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Sample size for monitoring sirex populations and their natural enemies

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Abstract - The woodwasp *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) was introduced in Brazil in 1988 and became the main pest in pine plantations. It spread to about 1.000.000 ha, at different population levels, in the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo and Minas Gerais. Wasp population control is done mainly by a nematode, *Deladenus siricidicola* Bedding (Nematoda: Neothylenchidae). Evaluation of natural enemies' efficiency is difficult because there are no appropriate sampling systems. This study tested a hierarchical sampling system to define sample size to monitor *S. noctilio* populations and therefore the efficiency of their natural enemies, which was found to be adequate.

Tamanho da amostra para monitorar a população de sirex e de seus inimigos naturais

Resumo - A vespa-da-madeira, *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) foi introduzida no Brasil em 1988 e tornou-se a principal praga dos plantios de pínus. Encontra-se distribuída em aproximadamente 1.000.000 de ha em diferentes níveis populacionais nos Estados do Rio Grande do Sul, Santa Catarina, Paraná, São Paulo e Minas Gerais. O controle da população da vespa-da-madeira é feito principalmente pela utilização do nematoide *Deladenus siricidicola* Bedding (Nematoda: Neothylenchidae). A avaliação da eficiência dos inimigos naturais é dificultada por não haver um sistema de amostragem apropriado. Este estudo testou o sistema de amostragem hierárquica para definir o tamanho da amostra para monitorar a população de *S. noctilio* e também a eficiência dos inimigos naturais, a qual mostrou-se adequada.

Introduction

Sirex noctilio Fabricius, the woodwasp, is native from Europe, Asia and North Africa and was first introduced in Brazil in 1988. It is estimate in up to US$ 42 million annually the potential losses due to this pest if no control measures are implemented soon after its detection, as recommended by National Woodwasp Control Programme (PNCVM).

The insect may develop inside trunks of several pine species. The most susceptible plants to be attacked are those under stress, for example, without thinning (Neumann et al., 1987). During oviposition females also deposit phytotoxic mucus and spores of a symbiotic fungus, *Amylostereum areolatum* (Chaillet ex Fr.), which is a source of nutrients for larvae. The fungus, along with the mucus causes physiological changes in the tree, causing its death (Coutts, 1969).

Besides forest management, biological control using parasites and the nematode *Deladenus siricidicola* Bedding is the most appropriate and efficient method to control this pest. Bedding & Akhurst (1974) reported that 70% of sirex females that emerged from trees inoculated with nematodes were parasitized. Zondag (1979) reported the occurrence of parasitism levels close to 90% in the forests of North Island, New Zealand. In Brazil, the nematode has been very effective in controlling the woodwasp, showing percentages close to 100% in many places. The parasitoid *Ibalia leucospoides* (Hochenwarth, 1785), naturally introduced with its host, has remained at average parasitism levels of 25%. The parasitoids *Megarhyssa nortoni* (Cresson, 1864) and

Rhyssa persuasoria (Linnaeus 1758) was introduced in Brazil between 1996 and 1998, and in 2003 from Australia (Iede et al., 2012), but only *M. nortoni* had its establishment confirmed in 2015.

The evaluation of the efficiency of natural enemies is important to determine the establishment, distribution and population levels of biological control agents, and the results should be used for planning control activities (Haugen, 1990). Conservation Forests & Lands (1989) recommended an annual assessment of effectiveness of nematode parasitism to check the efficiency of application techniques and its dissemination. However, the evaluation of efficiency has been hampered by the lack of an adequate sample system. There are few related works about this theme and there is a variation regarding the number, size and position of samples.

Haugen et al. (1990) recommended that in stands where less than 10.000 trees have been inoculated with the nematode, at least 5% of the inoculated trees and 25 no inoculated trees should be sampled and evaluated regarding the emerged *S. noctilio* adults. To assess the percentage of parasitism by the nematode Haugen & Underdown (1993) sampled 55 attacked trees, removing two logs (3 m long from the middle third of each tree), to evaluate emerged *S. noctilio* adults. Sirex Alert (2013) recommended that for every 20 inoculated trees, one should be collected and cut into billets of 0.9 m in length to evaluate emerged adults of the insect. Silva (1995) and Fenili et al. (2000) used samples of 0.80 m, collected in the middle third of five plants, to evaluate the parasitism of *D. siricidicola* and *I. leucospoides* by quantification and evaluation of emerged *S. noctilio* and *I. leucospoides* adults. Eager et al. (2011), studying the within-tree distributions of *S. noctilio* on *Pinus resinosa* and *P. sylvestris*, observed that a 2-bolt sampling method was statistically reliable and could be used to accurately extrapolate to whole-tree densities.

