (StatPoint, Inc., 2005),[35] and equations relating damage on fruits and leaf attack detected two weeks earlier showed coefficients of correlation higher or comparable to those calculated on the same date or one week earlier (see Results, section 3.3, for correlation between leaf and fruit infestation). To sum up, these models were used to set leaf ATs corresponding to 1 or 3% of fruit damage.

2.5 Enumerative sampling plan

The sampling plan was developed from datasets representative of the surveyed population and validated using independent datasets. The leaf infestation was estimated using sampling data previously described in the Section 2.3. Two fixed-precision sequential sampling plans were calculated using Green's[36] and Kuno's methods[37] from TPL and IPR coefficients, respectively. Both sampling plans were developed with 206 datasets collected in 2010 and validated with 318 datasets collected in 2011-2012. For both sampling plans, the desired levels of precision (D = SEM mean^{-1}) were 0.10 and 0.20, usually set up for intensive and extensive ecological studies, respectively.[38]

The optimum sample size (N) for Green's plan was calculated following the equation proposed by Karandinos:[39]

$$N = 1 / D^2 am^{(b-2)}$$

where N is the number of samples needed, D is the desired precision level, a and b are TPL coefficients obtained from data collected in 2010 and m is the mean number of mines per leaf. Stop lines for sequential sampling of *T. absoluta* mines on leaves were calculated as:

$$T_n \geq (an^{1-b} / D^2)^{1 / (2-b)}$$

where T_n is the cumulative number of mines sampled and n is the total number of sampled leaves. Values of T_n were plotted against n to generate the sequential sampling stop lines, which represent the number of mines required to estimate the *T. absoluta* density on leaves at a defined precision level.

The same process was applied for Kuno's method, using the IPR parameters α and β to calculate the optimum sample size:

$$N = 1 / D^2 [(\alpha + 1) / m + (\beta - 1)]$$

and the sequential sampling stop lines:

$$T_n \geq (\alpha + 1) / [D^2 - (\beta - 1) / n]$$

Kuno's stop line is subject to the restriction that $n > (\beta - 1) / D^2$.

Datasets collected in 2011 and 2012 were used to validate Green's and Kuno's sampling plans using the Resampling for Validation of Sample Plans (RVSP) software.[40] Each validation dataset was randomly sampled with replacement until crossing the sequential stop line limits. The minimum sample size was set at 10 leaves for Green's plan for both precision levels (0.10 and

0.20), while for Kuno's method the minimum sample size was set at 50 and 15 for $D = 0.10$ and 0.20, respectively. Calculations of mean precision and mean sample size for each dataset were performed using 500 iterative runs and values were used to calculate the overall mean precision and the overall mean sample size. Green's and Kuno's methods could be validated using datasets with a mean *T. absoluta* density > 0.10 (298 dataset). Overall mean sample sizes for each precision level were proposed as the recommended sample sizes to assess pest densities.

2.6 Binomial sampling plan

A binomial sequential sampling plan based on presence/absence of mines on leaves was generated from 524 datasets, relying on the relationship between the percentage of damaged fruits and the proportion of infested leaves. The resampling validation was performed separately for cultivars with big, medium and small fruits at the corresponding leaf ATs using 311, 48 and 165 datasets, respectively. The number of infested leaves was determined from greenhouse samplings mentioned above in the Section 2.3.

Stop lines for each action threshold were generated using the Wald's Sequential Probability Ratio Test (SPRT) by the RVSP software.[41,40] To generate the stop lines, SPRT requires a number of parameters, including the upper (θ_1) and lower (θ_2) boundaries for the decision action thresholds; α (type I) error, which occurs when a treat decision is made at a pest density below the action threshold; and β (type II) error, which indicates the probability of not treating when the pest density exceeds the AT.[42] The upper (θ_1) and lower (θ_2) boundaries were held at 10% above and below the AT, respectively, while α and β parameters were set at 0.10,[43,40] For each dataset, 500 resampling iterations with replacement were run, with a minimum sample size of 10 leaves. The tally threshold, representing the minimum number of mines per leaf at which the sample unit is classified infested by *T. absoluta*, was set at one mine per leaf.

The validation of binomial sampling plans was performed by calculating operating characteristic (OC) functions for each action threshold and determining the average sample number (ASN). The

OC function represents the probability of not taking action when the pest population reaches a defined density and is used to determine the accuracy of binomial sampling plans, whereas ASN indicates the sample size needed to make a decision and refers to the sampling plan efficiency.[44,45] The OC function was calculated by regressing the RVSP values against the proportion of infested leaves using CurveExpert, Version 1.4 (D. Hyams, Starkville, MS, USA).[46] The best equation fitting the OC data for all sampling plans was $y = 1 / (a + bx\sqrt{c})$. The actual α and β errors and the OC value at the preset leaf AT were calculated from OC functions.[47]

The precision of binomial sequential sampling plans was estimated with a decision probability matrix[48,49] that determines the probability of taking or not taking the correct pest management decision (hereafter called 'to treat or not treat', respectively, in accordance with the definition by Burkness and collaborators)[49] by comparing the observed proportion of infested samples (leaves with one or more mines) with the estimated proportion of damaged leaves obtained from the simulation at each AT. The matrix consists in four cells which represent the correct decisions to treat or not to treat (cells A and D, respectively) and the incorrect decisions to treat or not to treat (cells B and C, respectively). The decision to treat is correct if the observed and estimated pest densities are above the AT, while the correct decision not to treat occurs when both densities are below the AT.[49] The proper decision for each dataset is determined by the level of *T. absoluta* density (percentage of infested leaves) and must be made correct or incorrect, then either A + B or C + D equal 1 in the matrix.[49,50] Consequently, when the pest population density exceeds the AT, the probability of A is equal to 1 - OC and the probability of B is equal to the OC, when in contrast the infestation is too low to require a treatment, the probability of C = 1 - OC and the probability of D = OC.[49]

For each leaf AT, the probability of treating or not treating correctly can be generalized for all data sets as follows:

$$1 = \sum p_i (A_i + D_i) + \sum p_i (B_i + C_i)$$

where p_i is the proportion of n datasets represented by dataset i, A_i and D_i, are the probability of making a correct decision, and B_i and C_i are the probabilities of making an incorrect decision. The probability matrix was calculated for each sub-dataset of cultivars with big, medium and small fruits at the ATs of 36 and 48%, 43 and 56%, and 60 and 73%, respectively (see Results, section 3.3, for determination of ATs). The evaluation of the reliability of sampling plans considered both the overall probability of incorrect decisions (B + C) and the probability of not treating when the action threshold was exceeded (C).[49]

3 RESULTS

3.1 Within-plant distribution

Population densities of tomato leafminer observed in the canopy strata showed wide ranges of variation: 0.9-5.1, 0.5-6.4 and 0.1-35.1 mines per leaf in the lower, median and upper leaves, respectively. The mean pest abundance was lower than two mines per leaf until March and increased steadily during April-May reaching a density higher than 10 mines per leaf in June. Correlations between the number of mines per leaf and the number of mines with larvae per leaf were significant and showed high coefficients of determination on each strata (lower: $y = 0.26 + 0.33x$, $r^2 = 0.67$; $F = 53.8$; df = 1, 26; $P < 0.001$; median: $y = 0.31 + 0.39x$, $r^2 = 0.86$; $F = 159.3$; df = 1, 26; $P < 0.001$; upper: $y = 0.20 + 0.39x$, $r^2 = 0.97$; $F = 764.5$; df = 1, 26; $P < 0.001$), indicating that the count of mines in the field was a reliable density estimation of larvae on leaves.

