Bacteriophage effect on parasitism resistance

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May 9, 2022

Abstract

Many studies have shown that the protection of the host Acyrthosiphon pisum (Hemiptera, Aphididae) against the parasitoid Aphidius ervi (Hymenoptera, Braconidae) is conferred by the interaction between the secondary endosymbiont Hamiltonella defensa and the bacteriophage APSE (Acyrthosiphon pisum secondary endosymbiont). This interaction consists of the production of toxins by the endosymbiont’s molecular machinery, which is encoded by the inserted APSE genes. The toxins prevent the development of the parasitoid’s egg, conferring protection for the host. However, the effects of this microscopic interaction on host-parasitoid dynamics are still an open question. We presented a new mathematical model based on the bacteriophage effect on parasitism resistance. We identified that the vertical transmission of the bacteriophage and the host survival after the parasitoid attack are potential drivers of coexistence. Also, we showed that the vertical transmission of H. defensa is proportional to the time that the protected population became extinct. Our results showed that the protected and unprotected hosts’ survival after the parasitoid attack is fundamental to understanding the equilibrium of long host-parasitoid dynamics. Finally, we illustrated our model considering its parameters based on experiments performed with A. pisum biotypes Genista tinctoria and Medicago sativa.

1 Introduction

Symbiosis is a type of interaction that mutual benefits between two or more individuals who live close together [Moran 2006, Bourtzis and Miller 2008, Haine 2008, Martin and Schwab 2012]. It can be endosymbiotic when one of the individuals (symbiont) lives inside the body of the other individual (host), either inside their cells or extracellularly [Bourtzis and Miller 2008]. The interaction between symbiont and host may be mandatory when one or both individuals depend on each other for survival or reproduction or optional when the interaction is not strictly necessary [Moran 2006].
Bourtzis and Miller [2008] Hosokawa and Fukatsu [2020]. A mandatory endosymbiont will, in most cases, be present in all individuals of the host population since hosts who do not have the symbiont will not be able to survive [Hosokawa and Fukatsu 2020].

In insects, a well-known case of mandatory endosymbiosis is the interaction between aphids and the endosymbiotic bacterium Buchnera aphidicola [Gil and Latorre 2019]. The bacterium is strictly necessary for the production of amino acids that the aphid is unable to produce while benefiting from the resources used and processed by the aphid, characterizing the process with mutual benefits through interaction [Gil and Latorre 2019]. On the other hand, the result of this interaction can also imply antagonistic actions, depending essentially on how the benefits are obtained [Moran 2006, Haine 2008, Hosokawa and Fukatsu 2020].

During these interactions, the occurrence and permanence of symbionts in a host population are strongly dependent on how the endosymbionts are transmitted between individuals in the host population [Vorburger et al. 2017, Russell 2019, Hosokawa and Fukatsu 2020]. The transmission can be vertical when passed from mother to progeny, or horizontal, through direct contact between individuals, mediated by secretion or excrement and transmitted by parasitoids [Haine 2008, Gehrer and Vorburger 2012, Kaech and Vorburger 2021]. The transmission can also be a combination of these processes [Haine 2008, Gehrer and Vorburger 2012, Kaech and Vorburger 2021]. For optional vertically transmitted endosymbiont, the optional nature of the organism itself should influence its transmission rate [Bourtzis and Miller 2008]. Depending on the rate of transmission, the endosymbiont population may even be locally extinct in case the host is unable to pass it on to the next generations [Bourtzis and Miller 2008, Kwiatkowski and Vorburger 2012]. The study of mechanisms capable of influencing the success or extinction of endosymbionts in insects comprises an active area of research aimed at investigating the interactive dynamics of organisms [Haine 2008, Brownlie and Johnson 2009].

The dynamics of the interaction between a given host and its endosymbionts can also be important for understanding other trophic relationships in the community. A good example, particularly relevant for a scenario involving insects, is the parasitoid-symbiont-host system [Kwiatkowski and Vorburger 2012, Vorburger et al. 2013]. This system has already been investigated considering the host Acyrthosiphon pisum (Hemiptera, Aphididae), the parasitoid Aphidius ervi (Hymenoptera, Braconidae) and the secondary endosymbiont Hamiltonella defensa, a gamma-proteobacteria, [Kwiatkowski and Vorburger 2012, Kaech et al. 2021]. Many results have shown that the interaction between this endosymbiont and the bacteriophage named APSE (Acyrthosiphon pisum secondary endosymbiont) impacts the host protection against parasitoids [Oliver et al. 2009, Kaech et al. 2021]. This interaction consists of the production of toxins by the endosymbiont’s molecular machinery, which is encoded by the inserted APSE genes. The toxins prevent the development of the parasitoid’s egg, conferring protection for the host [Haine 2008, Leybourne et al. 2020, Kaech et al. 2021].

