



ALEXANDRE DOS SANTOS

**MORTALITY ESTIMATION OF SEEDLINGS AND
SAMPLING SUBTERRANEAN TERMITES IN
EUCALYPTUS PLANTATIONS**

LAVRAS - MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia, área de concentração em Entomologia, para a obtenção do título de Doutor.

Orientador
Dr. Ronald Zanetti

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2012**

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“O sábio não é o homem que dá as respostas certas e sim aquele que faz as perguntas certas.”

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RESUMO

Cupins subterrâneos são pragas em todas as fases de desenvolvimento em florestas de *Eucalyptus* no Brasil. A adoção de programas de manejo integrado de pragas (MIP) pode evitar perdas por esses cupins e reduzir a quantidade de inseticidas com benefícios econômicos e ambientais. No entanto, a implementação destes programas depende da quantificação dos danos e do conhecimento da distribuição espacial destes organismos. Este trabalho foi desenvolvido com duas espécies de cupins de importância econômica, *Syntermes molestus* que danifica mudas de eucalipto e *Heterotermes tenuis* que danifica a casca e o tronco de árvores adultas. Este trabalho foi desenvolvido em sete fazendas em região originalmente de Mata Atlântica em Minas Gerais, Brasil. No primeiro trabalho, a mortalidade de mudas de eucalipto foi correlacionada com orifícios de forrageamento de *S. molestus*, durante seis meses após o plantio, com o modelo probabilístico beta-binomial inflacionado com zeros. No segundo trabalho, a distribuição espacial conjunta de *S. molestus* e *H. tenuis* foi estudada, por meio da determinação de assembleias entre as duas espécies, para os fatores altitude e vegetação, com o método de classificação e análise espacial (CASA). O melhor modelo ajustado desconsiderou a inflação por zeros e apesar da incerteza apresentada, o modelo probabilístico beta-binomial permite prever o aumento de mortes de mudas em relação ao aumento do número de orifícios de forrageamento de *S. molestus*. O método CASA revelou cinco assembleias entre *S. molestus* e *H. tenuis*, que foram mapeadas e comparadas aos diferentes fatores analisados. Essa informação biológica permitiu a proposição de um plano de amostragem para esses cupins.

Palavras-chave: *Eucalyptus*. Proteção florestal. Distribuição espacial. Cupins-praga.

ABSTRACT

Subterranean termites are pests of all stages of the plant development in cultivated *Eucalyptus* in Brazil. Programs of integrated pest management (IPM) aims to avoid losses by termites and reduction on insecticides use, resulting in economic and environmental benefits. These programs require the quantification of damage and knowledge on the spatial distribution of these organisms. This study was conducted with two species of termites, both of economic importance, *Syntermes molestus* which damage seedling eucalyptus stage and *Heterotermes tenuis* which damage bark and trunk of mature trees. This study was conducted in seven commercial farms in a region originally of Mata Atlântica in Minas Gerais State, Brazil. In the first study, eucalyptus mortality was correlated with foraging holes of *S. molestus*, during six months after implantation, with zero inflated beta-binomial probability model. In the second work, spatial distribution of *S. molestus* and *H. tenuis* was studied, through the determination of assemblages between the two species related to altitude and vegetation factors, using the method of classification and spatial analysis (CASA). The best fitting model ignores the zero-inflation and despite the model uncertainty, the probabilistic beta-binomial model approach allows to predict the increase of seedlings deaths versus the increase of *S. molestus* foraging holes. The CASA method reveals five termites assemblages between *S. molestus* and *H. tenuis*, which was mapped and compared to the factors. This biological information allowed to propose a sampling plan for these termites.

Keywords: *Eucalyptus*. Forest protection. Spatial distribution. Termites pest.

1 INTRODUCTION

Exotic fast-growing species are planted in tropical and subtropical regions to reduce the impact of agro-industrial exploitation on native forests. Trees of the genus *Eucalyptus* are the most common, with more than 20 million hectares, of which, 53% in Brazil, China and India (IGLESIAS-TRABADO et al., 2009). Where these plantations were introduced, species of subterranean termites become severe pests (COWIE; LOGAN; WOOD, 1989).

Syntermes is the most important genus of termite pests of eucalyptus, due to ring-barking and root debarking, resulting in high seedling mortality in the first months after planting (WILCKEN; RAETANO; FORTI, 2002). *Heterotermes tenuis* is of secondary importance causing injuries in mature trees (DIETRICH, 1989) and seedlings (JUNQUEIRA, 1999), they may reduce wood production and may favour the trees to pathogen occurrence (RAETANO; WILCKEN; CROCOMO, 1997).

Termite is controlled in Brazil by immersion of seedlings in insecticides with long residual effects (fipronil, imidacloprid and thiametoxan) before planting (WILCKEN; RAETANO; FORTI, 2002). This systematic use of insecticides can have negative impacts on human populations and the environment. Additionally, it is contrary to current demands for the reduction and/or elimination of persistent chemicals substances (UNITED NATIONS ENVIRONMENT PROGRAMME, 2000).

Programs of integrated pest management (IPM) aims to avoid losses by termites and to reduce the insecticides use. These programs depends on the establishment of economic injury levels (EIL) and requires accurate sampling plans.

The relationship between the density of insect pests, the crop response to injury and the economic damages results in a bio-economic criteria denominated economic injury level and on which is based the choice of control tactics (PEDIGO; HIGLEY, 1996). The first step to satisfy the requisites of this criterion is the accurate representation of the relationship between insect density and injury. However, this correlation between eucalyptus seedlings and *Syntermes* is complex because, its subterranean nesting behaviour does not allow to quantify the number of nests. Additionally, this termite is a social organism, with density over 30,000 termites par nest (LEPAGE; DARLINGTON, 2000), this does not allow to establish the injury contribution per individual. In comparison with the injury of other insects which reduce vegetative material production or growth, *Syntermes* attack results in death of eucalyptus seedling.

The development of a sampling plans requires survey and the knowledge of spatial distribution of insects species (TAYLOR, 1984). *H. tenuis*, despite subterranean behaviour, can be easily collected with corrugated cardboard bait buried in the soil (ALMEIDA; ALVES, 1995), but *Syntermes* is not attracted (BEZERRA JÚNIOR; WILCKEN, 1998a). Foraging holes on the soil surface is the only indication of *Syntermes* presence (MARTIUS, 1998), but they are more difficult to be observed in the vegetation. The associations between the two species would be valuable for the pest management, since sampling *H. tenuis* with attractive baits demands less time and cost, compared to sampling foraging holes of *S. molestus*.

2 OBJECTIVES

The objectives were to estimate the mortality of eucalyptus seedlings by *S. molestus* and to explore joint spatial distribution of this termite with *H. tenuis* as function of altitude and vegetation, in eucalyptus plantations.

The present work is to elaborate a guide for risk evaluation in *Eucalyptus* plantation with these pest termites. But there are difficulties to be overtaken:

- difficulties of counting subterranean termites;
- the variable and often non-poisson spatial distribution of termite population;
- the highly variable (and often badly known) foraging behaviours of termites.

Field scale to assess the economic injury level (EIL) and especially the effect of termites density on death of seedlings and the probability that different termites species together could cause more damages, are complex and time consuming experiments. Models could allow to save time and to overtake practical difficulties at field scale. This models aim to predict a risk evaluation linked to *Syntermes molestus* activity.

Two approaches are proposed:

1) A general hierarchical model to consider three mains features on density of *S. molestus* on seedling death. Three main feature are:

- a zero-inflated distribution of sampling unit without seedling death in the presence of foraging holes of *S. molestus*;
- the non null probability, even if small, that dead with seedlings could be observed when there are no foraging holes;
- the high variability of response (dead seedlings) to the number of

foraging holes of *S. molestus*.

A general hierarchical model which carries uncertainty of the field observations was proposed.

2) An ecological investigation on the environmental factors that could enhance the combined actions of two termite species, one damaging seedlings (*S. molestus*) and another damaging trees (*Heterotermes tenuis*). A classification model is proposed to specify the location where the two termite species jointly could cause higher damages on plants. In this case, the risk evaluation is improved because the main two pests are considered jointly.

3 LITERATURE REVIEW

3.1 Importance of termites for forests plantation in Brazil

Cultivated forests present abundant resources for termites where some species assumed economic importance, due to the damage on seedlings or living trees. They may destroy root system and cause, death seedlings (BERTI FILHO, 1993), exposition to pathogens (KIRTON; CHENG, 2007) or reduction of the wood produced (ZANETTI et al., 2005a). However, most termites are beneficial (VARMA; SWARAN, 2007).

The severity of termites attack is usually higher in planted forests with exotic species than in native. Stressed plants by diseases, mechanical damage or prolonged drought are more susceptible to damage (LOGAN; COWIE; WOOD, 1990). Plantations can be attacked due to factors as termite species, population density, seasonal activity, accumulation of residues in soil, soil type, moisture conditions, plant species and physiological status (NAIR; VARMA, 1985).

Termites pests of eucalyptus plantations can be divided in seedlings and

heartwood feeders. The first group, which attacks plants up to one year, may destroy roots and/or cause ring barking, and causing plants death. Heartwood termites attack trees with more than two years, destroying wood with reduction in quantity and quality of wood (WILCKEN; RAETANO, 1995).

In Brazil, eucalyptus seedlings are damaged by: *Anoplotermes pacificus* Müller, 1873, *Anoplotermes* sp., *Armitermes euamignathus* Silvestri, 1901, *Armitermes* sp., *Cornitermes bequaerti* Emerson, 1952, *Cornitermes cumulans* (Kollar, 1832), *Cornitermes* spp., *Cylindrotermes* sp., *Embiratermes* sp., *Neocapritermes opacus* (Hagen, 1858), *Obtusitermes* sp., *Procornitermes araujo* Emerson, 1952, *P. striatus* (Hagen, 1858), *P. triacifer* (Silvestri, 1901), *Rhynchotermes* sp., *Subulitermes* sp., *Syntermes insidians* Silvestri, 1946, *S. molestus* (Burmeister, 1839) and *Syntermes* spp. (BERTI FILHO, 1995; WILCKEN; RAETANO, 1998).

