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Conference Paper · January 2013

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Integrated Protection and Production in Viticulture
IOBC-WPRS Bulletin Vol. 85, 2013
pp. 29-35

Research and management oriented sampling plans for vine inhabiting *Scaphoideus titanus* grape leafhopper nymphs

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Abstract: This paper summarizes the methodology used for describing the spatial distribution of the grape leafhopper *Scaphoideus titanus* in a vineyard located in Southern Switzerland and presents sampling plans for research and management purposes. The sampling technique consisted of repeated visual counts of *S. titanus* nymphs. In general, a vineyard is a highly structured environment whose influence on spatial distributions is studied by analyses of variance and quantified by regression models developed in a stratified and multi-stage sampling universe. First, a regression model was applied to the relationship between the proportion of infested trunk shoot leaves and the mean density of trunk shoot leaves. This allowed the translation of a critical density for entering the vineyard in an adaptive management (AM) program into a critical proportion. A sequential binomial sampling plan was developed to efficiently decide whether a vineyard should be included into the AM program or not. Second, the spatial distribution in the entire vine plant canopy was analysed. Since there were significant differences between densities on trunk shoots and productive shoots, two different sampling plans were designed. However, there were no significant differences between other strata (parts of the vineyard, leaf position within shoots), so that they were disregarded in sampling plan design. The mean crowding – mean regression model, with the intercept set to 0, indicated aggregated distributions at the vineyard, shoot type and shoot levels. On the basis of this statistics, enumerative and sequential sampling plans are proposed and implemented in the AM framework. The here presented sampling techniques are advantageous over the previously used beating tray method and the sampling plans are useful for research and management purposes.

Key words: Adaptive Management, trunk shoots, productive shoots, enumerative and sequential sampling plans, sequential sampling plan, enumerative sampling plan

Introduction

The leafhopper *Scaphoideus titanus* Ball is of North American origin and has accidentally been introduced into France from where it invaded Southwestern Europe. Currently it further expands its area of distribution as documented by reports from different European countries. The economic importance of *S. titanus* stems from the transmission of the *Candidatus* Phytoplasma vitis, a Phytoplasma of the Elm Yellows or 16Sr-V group, causing the Flavescence dorée (FD) disease. Both the disease, which is a quarantine organism, and the vector are generally subjected to mandatory control.

Knowledge on occurrence and densities of *S. titanus* life stages is important for timing control operations, for implementing monitoring schemes in supervised pest control, and finally, for implementing an Integrated Pest Management system (IPM). In the case of *S. titanus*, these activities are integrated into an Adaptive Management (AM) strategy explained by Jermini *et al.* (2013). An effective implementation of an AM strategy requires the availability of cost-efficient sampling techniques and adequate sampling plans.

Currently, the beating tray technique is often used to record the presence of nymphal instars. This technique provides a good insight into the population age structure and, hence, was satisfactory for monitoring phenological events in supervised *S. titanus* control and for the parameterization as well as the validation of a phenology model (Rigamonti *et al*., 2011). However, the technique is time consuming and proved to be inefficient for timing insecticide applications, particularly at low nymph densities. Moreover, the technique provides unreliable information of population densities and hence is of limited use in population dynamic studies and population management efforts.

To overcome these limitations, visual counts of nymphs on vine plant leaves were made in 2009 in a vineyard at Camorino, Southern Switzerland. The vineyard is a highly structured environment whose influence on the pest's spatial distributions should be studied before designing sampling plans.

The scope of this paper is to summarize the methodology used for describing the spatial distribution of *S. titanus* and to present sampling plans for research and management purposes. However, the presentation of the details of the statistical analysis and the parameter estimation procedures for the spatial distribution models goes beyond the scope of this paper and will be published elsewhere.

Material and methods

Introduction

Estimation of population parameters is indispensable for ecological studies and pest management activities. In general, an adequate knowledge on the spatial distribution of the population is required for the development of sampling plans that aim at obtaining estimates at predefined levels of reliability. The study of spatial distributions and the design of sampling plans is a challenging task in highly structured environments such as a vineyard.

