OXFORD UNIVERSITY PRESS | Journal of Economic Entomology

Spatial distribution and sampling plans for Grape vine plant canopy inhabiting *Scaphoideus titanus* (Hemiptera, Cicadellidae) nymphs

Journal:	*Journal of Economic Entomology*
Manuscript ID	Draft
Manuscript Type:	Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Rigamonti, Ivo; University of Milan, DeFENS Brambilla, Carla; CNR, IMATI - Istituto di Matematica Applicata e Tecnologie Informatiche Colleoni, Emanuele; University of Milan, DeFENS Jermini, Mauro; Agroscope, Research Centre Cadenazzo Trivellone, Valeria; Agroscope, Research Centre Cadenazzo Baumgärtner, Johann; CASAS, Center for the Analysis of Sustainable Agricultural Systems
Please choose a section from the list:	Sampling and Biostatistics
Field Keywords:	Agricultural Entomology, IPM-Agricultural, Sampling
Organism Keywords:	Cicadellidae

Rigamonti et al.: distribution and sampling plans for *S. titanus*

I. E. RIGAMONTI
University of Milan
DeFENS
via G. Celoria, 2
I-20133 Milan, Italy
Phone: 0039 02 5031 6752
Fax: 0039 02 5031 6748
E-mail: ivo.rigamonti@unimi.it

Journal of Economic Entomology
Sampling and Biostatistics

Spatial distribution and sampling plans for Grape vine plant canopy inhabiting *Scaphoideus titanus* (Hemiptera, Cicadellidae) nymphs

IVO ERCOLE RIGAMONTI, [1] CARLA BRAMBILLA, [2] EMANUELE COLLEONI, [3] MAURO JERMINI, [4] VALERIA TRIVELLONE, [4] AND JOHANN BAUMGÄRTNER [5]

[1] Department of Food, Environmental and Nutritional Sciences, University of Milan via G. Celoria, 2, I-20133 Milan, Italy,

[2] Istituto di Matematica Applicata e Tecnologie Informatiche, CNR, Dipartimento di Milano, via E. Bassini, 15, I-20133 Milan, Italy

[3] Via Manzoni, 22, I-24030 Mozzo, Italy

[4] Agroscope, Research Centre Cadenazzo (TI). A Ramél, 18, CH-6593 Cadenazzo, Switzerland,

[5] Center for the Analysis of Sustainable Agricultural Systems (CASAS), CA 94707 Kensington, USA

ABSTRACT The paper deals with the study of the spatial distribution and the design of sampling plans for estimating nymph densities of the Grape leafhopper *Scaphoideus titanus* Ball in vine plant canopies. In a reference vineyard, leaf samples were repeatedly taken according to a multi-stage stratified random sampling procedure and data were subjected to an ANOVA. There were no significant differences in density neither among the strata within the vineyard nor between the two strata with basal and apical leaves. The significant differences between densities on trunk and productive shoots lead instead to the adoption of two-stage (leaves, plants) and three-stage (leaves, shoots, plants) sampling plans for trunk shoots and productive shoots inhabiting individuals respectively. The mean crowding to mean relationship used to analyze the nymphs spatial distribution revealed aggregated distributions. In both the enumerative and the sequential enumerative sampling plans, the number of leaves of trunk shoots, and of leaves and shoots of productive shoots, was kept constant while the number of plants varied. In additional vineyards data were collected and used to test the applicability of the distribution model and the sampling plans. The tests confirmed the applicability i) of the mean crowding to mean regression model on the plant and leaf stages for representing trunk shoot inhabiting distributions, and on the plant, shoot and leaf stages for productive shoot inhabiting nymphs, ii) of the enumerative sampling plan, iii) of the sequential enumerative sampling plan. In general, sequential enumerative sampling was more cost-efficient than enumerative sampling.

KEY WORDS enumerative sampling plan, sequential enumerative sampling plan, mean crowding to mean regression, reliability levels, simulation

The North American leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) arrived in Europe in the 1950s to reach quickly the status of key pest in European vineyards (Bonfils and Schwester 1960, Schvester et al. 1961, 1962a, 1962b). It invaded Southwestern and Southern European grapevine growing areas from where it spread to Southeastern European regions. Currently, the area of distribution spreads from Portugal to Bulgaria and is still expanding (Chuche and Thiery 2014). *Scaphoideus titanus* is the vector of the *Candidatus* Phytoplasma vitis, a Phytoplasma of the Elm Yellows or 16Sr-V group, an economically important A2 quarantine pest for EPPO causing the Flavescence dorée (FD). To plan and carry out control operations, the occurrence of FD and its vector is monitored throughout invaded and prospective areas of distribution (EPPO/CABI 1996).

Beating tray and sticky trap techniques are satisfactory for monitoring *S. titanus* nymph and adult occurrences in the vine plant canopy and useful for validating short and long term phenology models (Rigamonti et al. 2011, 2013, 2014) but inadequate for obtaining intensities and absolute densities. Intensity estimates for vine plant leaf inhabiting life stages obtained through visual examination of leaves proved to be useful for between plant distribution studies, sampling plan design and canopy infestation model development (Lessio and Alma 2006, Maggi et al. 2013). To obtain absolute density estimates for nymphs, the proportion of nymphs inhabiting the vegetation of the vineyard floor and the number of leaves per plant has to be taken into account (Trivellone et al. 2011, 2013). Intensity estimates have long been used for analysing spatial distributions in heterogeneous sampling universes (Southwood 1978). They improve the insight into population processes in spatially structured environments and contribute to further developing supervised vector management programs (Pedigo and Buntin 1994).

Already in the 1950s, LeRoux and Reimer (1959) used stratified multi-stage sampling procedures in apple orchards. They randomly selected subsamples at different stages that they stratified for taking into account stage-specific spatial heterogeneities. The procedures have been detailed by Cochran (1977) and recently applied to spatial studies of vineyard insects by Ifoulis and Savopoulou-Soultani (2006). LeRoux and Reimer (1959) showed how to analyze the variance of within- and between plant components and opened the door to the development of spatial distribution models applicable to highly structured sampling universes, in that the variance or related statistics are often regressed on mean intensity (Taylor 1961, Iwao 1968, Kuno 1976). Since these models take into account the dependency of the variance on the mean density, they are generally valid on a wide range of densities (Davis, 1994).

For the estimation of the parameters of these models, quite large sets of data are required. Since, for a given scale, the parameters tend to be species-specific and constant through time and space, researchers rationalize data collection by applying repeated sampling procedures (Iwao and Kuno 1971, Hagihara 1976, Taylor and Woiwood 1980, Perry and Taylor 1986, Sawyer 1989).

The purpose of this paper is to apply multi-stage stratified random sampling procedures to analyze the spatial distribution patterns of *S. titanus* in a highly structured grape vine plant canopy of a FD free reference vineyard. Knowledge on the sources of spatial variations can be used to set the stage for adapting Kuno’s (1976) regression model and employing it for modeling the spatial distribution and designing sampling plans. A successful application of the distribution model and the sampling plans in additional vineyards is expected to improve the sampling strategy and to put adaptive *S. titanus* management in FD free areas on a more solid ground than available so far.

Materials and Methods

Study vineyards. In a reference vineyard located at Camorino (46°09’ N, 8°59’ E, 220 m a.s.l.) in Southern Switzerland, the number of *S. titanus* individuals was recorded repeatedly in 2009 to acquire data for analyzing the sources of variation, distribution model development and sampling plan design.

Additional vineyards were sampled at Arbedo (46°13’ N, 9°02’ E, 240 m a.s.l.), Bironico (46°07’ N, 8°55’ E, 490 m a.s.l.) and Contone (46°09’ N, 8°56’ E, 210 m a.s.l.) to acquire data for testing the applicability of the distribution model and the sampling plans

The plants were pruned to allow shoot development departing from 10 buds and trained according to the Guyot double system. These shoots are referred to as productive shoots that are different from the spontaneous unproductive trunk shoots. Occasionally, the productive shoots were topped and hedged. Spontaneous vegetation, occasionally mowed, covers the ground between the rows. Within the rows, herbicides were used to control weeds. Synthetic pesticides were applied against powdery and downy mildew but not against arthropods.

