Impact of fungi on control of bollworms *Chrysodeixis includens* and *Helicoverpa armigera*

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Outline

1. Introduction
2. Models and methods
3. Results and discussion
4. Final remarks

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Introduction

Entomological motivation

Species of interest:

- *Chrysodeixis includens*: the “soybean looper”;
- *Helicoverpa armigera*: the “cotton bollworm”;
- They feed feed on a wide range of plants, including many important cultivated crops (agronomic crops: soybean, cotton, maize, etc. and also vegetable and floricultural crops).

Biological pest control:

- Controlling pest population using other organisms;
- An important biological control agents are pathogenic fungi.

Research question:

- The inoculation of fungi in soybean plants may inhibit the development of bollworms *Chrysodeixis includens* and *Helicoverpa armigera*?

Design of the experiment

Treatments:

- Three species of fungi:
  Metarhizium anisopliae ESALQ-1638 (`Met 1638`);
  Beauveria bassiana ESALQ-3399 (`Bb 3399`); and
  Isaria fumosorosea ESALQ-3422 (`If 3422`);
- Control (Tween 80).

Experiment with whole plants:

- The fungi were inoculated on the commercial subtracts to cultivate the soybean plants.
- 30 bollworms (for each treatment) were confined in a pot with a plant, where the substrate was isolated.
- The plots were evaluated every three days during 18 days for *Chrysodeixis includens* and 21 days for *Helicoverpa armigera*.
- This design were repeated two times in different periods.

Outcomes:

- Weight of bollworms over time (longitudinal data) and
- Time to death of the bollworms (time-to-event data).

Design of the experiment

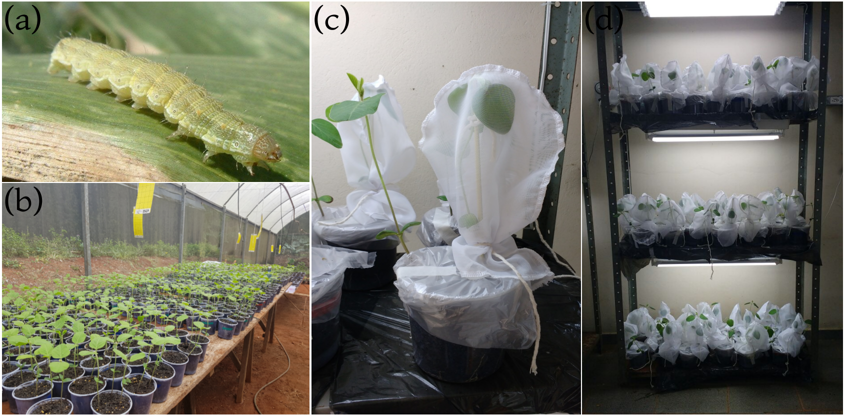
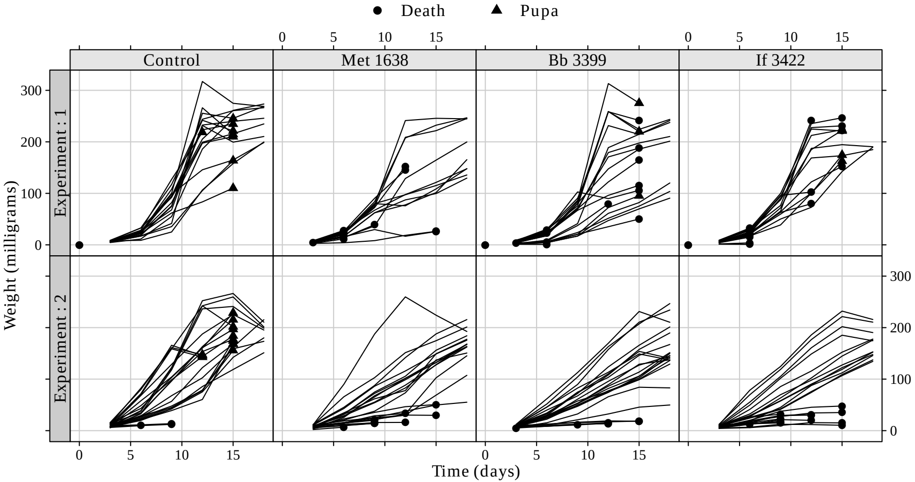


Figure: Pictures of the experiment: (a) *Helicoverpa armigera*, (b) soybean plants, (c)-(b) bollworms confined in the pots.

