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ARTICLE

Multistate model in the behavioral study of the parasitoid *Telenomus podisi* for biological soybean control

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Abstract

Biological pest control through the use of wasps are sustainable ways of protecting plants and, consequently, agricultural production. In this context, an entomological experiment is motivated, in which the forraging and reproductive behaviors of the parasitoid *Telenomus podisi* were qualified over time, considering non parasitized (sp0) and parasitized (sp1) of the brown stink bug, *Euschistus heros*. The experiment features a longitudinal study of continuous time with variable nominal qualitative response, grouped into four mutually exclusive categories (flying-walking, drumming, oviposition and marking). Continuous time transition models, also known as multistate models, are a viable alternative for analyzing data with these characteristics. Therefore, this class of models was considered in this work to study the behavior of the parasitoid, through the average length of stay in each of the response categories, as well as their preference probabilities. As a result, there was a significant effect of the treatment of host eggs (non parasitized or previously parasitized). When having contact with previously parasitized eggs, the female parasitoid *T. podisi* has preference for the categories of movement which are flying-walking and drumming. In contrast, when in contact with non parasitized eggs, the female *T. podisi* has a preference for oviposition and marking. The applied methodology proved to be adequate for this typical experimental situation in entomological studies associated with the description of the behavior of a parasitoid.

Keywords: Host-parasitoid interaction; Categorized longitudinal data; Maximum likelihood; Transition intensities.

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1. Introduction

In the agronomic area, the number of studies involving preventive measures for the protection of agricultural crops is growing. Care with the amount of sunlight, water, soil, winds and pest control are of paramount importance for the well-being of plants. Leite *et al.* (2021) studies the increase in height of the urochloa grass plant when applying nitrogen and phosphate fertilization. In particular, biological control is a safe and sustainable approach that combats insect pests using natural and cost-effective enemies. According to Gámez-Virués *et al.* (2009), there is a significant worldwide increase in defense of biological control, through the use of parasitoids as an effective strategy, since it eliminates the breeding of pests that attack plants without causing damage to the environment.

In this context, entomological experiments are designed to study and identify which parasitoid wasps perform better in protecting a particular plant species against insect pests. In some situations, parasitoid wasps have been used in biological pest control, including species diversity and different pest cultures (Wang *et al.*, 2019). In small farms or gardens, the use of predador social wasps has shown to be a competent agent for pest control (Prezoto *et al.*, 2019).

According to Embrapa (2019), Brazil is the second largest world producer of soybean, behind only the United States of America (USA). In the 2018/2019 harvest, the crop occupied an area of 35.82 million hectares, which totaled a production of 114.84 million tons. The average productivity of Brazilian soybean was 3,206 kg per hectare. Given this, it is important to study alternatives for pest prevention in this crop. The brown Sting bug, *E. heros* Fabricius (Hemiptera: Pentatomidae) is one of the most well-known pests in soybean cultivation and can cause great damage (Borges *et al.*, 1998). Correa-Ferreira and Azevedo (2002), point out the reduction in quality and weight of seeds as one of the main damages. The parasitoid *T. podisi* Ashmead (Hymenoptera: Scelionidae) is a natural agent frequently used to combat pests *E. heros*. The female locates and lays its eggs in the hosts, interrupting the development of the pest. In some studies, the researcher stores the pupa of the parasitoid, to later analyze its emergence, longevity and parasitism capacity in adulthood (Foerster *et al.*, 2004). Other researches verify the influence of the host (*E. heros*) on the behavior of the *T. podisi* (Tognon *et al.*, 2014).

In most cases, studies qualify the behavior of the microvespa, over a period of time and mutually exclusive categories (nominal or ordinal), technically called states of individuals. Statistical analysis of these data requires methods that take into account the time-state process. Among the possible models, the transition model stands out, which is a flexible technique for analyzing data categorized over time. Ware *et al.* (1988) classify this model as conditional because it takes into account past responses (individual's history) and a set of covariates. Molenberghs and Verbeke (2006), define this class as an extension of generalized linear models, being useful to describe the changes between the categories of individuals.