To facilitate the comparison with literature information, we simply refer to ‘densities’ rather than to ‘relative densities’ or ‘intensities’. When referring to subsampling, we refer to stages and levels in an interchangeable way.

Sampling procedures. LeRoux and Reimer’s (1959) procedure takes into account the spatial heterogeneity of the reference vineyard, the shoot type and the within-shoot leaf position, and enhances the sampling efficiency through sub-sampling the plant and the shoot. According to that procedure, to take into account the possible within-vineyard heterogeneity, we stratified the reference vineyard to obtain five plots of equal size, one at each side and one in the centre of the vineyard. In each stratum, five plants were selected at random. Sub-sampling of the plant was then carried out by randomly selecting three productive shoots and one trunk shoot. To take into account a possible heterogeneity within productive and trunk shoots, all shoots were divided into two equal strata, separating the leaves of the basal half from those of the apical half. In each shoot stratum, three leaves were selected at random. On each leaf, the number of *S. titanus* individuals was counted. The vineyard was sampled weekly from 27 May 2009 to 30 July 2009. The samples taken on 27 May, 10 June, 17 June, 25 June and 3 July contained a sufficient number of nymphs for conducting statistical analyses.

Sources of variation. To identify the sources of variation between sampling units in the reference vineyard, analyses of variance (ANOVA) were carried out as described by LeRoux and Reimer (1959). The between-plants variability is attributed to the differences between the strata at the vineyard level and between the plants. The within-plant variability is attributed to the differences between productive shoots and the trunk shoots, and to the differences between the strata within the shoots. The plant is considered as a random factor, as well as the productive shoots, while vineyard strata, shoot type and shoot strata are considered as fixed. We followed LeRoux and Reimer's (1959) recommendation of using untransformed counts.

Spatial distribution model. Iwao's (1968) model regresses Lloyd's (1967) mean crowding index $\overset{*}{m}$ on mean density *m*, i.e.

$$\overset{*}{m} = \alpha + \beta m \qquad [1]$$

where α and β have been defined as index of basic contagion and density-contagiousness coefficient, respectively, and qualified as meaningful indexes for the spatial characteristics of a population (Davis 1994). Thereby, the parameters are related to the basic population unit and to the aggregation of population units; the basic unit is a colony, an individual or individuals repelling each other if $\alpha>0$, $\alpha=0$ or $\alpha<0$, respectively, while the spatial distribution is aggregated, random or uniform if $\beta>1$, $\beta=1$, $\beta<1$, respectively. Theoretical and experimental information suggests that the regression parameters are species specific and constant through time and space, for a given scale and range of densities (Iwao and Kuno 1971, Hagihara 1976, Taylor and Woiwood 1980, Perry and Taylor 1986, Sawyer 1989). According to Kuno (1976), the model holds for habitats of any given size, for a whole area as well as for sub-samples.

Kuno (1976) used model [1] to represent the distribution of insects in two- and three stage random sampling programs. To facilitate the consultation of his paper, we use whenever possible his symbols and terminology. Since the shoot types are the most important source of variation (see the Results and Discussion section), separate analyses for trunk shoot and productive shoot inhabiting populations are carried out. The equation [1] then becomes

$$\overset{*}{m}_j = \alpha + \beta_j \, m \qquad \text{with } j=1,2 \text{ for trunk shoot distribution analyses} \qquad [2]$$

$$\overset{*}{m}_j = \alpha + \beta_j \, m \qquad \text{with } j=1,2,3 \text{ for productive shoot distribution analyses} \qquad [3]$$

In the case of trunk shoots, the model is applied on the level of the vineyard (j=1), with plants as primary sampling units (PSU), and on the level of the plant (j=2), with leaves as secondary sampling units (SSU) (eq. [2]). In the case of productive shoots, the model is applied on the level of the vineyard (j=1), with plants as primary sampling units (PSU), on the level of the plant (j=2), with shoots as secondary sampling units (SSU) and on the level of the shoot (j=3) with leaves as the tertiary sampling unit (TSU) (eq. [3]).

Empirical information suggests that individuals are the basic unit of the population. Waters *et al*. (2014) showed that modifying both the linear function and the statistical model to force the intercept or lower functional limit through the origin results in more intuitive biological interpretation of parameters and better sampling economy. Hence, the regression models were forced through the origin at all stages under consideration (α=0). The mean crowding values were calculated for between plants, between shoots and, for productive shoots only, within shoot relationships. The parameters β_1 and β_2 for trunk shoot inhabiting individuals, and β_1, β_2 and β_3 for productive shoots inhabiting individuals were obtained via least square regression analyses.

Reliability of density estimates and design of sampling plans. The variance of the density estimates for individuals living on trunk shoots and productive shoots can be calculated with eqs. [2] and [6] in Kuno's (1976) paper. To introduce the dependency of the variance to the mean, Kuno (1976) re-writes the variances in terms of the relevant mean crowing $\overset{*}{m}$ to mean regression models (eqs. [2, 3]). For assessing the reliability of ecological population estimates, the ratio *D* of the standard error *d* to the mean *m* is used (Karandinos 1976, Kuno 1976, Pedigo and Buntin 1994).

The number of sampled units (leaves, shoots, plants) is small relative to the total number of units, hence there is no need to make finite population corrections (Cochran 1977), and Kuno's (1976) eqs. [16] and [25] can be used to develop enumerative sampling plans.

For attaining a predefined reliability level D_0 with a fixed number k_0 of leaves per plant, the number *l* of plants to be sampled, expressed as a function of the mean *m* per leaf of individuals on trunk shoot leaves, is

$$l=\frac{\frac{1}{k_0}\left\{\frac{\alpha+1}{m}+\frac{\beta_1(\beta_2-1)}{\beta_2}\right\}+\frac{\beta_1-\beta_2}{\beta_2}}{D_0{}^2+\frac{1}{L}\frac{\beta_1-\beta_2}{\beta_2}} \qquad [4]$$

where α is the intercept, and β_1 and β_2 are the slopes of eq. [2], and in our study $k_0=6$. For attaining a predefined reliability level D_0 with a fixed number k_0 of leaves per shoot and a fixed number q_0 of shoots per plant, the number l of plants to be sampled, expressed as a function of the mean m per leaf of individuals on productive shoot leaves, is

$$l=\frac{\frac{1}{k_0}\left[\frac{\beta_1-\beta_3}{\beta_3}-\frac{\beta_1-\beta_2}{\beta_2}+\frac{1}{q_0}\left\{\frac{\alpha+1}{m}+\frac{\beta_1(\beta_3-1)}{\beta_3}\right\}\right]+\frac{\beta_1-\beta_2}{\beta_2}}{D_0{}^2+\frac{1}{L}\frac{\beta_1-\beta_2}{\beta_2}} \qquad [5]$$

where α is the intercept, and β_1, β_2 and β_3 are the slopes of eq. [3], and in our study $k_0=6$ and $q_0=3$.

The high total number L of plants in the vineyard under consideration reduces $1/L$ to zero and hence, the denominators to $D_0{}^2$. The computations are further facilitated taking into account α=0 as stated above.

The disregard of the finite population correction permits also the reference to Kuno's (1976) eqs. [27] and [34] to develop sequential enumerative sampling plans. According to this plan, the field work is terminated when the cumulative total count T_n, with n being the sample size, *i.e.* the number of leaves sampled, reaches a predetermined value.

