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RELAZIONE FINALE

Cox proportional-hazards model: evaluation of *Popillia japonica* survival after insecticide treatments

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Introduction

Aims of the study

Survival analysis is frequently used to assess the effectiveness of insecticide trials on insect survival (Caesar, 2003; Lopez & Sword, 2015; Ma, 2010; Moncharmont *et al.*, 2003). The target organism of this study is *Popillia japonica*, a pest currently causing considerable damage to various plant species in Northern Italy. Here, the effects of different insecticides, evaluated on insects by field trials in a peach orchard, were explored through non-parametric tests and semi-parametric marginal Cox proportional-hazard models. Adult insects were confined on peach plants in net cages containing 25 individuals each. Five insecticides (Abamectin, Acetamiprid, Deltamethrin, Phosmet and Sulfoxaflor) plus untreated control were tested with four net cages per treatment, following a completely randomized design. The non-parametric tests, performed both for right- and interval-censored data, and the marginal Cox model led to the same inferential conclusions. All the insecticides, in particular Abamectin, Acetamiprid, Deltamethrin and Phosmet, resulted to be effective in killing insects if compared to the untreated control.

Target organism

Popillia japonica Newman, 1841 (Coleoptera: Rutelidae) is a beetle native to Japan (Figure 1). Adult insects emerge in early summer and lay eggs near the soil surface continuously through their 30-day life span (Figure 2). An adult female lays 40-60 eggs in her lifetime. Within approximately two weeks, the eggs hatch and the larvae feed on fine roots and other organic material. As the larvae mature, they become c-shaped grubs which consume progressively coarser roots and may do economic damage to pasture and turf. Larvae hibernate in small cells in the soil, emerging in the spring when soil temperatures rise again. Within 4-6 weeks, the larvae pupate. Adults feed on leaf material above ground, using pheromones to attract other beetles and overwhelm plants, skeletonizing leaves from the top of the plant downward. The aggregation of beetles alternates daily between mating, feeding, and ovipositing (EFSA PLH Panel, 2018). *P. japonica* was recently recorded in Europe (2014, between Lombardy and

Piedmont Italian Regions). The insect is currently spreading from the Ticino Park, initial infested area, to the neighbouring areas causing considerable damage to crop, nursery and ornamental sectors due to its high polyphagia and adaptability. The pest is on the list of priority harmful organisms (delegated regulation (UE) 2019/1702). Therefore, the involved Regions activated all the mandatory control measures in order to eradicate and/or contain the insect. Nowadays, the pest outbreak affects more than 15000 km^2, in continuous expansion. The appearance of an alien organism requires the integration of agronomic, biological and chemical actions to protect the crops. However, during the early stages of infestation, the use of readily effective insecticides is essential to sustain the immediate needs of the crop production system. The current absence of registered products against the species requires experimental tests to evaluate the effectiveness of the active ingredients currently available on the market (EFSA PLH Panel, 2018).

Figure 1. *Popillia japonica* larvae (left) and adults (right).

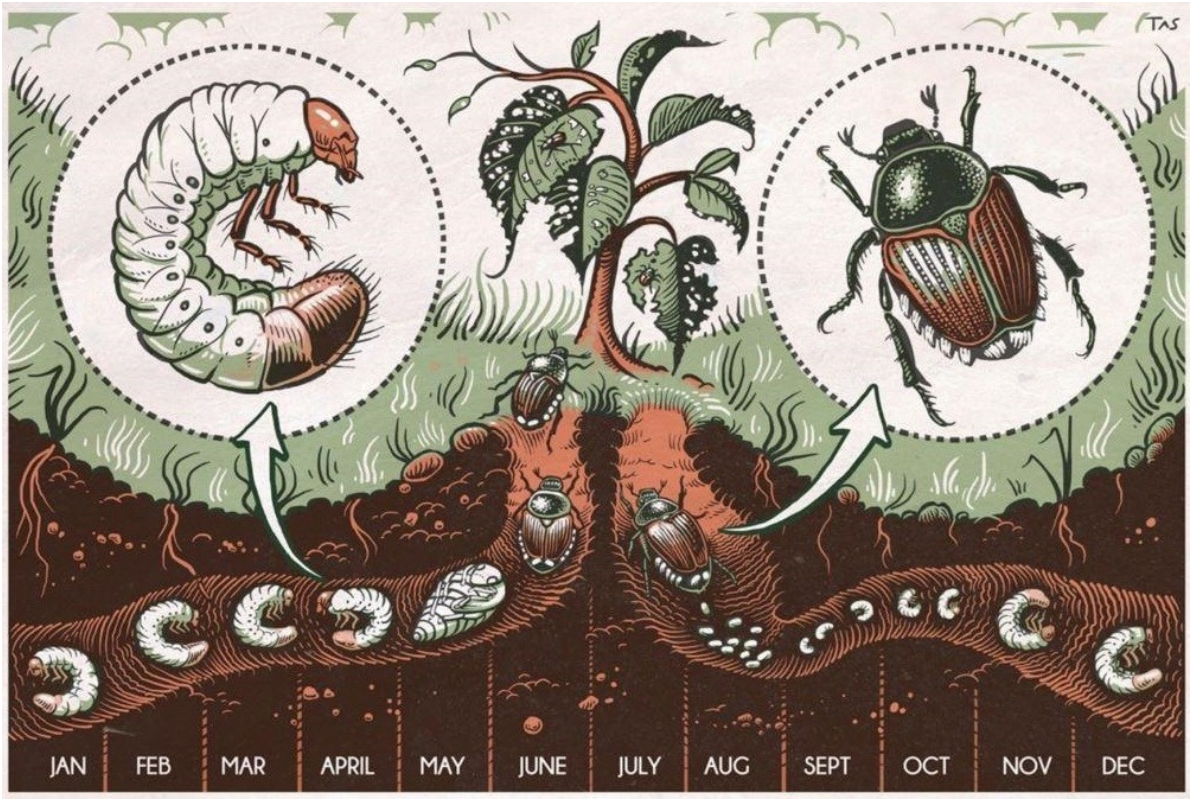

Figure 2. *Popillia japonica* lifecycle (Thomas Shahan, Oregon Department of Agriculture).