According to Lara *et al.* (2018), the transition model is based on stochastic processes, which can have discrete or continuous state space, just as time can be discrete or continuous, resulting in four different types of processes. Let S a espace state and τ a time when the process occur. It is noteworthy that, in categorized data, situations with discrete state space is common, i.e, $S = \{1, 2, \ldots, k\}$, which correspond to the categories of response and τ the set time, can be discrete or continuous. When the response is measured or observed over the time of an interval, we have a continuos time, i.e, τ is a measure of time. An example of a discrete-time study was analysis of the severity of injuries in pigs, when subjected to two rearing conditions: with and without environmental enrichment (Lara *et al.*, 2017). For models with τ observed over time Oliveira *et al.* (2018, 2013) and Meira-Machado *et al.* (2009), analyze the physical condition of patients about a certain disease.

The continuous time transition model is also known as the multistate model. In addition to the discrete time model, in this class, one can estimate the transition intensities, which are parameters associated with the probabilities of transitions, which are fundamental to simultaneously describe the behavioral changes of individuals as well as the speed with which this occurs over time (Kalbfleisch and Lawless, 1985). According to Gimenez *et al.* (2003), the multistate model is a powerful methodology, when one is interested in biological issues related to dispersion and/or individual variety in wild populations, as well illustrated in Conn and Cooch (2009), through a study of conjunctivitis in birds *Haemorhous mexicanus* (Birds: Fringillidae). When capturing and recapturing birds, the states of this disease and their rates of change over time are observed.

In this class of models, the time until the event occurs is also of interest, and time can be observed or censored. Censorship is described when the individual has his time until the occurrence is compromised (Klein and Moeschberger, 2006). The existence of this censored observation occurs due to several causes, which may or may not be related to the event of interest. The censored times should be used in the statistical analysis, because, even if they are incomplete, they provide information about the time of failure of the individuals and the non-use will lead to inaccurate results. In addition, multistate models can be considered as a generalization of survival models, once Meira-Machado *et al.* (2009) describe the mortality model as the simplest of the multistates for survival analysis.

In view of the importance of pest control through parasitoids and, considering the applicability of the transition model, the present work aims to extend this methodology to entomology, motivated by an experiment developed at the College of Agriculture “Luiz de Queiroz” - University of São Paulo. In order to analyze the preference of the female parasitoids *T. podisi* for eggs of *E. heros* (non parasitized or previously parasitized). This article is structured as follows: in section 2, a brief review of the continuous time stochastic process and the multistate model is made. In section 3, the motivation study and the methods to be used in the data analysis are presented. In section 4, the results of the data analysis and discussion are presented. Finally, in section 5 we have the final considerations of this article.

2. Stochastic processes and multistate models

A stochastic process is described as a random phenomenon that varies, over time and has unpredictable characteristics, once it can produce different results for each time the process is executed. According to Karlin and Taylor (1975), a stochastic process involves relations between random variables and is a sequence of events governed by probabilistic laws that can be of discrete or continuous time. In this context, consider individuals moving freely through k categories over time. A stochastic process can be denoted by the random variable Y_t, assuming values in the set $S = \{1, 2, \ldots, k\}$, called the process state space. The parameter t, in turn, assumes values in the set τ, which refer to the process time: if enumerable it defines the discrete time process, otherwise, there is the stochastic process of continuous time. However, in most applications in entomology, the stochastic process associated with the experiment is continuous time and, therefore, is the object of study in this work.