The sequential estimation of the density $m=T_n/n$ per leaf of trunk shoot inhabiting individuals is based on a constant number k_0 of leaves per plant. T_n is calculated after

$$T_n=\frac{\alpha+1}{D_0{}^2-\frac{1}{n}\left\{\frac{\beta_1(\beta_2-1)}{\beta_2}+\frac{\beta_1-\beta_2}{\beta_2}k_0\right\}} \qquad [6]$$

where $n= l\,k_0$, *l* being the number of plants, and $k_{0=}6$ in our study. For the sequential estimation of the density $m=T_n/n$ of productive shoot inhabiting individuals, the constancy of leaf numbers per shoot *(k_0)* and of shoot numbers per plant *(q_0)* leads to calculate T_n as follows

$$T_n = \frac{\alpha + 1}{D_0{}^2 - \frac{1}{n}\left\{\frac{\beta_1(\beta_3 - 1)}{\beta 3} + \frac{\beta_1(\beta_2 - \beta_3)}{\beta_2\beta_3} q_0 + \frac{\beta_1 - \beta_2}{\beta_2} k_0 q_0\right\}} \qquad [7]$$

where $n= l\,q_0\,k_0$, *l* being the number of plants, q_0=3 and $k_{0=}6$. In eq. [6] and eq. [7], the intercept α is set to 0.

Testing the applicability of the distribution model and the sampling plans

Sampling procedures. The sampling plans in eqs. [4, 5] were applied in the Arbedo, Bironico and Contone vineyards where 100, 90 and 20 plants were selected at random, respectively. The number of individuals was counted on six randomly selected trunk shoot leaves, if the total number of leaves per trunk equaled or exceeded 20. Trunks with a smaller number of leaves were disregarded and substituted by another trunk. Among productive shoots, three were selected at random on each plant, and nymphs were counted on six randomly selected leaves per shoot. The Arbedo vineyard was sampled on 24 May 2011, the Contone vineyard on 25 May 2011, and the Bironico vineyard on 12 June 2013 and on 1st July 2013. The visits yielded four data sets that are used here for testing the applicability of the spatial distribution model and the sampling plans.

Applicability of the distribution model. The sampling data obtained in the additional vineyards were used to estimate the mean crowding to mean regression statistics. Importantly, only a single sample was available in the Arbedo and Contone vineyards, whereas the Bironico vineyard was sampled twice. Since the four samples were treated separately, there were no replicates available for evaluating the slopes at the plant stage. The samples were used to calculate via least square linear regression analysis the parameters of eqs. [2, 3] with respective coefficient of determination R^2 and standard errors. The comparison with the reference vineyard statistics is made by evaluating the overlapping of confidence intervals at the probability level $\alpha/2=0.05$ according to Payton et al. (2003).

Applicability of the sampling plans. To test the applicability of the enumerative and sequential enumerative sampling plans, we calculated the precision levels on the basis of the aforementioned four data sets, as detailed below. In the following the index c was used to denote the precision levels (D_c) obtained.

To verify the applicability of the enumerative sampling plans, we computed hypothesized densities based on unpublished monitoring data collected in previous years in the additional vineyards by collaborators of the Agricultural Bureau of Canton Ticino and of the Agroscope centre of excellence for agricultural research in Switzerland. The resulting hypothesized densities were 0.36 and 0.26 individuals per leaf on trunk shoots and on productive shoots, respectively. For these densities the optimun sample size (OSS) for the $D_0=0.1$ and $D_0=0.3$ reliability levels, according to eqs. [4, 5], were 77 and 9 plants for trunk shoots and 69 and 8 plants for productive shoots respectively.

For each OSS, we carried out 1000 simulations with randomly selected plants in the Arbedo, Bironico and Contone data sets, and estimated the densities. Based on these densities and on the slopes β_I of the distribution model for the reference vineyard, 1000 reliability values D_c were then computed, to finally yield average values. The limited data set available for the Contone vineyard allowed the estimation of means and reliability levels D_c for productive shoots at $D_0=0.3$ only. For testing the enumerative sampling plan, the mean values D_c are compared with the predefined $D_0=0.1$ and $D_0=0.3$ reliability levels.

To verify the applicability of the sequential enumerative sampling plans the four data sets were used to simulate 1000 times the sequential sampling of nymphs on trunk and productive shoots at the two levels of reliability. In each of the 1000 simulations, the number of plants required to meet or exceed the stop line for estimates on trunk and productive shoots was obtained. As for the enumerative sampling plan, based on the estimates of the density and on the slopes β_l of the distribution model for the reference vineyard, 1000 reliability values D_c were then computed, to finally yield average values. For testing the reliability of the sequential sampling plan, the average D_c were compared with the predefined D_0=0.1 and D_0=0.3 reliability levels. The limited data set available from the Contone vineyard allowed the estimation of means and reliability levels D_c for productive shoots at D_0=0.3 only.

For testing the efficiency of the sequential enumerative sampling plan in relation to the enumerative sampling plan, the average number of plants examined for reaching or exceeding the stop line was calculated and compared to the number used in enumerative sampling plan.

Statistical analysis. All the statistical analysis were conducted using the IBM SPSS Statistics 22 software (IBM Software Group 2014, Chicago, IL).

Results and Discussion

Sources of variation in reference vineyard. According to Table 1, there were no significant differences between vineyard strata, indicating a homogeneous environment between plants. However, there was a significant difference between shoot types at the beginning of the growing season. This is explained by the behavior of nymphs. Newly hatched nymphs mainly inhabit trunk shoot leaves, while older nymphs disperse in the plant canopy, often after a passage through the vineyard floor vegetation (Trivellone et al. 2011, 2013). Unexpectedly, there was no significant difference within the shoot strata. Possibly, the topping of productive shoots induces the growth of secondary shoots and creates a sampling environment in that the stratification produced two subdivisions that differ in their distance from the trunk rather than grouping leaves according to their age, indicating that shoots form a homogeneous sampling environment.

Furthermore there were no significant differences between the grapevine plants. This was unexpected because the within-plant variance is often smaller than the between plant variance, allowing researchers to focus on plant samples and limit the number of within-plant samples (Southwood 1978, Lessio and Alma 2006). Nevertheless, the plants as primary units are retained in this and future works, because the interactions between grape vines, FD and *S. titanus* presumably occur on the plant level.

Based on the result of the ANOVA the stratification of the vineyard and the shoots could be disregarded in regression model development and in the design of the sampling plans. However, the difference between shoot types required a differentiation between them and to work with two-stage sampling plans for trunk shoot inhabiting individuals and with three-stage sampling plans for productive shoots inhabiting individuals.

The mean densities per leaf reported in Table 1 are similar to the ones observed by Lessio and Alma (2006) and by one of the authors of this paper (M. Jermini, personal communication) in many untreated vineyards with comparable architectures in Northwestern Italy and Southern Switzerland, respectively. Because of the occurrence of similar densities, canopy structures and management procedures, the Camorino reference vineyard is seen as representative for the many vineyards in an extensive FD-free grape growing region and possibly beyond.

Reference vineyard spatial distribution model. Figures 1 and 2 depict the mean crowding to mean relationship at the plant and leaf level for trunk shoot inhabiting *S. titanus* and at the plant, shoot and leaf level for productive shoot inhabiting *S. titanus,* respectively. By visual examination, Iwao's (1976) linear regression models, with intercept α set to 0, appeared to describe satisfactorily the relationships. The slopes β_1, β_2 and β_3 (in Fig. 2C only) were greater than 1 indicating an aggregated distribution of population individuals as previously observed by Lessio and Alma (2006). The aggregation on trunk shoot leaves was not unexpected since nymphs may first aggregate in a highly heterogeneous vineyard floor to feed on preferred plants and move to nearby vine plants thereafter (Trivellone et al. 2011, 2013).

Reference vineyard sampling plans. Figures 3 and 4 represent the enumerative sampling plan for *S. titanus* individuals inhabiting trunk shoots and productive shoots, respectively. The sampling plan referring to plants requires the taking of six leaves per plant for trunk shoots or six leaves on each of three shoots for productive shoots. The number of plants (*I*) depended on the density and the level of precision set to D_0=0.1, adequate for research purposes, or D_0=0.3, suitable for pest management activities.

This analysis deals with data obtained in vineyards in a FD free area. Presumably the densities occurring in FD invaded areas are much smaller, leading to excessive sample sizes for reliable population density estimates. For the time being this possible disadvantage has no practical implications because in FD invaded areas the mandatory treatments are undertaken without reference to population density estimates.