According to National Sirex Co-Ordination Committee (1991), the effectiveness of inoculation techniques can be obtained by comparing attacked trees considering the number of inoculated and non-inoculated trees. Neumann et al. (1987) recommend the evaluation of parasitism levels to determine their adaptation to different climatic conditions and whether new releases are required. Penteado et al. (2000), analyzing the distribution of woodwasp population along the trunk of *P. taeda*, found that the middle third and the lower portion of the upper third of the trunk are the most representative parts to be sampled.

Penteado et al. (2008) used sequential sampling to define the nematode efficiency and recommended the evaluation of a minimum of 68 insects per sampled trees and a maximum of 272.

According to Waters (1955), monitoring is the first step to minimize losses caused by insects in forest plantations. Different types of samples can be used, from a simple random selection of units to highly complex samples, involving multiple stages of selection with stratification of units from different groups (Ferber et al., 1980). However the primary factor in selecting a sampling method is that all individuals in the population have the same probability of being included in the sample. The sample method does not have to be exact but needs to be reliable. In many situations, the tolerable error depends on the expected result.

When a sample method is used to compare the efficiency of natural enemies of pests in different areas, it should be able to determine significant differences between populations and it should be easy to use, because sampling trees can consume too much time (Day et al., 1993).

In general, when more samples that are taken more precise the population estimates will be. However, time and costs are always constraining factors. Thus, the usual approach is to decide on the lowest number of samples that can be taken to achieve a reasonable population estimate within the established error limits (Leather, 2005).

Among the different sampling methods, Lima (1979) referred to a type of sampling called hierarchical sampling. According to this author, in hierarchical classification the population is divided into primary branches, and each primary branch divided into secondary branches, and so on until the final categories of the population are reached.

Thus, the objective of this study was to define an appropriate sampling methodology for monitoring *S. noctilio* population and the efficiency of its natural enemies in pine plantations, considering the operational facilities to carry on the activity.

Material and methods

The material used in this study was collected in a *Pinus taeda* plantation located in Encruzilhada do Sul, Rio Grande do Sul State and Lages, Santa Catarina State. At each place, it were selected at random five attacked trees inoculated with nematode *Deladenus siricidicola* according to the technique described by Penteado et al. (2015). Five months after inoculation, each tree was sectioned in small logs (0.50 m) and transferred to the laboratory. Each log was identified with location, tree number, log number and position in the tree (lower, middle and upper strata) and packed individually in cages. When the adults of *S. noctilio* began to emerge, all insects were collected, counted and dissected under a stereomicroscope with an increase of 40 times to evaluate the presence of nematodes in its reproductive system and calculate the percentage of parasitism. All adults of *Ibalia leucospoides* were also collected and counted to determine the percentage of parasitism.

As the trees had different heights, with average of 10.8 m and 14.6 m, from Encruzilhada do Sul and Lages, respectively, the first step was to perform ANOVA to standardize the number of logs by tree. Then, it was established that each tree would be represented by 21 logs. The logs were grouped into three equal portions (lower, middle and upper). For trees with more logs, a raffle for elimination of some logs was carried out, and for those containing less than 21 logs, the missing values were estimated by the average of their position (lower, middle and upper). ANOVA was based on the mixed model of hierarchical classification, in three stages, according to the methodology described by Snedecor & Cochran (1978) and Lima (1979). The model was defined by:

$$y_{ijk} = \mu + a_i + p_{ij} + t_{ijk}$$

where: y_{ijk} = observation of log k in position j of the tree i; μ = mean of population; a_i = effect of tree i (i = 1,2,3 ,.... 5); p_{ij} = effect of position j (j = lower, middle, upper) of tree i; and t_{ijk} = effect of log k (k = 1,2,3 ,...., 7) in position j of the tree i.

The components a_i and t_{ijk} were considered random, with mean and covariances equal to zero and variances equal to σ_a^2 and σ_t^2, respectively. The components *m* and *pij* were fixed effects.