Syntermes spp. causes most severe economic damages in eucalyptus plantations on young plants, with high mortality at the seedling stage (WILCKEN; RAETANO; FORTI, 2002). *S. insidians* and *S. molestus* damage seedlings to death by removing bark collar and roots. This leads to non-uniformity of stands making necessary replanting operation (ANJOS; SANTOS; ZANUNCIO, 1986). During the first half of the 20th century, 70% of planted eucalyptus were killed by *Syntermes* spp. (FONSECA, 1952). *Syntermes* species forage at night (MARTIUS; WELLER, 1998), feeding on grasses and dead leaves from deep nests (CONSTANTINO, 1995).

Heartwood termites are of minor importance compared to termites damaging seedlings. Species that attack eucalyptus trees includes *Coptotermes testaceus* (Linnaeus, 1758), *Cylindrotermes* sp., *Heterotermes tenuis* (Hagen 1858) and *Heterotermes* sp. (CONSTANTINO, 2002). The latter is the most commonly

found in eucalyptus plantations and is favoured by the large amount of branches and decaying trunks of eucalyptus (WILCKEN; RAETANO, 1998). This species can also attack the bark and trunk of living trees from nine months to eleven years (DIETRICH, 1989), and it is associated with eucalyptus canker disease (*Cryphonectria cubensis*) (RAETANO; WILCKEN; CROCOMO, 1997).

3.2 Spatial distribution of subterranean termites

Termites present regular distribution (DONOVAN et al., 2007; PRINGLE et al., 2010), determined by intra and interspecific competition and territorial behaviour (LEPAGE; DARLINGTON, 2000). However, this pattern depends on species, trophic group and environmental conditions such as vegetation and soil type (SANDS, 1965).

The spatial distribution of nests may change during time, with aggregate pattern for young nests and regular pattern for older nests (KORB; LINSENMAIR, 2001).

Mounds of 46 termites species in the Brazilian Cerrado were regular distributed when analysed all species together, but randomly individually. Grasses and leaves consumers that fed on soil organic matter exhibited aggregate pattern (GONTIJO; DOMINGOS, 1991).

Termite species showed aggregated dispersal pattern among forest fragments in central Amazônia because their foraging behaviour. Nests are mosaic distributed (SOUZA; BROWN, 1994), showing the correlation between distribution of termites and configuration of vegetation (MATHIEU et al., 2009). Aggregate pattern of Macrotermitinae has been also demonstrated in the African savannah. The pattern of *Odontotermes pauperans* was influenced by grass species distribution produced by changes in soil fertility (JOUQUET et al., 2004).

The spatial distribution of a species may change with sample size (DUNGAN et al., 2002). Termites of the genus *Syntermes* and *Cornitermes* showed aggregated spatial distribution in eucalyptus plantations in blocks of 1 and 10 ha (BEZERRA JÚNIOR; WILCKEN, 1998a). Sampling areas were proportional to the size of the block, where 40m² samples were used for 1ha blocks and 400m² ha for 10 ha blocks. *Cornitermes* sp. sampled with 400m² plots, was regularly distributed in grassland vegetation when *Brachiaria* sp. is predominant (CUNHA, 2011).

Aggregate pattern of *Syntermes* spp., *Cylindrotermes brevipilosus* and *H. tenuis* in eucalyptus plantation is also observed, when systematically sampled with samples distributed 50m apart, in a Cerrado region (SANTOS et al., 2011).

3.3 Sampling subterranean termites in eucalyptus plantations

Survey and sampling termite require great effort, especially because its cryptic behaviour. Manual collection is the most common technique for termites in dead wood, soil, mounds, under litter and stones, over and between vegetation and tree roots (DONOVAN; EGGLETON; MARTIN, 2002; JONES; EGGLETON, 2000); but it can also be done with attractive baits (COSTA-LEONARDO, 1997; HAVERTY, 2001).

Nests and foraging behaviour of *H. tenuis* are subterranean, with galleries scattered and diffuse into the soil, that makes difficult biological studies with this insect (ALMEIDA; ALVES, 2009). *Heterotermes tenuis* can be easily collected with corrugated cardboard bait buried in the soil (ALMEIDA; ALVES, 1995). This method is used in eucalyptus plantations to survey and sampling this species (JUNQUEIRA; DIEHL; BERTI FILHO, 2009; SANTOS et al., 2011).

Syntermes species are not attracted by attractive baits (BEZERRA

JÚNIOR; WILCKEN, 1998a). Foraging holes on soil surface (MARTIUS, 1998), with an estimated number of 35 holes/m²/year are the only evidence for the presence (MARTIUS; WELLER, 1998). These structures are commonly used to survey and to sample *Syntermes* spp. in forests plantations (BEZERRA JÚNIOR; WILCKEN, 1998a, 1998b; SANTOS et al., 2011).

A program for monitoring subterranean termites in eucalyptus plantations, indicates a minimum of a one cardboard bait/ha for sampling termites of the genus *Cornitermes* and a parcel of 36 m²/ha for *Syntermes* spp. (BEZERRA JÚNIOR; WILCKEN, 1998b).

Sampling plan based in presence and absence of termites have been proposed for different soils types in eucalyptus areas of the Cerrado region, with a sampling intensity of 1.36 cardboard baits/ha, randomly distributed in the field, to sample *C. brevipilosus* and *Heterotermes tenuis*, and five plots of 20m radius/ha for *Syntermes* spp. with 10% sampling error (SANTOS et al., 2011).

A sampling of the heartwood termite *Coptotermes* spp. was proposed, based on trunk damages in eucalyptus trees. Plots were established on a line of 40 plants and the minimum number defined for the sampling of termites was set on 29, 22 and two plots in João Pinheiro for *Eucalyptus camaldulensis*, *E. urophylla* and *Corymbia citriodora* and on four, seven and six plots for *E. camaldulensis*, *E. cloeziana* and *E. urophylla* in Bocaiúva (ZANETTI et al., 2005b).

3.4 Effect of *Syntermes molestus* on seedling death

The insects data are usually characterized by large proportion of zeros and overdispersion, making them intractable using standard distributions such as gaussian (SILESHI, 2006). Their analysis requires a statistical approach that incorporates zeros for not losing biological information (MARTINS et al., 2005),

associated with the probabilistic models to incorporate the uncertainty associated with the estimation of the response variable (PETERSON; HUNT, 2003).

Probabilistic models with large proportions of zeros are used in the study of insects, such as zero inflated Poisson (ZIP) for determining the oviposition pattern of *Curculio elephas* (Gyllenhal, 1836) (Coleoptera: Curculionidae) (DESOUHANT; DEBOUZIE; MENU, 1998) and survival of *Bemisia argentifolii* Bellows Perring, 1994 (Hemiptera: Aleyrodidae) submitted to imidacloprid (IERSEL; OETTING; HALL, 2000); zero inflated negative binomial (ZINB) for temporal prediction of oviposition in *Phenacoccus aceris* (Signoret, 1875) (Hemiptera: Pseudococcidae) (YESILOVA; KAYDAN; KAYA, 2010) and zero inflated beta-binomial (ZIBB) to determine the optimal number of *Trichogramma galloi* Zucchi, 1988 (Hymenoptera: Trichogrammatidae) for biological control (BORGATTO; DEMÉTRIO; LEANDRO, 2006).

The random response variable (Y) in the zero inflated beta-binomial model (ZIBB) can be represented by $Y = H(1 - Z)$ (GHOSH; MUKHOPADHYAY; LU, 2006), where Z is the response variable with Bernoulli distribution ($Ber(w)$) and H represents the random variable with beta-binomial distribution (BB), and w the probability of not occurrence of an event in the presence of the response variable. The probability function Y_i are given by :

$$P(Y_i = y_i) = \begin{cases} w_i + (1 - w_i) \frac{B(\frac{1}{\delta})B(\frac{1-p_1}{\delta} + \mu_1)}{B(\frac{1}{\delta} + \mu_i)B(\frac{1-p_1}{\delta})} & \text{if } y = 0 \\ (1 - w_i) \binom{\mu_i}{y_i} \frac{B(\frac{p_i}{\delta} + y_i)B(\frac{1-p_i}{\delta} + \mu_1 - y_i)B(\frac{1}{\delta})}{B(\frac{1}{\delta} + p_i)B(\frac{p_1}{\delta})B(\frac{1-p_1}{\delta})} & \text{if } y > 0 \end{cases}$$

where $0 \leq w_i < 1$, $0 \leq p_i < 1$ and $\delta > 0$. The expected value and variance of the model are given respectively by ZIBB: $E(Y_i) = (1 - w_i)m_i p_i$ and $Var(Y_i) = \mu_i[(1 - p_i)(\frac{m_i \delta + 1}{\delta + 1}) + (\frac{w_i}{1 - w_i})\mu_i]$.

The parameters $p = (p_1, \dots, p_n)$ and $\mu = (\mu_1, \dots, \mu_n)$ functions are

modeled by $\log\left(\frac{p_1}{1-p_1}\right) = G_i\gamma$ and $\log\left(\frac{\mu_1}{1-\mu_1}\right) = B_i\beta$, respectively.

The model and the estimation of the parameters should be adjusted and the next step is to select the best fitted model made through quantitative techniques as the Akaike information criterion (AIC). This criterion uses the discrepancy between the true model and the approximate model with maximum likelihood (AKAIKE, 1983), represented by the following expression: $AIC = -2\log L + 2K$, where L is the maximized likelihood model and K the number of parameters.