Figures 5 and 6 represent the stop lines for the sequential enumerative sampling for estimating the trunk shoot and productive shoot inhabiting individuals, respectively. The optimum sampling plan referring to plants is designed for the

D_0=0.1 and D_0=0.3 reliability levels and requires the examination of six leaves per plant or six leaves on each of three shoots for trunk shoot or productive shoots inhabiting individuals, respectively.

The users of the sequential enumerative sampling plan have to control a minimum number of plants, corresponding to the point at which the stop line reaches a positive value, and, if required, continue to examine randomly selected additional plants until the cumulative number of nymphs reaches the stop line. The minimum number of plants depends on the shoot type and the level of precision D_0 and is 31 (for D_0=0.1) or 4 (for D_0=0.3) when sampling trunk shoot inhabiting individuals, and 48 (for D_0=0.1) or 6 (for D_0=0.3) when sampling productive shoot inhabiting individuals. In both cases, the sequential method appears to be more efficient than the enumerative plan since it often leads to a reliable estimate with a smaller number of examined leaves.

Spatial distribution in additional vineyards. Table 2 shows the slopes with associated standard errors of the mean crowding to mean regressions at the different stages for trunk shoot and productive shoot inhabiting nymphs in the Bironico, Contone and Arbedo vineyards. The slopes representing Iwao's (1968) contagiousness show an aggregated distribution and moreover, little variability among the samples. The highest degree of aggregation occurred on the shoot stage for productive shoots, whereas the lowest degree of aggregation existed between leaves on trunk shoots. The degree of aggregation was intermediate on leaves of productive shoots.

The comparison of the average slopes given in Table 2 with means and confidence intervals of the reference data set reported in Table 3 shows that all means reported in Table 2 fall into the confidence interval obtained from Table 3. Moreover, the confidence intervals given in Table 2 overlap with the confidence intervals for the reference data set reported in Table 3. Hence, the parameter estimates obtained from the Arbedo, Bironico and Contone can be considered in agreement with the ones obtained in the Camorino reference vineyard.

Though the slope for densities on trunk shoots cannot be evaluated at the vineyard stage (Table 2), it can be concluded that the spatial distribution model is appropriate for the Arbedo, Bironico and Contone vineyards.

Applicability of the sampling plans. Table 4 shows that the number of plants, calculated by applying the enumerative sampling plan to a hypothesized density, yielded density estimates with mean reliability levels (columns 8 and 9) close to ones predefined in the sampling plan (columns 4 and 5). This result was unexpected since the hypothesized density (columns 2 and 3) used for determining the OSS was generally different from the mean density obtained through simulations (column 6 and 7). Apparently, the enumerative sampling plans yielded density estimates at satisfactory reliability levels even when using inaccurate hypothesized densities to define the OSS. This is due to generally similar and relatively high densities observed in the Arbedo, Bironico and Contone vineyards, a density range within that the OSS changes little.

Table 5 shows that the average reliability levels D_c obtained through 1000 simulations are in general close to the predefined reliability levels D_0, from which we can conclude that the sequential enumerative sampling plan with the statistics obtained from the Camorino reference data set yielded reliable estimates for the Arbedo, Bironico and Contone vineyards.

Table 5 also shows that, at least for the trunk shoot inhabiting nymphs, the number of plants required to obtain a reliable density estimate was often but not always lower than the number of plants used in the enumerative sampling plan. Hence, the sequential enumerative sampling plan was in general more efficient than the enumerative sampling plan. Table 5 also shows that the sequential enumerative sampling plan is less efficient if the hypotesized density is much higher than the estimated density.

The methodology presented shows how sampling procedures in a heterogeneous sampling universe can be structured and prepared for spatial distribution modeling. The study of the sources for the spatial variation within the canopy led to the design of a multi-stage stratified random sampling program that could be simplified further into a two- and a three stage random sampling program that did not require stratification any more.

Nevertheless, a brief review of the modern literature suggests that the approach outlined by Pottinger and Le Roux (1971), Kuno (1976), Cochran (1977) and Southwood (1978) three decades ago did not receive sufficient attention in spatial analyses and sampling plan design. Without studying the sources of spatial variation in detail, the authors of papers on regression models rely on assumptions to proceed to model development.

The mean crowding to mean regression model used at different stages in a highly structured vine plant canopy appears to be appropriate to describe the distribution of nymphs in the reference and additional vineyards. We presume that the model is also applicable to additional vineyards with similar canopy architecture, spontaneous vegetation and surroundings, but we see a need to evaluate the applicability in vineyards with different characteristics. The slopes of the mean crowding to mean regressions indicated an aggregated distribution on the plant, shoot and leaf levels. The results obtained here may provide some support to the claims that the regression parameters are constant through time and space, for a given scale and range of densities (Iwao and Kuno 1971, Hagihara 1976, Taylor and Woiwood 1980, Perry and Taylor 1986, Sawyer 1989), and the model holds for habitats of any given size, for a whole area as well as for sub-samples (Kuno 1976). Undoubtedly, more data should be analyzed to confirm the validity of this claim.

Moreover, the results show that both the enumerative and the sequential enumerative sampling plans can be applied to other vineyards with similar canopy architecture, spontaneous vegetation and surroundings. To be able to apply efficiently the enumerative sampling plan, the user should have some information on possible densities in the vineyard to be sampled. This information doesn’t have to be accurate, however, and could be acquired in a preliminary sampling program or, as done here, by approximating densities on the basis of previously conducted monitoring programs. The results show that satisfactory levels of reliability are reached even if the density estimate for determining the OSS is considerably different from the actual density. This result may be of interest to pest managers favoring enumerative over sequential enumerative sampling plans. As stated in the case of the distribution model, the results obtained here should be critically evaluated for other populations in different environments before drawing general conclusions.

The number of samples to be taken in the enumerative sampling program is higher than the number reported by Lessio and Alma (2006). To some extent, the difference may be due to different reliability levels for density estimates and the use of different regression models that both can be justified (Davis 1994). Indeed, in contrast to this work, Lessio and Alma (2006) relied on an application of Taylor’s (1961) power law. To a greater extent, however, the difference is due to the spatial variation under consideration. Whereas Lessio and Alma (2006) neglected within-plant sources of variability and focused on between-plant variation, the present findings show that the within plant variability is more important than the between-plant variability and should consequently be taken into account.

The sequential enumerative sampling plan can be applied to other vineyards with a similar canopy architecture, spontaneous vegetation and surroundings. As expected the sequential enumerative sampling plan is in general, but not always, more cost efficient than the enumerative sampling plan. In sequential enumerative density estimation, the applicability of the sampling plan doesn't require previous knowledge on infestation levels and the effort to estimate population density promises to be smaller than in the case of enumerative sampling. If, however, the accumulated number of samples exceeds the one found in the enumerative sampling plan, the user is advised to terminate the sampling activities and rely on the reliability of the enumerative sampling plan.

The successful application and the applicability of the sampling plans in additional vineyards put adaptive *S. titanus* management on more solid grounds than available so far. So far, the *S. titanus* adaptive management project emphasized the within vineyard population dynamics and the development of respective adaptive management strategies. For this purpose the enumerative and sequential enumerative sampling plans presented here are particularly appropriate and a reliability level of D_0=0.1 was considered adequate. In the context of regional population studies and disease transmission, however, the pest manager may be interested in regional sampling and management operations. For this purpose, we tentatively proposed a D_0=0.3 reliability level. As shown already by Sawyer and Haynes (1985), regional population estimations require the extension of the spatial model and the possible consideration of additional strata at the landscape level.