Methodology in survival analysis

The Cox model

The Cox model belongs to the survival model class of proportional-hazards models. These models relate the time that passes before an event occurs to one or more covariates that can be associated with time. In proportional-hazards models, the effect of a unit increase in a covariate is multiplicative with respect to the hazard rate or, alternatively, additive with respect to the log-hazard rate.

Survival models consist of two parts:

- The baseline hazard function (or hazard rate), $\lambda_0(t)$, outlining how the risk of event per time unit changes over time at baseline levels of covariates.
- The effect parameters, outlining how the hazard varies in response to explanatory covariates.

Covariates are multiplicatively related to the hazard.

As defined before, let X denote the time to an event. Data, based on a sample of size n, consists of the triplet $(T_j, \delta_j, \boldsymbol{Z}_j(t))$, $j = 1,\ldots, n$, where T_j is the time of study for the j^{th} subject, δ_j is the event indicator for the j^{th} subject (1 if the event has occurred, 0 if the lifetime is right-censored, *i.e.*, when a data point is above a certain time value but it is unknown by how much) and $\boldsymbol{Z}_j(t) = \boldsymbol{Z}_j = (Z_{j1},\ldots, Z_{jp})^T$ the vector of fixed-covariates, or risk factors, for the j^{th} subject at time t which may affect the survival distribution of X.

Let $\lambda(t)$ be the hazard function at time t for a subject with risk vector $\boldsymbol{Z}$. The Cox model is expressed as follows (Cox, 1972):

$$\lambda(t|\boldsymbol{Z}) = \lambda_0(t)e^{\boldsymbol{\beta}^T\boldsymbol{Z}} = \lambda_0(t)e^{\beta_1 Z_1+\cdots+\beta_p Z_p}$$

$\boldsymbol{\beta} = (\beta_1,\ldots, \beta_p)^T$ represents the vector of the p regression coefficients. In the parametric component of the model, $e^{\beta_1 Z_1+\cdots+\beta_p Z_p}$, the intercept does not appear, since it is represented by $\lambda_0(t)$. This is called a semi-parametric model because a parametric form is assumed only for the covariate effect. There are no distributive hypotheses on time.

The Cox model is called a proportional-hazards model because, looking at two subjects with covariate values $\boldsymbol{Z}$ and $\boldsymbol{Z}^*$, the ratio of their hazard rates is:

$$\frac{\lambda(t|\boldsymbol{Z})}{\lambda(t|\boldsymbol{Z}^*)} = \frac{\lambda_0(t)e^{\boldsymbol{\beta}^T\boldsymbol{Z}}}{\lambda_0(t)e^{\boldsymbol{\beta}^T\boldsymbol{Z}^*}} = \frac{\lambda_0(t)e^{\sum_{k=1}^{p}\beta_k Z_k}}{\lambda_0(t)e^{\sum_{k=1}^{p}\beta_k Z_k^*}} = e^{\sum_{k=1}^{p}\beta_k(Z_k - Z_k^*)}$$

which is a constant over time (*i.e.*, hazard rates are proportional). This ratio is the relative risk (hazard ratio) of a subject with risk factor $\boldsymbol{Z}$ having the event as compared to a subject with risk factor $\boldsymbol{Z}^*$. In particular, if Z_1 indicates the treatment effect ($Z_1 = 1$ if treatment and $Z_1 = 0$ if no treatment) and all other covariates have the same value, then $\lambda(t|\boldsymbol{Z})/\lambda(t|\boldsymbol{Z}^*) = e^{\beta_1}$ is the risk of having the event if the subject received the treatment relative to the risk of having the event if the subject did not receive the treatment.

The cumulative hazard function is defined as: $\Lambda(t|\boldsymbol{Z}) = \int_0^t \lambda(s|\boldsymbol{Z})ds = \Lambda_0(t)e^{\boldsymbol{\beta}^T\boldsymbol{Z}}$, where $\Lambda_0(t)$ represents the baseline cumulative hazard function. The survival function is defined as: $S(t|\boldsymbol{Z}) = S_0(t)^{e^{\boldsymbol{\beta}^T\boldsymbol{Z}}}$, where $S_0(t) = e^{-\Lambda_0(t)}$ represents the baseline survival function. Baselines are treated non-parametrically.