In the example mentioned, the protection that the host A. pisum receives only occurs when the endosymbiont H. defensa is infected with the bacteriophage, APSE (Acyrthosiphon pisum endosymbiont secondary) [Van Der Wilk et al. 1999, Rajarajan et al. 2011]. Thus, the presence of the bacteriophage is fundamental for encoding the gene that produces the toxins that protect the hosts [Oliver et al. 2009, Brandt et al. 2017, Oliver and Higashi 2019]. The bacteriophage belongs to the Podoviridae family [Van Der Wilk et al. 1999, Rajarajan et al. 2011] and different varieties of this bacteriophage confer different levels of protection given the type of toxin that is produced. The endosymbiotic molecular machinery, once infected by the variety APSE-2, produces a homologue of the cytolethal distention toxin (cdtB), causing the host protection of approximately 40% against the parasitoid attack. Whereas the variety APSE-3 encodes a YD-repeat that has
toxic proteins, and when the symbiosis occurs between the host and the infected endosymbiont, the protection against the parasitoid attack is approximately 85% [Oliver et al., 2009]. This protection variation is also found within different hosts biotypes [Sochard et al., 2020a].

This microbial interaction causes directly or indirectly interference in interspecific interactions within the community. In general, organisms can modify the use of host plants by phytophagous insects, provide resistance to natural enemies, and also reduce global genetic diversity or gene flow between populations within some species [Ferrari and Vavre, 2011] Frago et al., 2017]. These actions can occur through changes between the sex ratio of insects, caused, for example, by the bacteria of genera Wolbachia, or even by the history of genetic relationship between the host and the symbiont [Moran, 2006] [Bourtzis and Miller, 2008] [Ferrari and Vavre, 2011]. Actions of this nature are often highly relevant to the permanence of symbionts in their hosts. The implications of the symbiont’s permanence within the system, as well as their effect on the parasitoid-host relationship [Kwiatkowski and Vorburger, 2012], give the system significant complexity.

The complexity encountered at the system involving the aphid Acyrthosiphon pisum, the parasitoid Aphidius ervi, the endosymbiont H. defensa and the bacteriophage APSE have a direct impact on biological controls methods [Vorburger, 2018]. Given that releasing parasitoids at agroecosystems is a common procedure [Vorburger, 2018] Giunti et al., 2015 Parra and Coelho, 2019 Leung et al., 2020 to reduce pest population, the host protection against the parasitoids makes a significant impact on pest control [Godfray and Godfray, 1994] Giunti et al., 2015 Leung et al., 2020. Thus, understanding the host protection evolution is fundamental to optimising biological control methods and reducing economic damage caused by these pests. Also, this dependent protection provided by the microbial interactions turns pest control into a challenging research question.

The whole scenario involving the simultaneous occurrence of protected and unprotected hosts requires careful analysis for the correct interpretation of the processes and phenomena involved, lacking the use of analytical tools with the potential to describe the complex mechanisms involved in the system [Leung et al., 2020]. It can be observed in several scales [Rocha et al., 2018] and to understand this system. It is necessary to use appropriate tools to identify and interpret ecological patterns more effectively. Mathematical and computational models are commonly worn among the analytical tools available to investigate systems of this nature [Ferreira and Godoy, 2014]. The use of mathematical models to describe ecological patterns in dynamical systems has brought a significant scientific contribution due to the flexibility of these tools to develop algorithms capable of analyzing different ecological phenomena in time and space. Mathematical models can be used to describe ecological processes and predict population trends. The use of this resource has been increasing in recent decades in response to the growing demand for the formalization of population processes, with the possibility of simulating biological scenarios. The understanding of fundamental population aspects and community functions using ecological mathematical formalism has produced actual results to compose ingredients of ecological theory, capable of covering a broad spectrum of issues, ranging from ecological space and time patterns of populations and communities to epidemiological aspects of trophic networks [Ferreira and Godoy, 2014].

The perspective of mathematical modelling brings the idea of reorganizing dynamic systems considering dimensions and scales that can be investigated [Ferreira and Godoy, 2014]. This new condition under which systems can be visualized allows new interpretations in a gradual and oriented way so that the population variations of systemic members can be understood as coming from endogenous and exogenous forces governing the system as a whole [Ferreira and Godoy, 2014]. The expectation of understanding the influence of the endosymbiont on the host-parasitoid system
ecologically can be met through the use of mathematical models [Kwiatkowski and Vorburger 2012 (Jones and Boots, 2007; Kwiatkowski and Vorburger, 2012). Issues of theoretical and applied relevance can be investigated from models capable of analyzing the population dynamics of the system, including the influence of the vertical transmission of endosymbionts. Here we aim to study the influence of the bacteriophage APSE on the host-symbiont-parasitoid system and its impact on the protection evolution. Thus, we introduced a new mathematical model that contains the microbial interaction responsible for conferring protection to the host A. sifum against the parasitoid A. ervi.