Further improvements of the sampling strategy for adaptive *S. titanus* management can be made when re-orienting the work from research to adaptive management under specific agricultural conditions. For example, improvements are possible when focusing on initial densities on trunk shoots to decide on the necessity of including the vineyard in a monitoring program. If a threshold density for this decision can be given and the nymph density is at moderate levels, cost efficient presence-absence sampling plans with respect to a threshold can be developed and implemented (Wilson *et al*., 1983; Bianchi *et al*., 1989, Knapp *et al*., 2006). Further improvements are possible when focusing on specific developmental stages whose occurrence may trigger a chemical control operation (*e.g.* in Switzerland the appearance of third instar nymphs is used to determine the timing of the first mandatory treatment). Guided by the results of continuously adapted phenology simulations, the pest manager should be able to reduce the frequency of sampling operations (Jermini *et al*., 2013; Prevostini *et al*, 2013).

The model describes the intensity per leaf of nymphs only. The analysis of the spatial distribution of bark inhabiting eggs and mobile adults requires a different sampling technique and a revision of the model structure. To obtain absolute density estimates for all life stages from intensity estimates, the proportion of vineyard floor inhabiting nymphs, which depends from the botanical composition, and the number of leaves per plant has to be taken into account (Trivellone *et al*., 2013).

Acknowledgments

The editorial assistance of Dr. Fritz Schulthess is appreciated. The Agricultural Bureau of the Canton Ticino kindly made available monitoring data from the Arbedo, Bironico and Contone vineyards.

References cited

Bianchi G., J. Baumgärtner, V. Delucchi, N. Rahalivavololona, S. Skillman, and P. H. Zahner. 1989. Sampling egg batches of *Maliarpha separaella* RAG. (Lep., Pyralidae) in Madagascan rice fields. Trop. Pest Man., 35: 420-424.

Bonfils, J., and D. Schvester. 1960. Les cicadelles (Homoptera: Auchenorrhyncha) dans leurs rapports avec la vigne dans le Sud-Ouest de la France. Ann. Epiph. 9: 325-336.

Chuche J., and D. Thiéry. 2014. Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: a review. Agron. Sustain. Dev. 34 (2): 381-403.

Cochran, W. G. 1977. Sampling Techniques, 3rd ed. John Wiley and Sons, New York, NY.

Davis, P. M. 1994. Statistic for Describing Populations. Vol. 1 pp 33-55. *In* L. P. Pedigo and G. D. Buntin (eds.), Handbook of Sampling Methods for Arthropods in Agriculture. CRC Press, Boca Raton, FLA.

(EPPO/CABI) European and Mediterranean Plant Protection Organization. 1996. Grapevine flavescence dorée phytoplasma. pp. 1013-1021. *In* I. M. Smith, D. G. McNamara, P. R. Scott and M. Holderness (eds.), Quarantine Pests for Europe, 2nd edition. CAB International, Wallingford, United Kingdom.

Hagihara, A. 1976. The time sequence of the relation between mean crowding and mean density with some population processes. Res. Popul. Ecol., 17: 224-239.

IBM Software Group. 2014. IBM SPSS Statitistics user's manual, version 22nd ed. IBM Software Group, Chicago, IL.

Ifoulis, A. A., and M. Savopoulou-Soultani. 2006. Developing optimum sample size and multistage sampling plans for *Lobesia botrana* (Lepidoptera: Tortricidae) larval infestation and injury in northern Greece. J. Econ. Entomol., 99: 1890-1898.

Iwao, S. 1968. A new regression method for analyzing the aggregation pattern of animal populations, Res. Popul. Ecol., 10: 1-20.

Iwao, S., and E. Kuno. 1971. An approach to the analysis of aggregation pattern in biological populations. Vol. 1, pp 461-513. *In* G. P.Patil, E. C. Pielou and W. E. Waters (eds.), Spatial patterns and statistical distributions. Statistical ecology. Pennsylvania State University Press, Philadelphia, PA.

Jermini, M., V. Trivellone, C. Cara, and J. Baumgärtner. 2013. Marrying research and management activities: adaptive management of grape leafhopper *Scaphoideus titanus*. IOBC/ wprs Bull. 85: 49-56.

Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22: 417-421.

Knapp, M., I. Sarr, G. Gilioli, and J. Baumgärtner. 2006. Population models for threshold-based control of *Tetranychus urticae* in small-scale Kenyan tomato fields and for evaluating weather and host plant species effects. Exp. Appl. Acarol. 39: 195-212.

Kuno, E. 1976. Multi-stage sampling for population estimation. Res. Popul. Ecol. 18: 39-56.

LeRoux, E. J., and C. Reimer. 1959. Variation between samples of immature stage, and of mortalities from some factors, of the eye-spotted bud moth, *Spilonota ocellana* (D. and S.) (Lepidoptera: Olethreutidae), and the pistol casebearer, *Coleophora serratella* (L.) (Lepidoptera: Coleophoridae), on apple in Quebec. Can. Entomol. 91: 428-449.

Lessio, F., and A. Alma. 2006. Spatial distribution of nymphs of *Scaphoideus titanus* (Homoptera: Cicadellidae) in grapes, and evaluation of sequential sampling plans. J. Econ. Entomol. 99: 578-582.

Lloyd, M. 1967. Mean crowding. J. Anim. Ecol. 36: 1-30.

Maggi, F., C. Marzachì, and D. Bosco. 2013. A stage structured model of *Scaphoideus titanus* in vineyards. Env. Entomol. 42: 181-193.

Payton, M. E., M. H. Greenstone, and N. Schenker. 2003. Overlapping confidence intervals or standard error intervals: What do they mean in terms of statistical significance? J. Insect Sc. 3: 34. DOI: 10.1673/031.003.3401, http://jinsectscience.oxfordjournals.org/content/jis/3/1/34.full.pdf.

Pedigo, L. P., and G. D. Buntin. 1994. Handbook of Sampling Methods for Arthropods in Agriculture. CRC Press, Boca Raton, FLA.

Perry, J. N., and L. R. Taylor. 1986. Stability of real interacting populations in space and time: implications, alternatives and negative binomial. J. Anim. Ecol., 55: 1053–1068.

Pottinger, R. P., and E. J. LeRoux. 1971. The biology and dynamics of *Lithocolletis blancardella* (Lepidoptera: Gracillariidae) on apple in Quebec. Mem. Entomol. Soc. Canada 77: 1-437.

Prevostini, M., A V. Taddeo, K. Balac, I. Rigamonti, J. Baumgärtner, and M. Jermini. 2013. WAMS - an adaptive system for knowledge acquisition and decision support: the case of *Scaphoideus titanus*. IOBC/ wprs Bull. 85: 57-64.

Rigamonti, I. E., M. Jermini, D. Fuog, and J. Baumgärtner. 2011. Towards an improved understanding of the dynamics of vineyard infesting *Scaphoideus titanus* leafhopper populations for better timing of management activities. Pest Manag. Sc. 67: 1222–1229.

Rigamonti, I. E., V. Trivellone, M. Jermini, and J. Baumgärtner. 2013. Multiannual infestation patterns of grapevine plant canopy inhabiting *Scaphoideus titanus* Ball leafhoppers. IOBC/ wprs Bull. 85: 43-48.

Rigamonti, I. E., V. Trivellone, M. Jermini, D. Fuog, and J. Baumgärtner. 2014. Multiannual infestation patterns of grapevine plant inhabiting *Scaphoideus titanus* (Hemiptera: Cicadellidae) leafhoppers. Can. Entomol. 146: 67-79.

Sawyer, A. J. 1989. Inconstancy of Taylor's *b*: simulated sampling with different quadrat sizes and spatial distributions. Res. Popul. Ecol. , 31: 11-24.

Sawyer, A. J., and D. L. Haynes. 1985. Spatial analysis of cereal leaf beetle abundance in relation to regional habitat features. Env. Entomol. 14: 92–99.

Schvester, D., P. Carle, and G. Moutous. 1961. Sur la transmission de la Flavescence dorée de la vigne par une Cicadelle. Compte Rendu Acad. Agr. Fran. 67: 1021-1024.

Schvester, D., G. Moutous, J. Bonfils, and P. Carle. 1962a. Étude biologique des cicadelles de la vigne dans le sud-ouest de la France. Ann. Épiph. 18: 205-237.

