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# Generalized linear mixed models: an application in fungal occurrence data

Thiago P. Oliveira<sup>1</sup>, Rafael A. Moral<sup>1</sup>, John Hinde<sup>2</sup>, Clarice G.B. Demétrio<sup>1</sup>, Silvio S. Zocchi<sup>1</sup>

<sup>1</sup> Universidade de São Paulo, Piracicaba, Brazil

<sup>2</sup> National University of Ireland Galway, Galway, Ireland

E-mail for correspondence: [thiago.paula.oliveira@gmail.com](mailto:thiago.paula.oliveira@gmail.com)

**Abstract:** When analysing proportion data, a useful framework is that of generalized linear models. Random effects may be included in the linear predictor for different reasons, e.g., to incorporate correlation between observations taken within the same subject or to model overdispersion. In this work, we use binomial mixed models to model the occurrence of entomopathogenic fungi in five different Brazilian biomes in the dry and humid seasons of 2012. We add an observation-level random effect to incorporate overdispersion and test for the significance of the interaction effect between biome and season.

**Keywords:** Overdispersion, Biological control; `lme4` package; Logistic-normal model.

## 1 Introduction

In Brazil there are several ecosystems and living organisms that are associated to the climatic conditions, soil, water and other factors. A range of microorganisms live in the soil, amongst them the fungi, which may contribute to arthropod regulation. There are several products made with fungi used to control insects in different crops, e.g., *Beauveria bassiana*, *Isaria fumosorosea* and *Metarhizium anisopliae*, see Faria and Wraight (2007). In this context, it is important to understand the occurrence of these entomopathogenic fungi in different Brazilian biomes to plan preservation strategies and test their potential in controlling different pests.

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## 2 Material and methods

### 2.1 Case-study

To study the occurrence of entomopathogenic fungi, soil samples were collected in areas that represented the native vegetation of the Brazilian biomes Amazon, Caatinga, Atlantic Forest, Cerrado and the Pampas in the humid and dry seasons of 2012. Sampling was made in four different farms per biome and in each farm, six points were sampled, totaling 120 observations per season. Then, 10 larvae of *Galleria mellonella* and 10 larvae of *Tenebrio molitor* were exposed to each soil sample and after three weeks, the number of insects dead due to fungal infection was observed. This is a hierarchical design and observations within the same farm are correlated so this must be taken into account in the modelling process.

### 2.2 Statistical models

The data in this experiment consist of proportions so a reasonable assumption is that the number of insects dead due to fungal infection  $Y_{ijk} \sim \text{Bin}(m_{ijk}, \pi_{ijk})$ . An initial step was to fit a binomial generalized linear mixed model with logit link and the linear predictor

$$\text{logit}(\pi_{ijk}) = \mu + \alpha_i + \gamma_j + (\alpha\gamma)_{ij} + \sigma_Z z_{ij}, \quad (1)$$

where  $\mu$  is the intercept,  $\alpha_i$  is the effect of the  $i$ -th biome,  $\gamma_j$  is the effect of the  $j$ -th season,  $(\alpha\gamma)_{ij}$  is the interaction between the  $i$ -th biome and the  $j$ -th season,  $\sigma_Z^2$  is the variance of the random effect associated with the farms and  $Z_{ij}$  are standard normal random variables.

To model overdispersion, a random effect at the observation level was included in the linear predictor, so that model (1) became

$$\text{logit}(\pi_{ijk}) = \mu + \alpha_i + \gamma_j + (\alpha\gamma)_{ij} + \sigma_Z z_{ij} + \sigma_W w_{ijk}, \quad (2)$$

where  $\sigma_W^2$  is the variance of the random effect at the observation level and  $W_{ijk}$  are standard normal random variables. This model is also called a logistic-normal model, see Demétrio et al. (2014).

To test for the significance of fixed effects, we used likelihood-ratio tests for nested models. Goodness-of-fit was assessed via half-normal plots with simulation envelopes (Moral et al., 2014) using R (R Core Team, 2014).

## 3 Results and discussion

We verified that for both species model (1) did not fit well to the data, see Figures 1(a) and (c). So we studied the inclusion of an observation-level random effect (model (2)) to incorporate overdispersion, resulting in a better model fit, see Figures 1(b) and (d). The interaction between biome

and season was significant at 5% significance level for both species, see Table 1 for estimated parameters. It was found that a larger proportion of entomopathogenic fungi infected *G. mellonella* in the dry season when compared to the humid, however the opposite result was obtained for *T. molitor* in the Amazon, see Figure 2. Moreover, there is a clear difference between the proportion of infected insects for humid and dry seasons in the Caatinga biome for *G. mellonella*, which was not found for *T. molitor*. Apparently, the patterns are the same for both species in the biomes Cerrado, Atlantic Forest and Pampas. The data is zero-inflated for some biome $\times$ season combinations and this is subject of ongoing work.