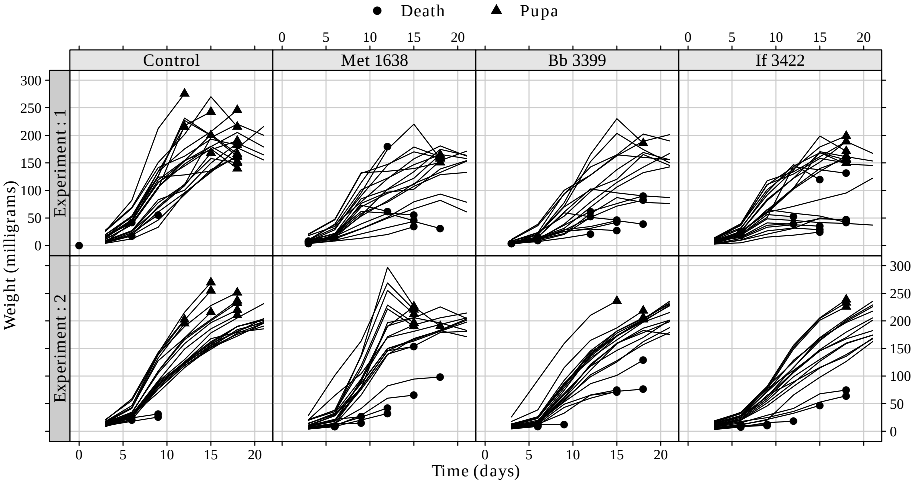
Descriptive analysis (longitudinal data)



(a) Specie: ***Chrysodeixis includens***

Figure: Data on weights of the *Chrysodeixis includens* bollworms over time. The symbols ● and ▲ indicate death and pupa stage, respectively.

Descriptive analysis (longitudinal data)



(b) Specie: ***Helicoverpa armigera***

Figure: Data on weights of the *Helicoverpa armigera* bollworms over time. The symbols ● and ▲ indicate death and pupa stage, respectively.

Descriptive analysis (time-to-event data)

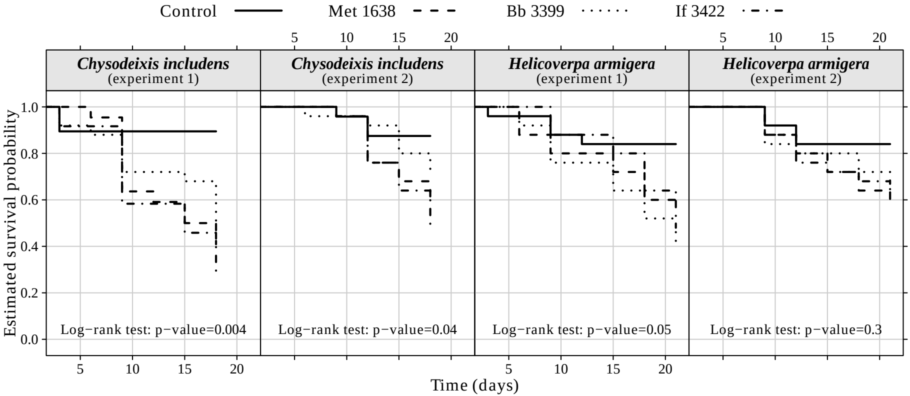


Figure: Kaplan-meier survivor function estimates for the times to death of the bollworms.

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Models and methods

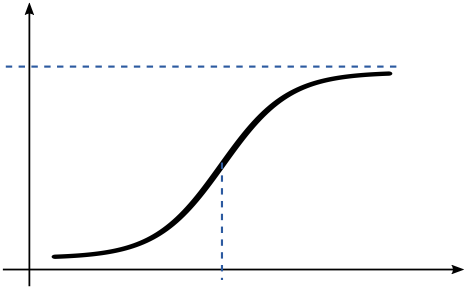
Non-linear models (logistic growth)

To model the weight growth of bollworms (Y) over time (t), we consider the logistic growth,

$$\mathrm{E}(Y) = f(t) = \frac{\theta_\mathrm{A}}{1 + \exp[(\theta_\mathrm{M} - t)/\theta_\mathrm{S}]},$$

where

- θ_A is the horizontal asymptote ($f(t)$ when $t \to \infty$, if $\theta_\mathrm{S} > 0$),
- θ_M is the inflection point of the curve and
- θ_S is the scale parameter.



Heteroscedastic non-linear mixed models

Statistical challenges

- Model the correlation between measures of the same bollworm and
- Model the heteroscedastic within-error.