2.1 Continuous time *Markov* chains

When $Y_t \in S$ is a continuous time stochastic process, $\tau = \{0 < s < t\}$, the probabilities of transition from state a to state b are defined by

$$\pi_{ab}(s, t) = P(Y_t = b | Y_s = a), \quad \forall \quad a, b \in S.$$

Matrixually, we have:

$$\boldsymbol{P}(s,t) = \begin{pmatrix} \pi_{11}(s,t) & \pi_{12}(s,t) & \dots & \pi_{1k}(s,t) \\ \pi_{21}(s,t) & \pi_{22}(s,t) & \dots & \pi_{2k}(s,t) \\ \vdots & \vdots & \ddots & \vdots \\ \pi_{k1}(s,t) & \pi_{k2}(s,t) & \dots & \pi_{kk}(s,t) \end{pmatrix}.$$

According to Lara *et al.* (2017), in a *Markovian* process, the probabilities can be classified as homogeneous, over time, which defines a stationary process. For the continuous case, we have:

$$\pi_{ab}(t) = P(Y_{t+s} = b | Y_s = a),$$

or, in matrix terms $\boldsymbol{P}(t) = \boldsymbol{P}(s, s+t)$. The $\boldsymbol{P}(t)$ transition matrix has elements that satisfy, $\pi_{ab}(t) \geq 0, \quad a, b = 1, 2, \dots, k$ and $\sum_{b=1}^{k} \pi_{ab}(t) = 1$. Under this condition, Karlin and Taylor (1975) show that if all states $y \in S$ are non-absorbent, then there must be a distribution function such that:

$$\frac{1 - F_y(t+s)}{1 - F_y(s)} = 1 - F_y(t), \qquad (1)$$

being an exponential distribution, with parameter q_y, it fully satisfies the equation (1), that is:

$$f_y(t) = q_y \exp(-q_y t) \qquad t \geq 0,$$

where $\mathrm{E}(Y) = \frac{1}{q_y}$ and $\mathrm{Var}(Y) = \frac{1}{q_y^2}$ correspond, respectively, to the mean and the variance of the distribution. If y is absorbent, there is $q_y = 0$. Thus, the stationary transition probabilities can be described as a function of the exponential distribution and, in addition, it is assumed that they are continuous and differentiable in time (Hoel textit *et al.*, 1972), we have:

$$\left[\frac{\partial \pi_{ab}(t)}{\partial t}\right]_{t=0} = q_{ab}(t),$$

which are the transition intensities of the *Markovian* process and it is further demonstrated that:

$$q_{ab}(t) = \lim_{\Delta_t \to 0} \frac{\pi_{ab}(t, t+\Delta_t)}{\Delta_t}.$$

The intensities can be interpreted as rates of transition of the process over time, and satisfy, $q_{aa} \leq 0$ with $a = 1, 2, \dots, k$, $q_{ab} \geq 0$ for $a \neq b$ and $\sum_{b=1}^{k} q_{ab}(t) = 0$. Matrixually, we have:

$$\mathbf{Q}(t) = \begin{pmatrix} q_{11}(t) & q_{12}(t) & \dots & q_{1k}(t) \\ q_{21}(t) & q_{22}(t) & \dots & q_{2k}(t) \\ \vdots & \vdots & \ddots & \vdots \\ q_{k1}(t) & q_{k2}(t) & \dots & q_{kk}(t) \end{pmatrix}.$$

where, for a stationary process, $\mathbf{Q}(t) = \mathbf{Q}(s, s+t)$. According to Singer and Spilerman (1976), intensities are related to transition probabilities and time, as follows: $\pi_{ab} = -\frac{q_{ab}}{q_{aa}}$ is the probability that an observation that is in the a state will move to the state b, given the occurrence of a transition; $-\frac{1}{q_{aa}}$ is the expected time of an observation that is in the state a stay in that state.