The Cox partial likelihood when there are no ties between events is defined as follow (Breslow, 1974). Let assume that censoring is noninformative in that, given $\boldsymbol{Z}_j$, the event and censoring time for the j^{th} subject are independent. Let $t_1 < t_2 < \ldots < t_D$ denote the D distinct, ordered, event times and $Z_{(i)k}$ be the k^{th} covariate associated with the subject whose failure time is t_i. Let R_i be the set of all subjects at risk just prior to t_i. The partial likelihood based on the hazard function is expressed by:

$$L(\boldsymbol{\beta}) = \prod_{i=1}^{D} \frac{e^{\boldsymbol{\beta}^T\boldsymbol{Z}_{(i)}}}{\sum_{j\in R_i} e^{\boldsymbol{\beta}^T\boldsymbol{Z}_j}} = \prod_{i=1}^{D} \frac{e^{\sum_{k=1}^{p}\beta_k Z_{(i)k}}}{\sum_{j\in R_i} e^{\sum_{k=1}^{p}\beta_k Z_{jk}}}$$

Often, ties between event times are found in the data. Alternate partial likelihoods have been provided by a variety of authors when ties are present (Klein & Moeschberger, 2006). Here, the Efron partial likelihood is proposed and used for data analysis, due to high computational efficiency and accuracy when dealing with many ties (Efron, 1977). Let d_i be the number of deaths at t_i and D_i the set of all subjects who die at time t_i. Let $\boldsymbol{s}_i$ be the sum of the vectors $\boldsymbol{Z}_j$ over all subjects who die at t_i (*i.e.*, $\boldsymbol{s}_i = \sum_{j\in D_i} \boldsymbol{Z}_j$). The partial likelihood is therefore expressed by:

$$L(\boldsymbol{\beta}) = \prod_{i=1}^{D} \frac{e^{\boldsymbol{\beta}^T \boldsymbol{s}_i}}{\prod_{j=1}^{d_i} \left[\sum_{k \in R_i} e^{\boldsymbol{\beta}^T \boldsymbol{z}_k} - \frac{j-1}{d_i} \sum_{k \in D_i} e^{\boldsymbol{\beta}^T \boldsymbol{z}_k} \right]}$$

The (partial) maximum likelihood estimates are found by maximizing $L(\boldsymbol{\beta})$. Further details can be found in Klein & Moeschberger (2006).

The standard asymptotic likelihood inference tests, the Wald, score (log-rank), and likelihood ratio, are also available for the Cox partial likelihood to test hypotheses about $\boldsymbol{\beta}$ (Therneau & Grambsch, 2000).

The marginal Cox model

In many survival studies there is a natural clustering of study subjects such that failure times within the same cluster may be correlated (Martinussen & Scheike, 2007). To compare the failure times of subjects across clusters the more direct approach is to apply so-called marginal models, where the covariate effects are specified unconditionally. For these models, the cluster structure is ignored when estimating the covariate effects and is used to derive valid estimates of standard errors to ensure correct inference. This approach is linked to the generalized estimating equation (GEE) methodology (Liang & Zeger, 1986) and has mostly been considered in the context of marginal proportional-hazards models. The ordinary Cox model estimate of variance for $\widehat{\boldsymbol{\beta}}$ treats each of the observations as independent. When a given subject may contribute multiple events or different subjects are clustered, this assumption obviously does not hold. In these cases, a robust estimate of variance, the so-called jackknife variance estimator, is used (Lin & Wei, 1989). Let define a jackknife influence value as $J_i = \widehat{\boldsymbol{\beta}}_{(i)} - \widehat{\boldsymbol{\beta}}$, where $\widehat{\boldsymbol{\beta}}_{(i)}$ is the result of a fit that includes all of the points except observation *i*. If *J* is the matrix of jackknife values (*i.e.*, the i^{th} row of *J* is $\widehat{\boldsymbol{\beta}}_{(i)} - \widehat{\boldsymbol{\beta}}$) then the jackknife estimate of variance can be written as the matrix product: $V_J = \frac{n-1}{n}(J - \bar{J})^T(J - \bar{J})$, where $\bar{J}$ is the matrix of column means of *J*. Let define a matrix $\mathbb{D}$ as the score residuals scaled by the variance of $\widehat{\boldsymbol{\beta}}$. The score residuals, detailed in Therneau & Grambsch (2000) and in Halabi *et al*. (2020), help to identify influential or extreme observations with respect to every covariate in the fitted model, and to determine which of the covariates do not fit well in the proportional-hazards model. A natural approximation to

V_J is $\mathbb{D}^T\mathbb{D}$, the matrix product of the approximate jackknife variances (ignoring the $(n-1)/n$ term). Lipsitz *et al*. (1990) found that, for small n, V_J significantly overestimated the variance, whereas $\mathbb{D}^T\mathbb{D}$ did quite well. $\mathbb{D}^T\mathbb{D}$ can be viewed as a sandwich estimator ABA, where A is the usual variance and B a correction term. Sandwich estimates are familiar from robust variance estimation in parametric models and in GEE methods. Lin & Wei (1989) showed that the estimate is consistent and robust to several possible misspecifications in the Cox model, including the lack of proportional hazards, incorrect functional form for the covariates, and omitted covariates. For a linear regression Gaussian model, the infinitesimal jackknife approach leads to the robust variance estimate: $\mathbb{D}^T\mathbb{D} = (X^TX)^{-1}X^T\boldsymbol{R}X(X^TX)^{-1}$ of White (1980; 1982), where $\boldsymbol{R}$ is a diagonal matrix containing the squared residuals ($\boldsymbol{R} = \hat{\sigma}^2 I$ if data are believed to be homoscedastic). Essentially, the idea is to fit the data as an ordinary Cox model, ignoring the possible correlation among subjects, and then replace the standard variance estimate with the robust one, which is corrected for the possible correlations. The jackknife will provide an honest estimate of variance for correlated data whenever the observations left out at any step are independent of the observations left in. For data in which the correlation is restricted to disjoint groups (*e.g.*, subjects grouped into clusters) the obvious choice is then a grouped jackknife estimate that leaves out one cluster at a time rather than one subject at a time. With correlated groups, the sandwich estimate $\mathbb{D}^T\mathbb{D}$ is often substantially larger than the model variance. All three of the usual tests, the Wald, score (log-rank), and likelihood ratio test, are then anticonservative. A robust Wald test is $\widehat{\boldsymbol{\beta}}^T\ (\mathbb{D}^T\mathbb{D})^{-1}\widehat{\boldsymbol{\beta}}$, where the usual variance is replaced with the sandwich estimate. Further details can be found in Therneau & Grambsch (2000).
The marginal Cox model analysis is carried out in R using 'coxph' function with the 'cluster' option in 'survival' package (Therneau & Lumley, 2020). The 'cluster' term in the model performs exactly the same operations as were needed when subjects are grouped into clusters, *i.e.*, the creation of the alternate variance $\mathbb{D}^T\mathbb{D}$, and incorporation of the result into the printout. The reported robust standard errors, used to compute z-values, are the proper standard errors derived above taking into account that observations within clusters cannot be considered as independent. The response variable is coded as 'Surv(time, event)', where 'time' is the follow up time for right-censored data and 'event' is the status indicator (0 = alive, 1 = dead). The function is not implemented for interval-censored data.