Schvester, D., G. Moutous, and P. Carle. 1962b. *Scaphoideus littoralis* Ball (Homopt. Jassidae) cicadelle vectrice de la Flavescence dorée de la vigne. Rev. Zool. Agric. Appl. 61 (10-12): 118-131.

Southwood, T. R. E. 1978. Ecological methods with particular reference to the study of insect population. 2nd Edition. Chapman and Hall, London, United Kingdom.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature (Lond.) 189: 732-735.

Taylor, L. R., and I. P. Woiwood. 1980. Temporal stability as a density dependent species characteristic. J. Anim. Ecol. 49: 209–224.

Trivellone, V., J. Baumgärtner, C. Linder, C. Cara, N. Delabays, and M. Jermini. 2011. Spatio-temporal distribution of *Scaphoideus titanus* Ball in Swiss vineyards. IOBC/WPRS Meeting of the Working Group "Integrated Protection and Production in Viticulture", Lacanau, 2-5 October, 2011 [online], Available from https://colloque4.inra.fr/var/iobc_wprs_bordeaux/storage/fckeditor/file/Trivelone.pdf [accessed 10 September 2014].

Trivellone, V., M. Jermini, C. Linder, C. Cara, N. Delabays, and J. Baumgärtner. 2013. Rôle de la flore du sol sur la distribution de *Scaphoideus titanus*. Rev. Suisse Vitic. Arboric. Hortic. 45: 222-228.

Waters, E. K., M. J. Furlong, K. K. Benke, J. R. Grove, and A. J. Hamilton. 2014. Iwao's patchiness regression through the origin: biological importance and efficiency of sampling applications. Popul. Ecol. 56: 393-399.

Wilson, L. T., C. Pickel, R. C. Mount, and F. G. Zalom. 1983. Presence-absence sequential sampling for cabbage aphid and green peach aphid (Homoptera: Aphididae) on Brussels sprouts. J. Econ. Entomol. 76: 476-479.

Table 1. Analysis of variance for visual *S. titanus* counts made on five sampling dates in a reference vineyard at Camorino (Switzerland) (DF = degrees of freedom, MS = observed mean squares, F = F-value; vineyard strata, shoot type and shoot strata are considered as fixed factors, while plants are considered as random factors; **: significant at *P*<0.01)

Sampling date (mean density)		May 27 (0.954)		June 10 (0.790)		June 17 (0.536)		June 25 (0.280)		July 3 (0.170)	
Source of variability	DF	MS	F	MS	F	MS	F	MS	F	MS	F
Between plants											
Vineyard strata	4	18.48	1.44	14.79	1.92	1.72	0.94	0.65	0.77	1.02	1.21
Plants	20	12.78	1.04	7.70	0.89	1.83	0.70	0.84	1.32	0.84	1.41
Within plants											
Shoot type	1	82.23	6.69**	178.20	20.64**	15.74	6.03**	0.04	0.06	1.62	2.07
Shoot strata	1	23.48	1.91	4.61	0.53	3.13	1.20	0.13	0.21	1.46	2.44
Residual	73	12.30		8.63		2.61		0.64		0.60	

Table 2. Mean crowding to mean regression statistics for *S. titanus* nymphs inhabiting trunk and productive shoots in the in the Arbedo, Bironico and Contone vineyards (for testing overlapping distributions, the confidence interval at the α/2=0.05 probability level is calculated after Payton et al. (2003)).

Sample	Slope and confidence interval for trunk shoot inhabiting individuals	Slope and confidence interval for productive shoot inhabiting individuals	
	Stage 1 Between leaf relationship	Stage 2 Between shoot relationship	Stage 1 Between leaf relationship
Arbedo vineyard	1.35 ± 0.1153	2.75 ± 0.4453	2.05 ± 0.1797
Bironico vineyard, 12June 2013	1.49 ± 0.1319	2.31 ± 0.3133	1.79 ± 0.1471
Bironico vineyard, 1st July 2013	1.36 ± 0.2144	2.34 ± 0.3958	1.95 ± 0.19661
Contone vineyard	1.28 ± 0.0208	2.42 ± 0.7099	1.67 ± 0.2988

Table 3. Mean crowding to mean regression statistics for *S. titanus* nymphs inhabiting trunk and productive shoots in the Camorino reference vineyard (for testing overlapping distributions, the confidence interval at the α/2=0.05 probability level is calculated after Payton et al. (2003)).

Stage	Slope	Standard error (SE)	R^2	n	Confidence interval
Trunk shoots					
Plant (β_1)	1.6784	0.1735	0.9045	5	0.3702
Leaf (β_2)	1.3603	0.7895	0.6344	79	1.3040
Productive shoots					
Plant (β_1)	2.8444	0.167	0.9563	5	0.3564
Shoot (β_2)	2.1325	0.960	0.6766	123	1.5777
Leaf (β_3)	1.8686	0.490	0.7398	296	0.8012

Table 4. The applicability of the enumerative sampling plan: the hypothesized mean densities with the required number of plants to be sampled, and means and reliability levels (mean values) obtained from 1000 simulated sampling procedures vineyards (nc = sample size too small for the calculations; the predefined reliability level D_0 is reported in parentheses).

Vineyard	Hypothesized density on		Number of plants selected for		Estimated mean (1000 simulations) on		Reliability D_c (1000 simulations), mean values, on	
	trunk shoots	productive shoots	trunk shoot estimates	productive shoot estimates	trunk shoots	productive shoots	trunk shoots	productive shoots
Arbedo	0.36	0.26	77 (0.1)	69 (0.1)	0.96	0.22	0.09	0.11
			9 (0.3)	8 (0.3)	0.96	0.22	0.25	0.32
Bironico 12 June 2013	0.36	0.26	77 (0.1)	69 (0.1)	0.83	0.26	0.10	0.12
			9 (0.3)	8 (0.3)	0.83	0.26	0.30	0.34
Bironico 1st July 2013[t]	0.36	0.26	77 (0.1)	69 (0.1)	0.17	0.18	0.16	0.10
			9 (0.3)	8 (0.3)	0.17	0.18	0.43	0.28
Contone	0.36	0.26	nc (0.1)	nc (0.1)	nc	nc	nc	nc
			9 (0.3)	8 (0.3)	nc	0.18	nc	0.33

Table 5. The applicability of the sequential enumerative sampling plan: the most frequent number of plants required to be sampled for reaching or exceeding the stop line and the reliability levels (mean values) obtained from 1000 simulated sampling procedures (nc = sample size too small for the calculations).

Vineyard	Shoot type	Most frequent number of plants required to meet the stop line	Calculated reliability (D_c), mean values
Predefined reliability $D_0 = 0.1$			
Arbedo	Trunk shoots	67	0.096
	Productive shoots	73	0.102
Bironico 12 June 2013	Trunk shoots	69	0.105
	Productive shoots	70	0.117
Bironico 1st July 2013	Trunk shoots	149	0.113
	Productive shoots	78	0.087
Contone	Trunk shoots	nc	nc
	Productive shoots	nc	nc
Predefined reliability $D_0 = 0.3$			
Arbedo	Trunk shoots	8	0.246
	Productive shoots	9	0.284
Bironico 12 June 2013	Trunk shoots	8	0.296
	Productive shoots	9	0.308
Bironico 1st July 2013	Trunk shoots	18	0.305
	Productive shoots	10	0.248
Contone	Trunk shoots	nc	nc
	Productive shoots	11	0.316

Fig. 1. Mean crowding ($\overset{*}{m}$) to mean (*m*) regression statistics (density-contagiousness coefficient β with SE = standard error, and coefficient of determination R^2) for *S. titanus* nymphs inhabiting trunk shoots of grapevine plants in the Camorino reference vineyard (A and B refer to the plant and leaf level, respectively).

Fig. 2. Mean crowding ($\overset{*}{m}$) to mean (*m*) regression statistics (density-contagiousness coefficient β with SE = standard error, and coefficient of determination R^2) for *S. titanus* nymphs inhabiting productive shoots of grapevine plants in the Camorino reference vineyard (A, B and C refer to the plant, shoot and leaf level, respectively).