Fitted model

- Let (y_{ijk}, t_{ijk}) denote the weight and time (days) of the i-th bollworm on the j-th treatment at the k-th time, the fitted model can be expressed as

$$y_{ijk} = \frac{\theta_{\mathrm{A}j} + b_{\mathrm{A}i}}{1 + \exp[(\theta_{\mathrm{M}j} + b_{\mathrm{M}i} - t_{ijk})/\theta_{\mathrm{S}j}]} + \varepsilon_{ijk},$$

Variance components

- $\begin{bmatrix} b_{\mathrm{A}i} \\ b_{\mathrm{M}i} \end{bmatrix} \sim \mathcal{N}\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \mathbf{\Sigma} = \begin{bmatrix} \sigma^2_{\mathrm{A}} & \sigma_{\mathrm{AM}} \\ \sigma_{\mathrm{AM}} & \sigma^2_{\mathrm{M}} \end{bmatrix}\right),$
- $\mathrm{Var}(\varepsilon_{ijk}) = \sigma^2 \delta^2_{1j} \delta^2_{2K}.$

Accelerated failure time models

Let T_{ij} be the time-to-death of the i-th treatment and j-th bollworm, the AFT model can be expressed (where $\omega = \log(\varepsilon)$) as

$$T_{ij} = \exp(\boldsymbol{x}_{ij}^\top \beta)\varepsilon^\sigma$$
$$\log(T_{ij}) = \boldsymbol{x}_{ij}^\top \beta + \sigma\omega.$$

The distributional assumption of T_{ij} implies distribution of ω (e.g. $T_{ij} \sim \text{Weibull}(\alpha, \lambda) \implies \omega \sim \text{E.V.}(\lambda, \alpha)$).

Censoring times

- The time of event endpoint is not observed exactly;
- Right-censoring: the censored time-to-death will be a time beyond the observed time.

$$\mathcal{L}(\boldsymbol{\theta} \mid \boldsymbol{t}) = \prod_{i=1}^{n} \left[f(t_i; \boldsymbol{\theta})\right]^{\mathsf{cens}_i} \left[S(t_i; \boldsymbol{\theta})\right]^{(1-\mathsf{cens}_i)},$$

- t_i is the i-th recorded time, $i = 1, \ldots, n$,
- $\mathsf{cens}_i = 0$, if t_i is a censored time and 1 otherwise.

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Results and discussion

Non-linear models (*Chrysodeixis includens*)

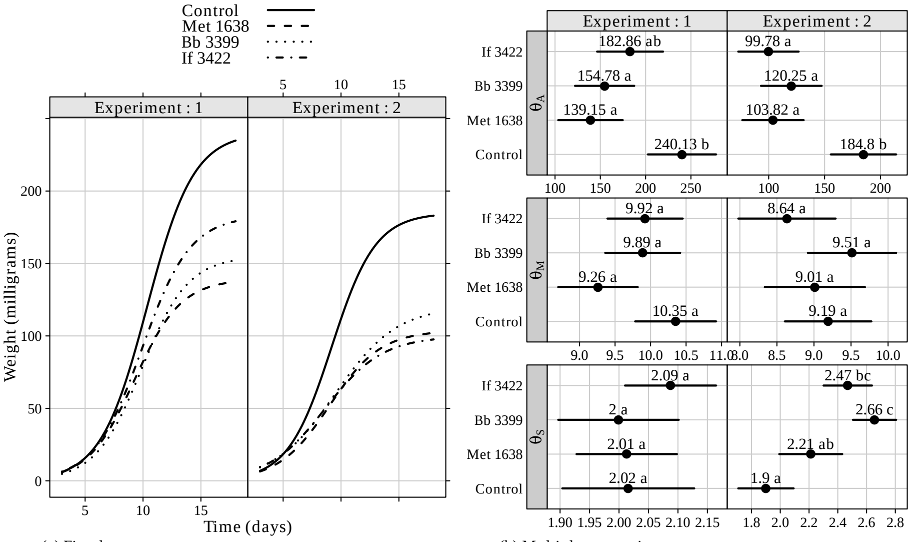
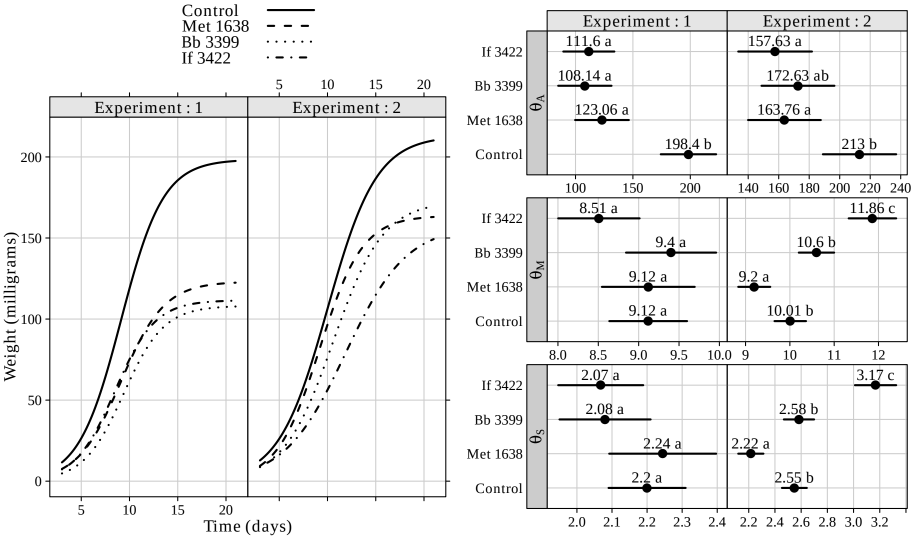