Cox model diagnostics

The (marginal) Cox model makes three assumptions:

- At any time *t*, all subjects are assumed to experience the same baseline hazard function $\lambda_0(t)$.
- The effect of the regression variables $\boldsymbol{Z}$ on the hazard experienced by a subject is assumed to remain constant over time.
- The regression coefficients $\boldsymbol{\beta}$ do not vary with time.

Schoenfeld residuals are used to validate the above assumptions made by the Cox model. When the assumption of proportional hazards is violated, it means that model coefficients are time-dependent: $\lambda(t|\boldsymbol{Z}) = \lambda_0(t)e^{\boldsymbol{\beta}(t)^T\boldsymbol{Z}}$. The coefficient $\boldsymbol{\beta}(t)$ expresses the time-varying effect of the $\boldsymbol{Z}$ variables on the hazard. If $\boldsymbol{\beta}(t) = \boldsymbol{\beta}$, hazards are proportional. Let consider the time-dependent coefficients: $\beta_k(t) = \beta_k + \theta_k g_k(t)$, k = 1,..., p, where $g_k(t)$ is a specific known time function (*e.g.*, $g_k(t) = t$ or $g_k(t) = \log(t)$). To obtain an estimate of $\beta_k(t)$, let introduce the scaled Schoenfeld residuals s^*_{ki} (Grambsch & Therneau, 1994), a matrix with a column for each variable (k = 1,..., p) and a row for each event time (t_1,..., t_i,..., t_D). It has been demonstrated that $E(s^*_{ki}) \approx \theta_k g_k(t) \rightarrow \beta_k(t) \approx \hat{\beta}_k + E(s^*_{ki})$. Hazard proportionality can be verified testing the null hypothesis $\theta_k = 0$, equivalent to $\beta_k(t) = \beta_k$, *i.e.*, estimating the regression coefficient θ_k for the residual regression s^*_{ki} on $g_k(t)$. The statistic approximates a χ^2_1 distribution (χ^2_p with a global test). Further details can be found in Martinussen & Scheike (2007). The test is carried out in R using 'cox.zph' function in 'survival' package (Therneau & Lumley, 2020).

The Cox & Snell (1968) residuals r_i can be used to assess the overall fit of a Cox proportional-hazards model. r_i give the overall goodness of fit of the model, without stating the causes of the violation. They are defined as: $r_i = \hat{\Lambda}_0(t_i)e^{\boldsymbol{\beta}^T\boldsymbol{Z_i}}$, i = 1,..., n. If the model is appropriate, the r_i residuals represent a censored random sample coming from an $Exp(1)$ distribution. In other words, if the model is appropriate $\hat{\Lambda}_r(r_i)$, the cumulative hazard function calculated on r_i, is the estimate of the cumulative hazard function of an $Exp(1)$ variable, *i.e.*, of $\Lambda_r(t) = t$. Departures from the exponential distribution may be partly due to the uncertainty in estimating $\boldsymbol{\beta}$ and Λ. This

uncertainty is the largest in the right-hand tail of the distribution and for small samples. Further details can be found in Klein & Moeschberger (2006).

Non-parametric analysis for right-censored data

The standard non-parametric estimator of the survival function, proposed by Kaplan & Meier (1958) and called Product-Limit estimator, is defined as follows:

$$\hat{S}(t) = \prod_{i:\, t_i \le t} 1 - \frac{d_i}{n_i}$$

where n_i is the number of all subjects at risk just prior to t_i and d_i/n_i is the estimate of the hazard function. The Product-Limit estimator is a step function with jumps at the observed event times. The size of these jumps depends not only on the number of events observed at each event time t_i, but also on the pattern of the censored observations prior to t_i. The variance of Product-Limit estimator is estimated by Greenwood's formula:

$$\hat{V}\left[\hat{S}(t)\right] = \hat{S}(t)^2 \sum_{i:\, t_i \le t} \frac{d_i}{n_i(n_i - d_i)}$$

The standard error is given by $\left\{\hat{V}\left[\hat{S}(t)\right]\right\}^{1/2}$. Further details can be found in Klein & Moeschberger (2006).