LeRoux & Reimer (1959) used analyses of variance to study the spatial distribution of insect populations in an apple orchard that is also a highly structured environment. In a series of papers, Iwao (1968), Iwao and Kuno (1971) and Kuno (1976) developed regression models relating Lloyd's (1964) mean crowding to the sample means as a comprehensive method to represent the spatial distribution of populations in structured environments. Both the analysis of variance and the regression models are used in this study to describe the spatial distribution of *S. titanus* as a basis for the design of sampling plans.

In this study, the reliability of the estimates is defined on the basis of formal probabilistic statements ($P = 0.05$) and the ratio of the standard error to the mean ($D_0 = 0.1, 0.3$) (Karandinos, 1976).

Simple random sampling on trunk shoot leaves

The relationship between the proportion p of infested suckers and the mean density m of nymphs on trunk shoot leaves is represented by Nachman's function (1984). This relationship allows the translation of a density m into a proportion p that can be estimated via sequential binomial sampling procedure. The sampling data are considered satisfactory for entering into the modeling process in AM, if m exceeds a critical density m_m that can be translated into a proportion p_m of infested leaves. In the ongoing AM project the simple relationship of Wilson *et al.* (1993) and Knapp *et al.* (2006) is considered as satisfactory. A sequential binomial sampling plan for identifying data sets with sufficient nymphal densities is described.

Description of the spatial distribution

For research purposes in particular, sampling procedures can be improved and additional insight into the distribution can be obtained by explicitly taking into account the spatial structure of the vineyard by applying stratification and multistage sampling procedures. Stratification allows the taking into account of heterogeneities in the sampling universe, while multistage procedures render sampling efficient by sub-sampling units arranged on stages. Analyses of variance allow the evaluation of strata at the levels of the vineyard (different parts of the vineyard), the shoot type (trunk shoots, productive shoots) and the leaf position within shoots (close to or distant from the trunk) and of differences between sampling units (plants, shoots, leaves) as sources of variability.

The method of Kuno (1976) is applied separately to counts on leaves of trunk shoots and leaves of productive shoots. In this analysis, counts from trunk shoot leaves are treated as a random sample taken among all trunk shoot leaves. Hence, a two-stage random sampling plan for trunk shoot populations is developed together with a three-stage sampling plan for productive shoot populations.

In the two-stage random sampling plan the plants are the Primary Sampling Unit (PSU) and leaves are the Secondary Sampling Unit (SSU). In the three-stage random sampling plan the plants are the PSU, the productive shoots are the SSU (three shoots per each plant) and the leaves are the Tertiary Sampling Unit (TSU) (six leaves per each shoot).

The mean crowding-mean relationship was used to describe the spatial distribution of *S. titanus* (Lloyd, 1967). Iwao (1968) and Iwao & Kuno (1971) showed that a simple linear regression is appropriate for describing the dependency of mean crowding on the mean. The intercept α and the slope β of the model are measures for the basic population unit and the aggregation of population units, respectively. In specific terms, mean crowding on mean regression models are developed for two relationships in the two-stage sampling and three relationships in the three stage sampling procedure. This is done here on the basis of two important assumptions: *i*) the intercepts α are equal to 0, and *ii*) the total number K and Q, respectively, of SSU's and TSU's comprising a PSU and a SSU are so large that the ratios k/K and q/Q are negligibly small (k and q = sampled units). The slopes $\beta_1$and β_2 for two-stage sampling and β_1 β_2 and β_3 for three-stage sampling are estimated with the Camorino field data. On these bases, an enumerative fixed sample size sampling plan and a sequential enumerative sampling plan are proposed.

Enumerative sampling plans for research purposes

The phenology and population dynamics of *S. titanus* is under detailed study in Swiss vineyards (Rigamonti *et al.*, 2011; Trivellone *et al.,* 2013). Reliable density estimates for grapevine inhabiting *S. titanus* individuals are required for research on the within- and between vineyard dynamics.

Kuno (1976) gives the optimum sample size for an enumerative sampling plan i.e. the number of PSU (plants) to be taken for attaining a predefined reliability level in terms of the ratio of the standard error to the mean (D_0) for the two-stage and the three-stage sampling plans. The enumerative fixed sample size sampling plan is constructed for $D_0 = 0.1$ and $D_0 = 0.3$.