3.5 Effect of environmental factors on *Syntermes molestus* and *Heterotermes tenuis* association

The method of classification and spatial analysis (CASA) can evaluate local interactions between species abundance in a specified sites (GEORGESCU et al., 2009). The CASA method allows the mapping of various assemblages types (assemblage= related species in the same community) (BEGON; TOWNSEND; HARPER, 2006), based on abundance. It despite the different types of assemblages their number and how a particular assemblage is organized in relation to others.

Let be X_i the abundance of species $s \in \{1, \dots, S\}$ for each location $i \in \{1, \dots, n\}$, where S and n are respectively, number and location of species studied. The classification of types of species assemblages will be given by describing the structure of dependence between the abundance of species, assuming a vector of abundance $X_i = (X_{i1}, \dots, X_{iS})$, considered independent in each site from a multivariate normal distribution (GEORGESCU et al., 2009), with:

$$X_i \sim \sum_{k=1}^K \tau_k \mathcal{N}(\mu_k, \Sigma_k)$$

where $k \in \{1, \dots, K\}$ is the mixture components, τ_k mixture proportions $\sum_{k=1}^K \tau_k = 1$, μ_k is the vector of means and Σ_k the covariance matrix of the k -th component.

The assemblages types are determined by fitting probabilistic models based on clustering, and abundances with the same mixed multivariate normal distribution are grouped within the same assemblage (GEORGESCU et al., 2009).

The selection of model parameters and the number of clusters that represents the assemblages are determined with quantitative techniques such as the Bayesian information criterion (BIC), being represented by the following expression (SCHWARZ, 1978): $BIC = 2\mathcal{L}_m^*(X,Z) - v_m \log(n)$, where $2\mathcal{L}_m^*(X,Z)$ is the maximized log-likelihoods model m , v_m the number of independent parameters estimated by the model m and n number of locations.

CONCLUSIONS

- Beta-binomial model provides a general structure and can be used in other entomological studies;
- Beta-binomial model would be easier to adjust if we had a better understanding of the temporal dynamics of *Syntermes*'s foraging holes, because there is probably a cumulative effect of the nocturnal behaviour of *S. molestus*. The measure once a month is probably not enough;
- The circular parcel in seedlings mortality essay should be taken larger because termites may be able to forage at long distance;
- We can study *S. molestus* and *H. tenuis* assemblages with CASA approach, but we should have better knowledge of the ecological factors (soil type, organic content, rain regime) to better use the classification;

- Better comprehension of termites spatial distribution can be done with stratification of sampling, but the exact position of each nest needs to be know.

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ARTICLE 1: Mortality estimation of *Eucalyptus* sp. seedlings for subterranean termite *Syntermes molestus* (Burmeister, 1839) (Isoptera: Termitidae) with hierarchical probability model approach

**Mortality estimation of *Eucalyptus* sp. seedlings for subterranean termite
Syntermes molestus (Burmeister, 1839) (Isoptera: Termitidae) with
hierarchical probability model approach**

Alexandre dos Santos¹, Samuel Soubeyrand², André Kretzchmar² and Ronald Zanetti¹

Abstract

Syntermes molestus is the termite that cause the most severe economic damages on *Eucalyptus* plantations at the time of the initial development of forests. The project of integrated pest management (IPM) aims to reduce losses caused by termites and to promote the reduction of insecticides uses. This program requires the quantification of termite damages. A model is proposed to predict the number of eucalyptus seedlings deaths depending on the number of foraging holes of *S. molestus*. We propose a hierarchical model in which the zero-inflation is driven by a Bernoulli while the effect of foraging holes on seedling death is depicted by a beta-binomial. Several structure of the hierarchical model are compared and AIC criterion is used for selection. The best fitting model ignores the zero-inflation and allows us to estimate the mortality of seedlings induced by termites. We discuss the properties of this model and the quite large part of the variability it does not explain, especially in regard to the difficulty to estimate the absence of death when there is no termite. Despite this uncertainty, the probabilistic beta-binomial model approach allows to predict the increase of seedlings deaths versus the increase of *S. molestus* foraging holes. This model aims to be a contribution to a risk assessment in the context of the pest management.

Keywords: *Eucalyptus*. Forest protection. Probabilist model. Termite damage.

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Resumo: Estimação da mortalidade de mudas de *Eucalyptus* sp. pelo cupim *Syntermes molestus* (Burmeister, 1839) (Isoptera: Termitidae) com a abordagem de modelos hierárquicos probabilísticos

Syntermes molestus é a espécie de cupim que causa o mais severo impacto econômico aos plantios com *Eucalyptus* em seu estágio inicial de desenvolvimento. O desenvolvimento de programas de manejo integrado de pragas (MIP) pode auxiliar na redução dos danos por cupins e promover uma redução na utilização de inseticidas. A implementação destes programas depende da quantificação dos danos causados por estes cupins. Um modelo foi proposto para predição do número de mudas mortas dependente do número de orifícios de forrageamento de *S. molestus*. Foi proposto um modelo hierárquico inflacionado de zeros, governado pela distribuição de Bernoulli, enquanto o efeito de orifícios de forrageamento sobre a morte de mudas é representado por uma beta-binomial. A estrutura de vários modelos hierárquicos são comparados e o critério AIC é usado para a seleção. O melhor modelo ajustado ignora a inflação por zeros e permite-nos estimar a mortalidade de mudas produzida por cupins. Foram discutidas as propriedades deste modelo e a larga parte da variabilidade não explicada, especialmente no que diz respeito à dificuldade de estimar a ausência de morte de mudas, quando não há cupins. Apesar dessa incerteza, o modelo probabilístico beta-binomial permite prever o aumento na mortalidade de mudas em relação ao aumento de orifícios de *S. molestus*. Este modelo pretende ser uma contribuição à avaliação de risco no contexto do manejo de pragas.

Palavras-chave: Cupins-praga. *Eucalyptus*. Modelo probabilístico. Proteção florestal.

1 INTRODUCTION

Termites are particularly important pests in the initial development of forests with *Eucalyptus* in Brazil (WILCKEN; RAETANO, 1998). This importance has increased with the advance of this forests on areas previously occupied by pastures, which have normally large populations of importance economic termites (FOWLER; FORTI, 1990). *Syntermes molestus* is the termite species which causes the most severe economic impact on *Eucalyptus* plantations by ring-barking and root debarking plants newly transplanted (ROULAND-LEFÈVRE, 2011), leading to high mortality of plants at the seedling stage (WILCKEN; RAETANO; FORTI, 2002).

In natural ecosystems, these termites have a foraging behaviour at night (MARTIUS; WELLER, 1998), feeding on grasses and dead leaves and living in subterranean deep nests (CONSTANTINO, 1995). The only evidence of this termites is the presence of foraging holes on soil surface (MARTIUS, 1998), with an average estimated number of 35 foraging holes/m²/year (MARTIUS; WELLER, 1998). These foraging structures are considering as population indice and they are used for survey and sampling *Syntermes* species in *Eucalyptus* plantations (BEZERRA JÚNIOR; WILCKEN, 1998; SANTOS et al., 2011).

The study of the relationships between the density of insect pests, the crop response to injury and the economic costs of these damages is needed so to make available a bio-economic criteria on which could be based the choice of pest control strategies (PEDIGO; HIGLEY, 1996). In order to satisfy the assumptions of this criteria, the first step is the adequate and quantitative estimation of the relationship between the density of termites and the damage caused to plantations at field scale. In this case, the objective of this study was to estimate the mortality

of eucalyptus seedlings for subterranean termite *Syntermes molestus* (Burmeister, 1839) (Isoptera: Termitidae) with hierarchical probability model approach.

However, in the case of termites of genus *Syntermes* and *Eucalyptus* seedlings, this relation is difficult to address for several reasons. Firstly, the termite density per area unit is almost impossible to quantify. This species lives in subterranean nests which does not allow a quantification of the number of nests per surface unit; additionally, an average of about 30,000 termites could be counted per nest with a high variability (LEPAGE; DARLINGTON, 2000). Consequently, termite density is only approximated through the number of foraging holes counted by surface unit.

Secondly, when counting dead seedlings, occurs the question of large proportion of zeros. This question is common in population spatial survey. Ignoring the zeros can result in loss of important biological information (MARTINS et al., 2005). In the hierarchical model proposed here, zero inflation is incorporated so to carry uncertainty into the damage estimation (PETERSON; HUNT, 2003).

Thirdly, unlike other pest insects inducing injury as a reduction in vegetative material of the eucalyptus seedlings, damages by *Syntermes* result in total loss of the young plants. As a main characteristic of the damages observed on *Eucalyptus* seedlings by *Syntermes*, it is noticeable that the number of dead plants related to the number of foraging holes for a given area shows a large over-dispersed distribution.

To cope with these difficulties, we proposed a hierarchical model which estimates the relationship between the number of foraging holes per surface unit and the cumulated number of dead seedlings and which takes into account both the zero-inflation and the over dispersion of cumulated number of dead seedlings.

2 MATERIAL AND METHODS

2.1 Study site

The sampling was performed in grassland area of 14.25ha, originally with *Brachiaria decumbens* Stapf. plants for eucalyptus implantation in Governador Valadares (latitude 18°51'04"S, longitude 41°56'58"W and 170 m altitude), Minas Gerais, Brazil, from January to June 2009. This region is tropical wet and dry (Aw) following the Köppen climate classification. The nodes of a regular square grid with 25m-mesh covering the study area were located with GPS (Global Position System). Around each node, a circular parcel with 10m-radius was marked; in total we had 108 parcels called thereafter sampling units. In each sampling unit, *S. molestus* foraging holes and the number of newly dead seedlings due to termites were counted once a month during six months after seedlings implantation. The initial number of plants in each sampling unit was also counted. To be able to study the termite effects, the plants did not receive preventive control with insecticide. Manual nocturnal collections were performed for identification of the *Syntermes* species present; the specimens were placed in 70% alcohol and sent to Dra. Eliana Marques Canello of MZUSP for identification.