2.2 Multistate model

The multistate model is a generalization of the continuous time stochastic process described in the section 2.1 with incorporated covariables in the analysis. This is possible, through regression models, for which a time probability distribution function is assumed, with the exponential distribution being the most usual by the identity relation (1). However, its use is not restricted, and *weibull* and log-normal distributions are other options. Consider a random sample of individuals with $i = 1, 2, \dots, n$ with variable response inherent to the stochastic process $Y_t \in S = \{1, 2, \dots, k\}, t \geq 0$. Also consider $\pi_{ab}(t)$ the probability of transition from a to b and $q_{ab}(t)$ the intensity with which this change occurs. According to Wreede *et al.* (2010), the multistate Cox model is the most well-known regression model and used in survival data studies, described as follows

$$q_a(t_a, \boldsymbol{x}_a) = q_a^{(0)}(t_a) \exp\{\boldsymbol{\beta}_a^\top \boldsymbol{x}_a\} \tag{2}$$

where $a \in \{1, \dots, k\}$ corresponds to the state of the transitions, $q_a^{(0)}(t_a)$ the risk or initial rate for the transition a, $\boldsymbol{\beta}_a$ is the vector of regression coefficients for each transition a, $\boldsymbol{x}_a$ is the vector of covariates, which can be specific to each transition or common to all transitions. The individual contribution to the model (2) is given by

$$q_{ai}(t_{ai}, \boldsymbol{x}_{ai}) = q_{ai}^{(0)}(t_{ai}) \exp\{\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai}(t_{ai})\}.$$

Putter (2011) and Wreede *et al.* (2011) describe that $\boldsymbol{x}_{ai}(t_{ai})$ allows each transition to have its own set of covariates or that each covariate in each transition has different coefficients. Furthermore, Rotolo (2013) describes that the proportionality of the rates of the different transitions can be specified by using the initial rate and by adding a dummy variable in $\boldsymbol{x}_{ai}(t_{ai})$. Consequently, the likelihood function of the model (2) is given by

$$L(\boldsymbol{\beta}, \boldsymbol{q}^{(0)}(\cdot)) = \prod_{i=1}^{n} \prod_{a=1}^{k} [q_{ai}(t_{ai})]^{\delta_{ai}} \frac{S_{ai}(t_{ai})}{S_{ai}(t_{ai}^{*})} \tag{3}$$

where $\boldsymbol{q}^{(0)}(\cdot) = (q_1^{(0)}(t), \dots, q_k^{(0)}(t))^\top$ represents the vector of the initial rates, $q_{ai}(t_{ai})$ the risk function or transition rate, δ_{ai} is the failure indicator (1, if t_{ai} is a time of failure in the transition a for the individual i or 0, if t_{ai} is a time of censorship in the transition a for the individual i), $S_{ai}(t_{ai})$ is the survival function considering the time of failure/censorship and $S_{ai}(t_{ai}^{*})$ the time of truncation to the left.

The survival function of the individual i in the a transition is defined by

$$\begin{aligned} S_{ai}(t_{ai}) &= \exp\{-\exp\{(\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai})\} q_a{}^{(0)}(t_{ai})\} \\ S_{ai}(t_{ai}^{*}) &= \exp\{-\exp\{(\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai})\} q_a{}^{(0)}(t_{ai}^{*})\}. \end{aligned} \tag{4}$$

Substituting the equations in (4) in (3), we have the following likelihood function

$$\begin{aligned} L(\boldsymbol{\beta}, \boldsymbol{q}^{(0)}(\cdot)) &= \prod_{i=1}^{n} \prod_{a=1}^{k} [q_{ai}^{(0)}(t_{ai}) \exp\{\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai}(t_{ai})\}]^{\delta_{ai}} \frac{\exp\{-\exp\{(\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai})\} q_j{}^{(0)}(t_{ai})\}}{\exp\{-\exp\{(\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai})\} q_a^{(0)}(t_{ai}^{*})\}} \\ &= \prod_{i=1}^{n} \prod_{a=1}^{k} [q_{ai}^{(0)}(t_{ai}) \exp\{\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai}(t_{ai})\}]^{\delta_{ai}} \exp\{-\exp\{\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai}\} \times \\ &\quad \times [q_{ai}^{(0)}(t_{ai}) - q_{ai}^{(0)}(t_{ai}^{*})]\}. \end{aligned} \tag{5}$$