For deduction of mathematical expectations, the following hypothesis was admitted:

$$\sum_i p_{ij} = \sum_j p_{ij} = \sum_i \sum_j p_{ij} = 0$$

The sample mean was defined as:

$$\bar{y} = \mu + \frac{1}{n_a}\sum_i a_i + \frac{1}{n_a n_p n_t}\sum_i \sum_j \sum_k t_{ijk}$$

And its variance:

$$\sigma_{\bar{y}}^2 = \frac{1}{n_a}\sigma_a^2 + \frac{1}{n_a n_p n_t}\sigma_t^2$$

Estimates of variance components, referring to the variation between trees s_a^2 and between logs s_t^2, were obtained by:

$$s_a^2 = \left(\frac{MS_a - MS_t}{n_p n_t}\right)$$

and

$$s_a^2 = MS_t$$

Where MS_a and MS_t are means squares of tree and log in position effects.

The estimated variance of the sample mean ($s_{\bar{y}}^2$) was obtained by the expression:

$$S_y^2 = \frac{1}{n_a}S_a^2 + \frac{1}{n_a n_p n_t}S_t^2$$

The coefficient of variation was calculated by using the following expression:

The data were transformed by $\sqrt{x + 0.5}$ and then analyzed according to the proposed model.

Results and discussion

Based on the proposed model, a double entry table was developed (Table 1), with coefficients of variation (CV) considering a variable number of trees (1 to 5) on lines, a variable number of logs (1 to 7) in columns, and fixed the position of the trunk, using the middle third of the plant, as recommended by Penteado et al. (2000).

Table 1. Coefficients of variation (%) related to the number of trees and logs of *Pinus taeda* attacked by *Sirex noctilio*.

Number of trees	Number of logs						
	1	2	3	4	5	6	7
1	43.17	35.85	33.04	31.55	30.62	29.98	29.52
2	30.53	25.35	23.36	22.31	21.65	21.20	20.87
3	24.94	20.71	19.09	18.23	17.69	17.33	17.06
4	21.59	17.92	16.52	15.77	15.31	14.99	14.76
5	19.34	16.06	14.81	14.15	13.73	13.45	13.24

Using table 1 it is possible to define the sample size, taking into account the operational facilities, the CV and to determine the number of trees to be sampled and the number of logs per tree to be collected. It is recommended to collect one sample per plot up to 50 ha to ensure the accuracy of the method. When the number of sampled trees or logs increases, the variation tends to become smaller and the results are more accurate.

It is possible to choose between a higher number of trees or logs, according to the operational facilities. Haugen et al. (1990) recommend a much larger number of logs and trees to be evaluated. Eager et al. (2011) refers to 2-bolt sampling method for estimating sirex population that was statistically reliable and could be used to accurately extrapolate to whole-tree densities.

Regarding the size of logs, it was found that for the purpose of this study, the use of 0.50 m logs was appropriate, since trees remained on the field until about 15 days before the emergence of adults. However, when the storage period exceeds 30 days, the use of logs of 0.80 m is recommended to prevent rapid moisture loss. Eager et al. (2011) recommend small logs of 0.50 m. Neumann et al. (1987) suggested the use of logs with 1.0 m, which seemed more appropriate to avoid losing moisture quickly. Haugen & Underdown (1993) used logs with 3.0 m; however, this size hampers storage. Silva (1995) and Fenili et al. (2000) used samples of 0.80 m.

To evaluate the parasitism by *I. leucospoides,* the same samples collected for nematode can be used or it can be collected a new sample (defined in Table 1) for each 50 ha of forest.

The logs should be stored in cages and all insects that emerge should be collected (*S. noctilio* and *I. leucospoides*). *Sirex* and *Ibalia* adults should be counted and the adults of parasitoid should be released back into the attacked pine plantation.

It is also important to evaluate the level of natural parasitism by nematode in trees that were not inoculated to check the establishment of nematode in the area. The samples can be collected during inoculation activities or later, when it will be necessary to fell attacked trees to collect logs. As the insects usually begin to emerge in late October, samples should be collected before that.

An important step of this process is the selection of trees to collect samples. In areas where nematode is already established, the number of insects per tree can go down and it is recommended during the samples selection to choose trees with a large number of resin droplets in order to ensure the presence of a sufficient number of insects per sample and accuracy in results. Penteado et al. (2008) recommend evaluation of a minimum of 68 insects per sample, what is considered necesssary to ensure reliable results.

Conclusions

Hierarchical sampling is adequate for definition of a sampling procedure to monitor woodwasp population and its natural enemies.

The coefficient of variation table allows the definition of more appropriate sample size to different situations, considering the accuracy of results, storage of samples and cost of operation.

The assessment should be carried out annually to set up the proper actions for biological control agents and to determine parasitism levels.

The sampling results are useful to evaluate nematodes inoculation process and it is also important for woodwasp controlling activities planning.

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