2.2 Model

We built a hierarchical model allowing the investigation of the link between the cumulated number Y_i of dead seedlings due to termites during the six months of the study and the number H_i of *S. molestus* foraging holes at a given month. The index i is the identifier of the sampling unit. Y_i is viewed as the response variable and H_i as the explanatory variable. The number of holes is used

as a proxy of the presence intensity of termites. In the more general model that we considered, the cumulated number of dead seedlings follows a zero-inflated beta-binomial distribution. The zero-inflation takes into account possible excess of zeros commonly encountered in ecological studies. The beta-binomial takes into account possible overdispersion of data also often encountered in ecology. Let N_i denote the initial number of seedlings in sampling unit i . Let Z_i denote an auxiliary binary variable used in the zero-inflation of the beta-binomial distribution. Under the more general model that is considered in this article, observations in different sampling units are independent; Z_i follows a Bernoulli distribution with success probability $p(H_i)$ depending on the number of holes:

$$Z_i = 1 \text{ with probability } p(H_i) = \text{logit}^{-1}(\alpha_1 + \alpha_2 H_i)$$

$$Z_i = 0 \text{ with probability } 1 - p(H_i)$$

$$\text{logit}^{-1}(u) = \frac{\exp(u)}{(1+\exp(u))}$$

The cumulated number of dead seedlings is either equal to zero if $Z_i = 0$ or follows a beta-binomial distribution with size N_i , success probability $q(H_i)$ and overdispersion parameter θ if $Z_i = 1$:

$$Y_i = 0 \text{ if } Z_i = 0$$

$$Y_i \sim \text{Beta} - \text{Binomial}(N_i, q(H_i), \theta) \text{ if } Z_i = 1$$

$$q(H_i) = \text{logit}^{-1}(\beta_1 + \beta_2 H_i).$$

The direct acyclic graph (Figure 1) shows the dependence structure of the hierarchical model built above. Appendix A provides the properties of the model, i.e. the probability distribution function of Y_i , its expectation and its variance. Sub-models can be derived from this general model. For examples, the zero-inflation may be removed or the probability can be constant (and not depend on H_i).

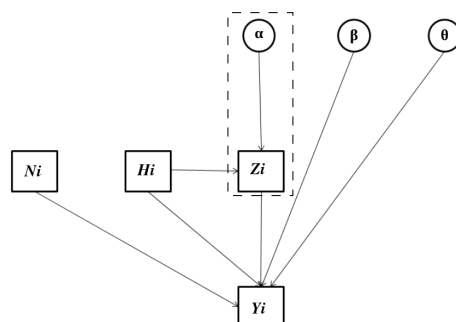


Figure 1 Directed acyclic graph (DAG) for hierarchical probability model proposed. Where: Y_i the death seedlings, N_i the number of seedlings, H_i the termite foraging holes, Z_i the binary variable and model parameters α , β and θ .

2.3 Parameter estimation and model selection

Model parameters (of the general model and its sub-models) were estimated with the maximum likelihood method. The maximization was carried out with the Nelder-Mead algorithm (NELDER; MEAD, 1965). The uncertainty about the parameters was assessed with non-parametric bootstrap using 5,000 resampling samples (EFRON; TIBSHIRANI, 1993). We used six different specifications for H_i , namely, for m in $\{1,2,\dots,6\}$, the number of holes in sampling unit i at month m after seedling implantations.

By considering the six different specifications for H_i and height specifications of the model corresponding to [M1: complete model], [M2: $\alpha_2=0$], [M3: $\alpha_2=\beta_2=0$], [M4 : no zero-inflation], [M5 : $\beta_2=0$ and no zero-inflation], [M6 : $\alpha_2=\beta_2=0$ and no overdispersion], [M7 : no zero-inflation and overdispersion], [M8 : $\beta_2=0$, no zero-inflation and overdispersion], we fitted to data 48 (= 6 times 8) different models. We used the Akaike information criterion (AIC, Burnham and Anderson) to select the more appropriate model.

This criterion takes into account both the likelihood and the parsimoniousness of the model. Analyses were performed with the statistical computer R program (R DEVELOPMENT CORE TEAM, 2005) and the emdbook package (BOLKER, 2011).

3 RESULTS

3.1 Mortality versus hole numbers

Cumulated mortality of seedlings by termites changed with time during the observations, showing an increasing pattern until the fourth month, when it reached a cumulative average of around 12,5%, and after this period no more attacks were observed on seedlings (Figure 2a). Despite *S. molestus* termites have stopped to induce mortality on plants, the number of foraging holes still increased up to the sixth month (Figure 2b).

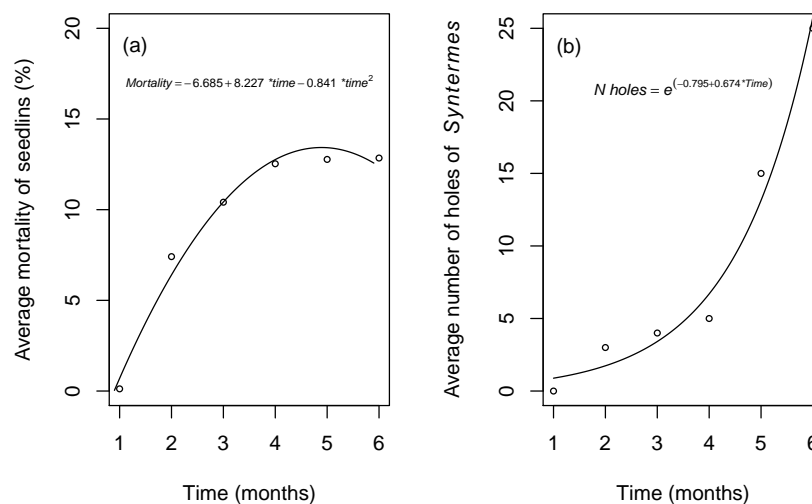


Figure 2 (a) Average mortality of *Eucalyptus* seedlings (%) in the time for six months. (b) Average number of foraging holes of *Syntermes molestus* in the time for six months.

With reference to the lowest value of Akaike criterion (AIC) (Table 1), only the model with β and θ parameters (beta-binomial) was used. The zero-inflation did not improve the model accuracy (the use of α parameter did not lead to lower AIC).

The model which is characterized by the lowest AIC criterion (374.2) (Table 1) specifies that, additionally to the irrelevance of zero-inflation, the number of dead seedlings Y_i depends, under the beta-binomial model, on the success probability $q(H_i)$ with both parameter β_1 and β_2 and on the over dispersion parameter θ .

Interestingly, the best prevision for seedling mortality is obtained with the number of holes observed at the the third month.

Table 1 Akaike Criterion (AIC) for various sets of parameters and various explanatory variables.

Optimized parameters	Model names	Number of <i>Syntermes</i> holes					
		month 1	month 2	month 3	month 4	month 5	month 6
$\beta_1, \theta, \beta_2, \alpha_1, \alpha_2$	M1	393.0	380.1	378.2	382.9	386.8	387.7
$\beta_1, \theta, \beta_2, \alpha_1$	M2	391.0	378.1	376.2	380.9	384.8	385.7
$\beta_1, \theta, \alpha_1$	M3	389.0	389.0	389.0	389.0	389.0	389.0
β_1, θ, β_2	M4	389.0	376.1	374.2	378.9	382.8	383.7
β_1, θ	M5	387.0	387.0	387.0	387.0	387.0	387.0
β_1, α_1	M6	541.9	541.9	541.9	541.9	541.9	541.9
β_1, β_2	M7	541.9	541.3	541.2	541.4	541.6	541.6
β_1	M8	539.9	539.9	539.9	539.9	539.9	539.9

Assessment of the goodness-of-fit of the beta-binomial model under the estimated values of the parameters

The model parametrized by $(\beta_1, \beta_2, \theta) = (-2.00, 0.0434, 79.7)$ was run 5000 times to simulate the numbers of deaths given the observed numbers of seedlings and the observed numbers of holes (Figure 3).

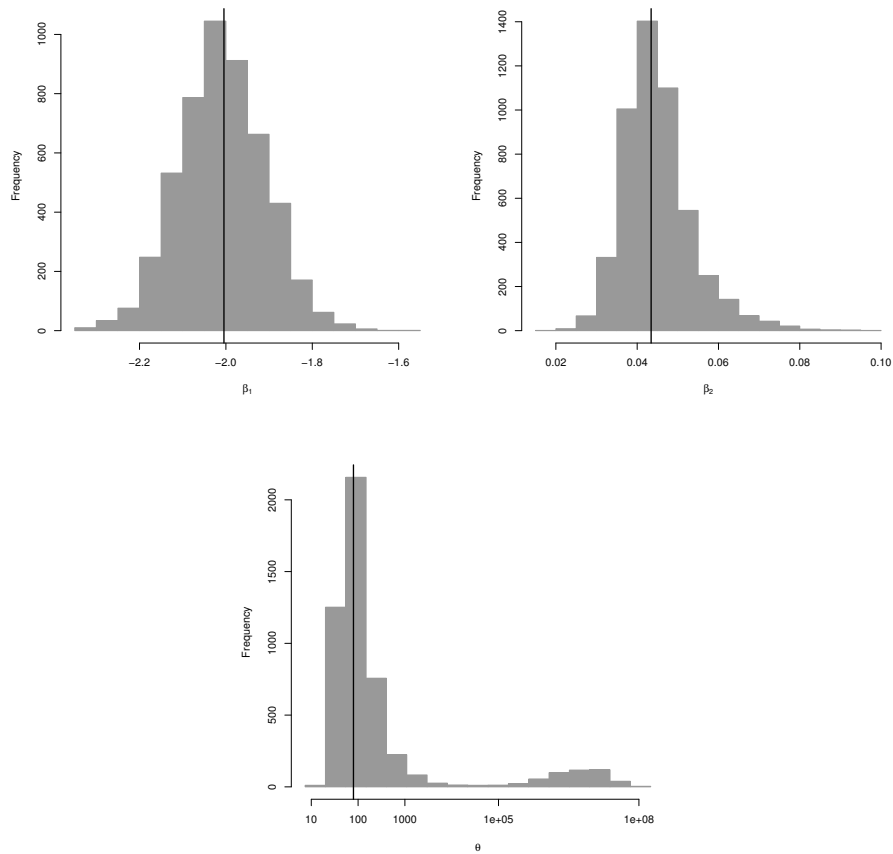


Figure 3 Bootstrap distributions (histograms) of parameter estimates (parameter theta is in log scale). The distributions were obtained by generating 5000 bootstrap resamples and estimating the parameters for all the resamples. Vertical lines are estimates obtained for the real data.