The percentage of mines per leaf was highest in the lower canopy at the beginning of the tomato cultivation and decreased during the growing season. Concurrently, the proportion of mines gradually increased in the median canopy until the last weeks of observations, when it sharply increased in the apical leaves (Fig. 1A). The spatial distribution of the percentage of mines with larvae in the three canopy strata was similar during the growing season (Fig. 1B), with a significantly higher proportion in the lower and median leaves (mean ± SEM = 40.4 ± 3.3% and 33.3 ± 1.9%, respectively) than in the upper canopy (26.3 ± 4.3%) ($F = 9.31$; df = 2,27; $P < 0.001$)

(Fig. 2A). The percentage of mines with larvae in the median canopy ranged from 12.7 to 52.4%, with a coefficient of variation (CV) of 0.30, in contrast a higher variability was observed in the lower leaves (range = 5.5-64.6%, CV = 0.43) and in the upper canopy (range = 5.1-81.8%, CV = 0.88) (Fig. 2B). The percentage (mean ± SEM) of empty mines in the lower third of the plant (57.5 ± 4.8%) was significantly higher than in the median (23.5 ± 1.9%) and in the upper (19.0 ± 4.7%) canopy (F = 17.38; df = 2,27; P < 0.001) (Fig. 2C). The proportion of empty mines throughout the tomato growing season was more variable in the upper leaves (CV = 1.32) than in the median and lower canopy (CV = 0.43 and 0.44, respectively) (Fig. 2D). Median leaves exhibited a not significant different proportion of mines with larvae and a significantly lower percentage of empty mines than bottom leaves, and the lowest coefficient of variation values, therefore appearing as the most suitable portion of the plant to sample in order to reliably assess the pest population in the field. In conclusion, the sampling unit used to develop and validate the sampling plans consisted in one randomly-chosen median leaf per plant in which the number of mines was counted.

3.2 Spatial distribution

The mean infestation density of tomato leafminer on leaves in the surveyed greenhouses was highly variable, ranging from 0.01 to 60.37 (Table 1). Taylor's power law and Iwao's patchiness regression models provided a strong correlation between mean and variance using the overall dataset (2010, 2011 and 2012), with high determination coefficients (Table 1). Slopes (b) of the regression lines were significantly > 1 (TPL: t = 27.56 df = 1, 522; P < 0.001; IPR: t = 26.31; df = 1, 522; P < 0.001), showing that *T. absoluta* populations had an aggregated distribution on tomato leaves in greenhouse crops. In order to develop and validate the enumerative sequential sampling plans, TPL and IPR parameters were calculated separately in 2010 and 2011-2012, respectively. Taylor's power law model fitted well the data both in 2010 and 2011-2012 (Table 1), showing values of b significantly > 1 (2010: t = 23.52; df = 1, 204; P < 0.001; 2011-2012: t = 15.72; df = 1, 316; P < 0.001). Similarly, IPR regression provided a strong linear correlation of data and showed values of β significantly > 1 (2010: $t = 21.07$; df = 1, 204; $P < 0.001$; 2011-2012: $t = 15.60$; df = 1, 316; $P < 0.001$).

3.3 Correlation between leaf infestation and fruit damage

The larval infestation on tomato plants started right after the transplant and increased on leaves and fruits during the crop cultivation, independently of growing season and cultivar. Exemplary infestation trends in tomato crops cultivated in the winter-spring and summer-winter seasons are presented in Figure 3.

In all fruit groups, the proportion of infested fruits (x) was significantly related with the percentage of infested leaves (y) recorded on the same sampling date and one or two weeks earlier through a squared (y) - square root (x) correlation (Table 2). In cultivars with big fruits, the coefficients of correlation were approximately 0.70, whereas cultivars with medium-size fruits showed increasing correlation values from 0 to 2-week delay. In contrast, in cultivars with small fruits, the correlations between leaf and fruit infestations were lower than in the other two groups and did not improve substantially when evaluated on the same date, one or two weeks earlier. The relationship between the percentage of infested fruits (x) and the number of mines per leaf (y) was best fitted by double squared functions in all fruit size groups (Table 3). Correlation coefficients were higher than 0.70 and did not show consistent variations at increasing time delay. Values of the percentage of infested leaves and the number of mines per leaf corresponding at fixed levels of infested fruits (ET = 1 or 3%) decreased at increasing time intervals (same sampling date, one week and two weeks earlier).

Relationships between pest infestation on fruits and leaves were used to define leaf ATs for enumerative and binomial sampling plans. Generally, coefficients of correlation between fruit damage and leaf infestation recorded two weeks earlier were higher or comparable to those calculated on the same date or one week earlier. In order to provide an early response to the increase of *T. absoluta* infestation and avoid reaching or exceeding the economic thresholds on fruits, ATs were chosen conservatively as leaf infestations observed two weeks before reaching ETs on fruits. Therefore, leaf ATs represent the potential economic damage on tomato fruits two weeks in advance, assisting growers to adopt appropriate and well-timed control measures against *T. absoluta*. As a consequence, the quantitative leaf ATs corresponding to ET = 1 and 3% of damaged fruits were 0.6 and 1 mine per leaf in cultivars with big fruits, 1 and 1.8 mines per leaf in cultivars with medium fruits, and 1.8 and 3 mines per leaf in small-fruit cultivars, respectively. For binomial sampling plans, leaf ATs were 36 and 48%, 43 and 56%, and 60 and 73% of infested leaves for cultivars with big, medium and small fruits for ET of 1 and 3%, respectively.

3.4 Enumerative sampling plan

Taylor's and Iwao's parameters, determined from leaf infestation data collected in 2010, were used to calculate the optimum sample size (N) (Fig. 4A and B) and the sequential sampling stop lines (Fig. 4C and D) calculated with Green's and Kuno's methods at D = 0.20 and 0.10. For both methods and precision levels, the number of sampled leaves decreased rapidly as the mean infestation increased. At a pest population density of 0.7 mines per leaf, both sampling plans required a sample size of 94 (D = 0.20) and 377 (D = 0.10) leaves (solid lines, Fig. 4A and B). Green's plan resulted in a lower N at a pest density < 0.7 mines per leaf, while Kuno's plan required a lower sample size at higher population levels. Indeed, the estimation of the AT of 0.6 mines per leaf that corresponded to the fruit ET of 1% in cultivars with big fruits, required N = 103 leaves at D = 0.20 and 412 leaves at D = 0.10 with Green's plan (Fig 4A and B), whereas with Kuno's plan N equaled 108 and 432 leaves at precision levels of 0.20 and 0.10, respectively (Fig 4A and B). When AT = 1 mine per leaf, corresponding to the ET of 3% for big-fruit cultivars and 1% for the medium-fruit group, the optimum sample sizes were 76 and 304 leaves for Green's plan and 69 and 278 leaves using Kuno's method at D = 0.20 and 0.10, respectively. The AT of 1.8 mines per leaf, corresponding at the ET of 3% of damaged fruits in cultivars with medium fruits and 1% in small-tomato cultivars, required sample sizes of 54 and 214 leaves with Green's plan and 44 and 176 leaves using Kuno's method at precision levels of 0.20 and 0.10, respectively. The leaf AT of 3.3

mines per leaf (small-fruit ET = 3%) resulted in N = 37 and 150 leaves using Green's plan and 29 and 117 leaves with Kuno's method at D = 0.20 and D = 0.10, respectively.

Sequential stop lines were used to determine the number of leaves to sample at the desired precision of D = 0.20 (Fig. 4C) or 0.10 (Fig. 4D) with Green's and Kuno's methods. As illustrative examples, at the leaf infestation of 0.6 mines per leaf, stop lines calculated with Green's model at D = 0.20 indicated that sampling would stop when 62 mines have been observed on 103 leaves (solid lines, Fig. 4C), while 245 mine counts on 412 leaves are needed at D = 0.10 (solid lines, Fig. 4D). Similarly, Kuno's sampling plan required the count of 64 mines on 108 leaves and 258 mines on 432 leaves to estimate a *T. absoluta* density of 0.6 mines per leaf at a precision of 0.20 and 0.10, respectively (Fig. 4C and D). A mean density of 1.8 mines per leaf could be estimated after a cumulative count of 79 mines on 44 leaves (dotted lines, Fig. 4C) or 316 mines on 176 leaves (dotted lines, Fig. 4D) using Kuno's plan and 97 mines on 54 leaves or 386 on 214 leaves using Green's method for precision levels of 0.20 and 0.10, respectively.

The validation of Green's sequential sampling plan at the fixed precision D = 0.20 and 0.10 produced an average precision of 0.195 and 0.099, respectively (Table 4; Fig. 5A and B), slightly better than the desired levels. Mean sample sizes calculated over 500 runs for each of the 298 datasets decreased at increasing infestation density (Fig. 5C) and the overall mean value resulted in 86.9 leaves at the preset precision of 0.20 (Table 4), increasing substantially to 342.5 leaves at D = 0.10 (Table 4; Fig. 5D). Similarly, resampling analysis of Kuno's sampling plan produced mean precision levels equal to 0.197 at D = 0.20 and 0.100 at D = 0.10 (Table 4; Fig. 6A and B). Overall mean sample sizes were 100.8 at D = 0.20 and 396.0 at the highest fixed-precision level (D = 0.10) (Table 4; Fig. 6C and D). Resampling validations of both methods provided actual precision levels ranging approximately from half to double the fixed precision value (from about 0.05 to 0.20 for D = 0.10 and from 0.10 to 0.40 for D = 0.20) (Table 4).

