The present study collects the results obtained in different agricultural farms of the Iberian Peninsula, demonstrating how right agricultural practices can also help to maintain biodiversity and favour its rapid increase, both qualitatively and quantitatively.

1.14 Applied statistics in field and semi-field studies with bees

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DOI 10.5073/jka.2020.465.014

Abstract

Field and semi-field studies are important tools in the ecotoxicological risk assessment of plant protection products for bees (honey bees, bumblebees and solitary bees). While these studies represent far more realistic conditions than laboratory tests, they also present a challenge for the analysis and interpretation due to the large and complex datasets. Therefore, in order to correctly answer the underlying ecotoxicological questions, it is crucial that these studies are not only thoroughly planned and conducted, it is also important that they are subjected to adequate statistical analysis. Our aim is to provide a better understanding on how to conduct and interpret statistical analyses in field and semi-field studies with bees made for regulatory purposes. An overview of how study design and statistics should be aligned with each other is given including the specific challenges of (semi-) field trials, as for instance how to address the problem of pseudoreplication if hives are regarded as experimental units. Different statistical tools are compared and their suitability for different data types and questions are discussed. Generalized Linear (Mixed) Models (GLMMs) are evaluated in more detail as they provide a flexible and robust tool for the analysis of honey bee (semi-) field data. Furthermore, some more light is shed on what p-values really tell us, how they can help to interpret data and how they should not be misinterpreted.

Keywords: Applied statistic, bees, field studies, plant protection products

Introduction

Field and semi-field studies are important tools in the ecotoxicological risk assessment of plant protection products for bees (honey bees, bumblebees and solitary bees). While these studies represent far more realistic conditions than laboratory tests, they also are a challenge for the analysis and interpretation due to the large and complex datasets. Therefore, in order to answer the underlying ecotoxicological questions correctly, it is crucial that these studies are not only thoroughly planned and conducted but also subjected to adequate statistical analyses. The choice of method for the analysis depends on the experimental setup, the consequential data set, and the possible effects. The steps that should be followed to obtain a satisfying and meaningful result and the challenges that have to be considered on the way are explained in the following.

Data exploration

Data exploration is a crucial step in analyzing the data that should preceed any further analysis. It intends to familiarize oneself with the data and getting to know its limitations. Data exploration includes the investigation of outliers, homogeneity, normality, zero observations, correlation between covariates (collinearity), nonlinear relationships among variables, temporal and spatial dependency (Zuur *et al*. 2010, Zuur *et al*. 2016).

Statistical methods

A key advice in statistics is to 'keep it simple', indicating that the simplest statistical test should be applied to the data but only if it is applied correctly. Often 'real world' data violate the assumptions of simple tests like ANOVA or linear regression (*i.e*., normality, homogeneity, independence of data).

Depending on the typology of the response variable and limitations detected during data exploration the adequate model is fitted: (G)LMM, beta regression model, Zero-inflated model or GAMM to name only the most common.

(Generalized) Linear (Mixed) Models ((G)L(M)Ms) are a flexible tool to apply more rigorous but more realistic statistical models to the data (Pirk *et al*. 2013).

There are multiple possible benefits that arise from using a (G)LMM.

Application to non-normally distributed data

Most ecological data are not normally distributed except weight data. Hypothesis tests such as the t-test rely on the normality assumption (although often these tests are quite robust against a violation of this assumption). The normality assumption states that the residuals of the tested data have to be normally distributed. If the test is a good fit, this corresponds to the data itself being normally distributed. However, if the data is not normally distributed, the test is not a good fit. The distribution determines which values can occur. The distribution of the data can be included into a GL(M)M by specifying the 'family' (a linear (mixed) model is used only for normally distributed data by setting the family to 'Gaussian').