Then, we could provide for each parcel a 95% prediction interval for the number of deaths and assess if this interval includes the corresponding observed number of deaths (Table 2). Percentage of 96.3% (92.7 ; 99.9) of the observed numbers of death are included in the 95% prediction intervals. Thus, the estimated model is able to reproduce the data variability.

Table 2 Maximum likelihood estimates of the parameters and 95% confidence intervals obtained with nonparametric bootstrap (the intervals are percentile intervals obtained with 5000 bootstrap resamples; Efron and Tibshirani, 1993).

Parameter	Estimate	95% confidence interval
β_1	-2.00	(-2.20 ; -1.81)
β_2	0.0434	(0.0314 ; 0.0668)
θ	79.7	(28.1 ; 11.9e ⁶)

For prediction of the number of deaths 1110 seedlings were uniformly spread into 46 parcels. One to one thousand holes were uniformly spread into the 46 parcels and for each total number of holes (from 1 to 1000), the theoretical expected total number of deaths was computed using $(\beta_1, \beta_2, \theta) = (-2.00, 0.0434, 79.7)$. Besides, the model was simulated once for each of the 5000 bootstrap estimates $(\beta_1, \beta_2, \theta)$ and, using the simulations, we computed the average total number of deaths and 95% confidence intervals for each total number of holes (Figure 4).

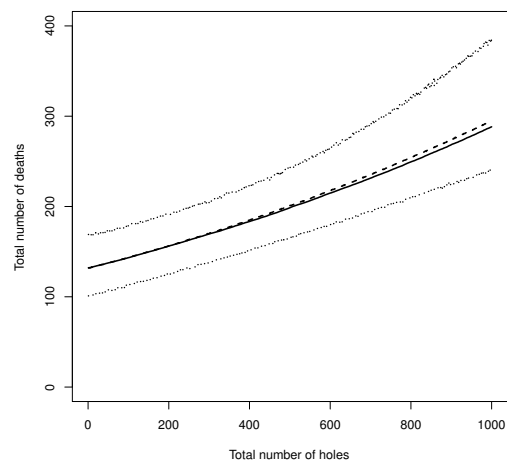


Figure 4 Prediction of the number of cumulated deaths with respect to the number of cumulated deaths. Theoretical expected curve (solid line) obtained with $(\beta_1, \beta_2, \theta) = (-2.00, 0.0434, 79.7)$; Empirical expected curve (dashed line) and point wise 95% confidence envelopes (dotted line) obtained from 5000 simulations carried out under the bootstrap distribution of the parameters.

4 DISCUSSION AND CONCLUSION

We can observed that the number of foraging holes of *S. molestus* in third month better explain the seedlings damage cumulated over six months.

As the cumulated number of death is almost constant after four months (Figure 2a), there is a delay in the effect of number of foraging holes on seedling death of about a month. Nevertheless, it also necessary to take into account the delay in the first damages done on plants by termites and the actual death of plants. It is then possible to consider the number of holes at the third month as the maximum hole density which leads to the maximum of dead plant. The mortality reduction of seedlings with time could be due to the increase in diameter

and thickness of bark of plants, making them more tolerant to termite attack. In this case, the increase in the number of termites won't increase the mortality of seedlings.

Due to the fast termite's foraging holes dynamic (Figure 2b), a description of foraging hole dynamics at a smaller interval of time could lead to a better understanding of this maximum threshold.

The model predicts an amount of dead seedling with no foraging holes, i.e. in absence of termites under our assumptions. Nevertheless this is not observed in our field works. Several reasons could be given to explain this model irrelevance. Additionally to the weakness of the model structure, it is possible that termites from outside the sampling area (the foraging holes of which were nor counted) created damages in the sampling areas. Finally damages might be caused by a hidden process not taking into account in our observations.

The model allowed us to estimate the damage that termites produce to seedlings, but a large part of the variance in the model remains to be explained (Figure 4). Again possible edge effect, due to termites located in the neighbourhood of the circular sub-parcels of 10m, could be the cause of this unexplained variance.

Unknown variance leads to concluded that in absence of *S. molestus* a mortality of 9% (100 plants) to 16% (175 plants) in *Eucalyptus* seedlings (Figure 4) can be expected. It is common in ecology studies to observe a over density of zero depicted the absence of the expected relationship (MARTINS et al., 2005) (here, the presence of termites versus the number of dead seedlings). Here the mode predict an over number of positive value which leads to a number of questions about the behaviour of the termites under studies. Mainly, it could be ask whether these termites forage at long distance from the holes they exhaust

from soil sub-surface.

Another factor which could contribute to the variance is the lack of relationship between the number of foraging holes and the colony size. Large colonies need more food and have greater potential for damage than smaller colonies. It is possible that same amount of foraging holes in a parcel represents a large colony or several small colonies. In this situation, the same number of foraging holes produces variation in mortality of seedlings.

The damage increase with the number of foraging holes indicates the areas with high activity of this termite was expect great losses of seedlings and for management insects, the areas with the presence of this species needs the using of preventive control of seedlings before implantation.

The hierarchical model initially proposed for comprise the zero-inflated of data showed that was not necessary considering the zeros (Z_i), damage of seedlings for termites are explain with the beta-binomial model using the variable number of plants (N_i) and foraging holes (H_i) (Figure 1), but this work encouraging the use of hierarchical model approach for others insect's species in applied entomology, where the zeros are not usually considered and only the occurrences are used. This because a large proportion of zeros values makes the data intractable with standard distributions (i.e. normal and Poisson) (MULLAHY, 1986; RIDONT; DEMÉTRIO; HINDE, 1998).

The beta-binomial model can be used for prediction the damage of *S. molestus* in *Eucalyptus* seedlings and improve biological inference of the termite behaviour. This probabilistic approach allows to know the level of risk associated with the mortality estimation that termites can be produce with gain in the context of the pest management purposes.

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We thank the Brazilian agencies CNPq and FAPEMIG and forestry company Celulose Nipo-Brasileira S/A for financial support. To CAPES and INRA (BioSP, France) for their support to organize the 6 month stay of Alexandre dos Santos in Avignon (France). To Dra. Eliana Marques Canello of MZUSP for termites identification. We are grateful to all colleagues who provided comments and discussions along this work. We thank the students André Luiz Evangelista, Lucas Willian Paiva e Gabriel Biagiotti for their assistance in data collection.

APPENDIX

Model properties

Probability distribution function f of a random variable U following the beta-binomial distribution with size N , success probability q and overdispersion parameter θ : for u in $\{0,1,2,\dots,N\}$,

$$\begin{aligned} f(u; N, q, \theta) &= Pr(U = u) \\ &= C(N, u) \frac{B(u + \theta q, N - u + \theta(1 - q))}{B(\theta q, \theta(1 - q))}, \end{aligned}$$

where $C(N, u)$ is a binomial coefficient and $B(a, b)$ is a beta function.

Probability distribution function of Y_i given H_i :

$$\begin{aligned} Pr(Y_i = 0 | H_i) &= (1 - p(H_i)) + f(0; N_i, q(H_i), \theta)p(H_i) \\ Pr(Y_i = y | H_i) &= f(y; N_i, q(H_i), \theta)p(H_i), \quad \text{for } y \text{ in } \{1, 2, \dots, N_i\}. \end{aligned}$$

Expectation and variance of Y_i given H_i :

$$E(Y_i|H_i) = N_i q(H_i) p(H_i)$$

$$Var(Y_i|H_i) = \frac{N_i(N_i + \theta)}{1 + \theta} q(H_i)(1 - q(H_i)) p(H_i)^2.$$

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ARTICLE 2: Assemblages of the termites *Syntermes molestus* (Burmeister, 1839) and *Heterotermes tenuis* (Hagen, 1858) using the classification and spatial analysis method

Assemblages of the termites *Syntermes molestus* (Burmeister, 1839) and *Heterotermes tenuis* (Hagen, 1858) using the classification and spatial analysis method

Alexandre dos Santos¹, Vera Georgescu², André Kretzchmar² and Ronald Zanetti¹

Abstract

Some termite species are important pests for eucalyptus trees at different stages of its development. The adoption of sampling plans in the context of integrated pest management (IPM) can reduce the use of insecticides by targeting the areas where the species are present, but the relationship between termite species and their spatial distribution needs to be elucidated first. This work focuses on two termite species of great economic importance in Brazil, *Syntermes molestus* (Burmeister, 1839), which attacks young eucalyptus plantations and *Heterotermes tenuis* (Hagen, 1858), which feed on older trees. We use the classification and spatial analysis (CASA) method on a termites abundance data set to explore how the joint spatial distribution of the *S. molestus* and *H. tenuis* termites relates to different altitudes (266 to 888m) and vegetation types (wood-dominated and grass-dominated). The study reveals 5 termites assemblages which are mapped and compared to the factors. The results suggest that *H. tenuis* is the only species capable of nidification at high altitudes, and coexistence of the two species is favoured by low altitude pastures.