3.5 Binomial sampling plan

Decision stop lines generated for the binomial sampling plans for each AT are depicted in Figure 7. Control measures should be applied when the cumulative number of infested leaves is above the upper decision line, whereas no treatment is needed if the cumulative number of leaves with at least one *T. absoluta* mine is below the lower threshold line. If the number of infested samples examined falls between the stop lines, additional samples are required before making a treatment decision. The intercept of the lower stop line on the *x*-axis (i.e., 7.1, 6.2, 5.6, 4.7, 4.3 and 2.8 leaves for leaf ATs of 36, 43, 48, 56, 60 and 73%, respectively) represents the minimum sample size to examine before adopting a management decision.

The OC and ASN values were determined separately for tomato cultivars with big, medium and small fruits by relating the obtained values to the observed percentage of infested sample units (Table 5). For big-fruit cultivars at the action thresholds of 36 and 48%, the OCs were 0.483 and 0.486, respectively (Table 5), suggesting that the binomial sampling plans were slightly conservative seeing that the decision to treat is more likely to occur than the decision not to treat at the AT.[51,50] The actual α and β values were lower than those entered in the RVSP program (0.10 for both parameters) (Table 5). Mean ASNs were 17.1 and 16.3 leaves at the action thresholds of 36 and 48%, respectively (Table 5). Similarly, in cultivars with medium-size fruits, OC values at the action thresholds of 43 and 56% corresponded to 0.483 and 0.492, respectively, while mean ASNs were 19.7 and 17.9 leaves, respectively (Table 5). The actual α and β values were lower than the preset error. OC values of small-fruit cultivars were neutral or slightly liberal at the action thresholds of 60 and 73%, being 0.500 and 0.503, respectively (Table 5), while mean sample sizes corresponded to 13.7 and 15.2 leaves, respectively. Similarly to the other ATs, values of actual α and β were < 0.10. In all fruit groups, maximum values of ASN were observed close to the ATs and ranged from 30 to 37 leaves, as the uncertainty of treating or not treating is higher around the threshold and more sample units must be observed. The probability of correct decisions to treat or not to treat (A + D) was high for all leaf ATs, ranging from 0.903 to 0.957 (Table 5). Conversely, the overall incorrect decisions (B + C) were always below 0.10. Moreover, it was noted that the incorrect decision not to treat when the pest density exceeded the AT (C) was always low and corresponded to 0.027, 0.048 and 0.035 in cultivars with small, medium and big fruits, respectively.

4 DISCUSSION

The tomato leafminer is a key pest of protected tomato crops because of its high reproductive potential and exponential population growth under optimal environmental conditions. Therefore, the establishment of appropriate sampling units is needed to define effective monitoring strategies for an early assessment of pest populations and develop reliable sampling plans in IPM programs, which are essential for informed decision-making and effective pest control.[52]

The analysis of the within-plant distribution of the tomato leafminer carried out in this study indicated that younger leaves were the preferred oviposition substrate and were more likely to harbor *T. absoluta* larvae, similarly with results obtained by Torres and collaborators in an open-sided greenhouse tomato crop in Brazil.[22] However, the upper portion of the canopy was not suitable for sampling, as apical leaves showed the highest variability in the proportion of mines with larvae. The median and lower canopy exhibited a similar density of occupied mines, whereas median leaves showed the lowest variation coefficients of empty and occupied mines and a higher correlation coefficient between the number of mines and mines with larvae. The highest proportion of abandoned mines was observed on basal leaves, which were the oldest and hence exposed to the pest for a longer period of time. These findings are in accordance with those reported by Gomide and collaborators,[23] who have found the highest correlations between the number of larvae and mines on the median portion of the canopy and the counts in the whole plant. They further suggested discarding basal leaves since they are more attacked by fungal pathogens, which make identifying any *T. absoluta* damage a difficult task. Similarly, other studies focused on the impact of *T. absoluta* infestations on yield loss in fresh-market tomato crops were carried out by sampling leaves from the median canopy.[25] Our study also depicted a strong relationship between the total number of mines per leaf and the total number of mines with larvae, which would suitably simplify the field sampling procedure as the count of mines on leaves is faster and easier than larvae detection. Accordingly, practical and non-destructive sampling plans established for growers should be reliably based on counts of *T. absoluta* leaf mines in the median portion of the canopy.

In a previous research, the tomato leafminer showed a clumped distribution pattern inside greenhouses, with higher abundance on plants close to openings (windows and doors), where moths immigrated from outside the greenhouses also in the presence of insect-proof screens.[10] Moreover, the spatial distribution of pests tends to be aggregated and is affected by different microclimatic conditions (e.g. sunlight exposure inside greenhouses) and control methods.[53,54,38] All these factors influence pest monitoring strategies to such an extent that samplings would be collected from the whole greenhouse. In the present work, Taylor's and Iwao's regression models were used to evaluate the spatial dispersion of *T. absoluta* mines on tomato leaves, indicating both an aggregated distribution and high coefficients of determination. These aggregation indexes provided basic information for designing reliable sampling programs to estimate the population density with Green's and Kuno's models, respectively.[55] Appropriate plans, as sequential sampling plans, minimize the effort to evaluate the pest density, being therefore cost-effective.[38] Finally, it is worthy to note that the required precision level (*D*) and the degree of aggregation affect the optimal sample size, that decreases at increasing pest population density.[56]

The prediction of fruit damage from leaf infestation seems to be a reliable tool to implement timely control measures. In the current research, the percentage of infested fruits was highly correlated with the leaf infestation observed two weeks earlier. Similarly, a strong correlation between the percentage of infested fruits and the density of larvae on leaves observed in the preceding week was also detected on *Keiferia lycopersicella* (Walshingam) (Lepidoptera: Gelechiidae) infesting tomato plants.[57] The strong relationship allowed defining different leaf ATs for cultivars with big, medium and small fruits from fruit ET. For economic thresholds of both 1 and 3% of infested fruits, the corresponding ATs were lower for big-fruit cultivars than those for cultivars with medium and small fruits, due to the fact that big-fruit cultivars produce a lower number of bigger tomatoes and can tolerate a lower pest population density before reaching the economic thresholds. The definition of specific leaf action thresholds for each fruit group and for different fruit damage levels allowed the development of sampling plans readily useful in IPM programs.

It was proven that the binomial sequential sampling plan is less labor intensive and provides a faster and more suitable method for assisting growers in decision making than the enumerative sampling. Indeed, the validation provided average sample sizes (14-20 leaves) many-fold lower than those of enumerative plans, nonetheless maintaining a high probability of making correct pest control decision. Operating characteristic values were constantly very close to 0.50, so slightly conservative or liberal, and an overall probability to make incorrect decisions to treat or not to treat (B + C) always < 0.10, indicating a lower-than-expected probability of making wrong choices. More importantly, the probability of incorrect decisions of not treating at a pest density higher than the action threshold (C), which is a critical decision owing to the high biotic potential of *T. absoluta*, was always < 5%. The binomial sampling, based on presence/absence of infested leaves, does not estimate the density of the tomato leafminer larvae, but rather indicates whether the pest density is above or below the AT.[44] This sampling method could be implemented as a practical monitoring tool in IPM programs and would require a minimum sample size ranging from 3 to 7 leaves, depending on leaf AT. The cumulative number of infested leaves could indefinitely fall between the upper decision line (treat) and the lower line (no treat), when the actual infestation is near the leaf AT.[44] In consequence, if a pest control decision could not be made after sampling the maximum sample size (30-37 leaves, depending on leaf AT), leaves should be sampled again at a later time. The resampling date should vary from 3-4 days to 1 week and should be determined depending on the predicted pest population growth and harvest time.

Enumerative sampling plans were more time-consuming than the binomial sampling, due to the higher time required to count all the mines of sampled leaves and for the wider sample size. However, they could be used effectively in all the studies where a quantitative assessment of the population density is required (e.g. ecological studies, comparative effectiveness of insecticides).

The validation of Green's and Kuno's plans showed precisions similar to the fixed levels, nevertheless the former method exhibited a lower overall mean sample size than the latter (i.e. 87 and 101 leaves, respectively, at the precision level of 0.20 required in extensive pest monitoring programs). Consequently, Green's sampling plan was more suitable to estimate *T. absoluta* infestation on leaves, requiring a lower sampling effort within the investigated pest density.