Finally, the logarithm of the likelihood function (log-likelihood) of the multistate Cox model is defined by

$$l(\boldsymbol{\beta}, \boldsymbol{q}^{(0)}(\cdot)) \quad = \quad \sum_{i=1}^{n} \sum_{a=1}^{k} \{\delta_{ai} \log[q_{ai}^{(0)}(t_{ai}) \exp(\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai}(t_{ai}))] - \exp(\boldsymbol{\beta}_a^\top \boldsymbol{x}_{ai})[q_{ai}^{(0)}(t_{ai}) - q_{ai}^{(0)}(t_{ai}^*)]\}.$$

The parameter estimates are obtained by maximizing the log-likelihood function, in which it is often to resort to an interactive process such as Newton-Raphson or Fisher's score.

3. Material and methods

3.1 Material

As a motivation study, an experiment was conducted in March 2020 at the Department of Entomology and Acarology (Laboratory of Biological Control of Insects) of the College of Agriculture "Luiz de Queiroz" - University of São Paulo, with the female parasitoid *T. podisi*. This agent locates the eggs of the host *E. heros* in which it oviposition in order to reproduce on the pest. Therefore, the objective was to study the behavior of the female parasitoid of *T. podisi* in healthy and previously parasitized eggs of hosts. Consequently, evaluate whether the parasitoids prevent intraspecific competition, that is, they recognize eggs already parasitized and also if time influences oviposition.

Euschistus heros were reared in plastic containers on a diet of fresh green beans (*Phaseolus vulgaris L.*), raw peanuts (*Arachis hypogaea L.*), and water. Twice a week, the diet was renewed. The eggs were collected daily and separated in petri dishes until nymphal eclosion. Colonies of *E. heros* were kept in a climatized room as a source of eggs. The parasitoid *T. podisi* was maintained in plastic bags on *E. heros* eggs (< 24h old) obtained from the colony. Pure honey was offered as a food source for adult females inside the plastic bags.

For the experiments, parasitized eggs of *E. heros* from the colony were individualized and separated in glass tubes (8.5 × 2.5 cm) until the emergency of parasitoids. The emerged parasitoids were sexed and couples were placed in glass tube (8.5 × 2.5 cm) for 24h, in which they fed on honey and were allowed to mate. After 24h, the female parasitoids were transferred to plastic petri dishes (9 × 1.5 cm) contained four non parasitized (sp0) and four parasitized (sp1) eggs of *E. heros*. The foraging behavior of females on *E. heros* eggs were observed for 10 minutes. The evaluated foraging behaviors were classified as: 1 - flying-walking, 2 - drumming, 3 - oviposition or 4 - marking. Were done 16 repetitions for each treatment. The experiments were carried out under controlled environmental conditions (25 ± 2°C, 70 ± 10% UR).

3.2 Methods

At first, an exploratory analysis was made using a contingency table for the four behaviors of the parasitoid (1 - flying-walking, 2 - drumming, 3 - oviposition or 4 - marking). The initial distribution of the process can be seen in Figure 1, with the possible paths to be measured and the treatment factor is ignored. The behaviors of the parasitoid were classified as recurrent or non-absorbent, that is, given that the parasitoid already occupied a category, the future probability of it coming to occupy that category again is different from zero.