Kuno (1976) also gives the stop line for an enumerative sequential sampling plan i.e. the cumulative number of individuals satisfying the reliability criteria in terms of the ratio of the standard error to the mean (D_0) for the two-stage sampling on trunk shoots and the three-stage sampling on productive shoots. The enumerative sequential sample size sampling plan is constructed for $D_0 = 0.1$ and $D_0 = 0.3$.

Results and discussion

Simple random sampling on trunk shoot leaves

The parameters estimate of the Nachman's (1984) equation for infested trunk shoot leaves and the mean density of *Scaphoideus titanus* nymphs on trunk shoot leaves are $a = 1.0325$, $b = 1.0251$. The critical mean density $m_m = 0.25$ translates into a critical proportion $p_m = 0.22$.

Figure 1 depicts the stop lines and the threshold proportion for the sequential binomial sampling plan used to estimate infestation levels of trunk shoot leaves infesting nymphs at the beginning of the infestation period. The user of this sampling plan starts with an initial sample composed of 12 leaves and checks additional leaves until the number of infested leaves reaches one of the two stop lines. If it reaches the lower stop line, the vineyard is dismissed from the pest management program. If the number reaches the upper stop line, the vineyard is subjected to supervised pest control. Note that this sampling plan is applicable to vineyards in which the FD disease is absent. Please also note that the critical density and proportion of infested leaves are not a damage or treatment thresholds. The sequential binomial sampling enables a cost-efficient decision on whether a vineyard is entered into the AM program or not (Prevostini *et al*., 2013; Jermini *et al*., 2013).

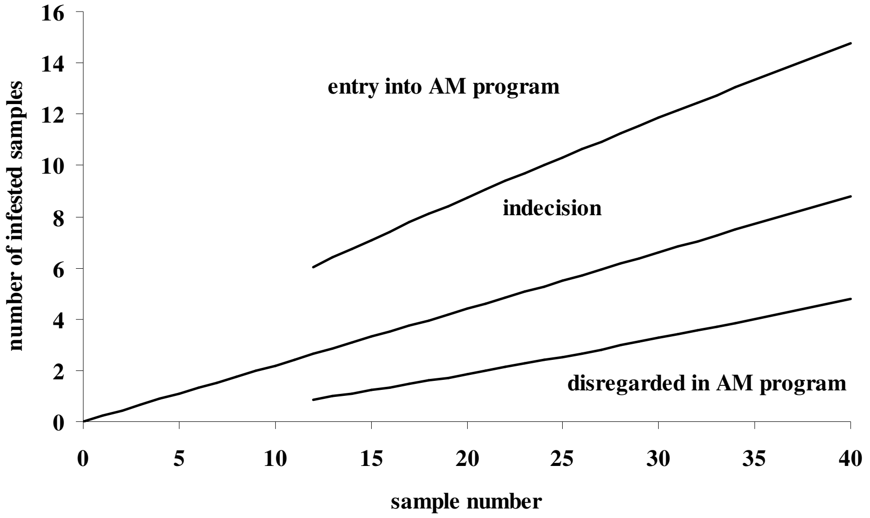

Figure 1. Stop lines in the sequential binomial sampling plan for identifying vineyards with sufficient *S. titanus* occurrences for insertion into the adaptive management (AM) program of Prevostini *et al*. (2013) and Jermini *et al*. (2013).

Description of the spatial distribution

The analysis of spatial distributions conducted in a vineyard at Camorino, Southern Switzerland, showed that first instar nymphs accumulate on trunk shoot leaves. To take into account this difference separate sampling plans are proposed for trunk shoot leaves and for productive shoot leaves. However, there were no significant differences between parts of the vineyard and between the leaf position within shoots. Hence, the stratification of the sampling universe is not necessary in the two sampling programs.

Enumerative sampling plans for research purposes

Figure 2 shows the curves representing the Optimal Sample Size (OSS) for a fixed enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (Figure 2a) and productive shoots (Figure 2b). The OSS is a function of the density of the insect in the vineyard. Please note that to achieve a precision level $D_0 = 0.1$ is necessary to sample a very high number of plants, so this precision level is useful only for research activities. The precision level $D_0 = 0.3$ requires a very lower workload and produces adequate information for pest management activities, so it is suited for routine monitoring purposes.