In conclusion, the spatial distribution of *T. absoluta* larvae on protected tomato crops was reliably estimated over a wide range of pest density from over 500 datasets, collected on 33 greenhouses cultivated with cultivars with different fruit sizes across several years and growing seasons and protected with various control methods. Leaf infestation was significantly related to fruit damage and action thresholds of 36, 43, and 60% of infested leaves in cultivars with big, medium and small fruits, respectively, corresponded to 1% of damaged fruits after two weeks. The enumerative sampling plan could be applied to evaluate the pest population density in extensive or intensive ecological studies by sampling 87 or 343 leaves, respectively (Green's method, D = 0.20 or 0.10).[38] On the other hand, the binomial sequential sampling plans required a much lower sampling effort and could be profitably implemented in IPM programs. In particular, no more than 35 leaves should be sampled and an average size of 17, 20, or 14 leaves should be observed in big-, medium- or small-fruit cultivars to take a pest control decision in order to avoid a fruit damage higher than 1%. The practical sampling protocols developed in this work would be suitable even in tomato-growing areas with different cultural conditions and would optimize monitoring and control measures against *T. absoluta* in commercial protected tomato crops.

ACKNOWLEDGEMENTS

We thank the 'Santa Margherita Terra e Sole' Cooperative and tomato growers for making their greenhouses available for this study. We also thank Francesco Serra for technical assistance and two anonymous reviewers for their constructive comments that substantially improved the manuscript. This research was supported in part by the Italian Ministry of Education, University and Research, project PRIN 2008 'Biology and integrated control of *Tuta absoluta* (Meyrick) in Italy'.

REFERENCES

1 Tropea Garzia G, Siscaro G, Biondi A and Zappala L, *Tuta absoluta*, a South American pest of tomato now in the EPPO region: biology, distribution and damage. *EPPO Bull* **42**:205-210 (2012).

2 Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA, González-Cabrera J, Ruescas DC, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T and Urbaneja A, Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospect for biological control. *J Pest Sci* **83**:197-215 (2010).

3 Desneux N, Luna MG, Guillemaud T and Urbaneja A, The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond. *J Pest Sci* **84**:403-408 (2011).

4 Cassino PR, Perusso JC, Rego LM and Sampaio HN, Proposta metodológica de monitoramento de pragas em tomateiro estaqueado. *An Soc Entomol Brasil* **24**:279-285 (1995).

5 Vercher Aznar R, Calabuig A and Felipe C, Ecología, muestreos y umbrales de *Tuta absoluta* (Meyrick). *Phytoma España* **217**:23-26 (2010).

6 Picanço MC, Guedes RNC, Leite GLD, Fontes PCR and Silva EA, Incidência de *Scrobipalpuloides absoluta* (Meyrick) (Lepidoptera: Gelechiidae) em tomateiro sob diferentes sistemas de tutoramento e controle químico de pragas. *Hortic Bras* **13**:180-183 (1995a).

7 Silva GA, Picanço MC, Bacci L, Crespo ALB, Rosado JF and Guedes RNC, Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. *Pest Manag Sci* **67**:913-920 (2011).

8 Haddi K, Berger M, Bielza P, Cifuentes D, Field LM, Gorman K, Rapisarda C, Williamson MS and Bass C, Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*). *Insect Biochem Mol Biol* **42**:506-513 (2012).

9 Urbaneja A, González-Cabrera J, Arnó J and Gabarra R, Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. *Pest Manag Sci* **68**:1215-1222 (2012).

10 Cocco A, Deliperi S and Delrio G, Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. *J Appl Entomol* **137**:16-28 (2013).

11 Bielza P, La resistencia a insecticidas en *Tuta absoluta*. *Phytoma España* **217**:103-106 (2010).

12 Roditakis E, Skarmoutsou C and Staurakaki M, Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. *Pest Manag Sci* **69**:834-840 (2013).

13 Zappalà L, Bernardo U, Biondi A, Cocco A, Deliperi S, Delrio G, Giorgini M, Pedata P, Rapisarda C, Tropea Garzia G and Siscaro G, Recruitment of native parasitoids by the exotic pest *Tuta absoluta* (Meyrick) in Southern Italy. *Bull Insectol* **65**:51-61 (2012).

14 Zappalà L, Biondi A, Alma A, Al-Jboory IJ, Arnò J, Bayram A, Chailleux A, El-Arnaouty A, Gerling D, Guenaoui Y, Shaltiel-Harpaz L, Siscaro G, Stavrinides M, Tavella L, Vercher Aznar R, Urbaneja A and Desneux N, Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle-East, and their potential use in pest control strategies. *J Pest Sci* **86**:635-647 (2013).

15 Biondi A, Desneux N, Siscaro G and Zappalà L, Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* **87**:803-812 (2012).

16 Biondi A, Zappalà L, Stark JD and Desneux N, Do biopesticides affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects? *PLoS ONE* **8(9)**:e76548 DOI: 10.1371/journal.pone.0076548 (2013).

17 Smagghe G, Deknopper J, Meeus I and Mommaerts V, Dietary chlorantraniliprole suppresses reproduction in worker bumblebees. *Pest Manag Sci* **69**:787-791 (2013).

18 European Union, Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. *Off J Eur Commun L* **309**:71-86 (2012).

19 Leite GLD, Picanço MC, Silva DJH, Mata AC and Jham GN, Distribuição de oviposição de *Scrobipalpuloides absoluta* no dossel de *Lycopersicon esculentum*, *L. hirsutum* e *L. peruvianum*. *Hortic Bras* **13**:47-51 (1995).

20 Haji FNP, Oliveira CAV, Amorim Neto MS and Batista JGS, Flutuação populacional da traça do tomateiro no Submédio São Francisco. *Pesq Agropec Bras* **23**:7-14 (1988).

21 Picanço MC, Silva DJH, Leite GLD, Mata AC and Jham GN, Intensidade de ataque de *Scrobipalpula absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) ao dossel de três espécies de tomateiro. *Pesq Agropec Bras* **30**:429-433 (1995b).

22 Torres JB, Faria CA, Evangelista WS and Pratissoli D, Within-plant distribution of the leaf miner *Tuta absoluta* (Meyrick) immatures in processing tomatoes, with notes on plant phenology. *Int J Pest Manag* **47**:173-178 (2001).

23 Gomide EVA, Vilela EF and Picanço M, Comparação de procedimentos de amostragem de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) em tomateiro estaqueado. *Neotrop Entomol* **30**:697-705 (2001).

24 Monserrat A, *La Polilla del Tomate* Tuta absoluta *en la Región de Murcia: Bases Para su Control.* Consejería de Agricultura y Agua, Región de Murcia, Murcia, Spain (2009).

25 Benvenga SR, Fernandes OA and Gravena S, Tomada de decisão de controle da traça-do-tomateiro através de armadilhas com feromônio sexual. *Hortic Bras* **25**:164-169 (2007).

26 Delrio G, Cocco A and Deliperi S, Prospettive e limiti di impiego di feromoni sessuali per la lotta contro la tignola del pomodoro. Atti Accademia Nazionale Italiana di Entomologia, **LX**:103-109 (2012).

27 Abbes K and Chermiti B, Comparison of two marks of sex pheromone dispensers commercialized in Tunisia for their efficiency to monitor and to control by mass-trapping *Tuta absoluta* under greenhouses. *Tunisian J Plant Prot* **6**:133-148 (2011).

28 SAS Institute, *SAS/ETS® 9.2 User's Guide*. SAS Institute Inc., Cary, NC (2008).

29 Ipe D, Performing the Friedman test and the associated multiple comparison test using PROC GLM. *Proceedings of the 12th Annual SAS Users Group International Conference* **12**:1146-1148 (1987).

30 Taylor LR, Aggregation, variance and the mean. *Nature* **189**:732-735 (1961).

31 Lloyd M, Mean crowding. *J Anim Ecol* **36**:1-30 (1967).

32 Iwao SA, new regression method for analyzing the aggregation pattern of animal populations. *Res Popul Ecol* **10**:1-20 (1968).

33 Myers JH, Selecting a measure of dispersion of individuals. *Environ Entomol* **7**:619-621 (1978).

34 Butler CD and Trumble JT, Spatial dispersion and binomial sequential sampling for the potato psyllid (Hemiptera: Triozidae) on potato. *Pest Manag Sci* **68**:865-869 (2012).