Fig. 3. Enumerative sampling plan with optimal sample size for *S. titanus* nymphs inhabiting trunk shoot leaves. The solid and dotted lines represent the number of plants to be sampled in relation to *S. titanus* density, at precision levels D_0=0.1 and D_0=0.3 respectively.

Fig. 4. Enumerative sampling plan with optimal sample size for *S. titanus* nymphs inhabiting productive shoot leaves. The solid and dotted lines represent the number of plants to be sampled in relation to *S. titanus* density, at precision levels D_0=0.1 and D_0=0.3 respectively.

Fig. 5. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (A: stop line for D_0=0.1; B: stop line for D_0=0.3).

Fig. 6. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting productive shoots (A: stop line for D_0=0.1; B: stop line for D_0=0.3).

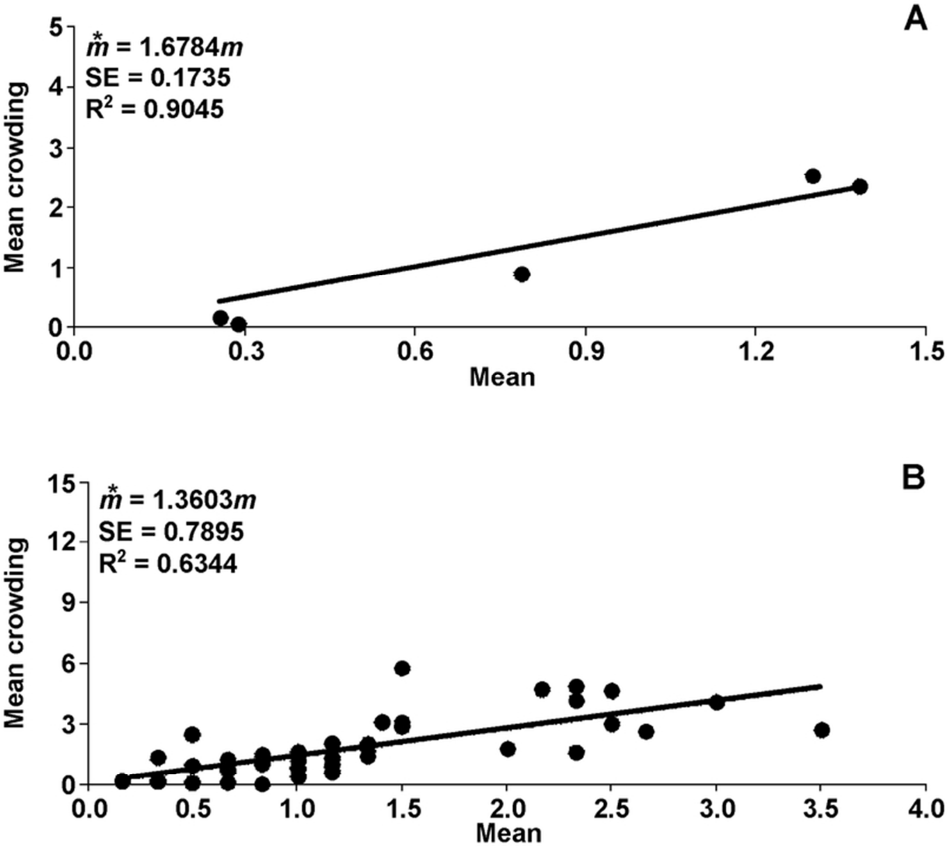

Fig. 1. Mean crowding (m^*) to mean (m) regression statistics (density-contagiousness coefficient ββwith SE = standard error, and coefficient of determination R^2) for *S. titanus* nymphs inhabiting trunk shoots of grapevine plants in the Camorino reference vineyard (A and B refer to the plant and leaf level, respectively).
71x64mm (300 x 300 DPI)

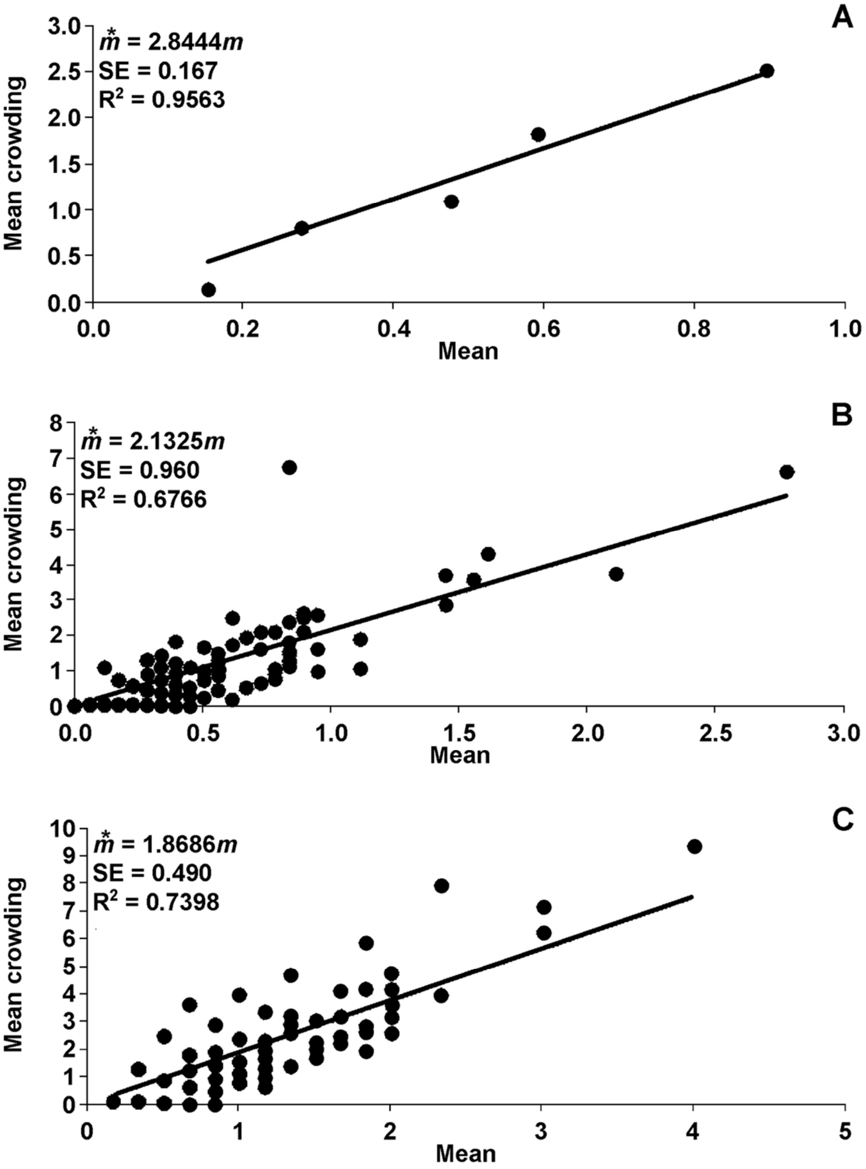

Fig. 2. Mean crowding (m^*) to mean (m) regression statistics (density-contagiousness coefficient β with SE = standard error, and coefficient of determination R^2) for *S. titanus* nymphs inhabiting productive shoots of grapevine plants in the Camorino reference vineyard (A, B and C refer to the plant, shoot and leaf level, respectively).
105x138mm (300 x 300 DPI)