The log-rank test, also known as Mantel-Cox test, is a non-parametric hypothesis test to compare the survival distributions of two samples. It compares estimates of the hazard functions of the two groups of the two groups (j = 1, 2) at each observed event time i. Given $d_i = d_{1i} + d_{2i}$ and $n_i = n_{1i} + n_{2i}$, let define $Z = \sum_{i=1}^{D} Z_i = \sum_{i=1}^{D}(d_{2i} - (n_{2i}/n_i)d_i)$ and $W = Var(d_{2i}|d_i) = \sum_{i=1}^{D}\{[n_{2i}(n_i - n_{2i})d_i(n_i - d_i)]/[{n_i}^2(n_i - 1)]\}$. Under the null hypothesis that the two groups have identical hazard functions, the statistic Z^2/W approximates a χ_1^2 distribution. The log-rank test is carried out in R using 'survdiff' function in 'survival' package (Therneau & Lumley, 2020). The response variable is coded as in 'coxph' function.

Non-parametric analysis for interval-censored data

A non-parametric comparison of two survival distributions under interval censoring (*i.e.*, a data point is somewhere on an interval between two time values) is comprehensively treated with the 'ictest' function of the R 'interval' package (Fai, 2020). The function allows to perform directly the generalized log-rank test with scores and other tests. The response variable is coded as 'Surv(lower, upper)', where 'lower' and 'upper' are numeric vectors of left and right time endpoints of censoring interval, respectively. Details are reported in Bogaerts *et al.* (2017).

Application to the *Popillia japonica* case study: Materials & Methods

Study area

The trial was carried out in Lombardy Region near the Ticino River in a peach orchard (Varese province). The peach plants were 7 years old, Redhaven cultivar, with 6 m spacing between the rows and 4 m spacing within the row. The climate of the region is mainly humid subtropical, with a high seasonal temperature variation: the average temperature is 2.5 °C in January and 24 °C in July. The total annual rainfall is on average 827 mm.

Experimental design

Twenty-four peach plants of similar canopy size were selected. In each plant, a single branch of the canopy was confined in an insect-proof net cage made of tulle (70 x 100 cm, Figure 3). Five insecticides plus untreated control were tested with four replicates per treatment. Each replicate consisted in the net cage where 25 *P. japonica* adults were introduced (*i.e.*, 100 insects per treatment). The insects were collected on wild plants, growing at the borders of the orchard, immediately before the experiment by sweep entomological net and pooter. The size of the branches confined in the cages guaranteed a sufficient amount of food for the insects throughout the entire trial period. The units (*i.e.*, peach plants) were randomly assigned to a treatment. A completely randomized design was chosen since the plants were fairly homogeneous (Davison, 2003). This meant that the effect of the treatments can be considered far greater than other "plant-induced" effects that might affect the insect survival (*e.g.*, leaf quality as food). The main advantages of this design is that the analysis is simplest as compared to any other design, and that is provided a maximum degree of freedom for error (Freund *et al.*, 2010). An example of a completely randomized design is reported in Figure 4.

Figure 3. Peach plants with insect-proof net cages.

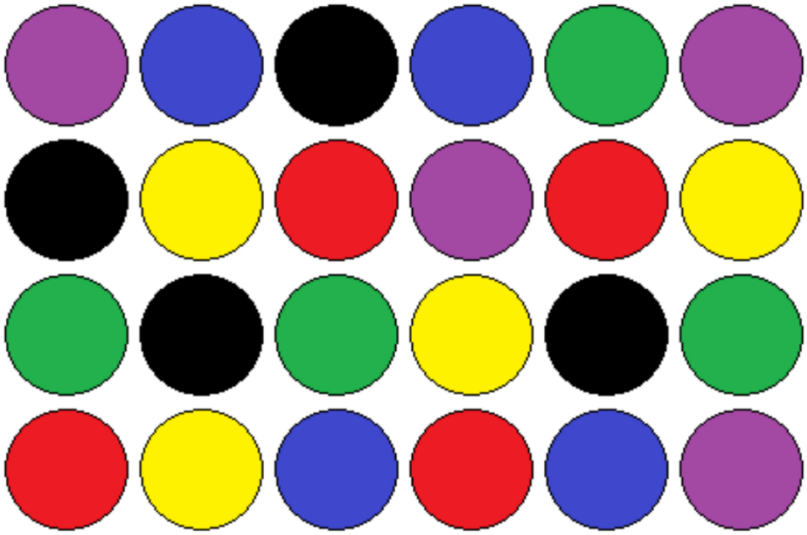

Figure 4. Example of a completely randomized design. Circles of the same color represent peach plants subjected to the same treatment (six treatments with four replicates each).

Tested treatments

Five insecticides registered for beetle management in peach orchard and the untreated control were tested (Table 1).

Table 1. List of the tested products. Classification provided by Insecticide Resistance Action Committee (IRAC).

Active ingredient	Chemical group	Mode of Action (IRAC)	Commercial formulation	Dose
Acetamiprid	Neonicotinoids	4A	Epik® SL	200 mL/hL
Deltamethrin	Pyrethroids	3A	Decis® EVO	50 mL/hL
Sulfoxaflor	Sulfoximines	4C	Closer™	40 mL/hL
Abamectin	Avermectins	6	Vertimec® EC	100 mL/hL
Phosmet	Phosphorganics	1B	Spada® 200 EC	375 mL/hL
Water	Untreated control			

Field applications

Insecticide applications were performed by spraying the entire canopy of the plants until dripping point on 30th June 2020. Peaches were sampled before the applications. The phenological stage of the orchard was BBCH code 87 ("Fruit ripe for picking"; Meier, 2001). Tap water acted as untreated control. A motorized sprayer with a disc core nozzle was used for the foliar applications, with 12 bar of pressure, and a water volume of 1000 L/ha (insecticide doses reported in Table 1). Insecticide effectiveness was determined by scoring insect mortality, *i.e.*, number of dead insects in each cage, at 1, 3, 7, 10, 14, and 21 days after treatment (DAT). The experiment ended at 21 DAT. All the insects survived at this date were considered as right-censored data.