35 StatPoint Inc, *The User's Guide to Statgraphics® Centurion XV*. StatPoint Technologies Inc., Warrenton, VA (2005).

36 Green RH, On fixed precision level sequential sampling. *Res Popul Ecol* **12**:249-241 (1970).

37 Kuno E, A new method of sequential sampling to obtain the population estimates with a fixed level of precision. *Res Popul Ecol* **11**:127-136 (1969).

38 Southwood TRE and Henderson PA, *Ecological methods, 3rd. ed*. Blackwell, Oxford, UK (2000).

39 Karandinos MG, Optimum sample size and comments on some published formulae. *Bull Entomol Soc Amer* **22**:417-421 (1976).

40 Naranjo SE and Hutchison WD, Validation of arthropod sampling plans using a resampling approach: software and analysis. *Am Entomol* **43**:48-57 (1997).

41 Wald A, *Sequential Analysis*. Wiley, New York, NY (1947).

42 Jones VP, Sequential estimation and classification procedures for binomial counts, in *Handbook of Sampling Methods for Arthropods in Agriculture*, ed. by Pedigo L and Buntin G, CRC Press, Boca Raton, FL, pp. 175-205 (1994).

43 Binns MR, Sequential sampling for classifying pest status, in *Handbook of Sampling Methods for Arthropods in Agriculture*, ed. by Pedigo L and Buntin G, CRC Press, Boca Raton, FL, pp. 137-174 (1994).

44 Onsager JA, The rationale of sequential sampling, with emphasis on its use in pest management. *USDA Tech Bull* **1526**:1-19 (1976).

45 Binns MR and Nyrop JP, Sampling insect populations for the purpose of IPM decision making. *Annu Rev Entomol* **37**:427-453 (1992).

46 Burkness, EC and Hutchison WD, Implementing reduced-risk integrated pest management in fresh-market cabbage: influence of sampling parameters, and validation of binomial sequential sampling plans for the cabbage looper (Lepidoptera: Noctuidae). *J Econ Entomol* **102**:1874-1883 (2009).

47 Subramanyam BH, Hagstrum DW, Meagher RL, Burkness EC, Hutchinson WD and Naranjo SE, Development and evaluation of sequential sampling plans for *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) infesting farmstored wheat. *J Stored Prod Res* **33**:321-329 (1997).

48 Calvin DD, Knapp MC, Xingquam K, Poston FL and Welch SM, Using a decision model to optimize European corn borer (Lepidoptera: Pyralidae) eggmass sampling. *Environ Entomol* **15**:1212-1219 (1986).

49 Burkness EC, Venette RC, O'Rourke PK and Hutchison WD, Binomial sequential sampling for management of aster leafhopper (Homoptera: Cicadellidae) and aster yellows phytoplasma in carrot: impact of tally threshold on the accuracy of treatment decisions. *Environ Entomol* **28**:851-857 (1999).

50 Galvan TL, Burkness EC and Hutchison WD, Enumerative and binomial sequential sampling plans for the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in wine grapes. *J Econ Entomol* **100**:1000-1010 (2007).

51 Binns MR, Nyrop JP and van der Werf W, *Sampling and Monitoring in Crop Protection. Theoretical Basis for Developing Practical Decision Guides*. CABI Publishing, Wallingford, UK (2000).

52 Castle SJ and Naranjo SE, Sampling plans, selective insecticides and sustainability: the case for IPM as 'informed pest management'. *Pest Manag Sci* **65**:1321-1328 (2009).

53 Trumble JT, Implications of changes in arthropod distribution following chemical application. *Res Popul Ecol* **27**:277-285 (1985).

54 Taylor RAJ, On the accuracy of insecticide efficacy reports. *Environ Entomol* **16**:1-8 (1987).

55 Kuno E, Sampling and analysis of insect populations. *Annu Rev Entomol* **36**:285-304 (1991).

56 Buntin GD, Developing primary sampling program, in *Handbook of Sampling Methods for Arthropods in Agriculture*, ed. by Pedigo L and Buntin G, CRC Press, Boca Raton, FL, pp. 99-115 (1994).

57 Van Steenwyk RA, Oatman ER and Wyman JA, Density treatment level for tomato pinworm (Lepidoptera: Gelechiidae) based on pheromone trap catches. *J Econ Entomol* **76**:440-445 (1983).

Table 1. Dispersion indices for *Tuta absoluta* on tomato leaves in greenhouse crops in 2010-2012

Year	Dataset (n)	Pest density range (mines leaf $^{-1}$)	Taylor's power law				Iwao's patchiness regression		
			ln(a) ± SEM	a	b ± SEM	r^2	α ± SEM	β ± SEM	r^2
Overall (2010-2012)	524	0.01 - 60.37	1.09 ± 0.02	2.97	1.36 ± 0.01	0.95	1.43 ± 0.12	1.47 ± 0.02	0.93
Model (2010)	206	0.01 - 60.37	1.11 ± 0.03	3.04	1.41 ± 0.02	0.97	1.30 ± 0.19	1.47 ± 0.02	0.95
Validation (2011-12)	318	0.01 - 47.64	1.08 ± 0.03	2.96	1.30 ± 0.02	0.94	1.53 ± 0.16	1.45 ± 0.03	0.89

Table 2. Relationship between the percentage of tomato fruits damaged by *Tuta absoluta* and the percentage of infested leaves (leaves with one or more mines over the total leaves sampled) recorded on the same sampling date, one or two weeks earlier in cultivars with big, medium and small fruits

	Dataset (n)	Regression model	r^2	F	*P*
Cultivars with big fruits					
Same sampling date	123	$y = \sqrt{(174.814 + 1962.560 \times \sqrt{(x)})}$	0.72	315.12	< 0.001
1 week earlier	120	$y = \sqrt{(-58.039 + 1653.960 \times \sqrt{(x)})}$	0.72	298.86	< 0.001
2 weeks earlier	120	$y = \sqrt{(-100.477 + 1401.280 \times \sqrt{(x)})}$	0.69	258.86	< 0.001
Cultivars with medium fruits					
Same sampling date	24	$y = \sqrt{(1792.300 + 1823.120 \times \sqrt{(x)})}$	0.56	28.61	< 0.001
1 week earlier	23	$y = \sqrt{(915.665 + 1877.320 \times \sqrt{(x)})}$	0.70	49.18	< 0.001
2 weeks earlier	23	$y = \sqrt{(116.632 + 1725.560 \times \sqrt{(x)})}$	0.78	73.2	< 0.001
Cultivars with small fruits					
Same sampling date	64	$y = \sqrt{(3002.860 + 2092.150 \times \sqrt{(x)})}$	0.53	70.41	< 0.001
1 week earlier	60	$y = \sqrt{(1958.410 + 2297.670 \times \sqrt{(x)})}$	0.58	80.91	< 0.001
2 weeks earlier	59	$y = \sqrt{(1186.810 + 2355.620 \times \sqrt{(x)})}$	0.57	76.59	< 0.001

Table 3. Relationship between the percentage of tomato fruits damaged by *Tuta absoluta* and the number of mines per leaf recorded on the same sampling date, one or two weeks earlier in cultivars with big, medium and small fruits

	Dataset (n)	Regression model	r^2	F	*P*
Cultivars with big fruits					
Same sampling date	123	$y = (0.467 + 0.556 \times \sqrt{(x)})^2$	0.72	318.26	< 0.001
1 week earlier	120	$y = (0.459 + 0.432 \times \sqrt{(x)})^2$	0.73	323.42	< 0.001
2 weeks earlier	120	$y = (0.451 + 0.349 \times \sqrt{(x)})^2$	0.70	271.82	< 0.001
Cultivars with medium fruits					
Same sampling date	24	$y = (0.765 + 0.698 \times \sqrt{(x)})^2$	0.74	64.27	< 0.001
1 week earlier	23	$y = (0.644 + 0.576 \times \sqrt{(x)})^2$	0.81	92.32	< 0.001
2 weeks earlier	23	$y = (0.487 + 0.509 \times \sqrt{(x)})^2$	0.80	85.39	< 0.001
Cultivars with small fruits					
Same sampling date	64	$y = (0.804 + 0.967 \times \sqrt{(x)})^2$	0.75	187.87	< 0.001
1 week earlier	60	$y = (0.660 + 0.830 \times \sqrt{(x)})^2$	0.76	188.59	< 0.001
2 weeks earlier	59	$y = (0.718 + 0.632 \times \sqrt{(x)})^2$	0.73	158.60	< 0.001

Table 4. Resampling validation of Green's and Kuno's sequential sampling plans at fixed-precision levels of 0.20 and 0.10