Figure: Results for *Helicoverpa armigera*. (a) fitted logistic curves and (b) parameter estimates and multiple comparisons (5% significance level).

Non-linear models (*Helicoverpa armigera*)



(a) Fitted curves (b) Multiple comparisons

Figure: Results for *Helicoverpa armigera*. (a) fitted logistic curves and (b) parameter estimates and multiple comparisons (5% significance level).

AFT models

Table: Analysis of deviance for the models fitted to the time-to-death of the bollworms *Chrysodeixis includens* and *Helicoverpa armigera*.

Specie	Effect	df	Deviance	Diff df	2 logLik	p-value
Chrysodeixis	Null			187	679.9222	
	Treatment	3	23.4077	184	656.5144	0.00003
	Experiment	1	7.7327	183	648.7817	0.00542
	Interaction	3	1.1316	180	647.6501	0.76946
Helicoverpa	Null			198	693.8224	
	Treatment	3	11.3926	195	682.4298	0.00978
	Experiment	1	1.6096	194	680.8202	0.20455
	Interaction	3	1.2429	191	679.5772	0.74272

AFT models

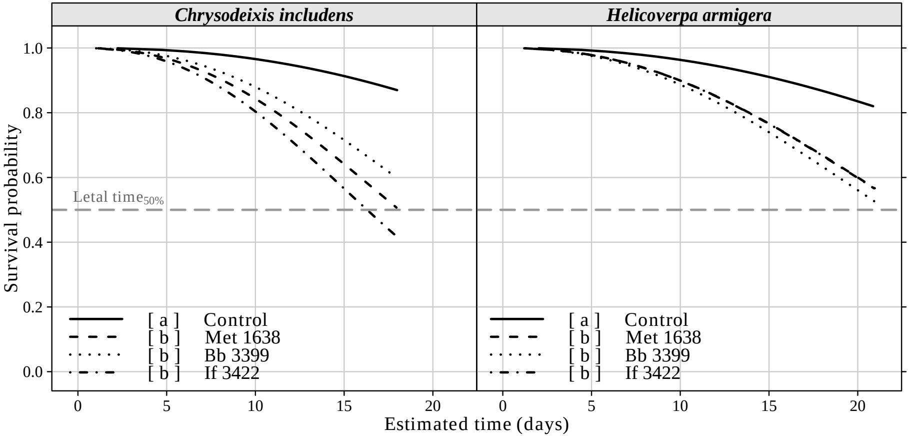


Figure: Survival curves for the estimated times to death.

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Final remarks

Data analysis results

- In this work, we evaluated the effect of fungus inoculation in soybean plants on delayed development of bollworms;
- The data analysis has shown that inoculation of fungi may delay the bollworm development.
  The weight gain and time-to-death were lower for bollworms fed with fungus inoculated plants.

Methodological contributions

- Use of non-linear models with interpretive parameters and multiple comparison tests for each parameter;
- Use of parametric models for time-to-event data with comparison of the survival curves by using the likelihood ratio test.

Future research

- Joint modelling longitudinal outcomes and time-to-event data (Rizopoulos 2012).

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