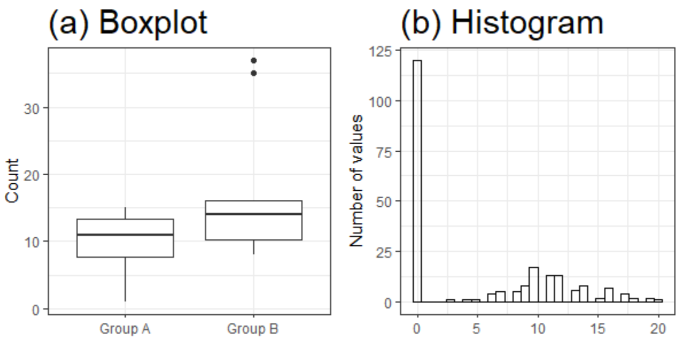

Fig. 1 (a) Examplary boxplot indicating two outliers per group. (b) Historgram of exemplary count data indicating zero-inflation.

I. Inclusion of multiple, interacting explanatory variables
Depending on the endpoint and the test system, more than one explanatory variable might influence the outcome of the test. Assessing the same parameter at different days can result in a time related influence. A treatment effect might only show up during some days of the assessment period. Another example is a treatment effect that is limited to one sex. All these variables can be included into a (G)L(M)M either independently or as an interaction between multiple variables. Furthermore, explanatory variables, which are known to influence an endpoint (*e.g.*, temperature and development), can be included into the model to reduce the amount of unexplained variability.

II. Application to dependent data (pseudoreplication) and random effects
The experimental setup of field studies often results in one major challenge during analysis: the lack of statistical independence in the replicates of field studies (Hurlbert 1984). In the case of full field honey bee studies this pseudoreplication arises from for example repeated sampling of individual hives and/or a study set up with several hives per study field. These study designs lead to biased parameter estimates and increased type I errors in regression models if not handled appropriately. This kind of pseudoreplication can be dealt with by applying multilevel models (*e.g.*, generalized mixed-effects models (GLMMs and GAMMs) (Pinheiro and Bates 2000)).
Random effects can be included into mixed models to account for differences between groups (*e.g.*, tunnel or field specific effects) and dependencies in the data. While a fixed effect applies to all groups, a random effect may vary across groups. An example is a field study with several colonies per field. The colony size is assessed multiple times throughout the study period and therefore, the data are not independent. A nested random effect can

be included into the mixed model to relate a) the data of the colonies located on one field and b) the data of one single colony over time.

III. Handling of zero-inflated data
In data from ecological studies, the occurrence of zeros is common (*e.g.*, occurence of a rare species, occupancy of nesting hole at low population densities). If the proportion of zeros to non-zero values is high (*i.e.*, higher than expected from the data distribution family, see I.), this is called zero-inflation. Several types of statistical models have been developed that can handle this situation, like zero-inflated GL(M)Ms or hurdle models.

Validation of the statistical model

Once the adequate model is selected and fitted to the data, it has to be validated. Model validation is important to verify that assumptions such as independence and absence of residual patterns are not violated (Zuur *et al.* 2016). Fitted models are validated by plotting standardized or Pearson residuals against fitted values, against each covariate in the model and against each covariate not in the model. To add a regression line aids visual interpretation. If the data include temporal (or spatial) aspects, autocorrelation functions and/or variograms should be used to assess independence of residuals.

Presentation of the results

Grasping the biological relevance of the numerical output of a statistical model may be a challenge for readers. To facilitate comparability the following information should be given: parameter estimates, standard errors, t-values, R^2 and the estimated variance. Whether p-values should be included is an ongoing debate. In the recommended techniques, p-values are approximate at best and should be interpreted with care. It is important to notice, that p-values do not show how well the model explains the data, do not give any estimate on the effect size and do not represent the likelihood of any hypotheses to be true. They show how often after infinite repetitions of the experiment an effect as observed (or greater) would occur by chance. The value of 0.05 (5%) is a convention. An alternative for the use of p-values is to present 95% confidence intervals for the regression parameters and effect size estimates and their precision.

Plotting results facilitates comprehension, as graphs are more effective at imparting information, especially if interactions are included in the model. For models with multiple covariates and interactions multipanel plots proved to be useful.