Pest density range (mines leaf $^{-1}$)	Dataset (n)	Fixed-precision level = 0.20		Fixed-precision level = 0.10	
		Mean precision (range)	Mean sample size (range)	Mean precision (range)	Mean sample size (range)
		Green's plan			
0.10 - 47.50	298	0.195 (0.085 - 0.443)	86.9 (10 - 273)	0.099 (0.043 - 0.226)	342.5 (32 - 1072)
		Kuno's plan			
		0.197 (0.087 - 0.389)	100.8 (15 - 501)	0.100 (0.043 - 0.203)	396.0 (53 - 1976)

Table 5. Comparison of operation characteristics and probabilities of correct and incorrect pest control decisions for sequential binomial sampling plans for *Tuta absoluta* on tomato cultivars with big, medium and small fruits at different leaf action thresholds

Leaf AT (%)[a]	Dataset (n)	OC value[b]	Actual α[c]	Actual β[c]	Mean ASN (range)[d]	A[e]	D[f]	A + D[g]	B[h]	C[i]	B + C[j]
Cultivars with big fruits											
36	311	0.483	0.059	0.096	17.1 (10 - 35)	0.395	0.537	0.932	0.033	0.035	0.068
48	311	0.486	0.070	0.097	16.3 (10 - 37)	0.269	0.676	0.945	0.020	0.034	0.055
Cultivars with medium fruits											
43	48	0.483	0.067	0.096	19.7 (10 - 35)	0.492	0.411	0.903	0.050	0.048	0.097
56	48	0.492	0.072	0.097	17.9 (10 - 36)	0.349	0.588	0.937	0.047	0.016	0.063
Cultivars with small fruits											
60	165	0.500	0.065	0.092	13.7 (10 - 35)	0.415	0.542	0.957	0.022	0.022	0.043
73	165	0.503	0.068	0.092	15.2 (10 - 30)	0.310	0.646	0.956	0.017	0.027	0.044

[a] Leaf Action Thresholds.
[b] Operating Characteristic value: probability of not treating when the pest population density reaches the AT.
[c] α error: probability to treat when the pest density is below the AT (type I error); β error: probability of not treating when the pest density is above the AT (type II error). α and β values were preset at 0.1 to generate binomial sequential sampling stop lines; actual α and β were calculated from OC curves for resampling validations.
[d] Average Sample Number: number of samples required to make a pest control decision (i.e., to treat or not to treat).
[e] A: correct decision to treat.
[f] D: correct decision not to treat.
[g] A + D: overall probability to make a correct pest control decision (i.e., to treat or not to treat).
[h] B: incorrect decision to treat.
[i] C: incorrect decision not to treat.
[j] B +C: overall probability to make an incorrect pest control decision (i.e., to treat or not to treat).

Figure legends

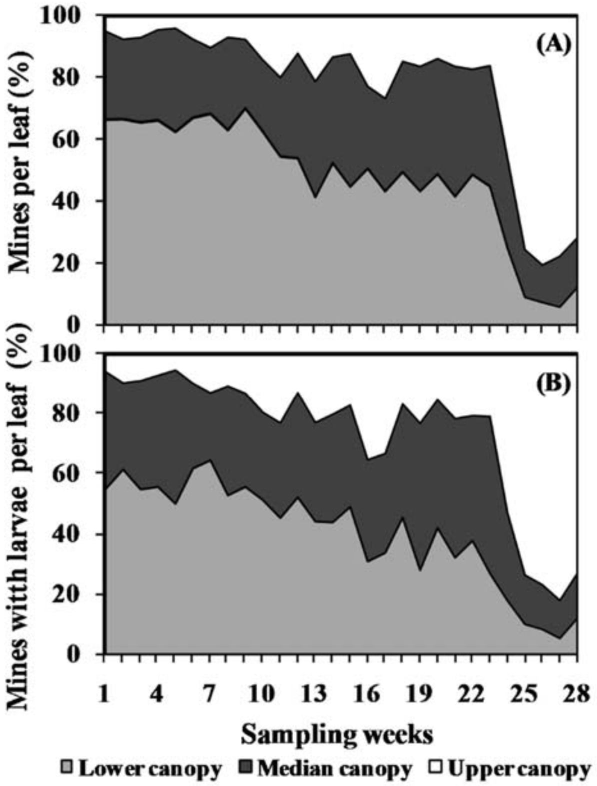

Figure 1. Patterns of within-plant distributions of *Tuta absoluta* mines (A) and mines with larvae (B) in the tomato canopy strata throughout the growing season period.

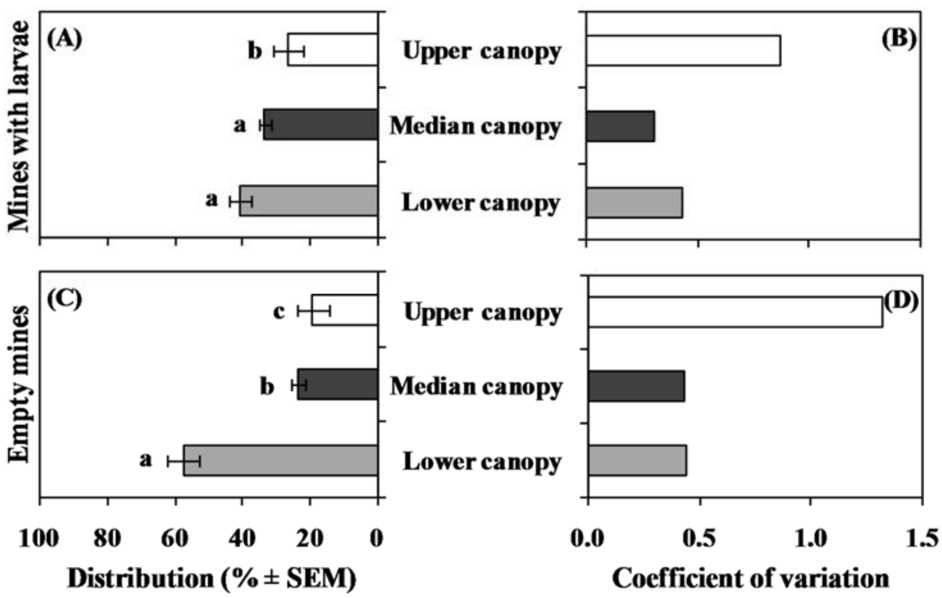

Figure 2. Within-plant distributions of *Tuta absoluta* mines with larvae (A) and empty mines (C) in the canopy strata and associated coefficients of variation (standard deviation mean^{-1}) (B and D). Bars with different letters are significantly different by Friedman test and rank sum multiple comparison test ($P < 0.05$).

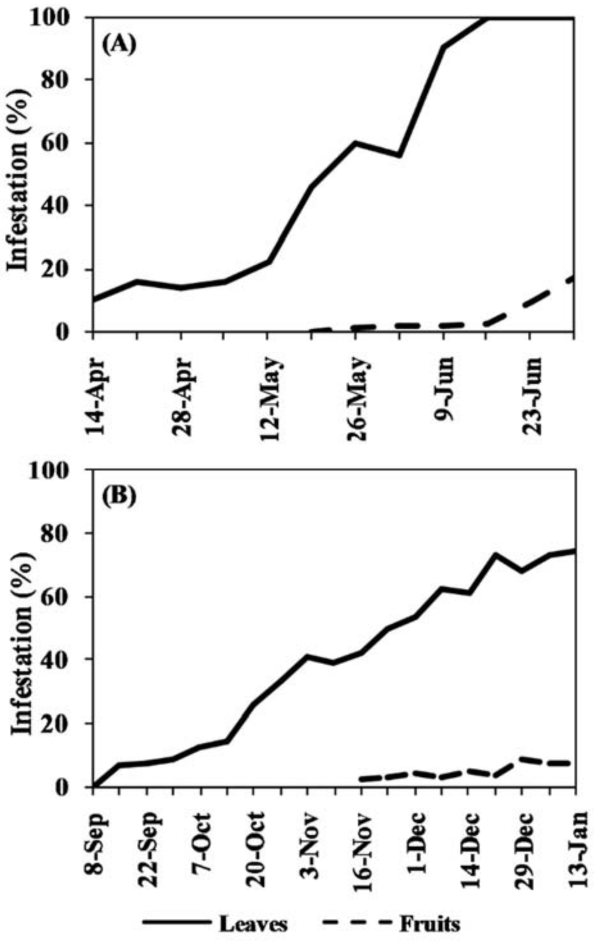

Figure 3. Percentage of tomato leaves and fruits infested by *Tuta absoluta* in a cultivar with big fruits cultivated in the winter-spring season (A) and in a small-fruit cultivar grown in the summer-winter season (B).