Data analysis

The effectiveness of the insecticide treatments was assessed through a survival analysis. This analysis was more suitable than a generalized linear (mixed) model since right-censored data were present. Moreover, the survival analysis can highlight the presence of lower/greater risk of mortality over time through the survival or hazard function. Survival curves were estimated by the Kaplan-Meier method for right-censored data. Pairwise comparisons among treatments were estimated through non-parametric log-rank tests both for right- and interval-censored data, in order to detect any differences between the two approaches. P-values were adjusted with Bonferroni correction. To account for possible insect intra-cluster dependence, a marginal Cox proportional-hazards model was applied where robust standard errors were obtained to adjust for such dependence (Martinussen & Scheike, 2007; Therneau & Grambsch, 2000). The cluster factor was the cage identity. Efron approximation for ties was adopted. The Cox model was validated by checking the proportional hazards assumptions with Schoenfeld and Cox-Snell residual analyses (Klein & Moeschberger, 2006; Martinussen & Scheike, 2007). The dependent variable was the lifetime of each insect; the categorical explanatory variable was the treatment, composed by five insecticide products and the untreated control. Pairwise comparisons among treatments were estimated running the Cox model with different baselines and adjusting the p-values with Bonferroni correction.

R packages

All analyses were performed in R 3.5.1 (R Core Team, 2018). Non-parametric right-censored analysis and Cox model were run using 'survival' package (Therneau & Lumley, 2020). Non-parametric interval censored analysis was run using 'interval' package (Fay, 2020). Pairwise comparisons for the Cox model were run using 'emmeans' package (Lenth *et al.*, 2020). Kaplan-Meier curves were plotted using 'survminer' package (Kassambara *et al.*, 2020).

Results

Non-parametric analyses

All the insecticides resulted effective if compared with untreated control (Figure 5). Acetamiprid, Deltamethrin and Phosmet (equally effective) had a stronger effect if compared to the other treatments, reducing survival under 15% at 1 DAT and killing all the insects at 3 DAT. 43% of untreated insects and 13% of insects treated with Sulfoxaflor were still alive at the end of the study (Table 2). Right- and interval-censored log-rank tests led to the same inferential conclusions. Indeed, the p-values obtained by each of the 15 pairwise comparisons among treatments were almost equal between the two methods. This justified the use of a semi-parametric model for right-censored data (instead of a more complex one for interval-censored data) despite the presence of intervals of two up to seven days between survey times. The output of the 30 log-rank tests was not reported since it led to the same inferential conclusions of the later discussed marginal Cox model.

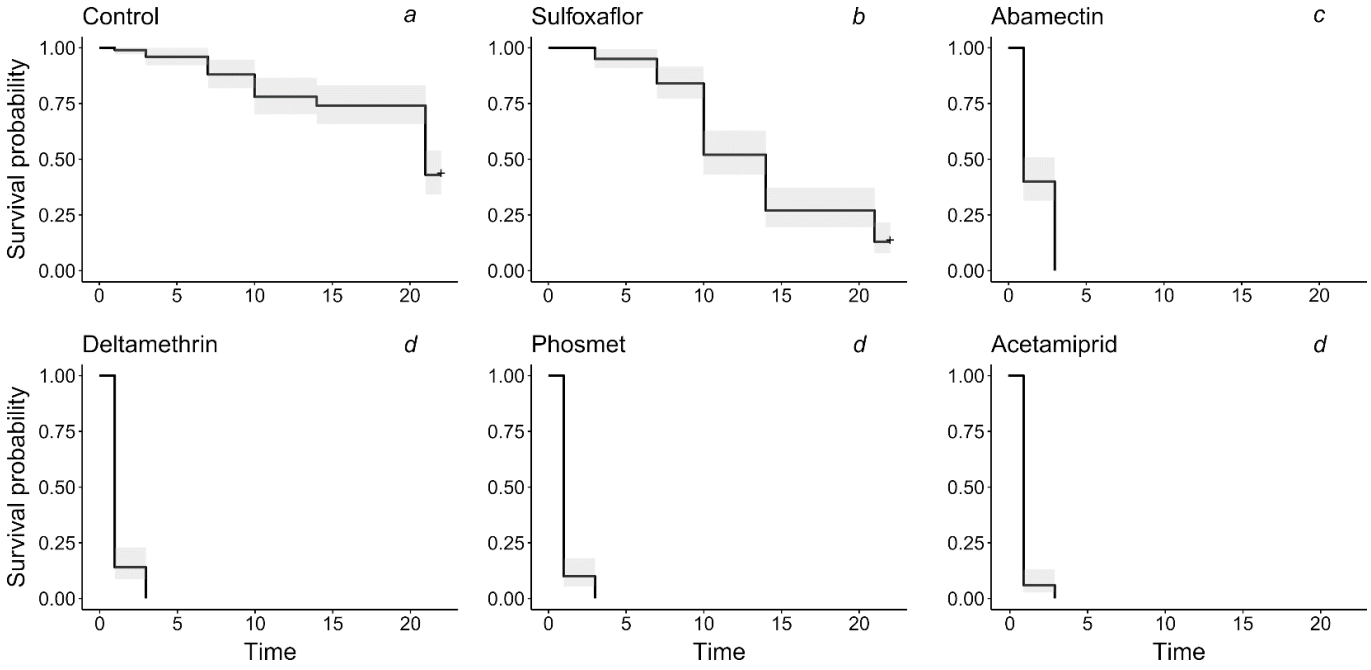

Figure 5. Survival curves estimated by the Kaplan-Meier method for right-censored data, with 95% confidence intervals (grey shading). Censored data are marked with a plus (+). Different letters (a-d) indicate significant differences among treatments ($p < 0.05$ after Bonferroni correction).