Keywords: Coexistence. Eucalyptus. Pests. Spatial distribution.

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Resumo: Assembleias dos cupins *Syntermes molestus* (Burmeister, 1839) e *Heterotermes tenuis* (Hagen, 1858) usando o método de classificação e análise espacial

Algumas espécies de cupins são importantes pragas de *Eucalyptus* em diferentes estágios de seu desenvolvimento. A adoção de planos de amostragem, no contexto do manejo integrado de pragas (MIP), pode reduzir o uso de inseticidas somente em áreas onde estas espécies encontram-se presentes, mas a relação entre as espécies de cupins e sua distribuição espacial precisam ser inicialmente elucidadas. O presente trabalho foca em duas espécies de cupins, ambas de importância econômica no Brasil, *Syntermes molestus* (Burmeister, 1839), a qual ataca cultivos de eucalipto em seu estágio jovem e *Heterotermes tenuis* (Hagen, 1858), a qual se alimenta de árvores adultas. Utilizou-se o método de classificação e análise espacial (CASA) sobre os dados de abundância de cupins para explorar como relacionar a distribuição espacial de *S. molestus* e *H. tenuis* em diferentes altitudes (266 a 888 m) e tipo de vegetação (dominada por madeira e pastagem). O estudo revelou 5 assembleias de cupins que foram mapeadas e comparadas com os fatores. Os resultados sugerem que *H. tenuis* é a única espécie capaz de nidificar em altas altitudes, e a coexistência entre as duas espécies é favorecida em pastagem em baixa altitude.

Palavras-chave: Coexistência. *Eucalyptus*. Pragas. Distribuição espacial.

1 INTRODUCTION

Some termite species become severe pests where exotic fast-growing forest species are introduced (COWIE; LOGAN; WOOD, 1989), although most

species of termites developed an important ecological role in these forests by improving soil characteristics (VARMA; SWARAN, 2007). Among these fast-growing forest species, trees of the genus *Eucalyptus* are the most common, with more than 20 million hectares planted around the world, of which 53% in Brazil, China and India (IGLESIAS-TRABADO et al., 2009).

Eucalyptus has a susceptibility for termite attacks at different stages of its development: in the first months after planting (NAIR; VARMA, 1985), due to the destruction of the root system and/or ring barking around the collar region (WILCKEN; RAETANO; FORTI, 2002), but also in mature trees, where these insects cause injuries to the bark and wood (VERMA, 1993; MORAES et al., 2002). In Brazil, eucalyptus trees are attacked by several termite species (BERTI FILHO, 1995; WILCKEN; RAETANO, 1998). The present work focuses only on the two termite species *Syntermes molestus* (Burmeister, 1839) and *Heterotermes tenuis* (Hagen, 1858), both of great economic importance in Brazil.

Syntermes molestus is the termite species that has the most severe economic impact on young eucalyptus plantations. This species causes high mortality during the plant seedling stage (WILCKEN; RAETANO; FORTI, 2002). In natural ecosystems, these termites have nocturnal foraging behavior (BARBOSA, 1993), feed on leaves or grass-litter and live in deep subterranean nests (CONSTANTINO, 1995). The only visible presence of the nests in the soil are the foraging holes (MARTIUS, 1998), with an estimated number of 35 foraging holes/m²/year (MARTIUS; WELLER, 1998). These foraging holes are normally used in studies for survey and sampling purposes, but the sampling effort required to obtain all foraging holes at a large scale is too expensive for insect management purposes. Termites of the genus *Syntermes* have a random spatial distribution in the natural environment (GONTIJO; DOMINGOS, 1991), but they

show an aggregated distribution in the *Eucalyptus* commercial forests (BEZERRA JÚNIOR; WILCKEN, 1998; SANTOS et al., 2011).

Heterotermes tenuis is also an important pest of eucalyptus forests (CONSTANTINO, 2002). These termites are xylophagous therefore attacks by this termite species occur in the bark in the collar region of eucalyptus trees aged between 9 months to 11 years (DIETRICH, 1989). Nests and foraging behavior of this termite species are cryptic with sparse and diffuse galleries in the soil, which makes biological studies difficult (ALMEIDA; ALVES, 2009).

The most largely used method to prevent attacks by *S. molestus* on *Eucalyptus* is the immersion of all plants before plantation in chemical substances (fipronil, imidacloprid or thiametoxan) at risk of high level residues (WILCKEN; RAETANO; FORTI, 2002). There is no preventive control method against the *H. tenuis* species, but a curative method has been proposed by Raetano, Wilcken and Crocomo (1997), which consists in applying fipronil at 0.30m around the trunk of *Eucalyptus* trees. The preventive control practice against *S. molestus*, systematically used, increases the risk of negative impacts on humans and on the environment and is in disagreement with the actual policy of reduction and elimination of persistent insecticides (UNITED NATIONS ENVIRONMENT PROGRAMME, 2000).

To reduce the application of insecticides against termites is necessary to develop sampling programs to identify the areas where *S. molestus* and *H. tenuis* occurs in pest status, but the relationship between these species of termites and their spatial distribution in the forest or pasture must to be elucidated first (PEDIGO; RICE, 2006; TAYLOR, 1984).

According to Constantino (1995) the genus *Heterotermes* was found in association with the species *Syntermes dirus*, which indicates that there is no

antagonistic relationship between the genus *Heterotermes* and *Syntermes*. To our knowledge, there have been no studies on the possible relationship between these termite genus. Such a study may be helpful at least for sampling purposes. *H. tenuis* can be easily collected by burying attractive traps of corrugated cardboard in the soil (ALMEIDA; ALVES, 1995). This method was used in *Eucalyptus* forest areas (BEZERRA JÚNIOR; WILCKEN, 1998; SANTOS et al., 2011). Indeed, since *Heterotermes* are much easier to collect than *Syntermes*, it would be convenient if the abundance of *Heterotermes* gave an indication on the abundances or on presence/absence of *Syntermes*.

In this paper, we explore the existence of spatial assemblages of these two termite species in *Eucalyptus* forest and pasture ecosystems. We use the classification and spatial analysis (CASA) method (GEORGESCU et al., 2009) on a termites abundance data set to explore how the joint spatial distribution of the *S. molestus* and *H. tenuis* termites relates to different altitudes (266 to 888m) and vegetation types (wood-dominated and grass-dominated) in the Atlantic forest region in the state of Minas Gerais, Brazil.

2 MATERIAL AND METHODS

2.1 Data collection

The data collection was performed from March 2008 to August 2010 on 6 farms of the Atlantic forest region, in the municipalities of Belo Oriente (19°13'12"S latitude, 42°29'01"W longitude and 300m altitude); Guanhães (18°46'30"S latitude, 42°55'57"W longitude and 800m altitude); Governador Valadares (18°51'04"S latitude, 41°56'58"W longitude and 170m altitude) and Pingo d'Água (19°43'39"S latitude, 42°24'29"W longitude and 200m altitude),

Estate of Minas Gerais, Brazil.

The 6 farms (see Table 3) contained a total of 14 blocks, of which 50.9 ha of harvested eucalyptus stands in pre-planting stage (wood-dominated), and 37.6 ha of pastures (grass-dominated), which consists predominantly of *Brachiaria decumbens* Stapf.

Table 3 Farms, number of blocks, area (ha), type of vegetation and altitude (m) where *Syntermes molestus* and *Heterotermes tenuis* were sampled, in Minas Gerais, Brazil.

Farm	N blocks	Area (ha)	Vegetation	Altitude (m)
Bela Vista	1	9.00	pasture	294
Cajá	1	6.25	forest	266
Lagoa Redonda	4	27.56	forest	273
Pitanga	3	14.69	pasture	820
Recanto do Aconchego	3	13.93	pasture	300
Três Morros	3	17.19	forest	888

Each block was divided into parcels of 25 x 25m, with a GPS device (Global Position System). The attracting bait for *Heterotermes* was composed of a corrugated cardboard roll, with 25cm x 5cm diameter, waterlogged, and buried at the center of each parcel, where the subterranean termites were sampled. Circular sub-parcels of 10m radius were marked around the bait where *Syntermes* foraging holes were counted, because this species is not attract to cardboard baits. The baits were removed after 30 days. Manual nocturnal collections were performed for identification of the *Syntermes* species present. Termites were collected, counted, preserved in 70% of alcohol and sent to Dra. Eliana Marques Canello of MZUSP, Brazil, for identification.

2.2 Data analysis

The termites abundances dataset was analyzed with the classification and spatial analysis method (CASA) proposed by Georgescu et al. (2009). This method was created to explore multivariate spatial data, when several variables (such as species abundances) are measured at the same sites. CASA explores the joint spatial distribution of several species abundances by providing first a classification of the species abundances data (using multivariate Gaussian mixture models), followed by a spatial analysis of the classes.

The first stage of CASA (the classification stage) groups together similar observations. This clustering procedure is not spatial, in the sense that the observations being clustered are vectors of abundances observed at each site, without any information on the spatial proximity between sites. Considering the scatterplot of the species abundances, the clustering procedure seeks the number of Gaussian distributions (and their parameters) that best fit the data. The optimal number of clusters is chosen according to a model selection criterion (Bayesian information criterion - BIC or Integrated Classification Likelihood - ICL). For each observation we obtain a probability to belong to each class, therefore the observations can be affected to the most probable class, but these probabilities can also be studied to refine the analysis. A given class is called a species assemblage. The sites of a given class contain similar abundances for each species (e.g. high abundances for species 1 and low abundances for species 2).