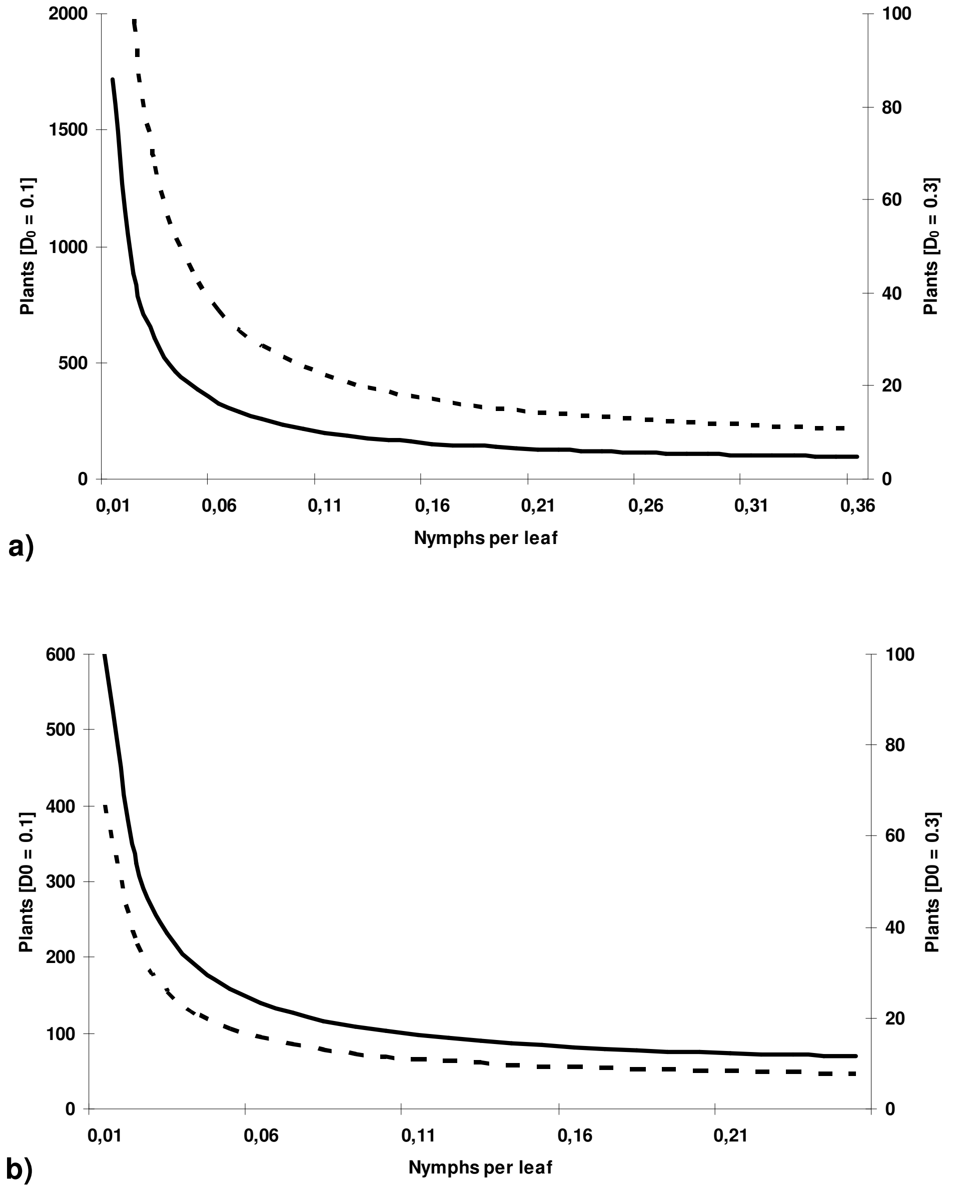

Figure 2. Fixed enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (a) and productive shoots (b). Solid line $D_0 = 0.1$, dotted line $D_0 = 0.3$.

Figure 3 shows the curves representing the Stop Line for a sequential enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (Figure 3a, 3b) and productive shoots (Figure 3c, 3d). The sequential sampling ends when the cumulative number of observed *S. titanus* nymphs reaches the Stop Line, thus allowing ascertaining the density of the pest in the field.