In sequence, multistate models were adjusted, under the hypothesis of a *Markovian* process with probabilities and stationary intensities in time, without and with the treatment effect (sp0 and sp1). In all models, a *Markovian* chain of reach one was considered, that is, in which the previous category is additionally considered in the linear predictor, as is classic in multistate models. To adjust these models, the maximum likelihood method was used and the exponential distribution for the period of time between transitions was considered as described in the equation (1). As the models are

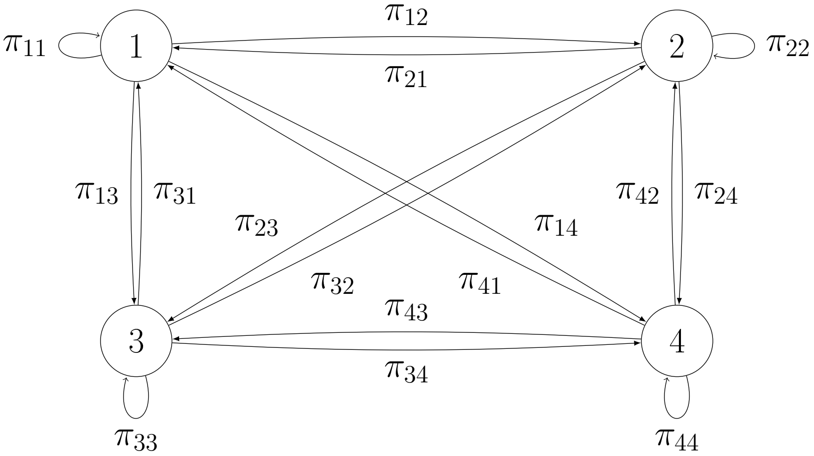


Figure 1. Scheme of the stochastic process with the four states of the behavior of the parasitoid *T. podisi*, in which 1 - flying-walking, 2 - drumming, 3 - oviposition and 4 - marking.

considered docked, the treatment effect was verified, using the likelihood ratio test, whose statistics are given by

$$\Lambda = -2[\log(L(\widehat{\theta}, x)) - \log(L(\widetilde{\theta}, x))], \tag{6}$$

$L(\widehat{\theta}, x)$ and $L(\widetilde{\theta}, x)$ correspond to the likelihoods of the restricted (no treatment effect) and unrestricted (with treatment effect) models, respectively. The null hypothesis that there is no treatment effect is rejected if the value of the statistic (6) is greater than the quantile of the distribution χ^2_ν, with $\nu = dim(\widetilde{\theta}) - dim(\widehat{\theta})$ degrees of freedom. If the likelihood ratio test leads to the rejection of the null hypothesis, the probability and intensities matrices of the multistate model will be estimated, for each treatment effect (sp0 and sp1), allowing the differentiation between their levels (1 - flying-walking, 2 - patter, 3 - oviposition and 4 - marking). In practical terms, the adjustment of the model by the maximum likelihood method allows to estimate the intensities of each behavior and, by the invariance principle, the transition probabilities are obtained. Data analysis was performed with the aid of the `msm` package (Jackson, 2018) available in the `R` software (R Core team, 2020).

4. Results and discussions

Initially, an exploratory analysis was carried out disregarding the treatment effect (non parasitized and previously parasitized eggs), using a contingency table. The frequencies of the transitions, the response variable behavior of the female parasitoid *T. podisi* are presented in Table 1. A total of 128 transitions are observed, of which 54 come from previous response 1 (flying-walking), 23 from previous response 2 (drumming), 45 from previous response 3 (oviposition) and 6 from previous response 4 (marking). Regarding the previous response, it is noted that there was less frequency of transition in the marking behavior and higher frequency in the flying-walking category. In addition, the probabilities of the parasitoid remaining in the flying-walking (0.61) and oviposition (0.64) flight response were higher when compared to the drumming (0.39) and marking (0.00) states. However, this analysis is only exploratory and the fact that *T. podisi* stay in a given category does not imply an absence of transition.