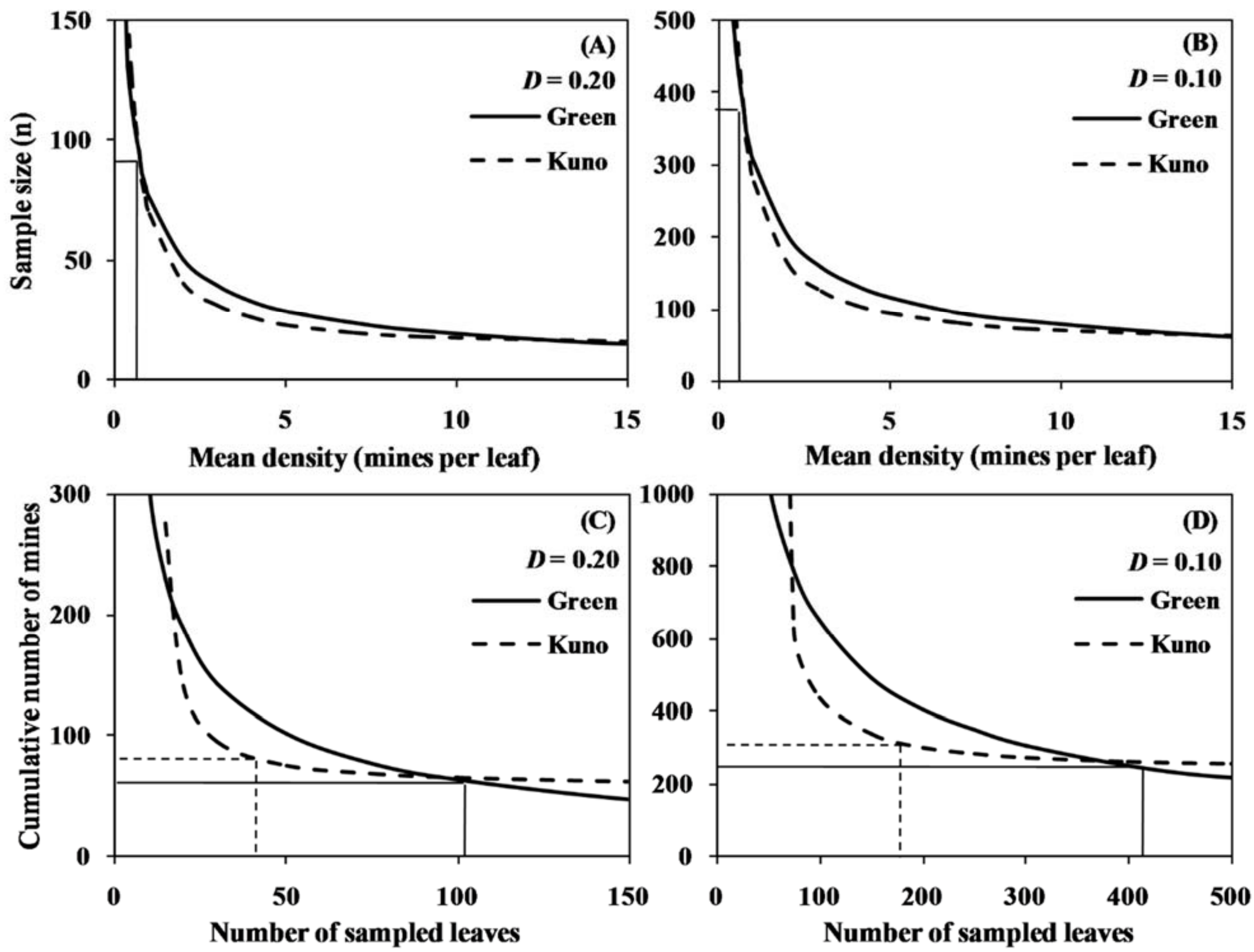

Figure 4. Optimum sample sizes (A and B) and sequential stop lines (C and D) for the assessment of *Tuta absoluta* density on tomato leaves using Green's and Kuno's methods at D = 0.20 (A and C) and D = 0.10 (B and D). Note the different axis scales.

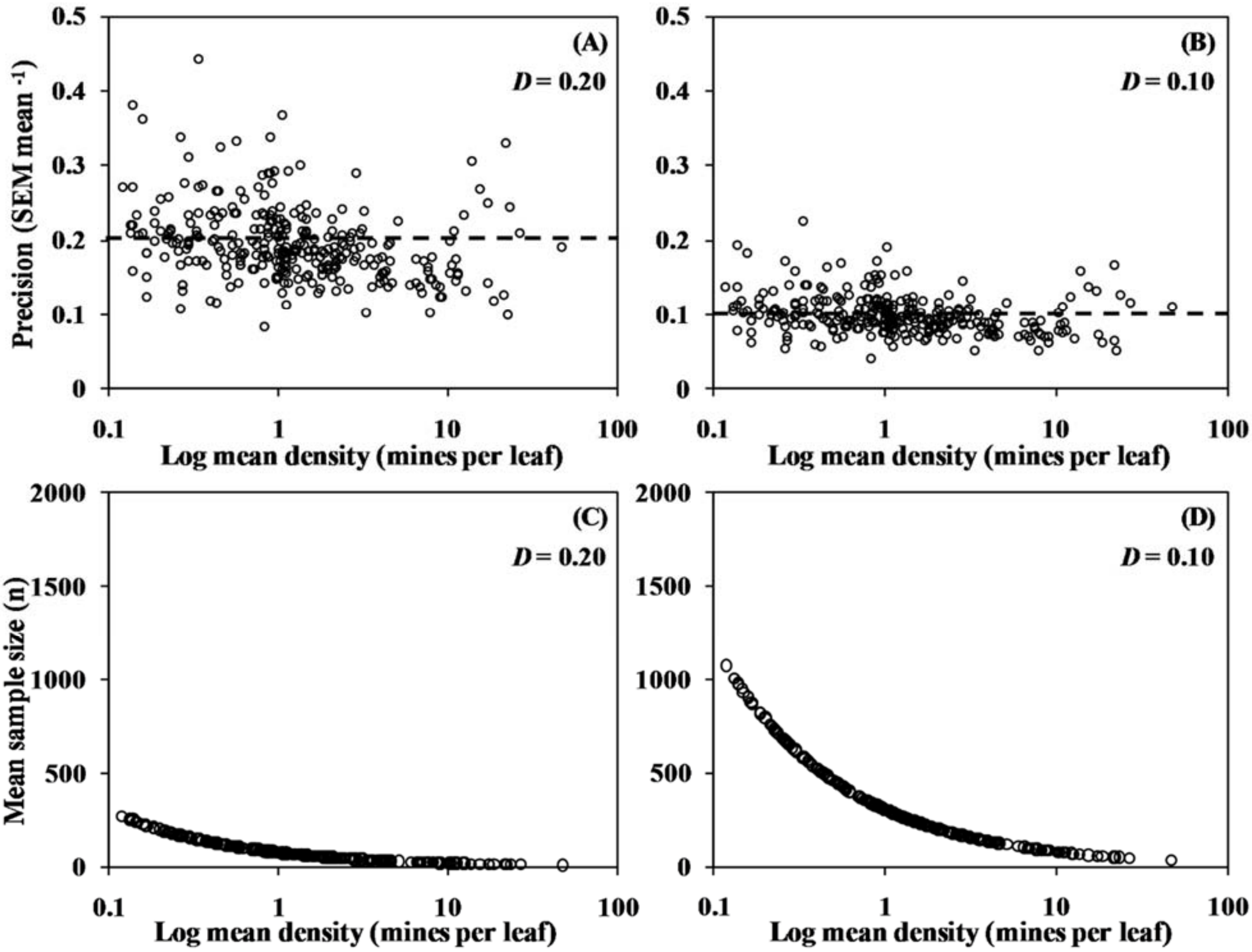

Figure 5. Validation of enumerative sampling plans for estimating leaf infestation of *Tuta absoluta* on protected tomato crops based on Green's plan showing actual precision levels (A and B) and sample sizes (C and D) calculated at the fixed level of 0.20 (A and C) and 0.10 (B and D). Dotted lines indicate the desired precision levels *D* = 0.10 (A) and 0.20 (B).