TABLE 1. Parameter estimates for model (2) fitted to both species' data.

Parameter	Species	
	<i>G. mellonella</i>	<i>T. molitor</i>
Intercept ( $\mu$ )	-0.28 (0.30)	-3.88 (0.49)
Caatinga ( $\alpha_2$ )	1.24 (0.43)	1.09 (0.62)
Cerrado ( $\alpha_3$ )	-1.89 (0.46)	1.46 (0.61)
Atlantic Forest ( $\alpha_4$ )	-1.66 (0.46)	-0.15 (0.69)
Pampas ( $\alpha_5$ )	-1.92 (0.46)	4.92 (0.61)
Humid season ( $\gamma_2$ )	-3.34 (0.55)	1.78 (0.60)
Caatinga $\times$ humid season ( $\alpha\gamma_{22}$ )	3.74 (1.21)	-1.42 (0.82)
Cerrado $\times$ humid season ( $\alpha\gamma_{32}$ )	5.96 (0.73)	0.61 (0.78)
Atlantic Forest $\times$ humid season ( $\alpha\gamma_{42}$ )	1.75 (0.77)	-2.53 (0.99)
Pampas $\times$ humid season ( $\alpha\gamma_{52}$ )	3.81 (0.73)	-1.99 (0.77)
$\sigma_Z$	0.0143	0.0898
$\sigma_W$	1.2766	1.4082

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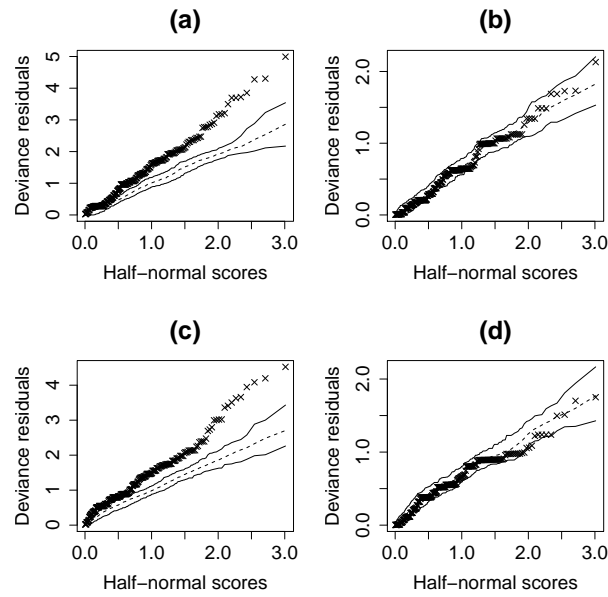


FIGURE 1. Half-normal plots with simulation envelopes for (a) model (1) and (b) model (2) for *G. mellonella* and (c) model (1) and (d) model (2) for *T. molitor*.

with simulation envelopes. R package version 1.0. URL <http://CRAN.R-project.org/package=hnp>.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

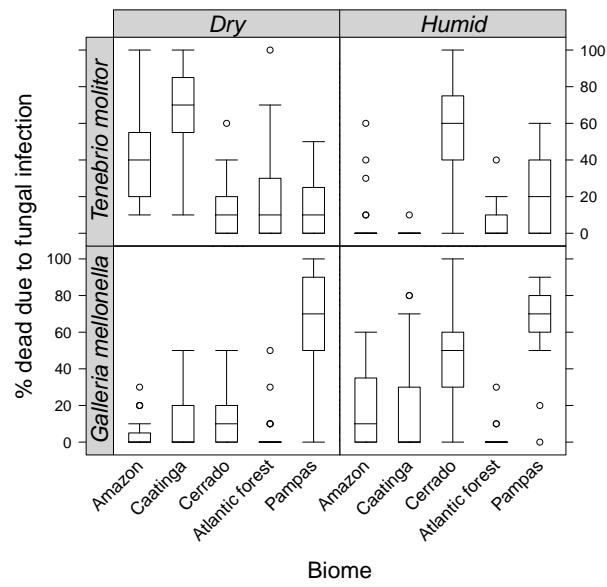


FIGURE 2. Box-plots for each biome×season combination for both species.