Table 1. Observed frequency and proportion (in parentheses) of the parasitoid *T. podisi* regarding changes in their responses

Previous	Future response				
response	1	2	3	4	Total
1	33	13	8	0	54
	(0.6111)	(0.2407)	(0.1481)	(0.0000)	
2	4	9	10	0	23
	(0.1739)	(0.3913)	(0.4348)	(0.0000)	
3	1	8	29	7	45
	(0.0222)	(0.1777)	(0.6444)	(0.1555)	
4	0	0	6	0	6
	(0.0000)	(0.000)	(1.0000)	(0.0000)	
Total	38	30	53	7	128

The restricted multistate models (without treatment effect) and unrestricted (with treatment effect) models were adjusted to the data to measure the average length of stay, in the behaviors of the parasitoid *T. podisi*. Based on the likelihood ratio test, whose test statistic was $\Lambda = 37.6790$ associated with 11 degrees of freedom, the effect of the treatments (sp0 and sp1) was verified (p - value = 0.0001). Estimates of the intensity of behavioral transitions for each treatment are presented in Table 2. On the diagonal of each matrix, there is the transition intensity associated with the permanence in each state. The higher the rate, the shorter your average length of stay in the state. For example, for the non parasitized egg (sp0), the average time associated with the marking state was approximately 31 seconds ($\frac{-1}{-0.0322}$), while the average time associated with the previously parasitized (sp1) was approximately 1 second ($\frac{-1}{-1.3367}$). It is worth mentioning that a zero transition intensity does not imply an absence of intensities in other behaviors.

Table 2. Intensities of the behavioral transitions of the parasitoid *T. podisi*, in which 1: flying-walking, 2: drumming, 3: oviposition and 4: marking, according to each treatment (sp0 and sp1).

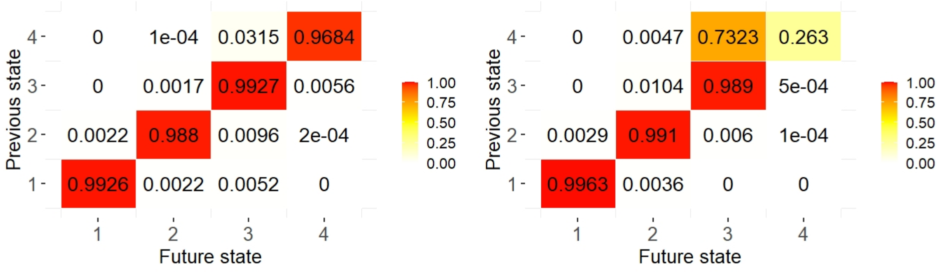
	Previous	Future response			
Treatment	response	1	2	3	4
sp0	1	-0.0075	0.0023	0.0052	0,0000
	2	0.0022	-0.0120	0.0097	0.0001
	3	0.0000	0.0017	-0.0074	0.0057
	4	0.0000	0.0001	0.0321	-0.0322
sp1	1	-0.0037	0.0037	0.0000	0.0000
	2	0.0029	-0.0091	0.0060	0.0002
	3	0.0000	0.0106	-0.0115	0.0010
	4	0.0000	0.0001	1.3366	-1.3367

The average times and respective standard deviations for each behavior are shown in Table 3. It was observed that the stays for the oviposition and marking behaviors are higher when the parasitoids were subjected to non parasitized eggs (sp0). For previously parasitized eggs (sp1), the average times were longer in the flying-walking and drumming states. It should be noted that in the oviposition category the standard deviation was lower for the treatment of non parasitized eggs (sp0).

Table 3. Average and standard deviation of stays times in each of the behaviors of the parasitoid *T. podisi*, in which 1: flying-walking, 2: drumming, 3: oviposition and 4: marking, considering each treatment (sp0 and sp1).

Treatment	Measures	Behaviors			
		1	2	3	4
sp0	Average	134.1266	82.9912	134.4171	31.0951
	Standard deviation	48.1854	44.8590	101.2770	30.2004
sp1	Average	271.8204	110.1277	86.6757	0.7481
	Standard deviation	86.5264	49.5328	530.8702	53.6810

With the estimation of the transition intensities, the probabilities were obtained, which are presented in Figure 2. The stronger colors indicate greater transition probabilities, visually verifying that the highest probabilities refer to stays in the same state for both treatments. It is also noteworthy that in the sp1 treatment (previously parasitized eggs), the probability of transition from the category marking to oviposition was 0.7323.