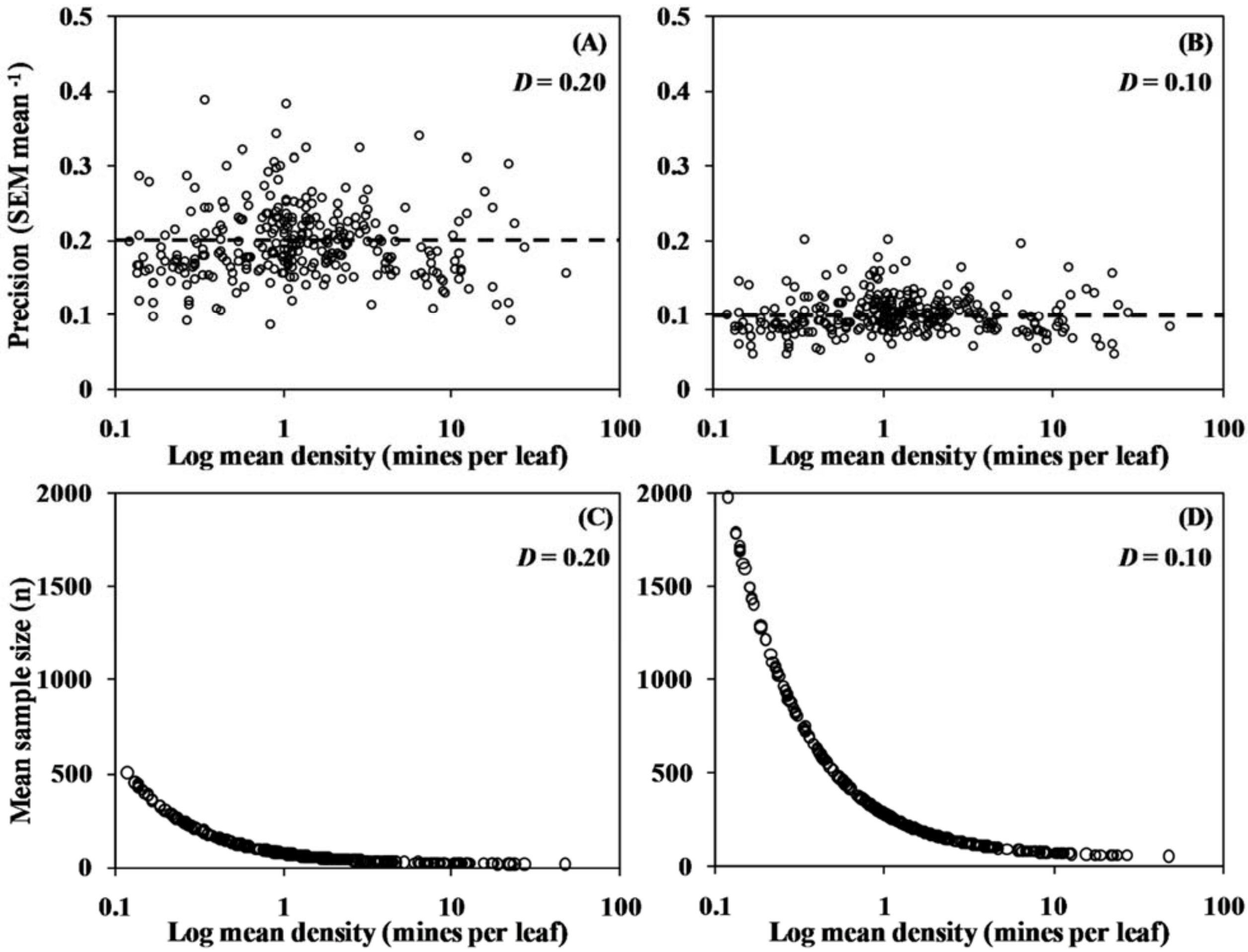

Figure 6. Validation of enumerative sampling plans for estimating leaf infestation of *Tuta absoluta* on protected tomato crops based on Kuno's plan showing actual precision levels (A and B) and sample sizes (C and D) calculated at the fixed level of 0.20 (A and C) and 0.10 (B and D). Dotted lines indicate the desired precision levels D = 0.10 (A) and 0.20 (B).

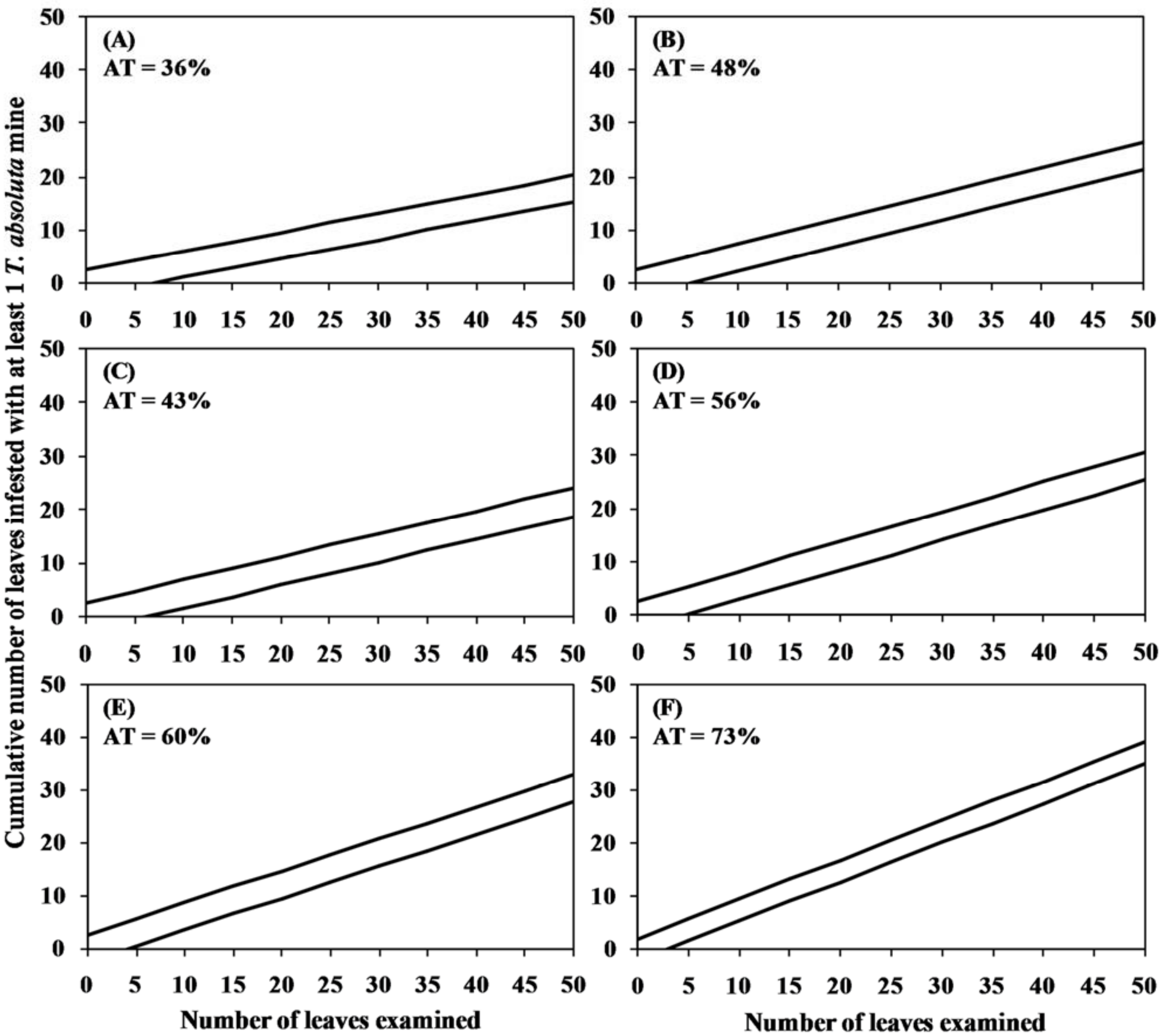

Figure 7. Decision stop lines for *Tuta absoluta* binomial sequential sampling plans in tomato cultivars with big (A and B), medium (C and D) and small (E and F) fruits. Binomial plans were obtained from resampling validation analysis based on the action thresholds of 36% (A), 48% (B), 43% (C), 56% (D), 60% (E) and 73% (F) of infested sample units, α and β = 0.1 and a tally threshold of 1 mine per leaf.