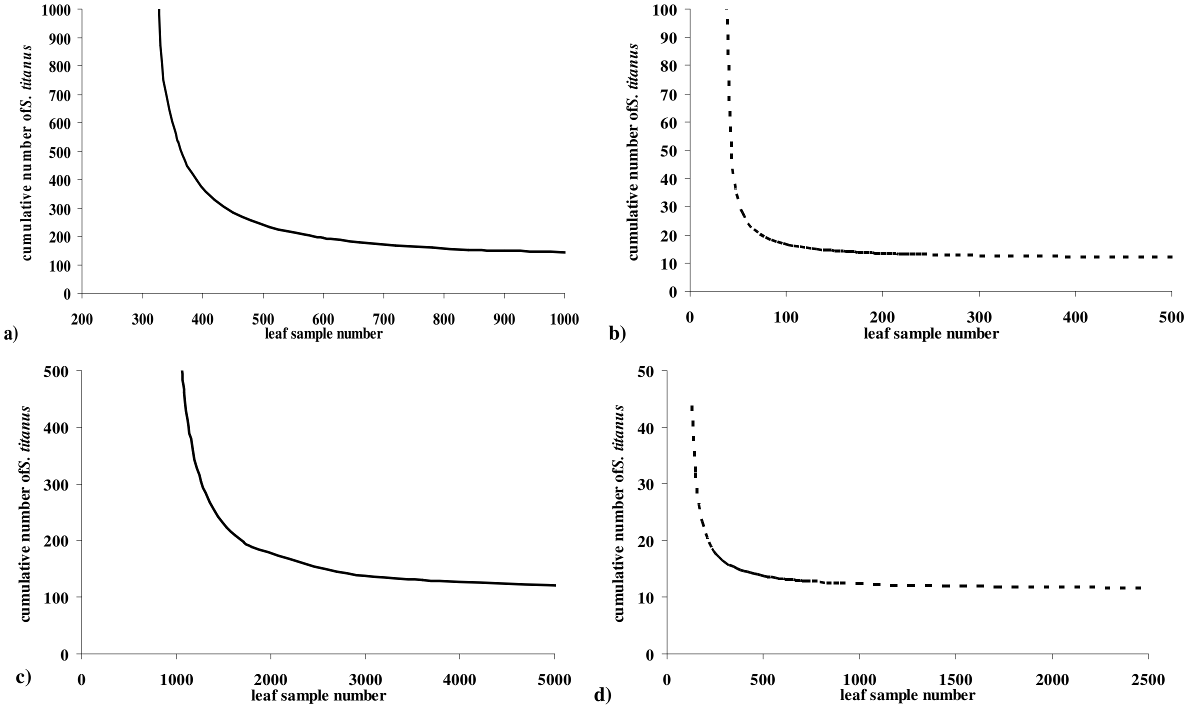

Figure 3. Sequential enumerative sampling plan for *S. titanus* nymphs inhabiting trunk shoots (a, b) and productive shoots (c, d) for two reliability levels defined for P = 0.05 and the ratio of the standard error to the mean (Solid line $D_0 = 0.1$, dotted line $D_0 = 0.3$).

The here proposed sampling plans allow the estimating of pest occurrences with respect to thresholds, that are often of the utmost importance in IPM programs. They have been developed for vineyards without FD presence. However, the knowledge on spatial distributions may be useful for managing the vector-FD disease system in FD infested vineyards. For example this knowledge could facilitate the development and selection of management options different from insecticide treatments.

In the here applied AM framework, sampling data are continuously used to improve parameter estimates for enhancing explanatory and predictive capabilities of models. AM serves for the purpose of improving the methodology, for obtaining better insight into the *S. titanus* infestation patterns, and for rationalizing management decisions (Prevostini *et al.*, 2013; Jermini *et al.*, 2013). Within the AM framework, the here presented sampling techniques are advantageous over the previously used beating tray method and the sampling plans are useful for research and management purposes.

The visual count technique used here is useful for recording grapevine plant inhabiting nymphs and for deciding on management operations in relation to infestation patterns. However, Trivellone *et al.* (2013) observed that a substantial part of the population inhabits the herbaceous vegetation of the vineyard floor. Hence, a study on population dynamics has to take into account both the vine plant canopy and the vineyard floor vegetation. For this purpose, the visual sampling technique should be complemented with suction sampling (Trivellone *et al*., 2013).

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