(a) Treatment sp0 **(b)** Treatment sp1

Figure 2. Estimates of the probabilities of the parasitoid *T. podisi*, where 1: flying-walking, 2: drumming, 3: oviposition and 4: marking, considering each treatment (sp0 and sp1).

In order to have a better view of these behaviors, the probabilities of transition of the parasitoids *T. podisi* were obtained considering the passage of time in seconds (Figure 3). It was found that, in all transitions, the probabilities of oviposition of the parasitoids were higher for healthy eggs. When calculating the odds ratio, considering the flying-walking change to oviposition, the value of approximately 13 times greater was obtained, when subjected to non parasitized eggs, when spending 50 seconds. When spending 200 seconds and considering the change in behavior from drumming to oviposition, a value of approximately 5 times greater was obtained when exposed to healthy eggs, thus indicating greater chances, when the parasitoid *T. podisi* is submitted to sp0 treatment (non parasitized egg).

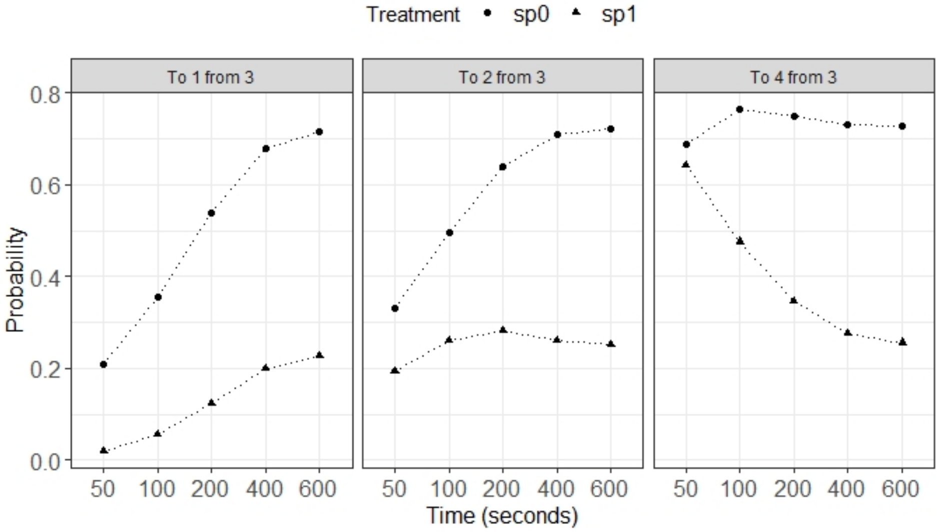


Figure 3. Estimates of the probabilities of the parasitoid *T. podisi* from state 1 to 3, 2 to 3 and 4 to 3, considering each treatment (sp0 and sp1) and the change in time.

Conclusions

Multistate models are flexible to study longitudinal categorical dependent variables. In these cases, it is possible to obtain the transition and intensity matrices, which greatly summarize the continuous time study.

In this work, the use of this methodology made it possible to analyze a typical situation in entomological studies, in which there was interest in describing the behavior of the parasitoid *T. podisi* under two treatments. No censorship data was observed in this study. However, even under this restriction, the methodology was versatile. The results indicate a greater performance and chances of oviposition in the female, when submitted to sp0 treatment (non parasitized eggs), indicating that the parasitoids *T. podisi* prevent intraspecific competition, and are also excellent agents in the biological control of the *E. heros*.

As further studies, the need to develop methods to assess the stationarity or not of these processes is emphasized, as this is a factor that can change the conclusions.

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Conflicts of Interest

The authors declare no conflict of interest.

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