The spatial analysis, performed in the second stage of CASA, can answer questions such as: “Are similar observations clustered?” (by testing if the classes obtained in the first stage are clustered), “Do other environmental factors explain the distribution of similar observations?” (by comparing the distributions of a class with the distribution of an environmental factor).

In our case, a site is a 25×25 parcel and an observation corresponds to the two species abundances (*H. tenuis* and *S. molestus*) measured in a parcel. The parcels where both species were absent were excluded of the analysis.

Analyses were performed with the RcodeCASA (GEORGESCU et al., 2009), which uses the mclust package (FRALEY; RAFTERY, 2006), with the statistical computer program R (R DEVELOPMENT CORE TEAM, 2005).

3 RESULTS

The exclusion of parcels where both species were absent resulted in 605 non-empty plots (Figure 5 (left)). The logarithmic transformation of abundance for both species is shown in Figure 5(right).

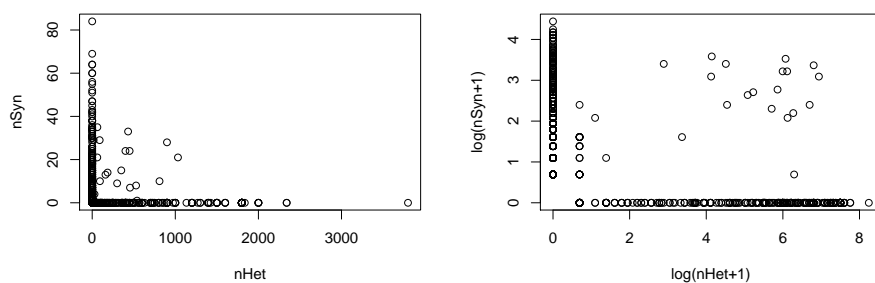


Figure 5 Scatterplot of the abundances of *Heterotermes tenuis* (nHet) and *Syntermes molestus* (nSyn) (left) and of the log-transformed abundances (right). (NB: Many points are superposed on this Figure, when there are identical observations at different sites)

The classification stage of CASA provided 5 optimal classes with the BIC criterion (which corresponds to the maximum in Figure 6(a)).

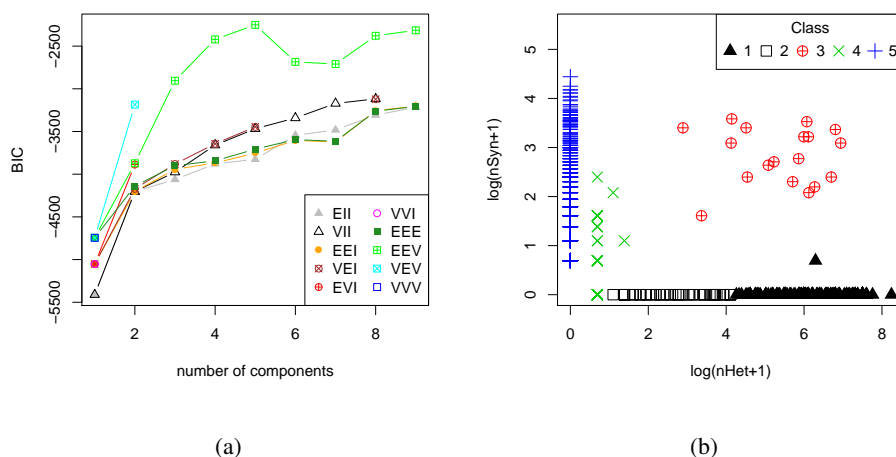


Figure 6 Classification stage of CASA: (a) BIC criterion for different number of clusters and constraints of the model (for details on different models represents for E, I and V combination see Fraley and Raftery, 2006). (b) Classification on the log-transformed abundances of *Heterotermes tenuis* (nHet) and *Syntermes molestus* (nSyn).

We choose the first peak of the BIC and do not investigate further (even though we can see that the BIC criterion increases slowly, so there might be another peak further) because the number of classes should be limited in order to be interpretable. The ICL criterion (BIERNACKI; CELEUX; GOVAERT, 2000) provides the same number of classes. The 5 classes (types of termites assemblages) are represented Figure 6(b). Classes 1 and 2 represent sites infested by *H. tenuis* alone, as opposed to class 5 with parcels infested only by *S. molestus*. The two species coexist in classes 3 and 4, both species being more abundant in class 3. Note that there are several exceptions to this description of the classes: 1 observation in class 1 where *Syntermes* is present (nSyn=1) and 23 observations in class 4 where *Syntermes* are absent (nHet=1 and nSyn=0). The uncertainty for this classification is generally very low (close to 0) except for a few observations

between the classes 1 and 2 (where probabilities of belonging to either of the two classes is close to 0.5) and for the 23 observations in class 4 where *Syntermes* are absent (nHet=1 and nSyn=0), which were affected to class 4 with probability 0.74 but their probability to belong to class 2 is 0.26. In order to simplify the interpretation of these classes we affected the latter 23 observations to class 2 (*Heterotermes* alone, low abundances). We obtain the modified classification in Figure 7, with the following simplified description :

- Class 1 (▲): *Heterotermes* alone, high abundances (70 to 3800),
- Class 2 (□): *Heterotermes* alone, low abundances (1 to 60),
- Class 3 (⊕): both species, high abundances (nHet: 17 to 1032, nSyn: 4 to 35),
- Class 4 (×): both species, low abundances (nHet: 1 to 3, nSyn: 1 to 10),
- Class 5 (+): *Syntermes* alone (1 to 84).

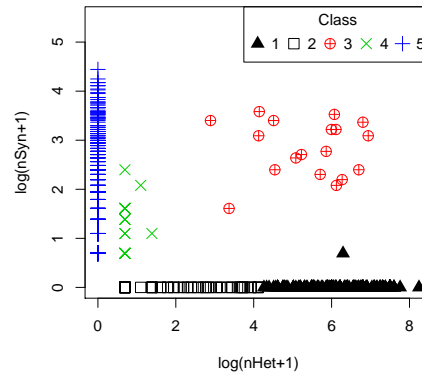
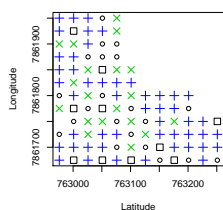
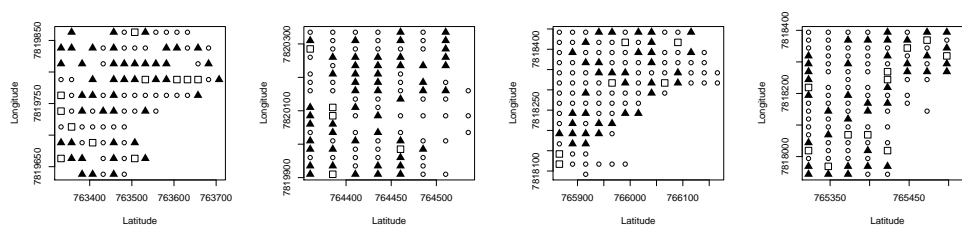


Figure 7 Classification into 5 types of assemblages on the log-transformed abundances. Class 1 (▲): *Heterotermes* alone, high abundances (70 to 3800), Class 2 (□): *Heterotermes* alone, low abundances (1 to 60), Class 3 (⊕): both species, high abundances (nHet: 17 to 1032, nSyn: 4 to 35), Class 4 (×): both species, low abundances (nHet: 1 to 3, nSyn: 1 to 10) and Class 5 (+): *Syntermes* alone (1 to 84).

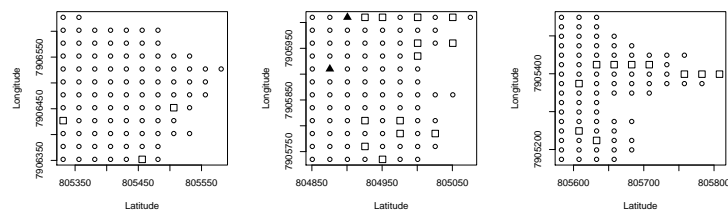
Figures 8 and Figure 9 represent the maps of the 5 termites assemblages over the 6 farms of this study. As expected, *Heterotermes* is found preferentially in forest vegetation and *Syntermes* in pasture vegetation. Even though the two species coexist as well in the forest vegetation of Cajá farm (Figure 8(a)) as in the pasture vegetation of Recanto farm (Figure 9(b)), species are both abundant only in the low-altitude pasture of Recanto farm, which is the only block where class 3 (⊕) is present.



(a) Cajá: forest, 266m



(b) Lagoa Redonda: forest, 273m



(c) Três Morros: forest, 888m

Figure 8 Wood-dominated farms: (a)–(c) Maps of the types of assemblages of *S. molestus* and *H. tenuis*. Classification into 5 types of assemblages on the log-transformed abundances. Class 1 (▲): *Heterotermes* alone, high abundances (70 to 3800), Class 2 (□): *Heterotermes* alone, low abundances (1 to 60), Class 4 (×): both species, low abundances (nHet: 1 to 3, nSyn: 1 to 10) and Class 5 (+): *Syntermes* alone (1 to 84). Empty circles are observations where termites were absent.