Table 2. Life-table using the Kaplan-Meier method for right-censored data. For each treatment, *time* is the survey time (DAT), *n* is the number of insects at risk just prior to *time*, *d* is the number of events (deaths) at *time,* $\hat{S}(t)$ is the Product-Limit estimator of the survival function, *SE* is the standard error.

Control				
time	*n*	*d*	$\hat{S}(t)$	*SE*
1	100	1	0.99	0.010
3	99	3	0.96	0.020
7	96	8	0.88	0.033
10	88	10	0.78	0.041
14	78	4	0.74	0.044
21	74	31	0.43	0.050
Abamectin				
time	*n*	*d*	$\hat{S}(t)$	*SE*
1	100	60	0.40	0.049
3	40	40	0.00	NA
Acetamiprid				
time	*n*	*d*	$\hat{S}(t)$	*SE*
1	100	94	0.06	0.024
3	6	6	0.00	NA
Deltamethrin				
time	*n*	*d*	$\hat{S}(t)$	*SE*
1	100	86	0.14	0.035
3	14	14	0.00	NA
Phosmet				
time	*n*	*d*	$\hat{S}(t)$	*SE*
1	100	90	0.10	0.030
3	10	10	0.00	NA
Sulfoxaflor				
time	*n*	*d*	$\hat{S}(t)$	*SE*
3	100	5	0.95	0.022
7	95	11	0.84	0.037
10	84	32	0.52	0.050
14	52	25	0.27	0.044
21	27	14	0.13	0.034

Marginal Cox model analysis

As mentioned before, all the insecticides resulted effective if compared with untreated control (Table 3). Acetamiprid, Deltamethrin and Phosmet were the most effective ($p < 0.0001$). In particular, an insect exposed to one of these three insecticides had a death risk about 300 times higher than an untreated insect (*i.e.*, hazard ratio ≈ 300). Pairwise comparisons confirmed the equal effectiveness ($p > 0.05$) of the three insecticides (Table 4). Abamectin showed a high effectiveness too ($p < 0.0001$), despite lower than the above mentioned insecticides at 1 DAT. Sulfoxaflor was the only insecticide unable to kill all the insects at the end of the study. Robust standard errors were quite close to the naive estimates, suggesting independence among intra-cluster subjects. Schoenfeld residual analysis (Table 5) supported the null hypothesis of hazard proportionality (global test: $p > 0.05$) despite a slight violation caused by Abamectin *versus* untreated control ($p = 0.01$). Cox-Snell residual analysis (Figure 6) showed a barely good fit of the model, since the cumulative hazard function calculated on residuals did not closely follow the plot bisector $\Lambda_r(t) = t$. Nevertheless, the residual trend was acceptable. Running the analysis without Abamectin led to a similar plot.

Table 3. R summary output of the marginal Cox proportional-hazards model. Untreated control is used as baseline. The likelihood ratio and score tests assume independence of observations within a cluster, the Wald and robust score tests do not.

Insecticides	β	e^{β}	*SE*	*Robust SE*	*z-value*	*p-value*
Abamectin	5.0862	161.7757	0.3910	0.2970	17.127	< 0.0001
Acetamiprid	5.8016	330.8255	0.3960	0.3079	18.840	< 0.0001
Deltamethrin	5.5818	265.5369	0.3937	0.3083	18.107	< 0.0001
Phosmet	5.6856	294.6074	0.3947	0.3186	17.846	< 0.0001
Sulfoxaflor	0.9755	2.6525	0.1743	0.1726	5.651	< 0.0001

$n = 600$, $d = 544$
Concordance = 0.897 ($SE = 0.009$)
Likelihood ratio test = 744.7 on 5 *df*, $p < 0.0001$
Wald test = 393.5 on 5 *df*, $p < 0.0001$
Score (log-rank) test = 584.9 on 5 *df*, $p < 0.0001$; Robust = 23.61, $p = 0.0003$

Table 4. R output of the pairwise comparisons among treatments for the marginal Cox model. P-values were adjusted with Bonferroni correction for 15 tests.

Contrasts	*β*	*Robust SE*	*z-value*	*p-value*
Control - Abamectin	-5.0862	0.2970	-17.127	< 0.0001
Control - Acetamiprid	-5.8016	0.3079	-18.840	< 0.0001
Control - Deltamethrin	-5.5818	0.3083	-18.107	< 0.0001
Control - Phosmet	-5.6856	0.3186	-17.846	< 0.0001
Control - Sulfoxaflor	-0.9755	0.1726	-5.651	< 0.0001
Abamectin - Acetamiprid	-0.7154	0.0911	-7.856	< 0.0001
Abamectin - Deltamethrin	-0.4955	0.1021	-4.853	< 0.0001
Abamectin - Phosmet	-0.5994	0.1281	-4.678	< 0.0001
Abamectin - Sulfoxaflor	4.1107	0.3244	12.671	< 0.0001
Acetamiprid - Deltamethrin	0.2198	0.1134	1.938	0.7896
Acetamiprid - Phosmet	0.1159	0.1316	0.881	1.0000
Acetamiprid - Sulfoxaflor	4.8261	0.3346	14.423	< 0.0001
Deltamethrin - Phosmet	-0.1039	0.1444	-0.719	1.0000
Deltamethrin - Sulfoxaflor	4.6063	0.3349	13.755	< 0.0001
Phosmet - Sulfoxaflor	4.7101	0.3444	13.675	< 0.0001

Table 5. R output of the Schoenfeld residual analysis to test the proportional hazards assumption for a marginal Cox model fit.