Conclusion

In ecotoxicological field and semi-field studies, increasingly complex data sets are obtained for which sophisticated statistical approaches are required. Statistical models form a set of methods to handle different types of challenges that come with this kind of data. They are able to handle non-normality, pseudoreplication and dependent data, zero-inflation and the inclusion of multiple possible explanatory variables. However, their application depends on the particular dataset. Before starting the statistical analysis, the characteristics of the data need to be explored. After the analysis with a statistical model, the model has to be validated to show its accordance with the model assumptions. The results should be presented in a comprehensive way but still include all necessary information.

If performed and interpreted correctly, data analysis of field and semi-field studies with statistical models is a powerful tool to identify the risk to bees in ecotoxicological risk assessment of plant protection products.

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1.15 ICPPR WG Semi-field and field Report and Discussion

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DOI 10.5073/jka.2020.465.015

Abstract

The ICPPR Semi-Field/Field Testing (SF/FT) workgroup consists of several 'writing groups' that are focused developing technical guidance that is focused on 4 separate but related topics: 1) designing and conducting pollen and nectar residue studies, 2) conducting large scale colony feeding studies, 3) updating guidance for conducting semi-field tunnel studies, and 4) design and interpretation of full field studies with bees. What follows is the current status of each of these activities.

Bee-Relevant Field Residue Studies. At the present time, detailed regulatory guidance for conducting field studies of pesticide residues in with pollen and nectar is lacking. Therefore, the Residue Study Writing Group is drafting guidance that is designed to increase the consistency, defensibility and utility of bee-relevant residue studies for use in regulatory risk assessment. Importantly, this guidance is being tailored to address specific regulatory objectives of bee-relevant residue studies which may vary among pesticides and regulatory authorities. Areas of focus include guidance on:

Spatial Scale: (*e.g.,* defining representative sites, minimum # of sites to include)
Temporal Scale: (*e.g.,* sample timing, intervals, number of samples, # replicates)
Crop Selection & Sampling Methods: (*e.g.,* selecting appropriate crops and matrices for sampling, choosing sampling methods)
Pesticide Application: (*e.g.,* determining the appropriate application timing, rate, intervals)
Analytical methods: (*e.g.,* methods validation/recovery, LOQ/LOD)
Statistical analysis: (determination of DT50s, consideration of outliers)

To date, existing regulatory guidance relating to bee-relevant residue studies has been compiled and summarized, in addition to common regulatory objectives of such studies. Based on these objectives, technical guidance on the aforementioned topics is being drafted. In addition, bee-relevant residue data are from EPA and EFSA sources being compiled into a common database for additional analysis. Draft guidance for review by the SF/FT is expected during the summer of 2020 with a final guidance being drafted by the end of 2020.

Current Residue Writing Group Members: Keith Sappington (chair), Jeremey Barnekow, Sigrun Bocksch, Silvia Hinarejos, Stefan Kimmel, Silvio Knäbe, Raj Singh

Large-Scale Colony Feeding Studies. Within the last decade, regulatory authorities in Europe, North America, and elsewhere have greatly expanded their procedures for quantifying pesticide risks to bees to include a tiered approach. As a higher tier level approach, regulatory authorities in North America have quantitatively used results from "Large Scale Colony Feeding Studies" (LSCFS) to associate honey bee colony-level impacts with exposure to pesticides mostly via in-hive sucrose solution in a concentration-dependent manner. Examples of LSCFS with exposure to pesticides via pollen patties are more limited. Because of its design, the LSCFS is not specific to any particular crop and can be directly compared to nectar and pollen residues from multiple crops. The LSCFS design involves a relatively large number of replicates (*e.g.*, 12 separate replicate/apiaries), multiple (*e.g.*, five) treatment levels, and periodic colony condition assessments (*e.g.,* 8-9 assessments over 12+ months, including pre-exposure, exposure and post-exposure periods). Despite its continued use in regulatory risk assessments, no formal regulatory protocol exists for conducting the LSCFS.