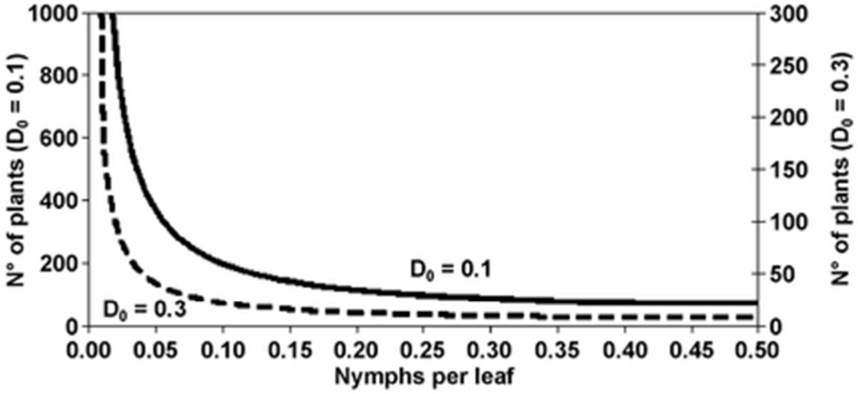

Fig. 3. Enumerative sampling plan with optimal sample size for *S. titanus* nymphs inhabiting trunk shoot leaves. The solid and dotted lines represent the number of plants to be sampled in relation to *S. titanus* density, at precision levels D_0=0.1 and D_0=0.3 respectively.
36x17mm (300 x 300 DPI)

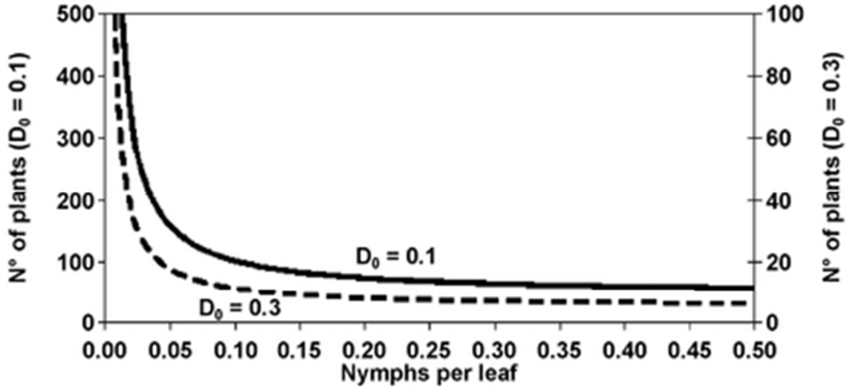

Fig. 4. Enumerative sampling plan with optimal sample size for *S. titanus* nymphs inhabiting productive shoot leaves. The solid and dotted lines represent the number of plants to be sampled in relation to *S. titanus* density, at precision levels D_0=0.1 and D_0=0.3 respectively.
36x16mm (300 x 300 DPI)

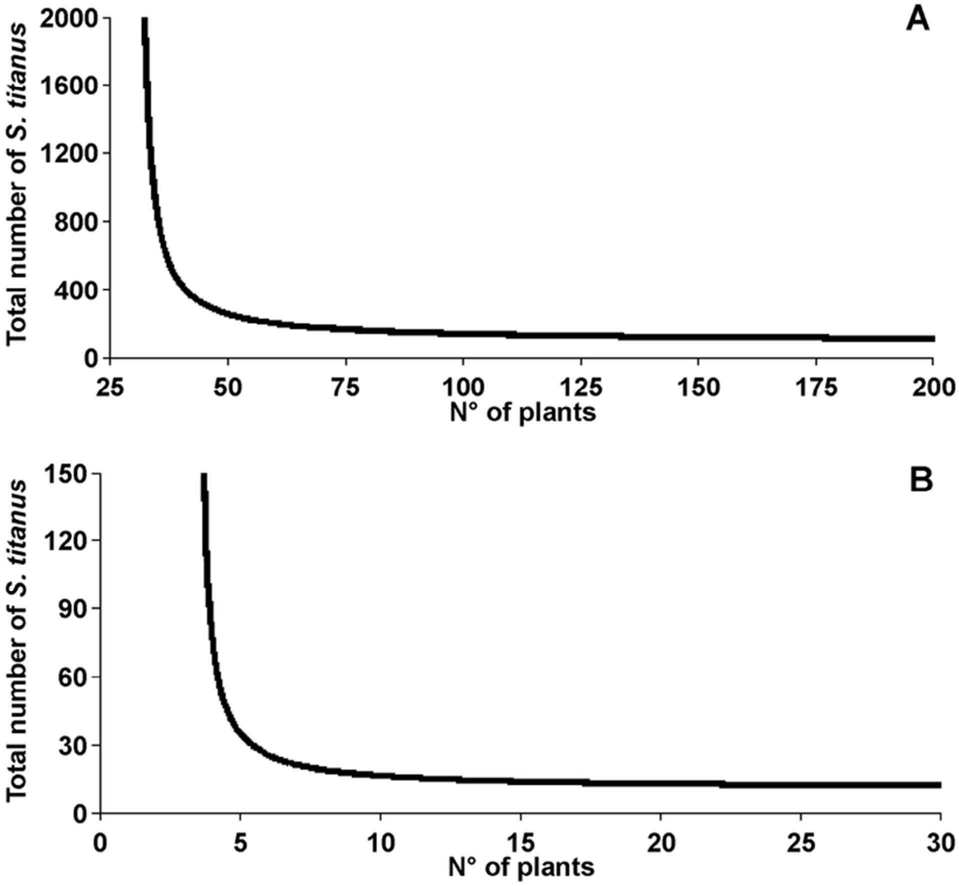

Fig. 5. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (A: stop line for D_0=0.1; B: stop line for D_0=0.3).
72x65mm (300 x 300 DPI)

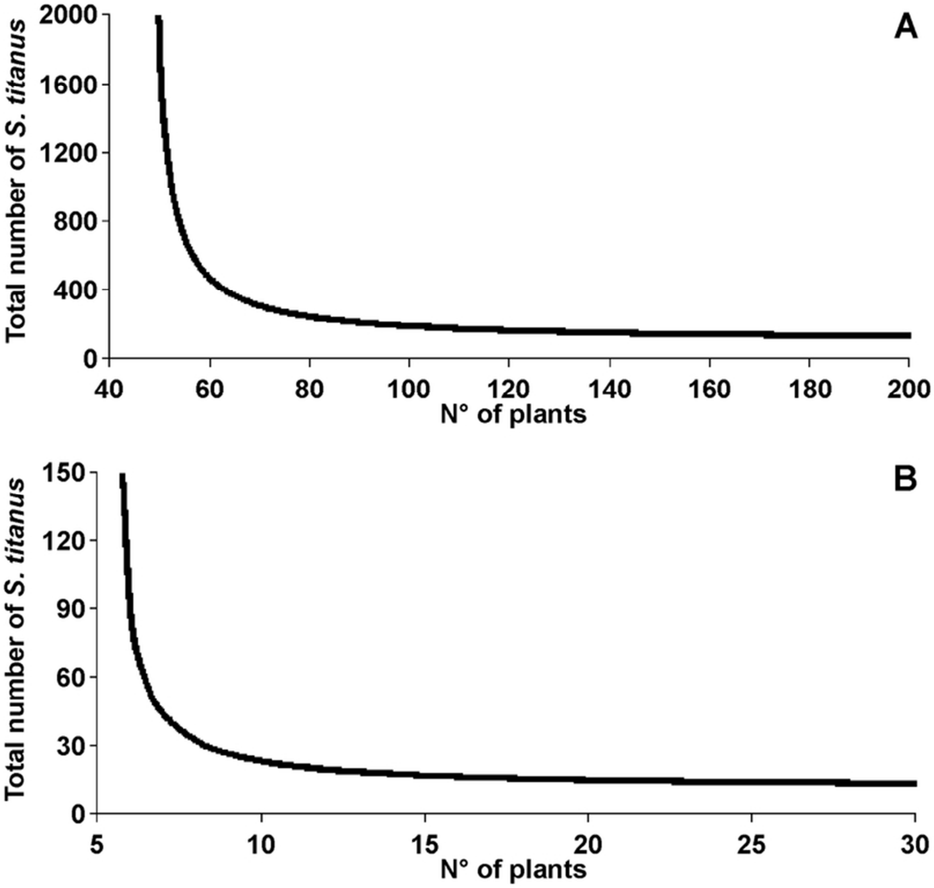

Fig. 6. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting productive shoots (A: stop line for D_0=0.1; B: stop line for D_0=0.3).
73x67mm (300 x 300 DPI)