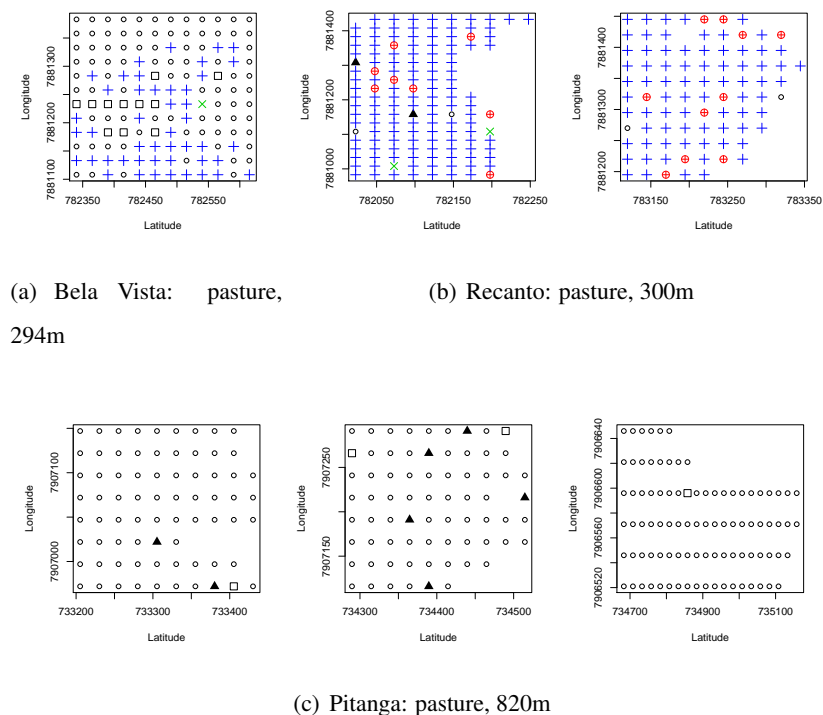


Figure 9 Maps of the types of assemblages of *S. molestus* and *H. tenuis* over the grass-dominated farms. Classification into 5 types of assemblages on the log-transformed abundances. Class 1 (\blacktriangle): *Heterotermes* alone, high abundances (70 to 3800), Class 2 (\square): *Heterotermes* alone, low abundances (1 to 60), Class 3 (\oplus): both species, high abundances (nHet: 17 to 1032, nSyn: 4 to 35), Class 4 (\times): both species, low abundances (nHet: 1 to 3, nSyn: 1 to 10) and Class 5 ($+$): *Syntermes* alone (1 to 84). Empty circles are observations where termites were absent.

Table 4 sums up the types of vegetation and altitudes for each class. It shows that classes 1 and 2, corresponding to *Heterotermes* alone, are the most diverse in terms of vegetation and altitude, which implies that *H. tenuis* can survive at high or low altitudes in forest or pasture vegetation, although it has a preference

Table 4 Classification in the classes for vegetation, altitude (m), numbers of observations (Nobs) and farms for *Syntermes molestus* and *Heterotermes tenuis* abundance. Vegetation, altitude, number of observations (N) and farms for the 5 classes (types of species assemblages). Class 1 (▲): *Heterotermes* alone, high abundances (70 to 3800), Class 2 (□): *Heterotermes* alone, low abundances (1 to 60), Class 3 (⊕): both species, high abundances (nHet: 17 to 1032, nSyn: 4 to 35), Class 4 (×): both species, low abundances (nHet: 1 to 3, nSyn: 1 to 10) and Class 5 (+): *Syntermes* alone (1 to 84).

Class	N (%)	Vegetation	Altitude (m)	N	Farm
1 ▲	185 (30%)	forest	low (273)	174	Lagoa Redonda
			high (888)	2	Três Morros
		pasture	low (300)	2	Recanto
			high (820)	7	Pitanga
2 □	84 (14%)	forest	low (266-273)	43	Cajá & Lagoa Redonda
			high (888)	26	Três Morros
		pasture	low (294)	11	Bela Vista
			high (820)	4	Pitanga
3 ⊕	18 (3%)	pasture	low (300)	18	Recanto
4 ×	22 (4%)	forest	low (266)	19	Cajá
		pasture	low (294-300)	3	Bela Vista & Recanto
5 +	296 (49%)	forest	low (266)	49	Cajá
		pasture	low (294-300)	247	Bela Vista & Recanto

for forest vegetation at low altitudes. Conversely, class 3 is found only in pastures at low altitudes, which suggests that this type of environment favours coexistence of the two species. Species coexist also in class 4 but are both less abundant. This class can be found in low altitude pastures (Figure 9(a), 9(b)) but also in low altitude forests (Figure 8(a)). Class 5 (*Syntermes* alone) is found only at low altitudes with a preference for pasture. The vegetation preferred by each termite species is not surprising given their feeding habits.

Table 5 summarizes the number of observations and the proportion affected to each of the classes for each category of the two variables vegetation

and altitude (in a sort of contingency table). This table shows another aspect of the results, namely the prediction quality of the two external factors considered in this study (vegetation and altitude) or, in other words, what classes and in what proportion are expected in each environment (combination of factors). For example in high altitude forests we expect to find *Heterotermes* alone, with a majority of observations in class 2 (less than 60). These factors are particularly good predictors for class 3 (⊕), expected only in low altitude pastures.

Table 5 Are vegetation and altitude good predictors for termites assemblages?: Number of observations and proportion affected to each of the classes for each category of the variables vegetation and altitude. Class 1 (▲): *Heterotermes* alone, high abundances (70 to 3800), Class 2 (□): *Heterotermes* alone, low abundances (1 to 60), Class 3 (⊕): both species, high abundances (nHet: 17 to 1032, nSyn: 4 to 35), Class 4 (×): both species, low abundances (nHet: 1 to 3, nSyn: 1 to 10) and Class 5 (+): *Syntermes* alone (1 to 84).

		Altitude	
		low	high
Vegetation	forest	285 sites	28 sites
		61% ▲, 17% +, 15% □, 7% ×	93% □, 7% ▲
	pasture	281 sites	11 sites
		88% +, 6% ⊕, 4% □, 1% ▲, 1% ×	64% ▲, 36% □

4 DISCUSSION AND CONCLUSION

Vegetation and altitude play an important role on the distribution of *H. tenuis* and *S. molestus*. Knowing how these two factors (and other factors) influence the distribution of these termite species can be useful for improving the designs for survey and sampling purposes. In terms of vegetation, the result of this study reflects well the feeding preferences of the two species: grass and

leaves for *S. molestus*, which prefers pastures, versus bark and wood for *H. tenuis*, which prefers forests (BIGNELL; EGGLETON, 2000). The altitudinal factor is negatively correlated with termites abundance (COLLINS, 1980), but wood feeders seem to be less affected than other groups (PALIN et al., 2010). Indeed, we observed less infested samples when the altitude for *H. tenuis* increases (Figure 8 and Figure 9). *S. molestus* are absent in altitudes greater than 300m, but can be abundant in low altitudes.

The two external factors we observed, vegetation (V) and altitude (A), can play the role of predictors for the abundances of the two termite species. The following conclusions can be drawn:

1. *S. molestus*

- (A) *S. molestus* is not expected at high altitude (never in our data),
- (V) When present, *S. molestus* is not abundant in forest vegetation (less than 10 in our observations).

2. *H. tenuis*

- (A) The distribution of *H. tenuis* seems to be affected by altitude: even though abundances of *H. tenuis* can be high at high altitudes (cf. Figure 9(c), 8(c)), the number of infested sites is considerably lower than those at low altitudes (cf. Figure 8(b), 9(b), Table 3),
- (V) The abundance of *H. tenuis* does not seem to be affected by vegetation: it can be abundant in pastures (cf. Pitanga Figure 9(c) and Recanto Figure 9(b)) as well as in forests (Figure 8(b) and 8(c)).

3. Coexistence :

- (A) Coexistence of the two species occurs only at low altitudes,

- (V) High abundances for both species is expected only in a pasture vegetation.

More data is needed to confirm these conclusions. These results suggest several orientations for future studies. In order to refine the study of the relationship between the two species, sampling effort should be concentrated on low altitude pastures, since these seem to favor coexistence of the species. Other external factors (such as temperature, humidity, soil characteristics as diverse as organic content, biological activity, pedo-chemical parameters) should be used to improve the prediction of classes or the prediction of the abundance of *S. molestus* given the abundance of *H. tenuis* (which is easy to sample). A special attention could be brought to the comparison of the pastures at Bela Vista and Recanto (Fig 9(a) and 9(b)) in order to examine the possible factors which seem to favour the coexistence of both species with high abundance in Recanto pasture. The same focus should be brought to the comparison between Cajá (forest, low altitude) and Recanto (pasture, low altitude) to understand if the type of vegetation is the only factor that limits the abundance of coexistent species in Cajá forest.

Beyond the biological and ecological interests of this study to understand the factors that govern the coexistence of the two major pests for eucalyptus plantation, we propose, based on our results, a practical improvement of the current pest management practices in order to reduce the use of pesticides. Firstly, the preventive control of eucalyptus plants against *S. molestus* should not be used at high altitudes, where this species has not been found. At low altitudes, we propose an alternative sampling strategy for pest management purposes in order to save time in the sampling of *S. molestus*. *H. tenuis* needs to be evaluated with cardboard baits for all vegetation types and at all altitudes because it is the only species expected in all locations. In pre-planting stage of eucalyptus forests and mainly in

pastures at low altitudes, we propose a sampling procedure in two phases. Firstly, a systematic plan for sampling *H. tenuis* with cardboard baits has to be done on a grid sampling pattern with node distance fixed at 25m. At the time of bait removal (after 30 days), *S. molestus* could be sampled by counting the foraging holes within a circular sub-parcel (10m radius) centered on grid nodes. Starting from one hedge corner of the studied area, sampling could proceed systematically until *S. molestus* foraging holes are observed. As observed in our data, when coexistence is encountered, it is wide spread, at low or high level. To evaluate the level at which the two species are found coexisting, few spots will be necessary. In any case, the whole area does not have to be sampled.

Classification and spatial analysis with CASA can contribute to the better understanding of the ecological factors responsible of coexistence of the species and could thus contribute to the improvement of pest control strategies.

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