Insecticides	χ^2	*df*	*p-value*
Abametin	6.55	1	0.010
Acetamiprid	1.59	1	0.207
Deltamethrin	1.19	1	0.276
Phosmet	1.64	1	0.201
Sulfoxaflor	1.59	1	0.207
GLOBAL	9.58	5	0.088

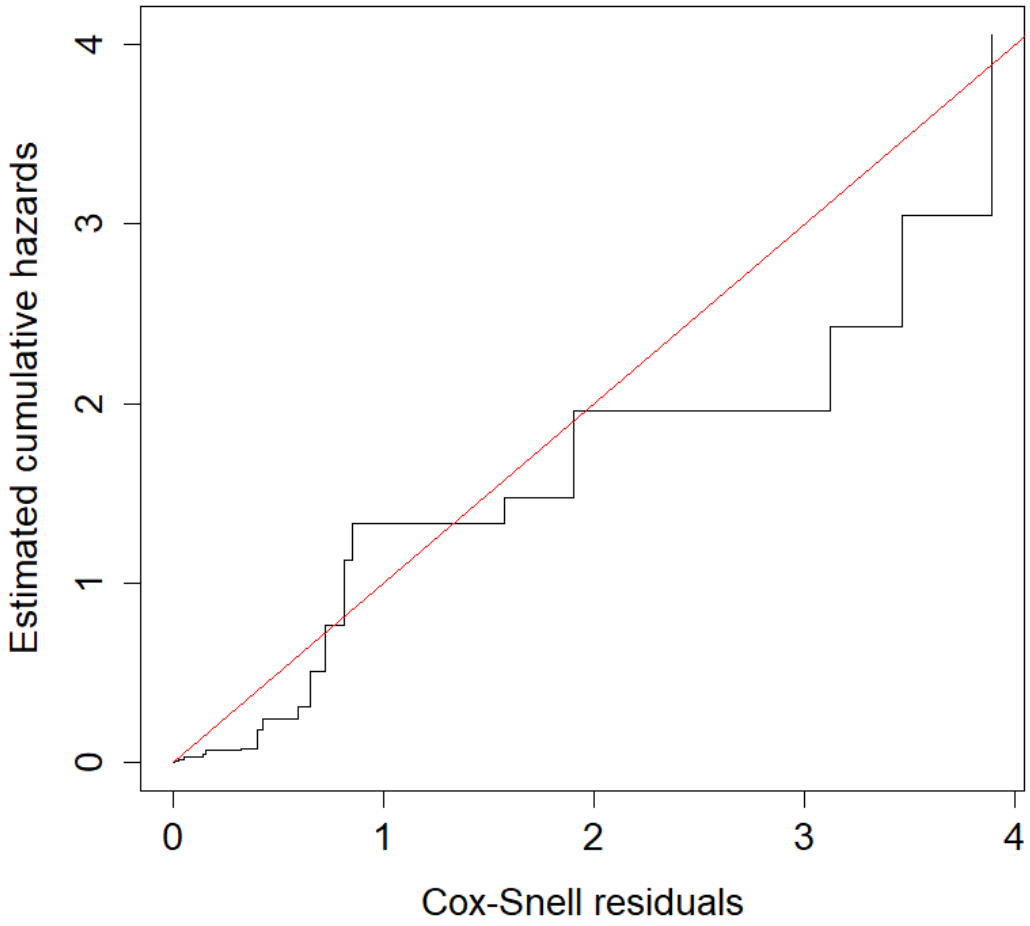

Figure 6. Plot of the cumulative hazard function calculated on Cox-Snell residuals *versus* Cox-Snell residuals. The red line is the plot bisector.

Discussion & Conclusions

Non-parametric (right- and interval-censored) and semi-parametric (marginal Cox model) analyses led to equal inferential conclusions. The marginal Cox proportional-hazards model for right-censored data proved to be suitable for the analysis, even if interval-censored observations were present. It will be interesting to evaluate a parametric approach to data analysis, in order to evaluate possible differences with the approaches adopted in this study. An example could be the implementation of an accelerated failure time model. This model assumes that the effect of a covariate is to accelerate or decelerate the lifetime by some constant. Unlike classical proportional-hazards models such as Cox models, the regression parameter estimates from parametric survival models are robust to omitted covariates. They are also less affected by the choice of probability distribution. However, the assumptions are stronger since a probability distribution is specified for the time. Parametric survival models are detailed in Klein & Moeschberger (2006) and Martinussen & Scheike (2007), and carried out in R using 'survreg' function in 'survival' package (Therneau & Lumley, 2020).

The tested insecticides were effective in reducing *P. japonica* survival. Abamectin, Acetamiprid, Deltamethrin and Phosmet killed all the insects at 3 DAT. They are therefore recommended to counteract the outbreak of the species in Northern Italy in peach orchards. Further insecticide trials demonstered that these products are effective in killing the species in most of the commercial crops and nursery plants present in the study area (Marianelli *et al.*, 2019; Mori *et al.*, unpublished; Santoiemma *et al.*, unpublished) and in other parts of the world (Koppenhöfer *et al.*, 2002; Morales-Rodriguez & Peck, 2009; Pfeiffer, 2012). Nevertheless, given the non-negligible environmental impact and toxicological profile of these active ingredients, an integrated pest management approach based on biological control should be implemented in the near future.

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