2 Methods

Model formulation

To model the system involving the aphid Acyrthosiphon pisum and the microorganisms responsible for its protection against the parasitoid Aphidius ervi [Oliver et al. 2009] we considered the results provided by the Oliver et al. [2009] and added the microorganisms interaction by including the bacteriophage APSE and the secondary symbiont Hamiltonela defensa on the host-parasitoid system. To consider these elements, the model consists of three differential equations representing the host population of Acyrthosiphon pisum which is infected with the endosymbiont Hamiltonela defensa (H), population of Acyrthosiphon pisum which is infected with the endosymbiont Hamiltonela defensa plus the bacteriophage APSE (V) and the non-infected population of Acyrthosiphon pisum (S). The model is composed of the following system:

\[
\begin{align*}
\frac{dS}{dt} &= b_S S \left(1 - \frac{N}{K}\right) \exp\left(\frac{\alpha P}{\beta N + P}\right) + (1 - \tau_H) b_H H \left(1 - \frac{N}{K}\right) \exp\left(\frac{\alpha P}{\beta N + P}\right) \\
&\quad + (1 - \tau_V) b_V V \left(1 - \frac{N}{K}\right) \exp\left(\frac{\alpha P}{\beta N + P}\right), \\
\frac{dH}{dt} &= \tau_H b_H H \left(1 - \frac{N}{K}\right) \exp\left(\frac{\alpha P}{\beta N + P}\right) - \mu H \\
&\quad + (1 - \tau_X) \tau_V b_V V \left(1 - \frac{N}{K}\right) \exp\left(\frac{\alpha P}{\beta N + P}\right), \\
\frac{dV}{dt} &= \tau_X \tau_V b_V V \left(1 - \frac{N}{K}\right) \exp\left(\frac{\alpha P}{\beta N + P}\right) - \mu V.
\end{align*}
\]

We considered the logistic growth \(b_S \left(1 - \frac{N}{K}\right)\), where \(b_s\), \(b_h\) and \(b_v\) are growth rates, \(N = S + H + V\) is the total of hosts and \(K\) is the carrying capacity for the system. We consider the logistic growth based on the fact that the population density of hosts depends on the presence of resources and the space restriction encountered during its life cycle. To indicate the protection against parasitoids, we included the survival rate as \(\exp\left(\frac{\alpha P}{\beta N + P}\right)\), where \(P\) is the number of parasitoids, \(\alpha\) is the percentage of unprotected hosts (S and H) killed by its attack, and \(\beta\) is the oviposition ratio. For the hosts that carry both APSE plus Hamiltonela defensa, we used the parameter \(\theta\) representing the percentage of protected hosts (V) killed by parasitoids. To represent the protection of this population, we set \(\theta \geq \alpha\).
The vertical transmission of *Hamiltonela defensa* can occur in two forms by our model. The first considers that the secondary endosymbiont can be transmitted to an offspring by adults that has only *Hamiltonela defensa* ($\tau_H$) or by adults that has both *Hamiltonela defensa* plus the bacteriophage APSE ($\tau_V$). We also considered the vertical transmission of the bacteriophage APSE by the parameter $\tau_X$. These vertical transmissions are based on the anatomy of *Acyrthosiphon pisum* that indicates these possible types of transmissions. More specifically, we are considering that during the process of nymphs birth, the aphid embryo can touch or not the mycetocytes (Cells carrying secondary endosymbiont and the bacteriophage) to receive these microorganisms [Van Der Wilk et al., 1999]. These transmissions can be clarified by the figure 1 where we represent all possibilities of microorganisms’ vertical transmissions and other components of our model. Finally, we considered that the natural mortality of each population of hosts is the same $\mu$ based on the fact that the main difference between these populations only occurs with parasitoids.

Figure 1: Diagram representing the ingredients of the model. Only the blue boxes are dynamic variables. Full lines represent reproduction and maturation, dotted ones represent mortality.

**Parameter setting**

The growth rate of each population $b_s, b_h$ and $b_v$ are here determined by *Acyrthosiphon pisum* biology and the constitutive and induced cost of the harbouring the endosymbiont [Hutchison and Hogg, 1985, Lu and Kuo, 2008, Weldon et al. 2013, Kaech et al. 2022]. Thus, we set $b_s = 0.4$, $b_v = 0.3$ and $b_h = 0.2$ considering a constitutive cost of $c_c = \frac{b_v}{b_s} = 0.5$ and a induced cost $c_i = \frac{b_v - b_s}{b_s} = 0.25$. We also set the carrying capacity $k = 15,000$ [Kwiatkowski and Vorburger 2012] and the death rate $\mu = 0.05$ [Hutchison and Hogg 1985, Lu and Kuo 2008]. The vertical
transmission rates $0 \leq \tau_H \leq 1$, $0 \leq \tau_V \leq 1$, $0 \leq \tau_X \leq 1$ are positive parameters. Here we set $\tau_H = 0.995$ based on the feasibility of vertical transmission of $H. \text{defensa}$ [Vorburger et al., 2017]. We also considered $\tau_V \leq \tau_H$ based on the fact that the bacteriophage can kill some symbionts and create difficulties during the vertical transmission of the endosymbiont. Considering the vertical transmission, the anatomy of the hosts suggests that these vertical transmission parameters are closed, thus we set $\tau_V = 0.95$ and $\tau_X = 0.995$. However we could not find it in literature and we selected this parameter empirically based on the insect biology. To overcome this problem, we analyzed the parameters space of $\tau_X$ and $\tau_V$ by bifurcation analysis.

We considered two biotypes of the host Acyrthosiphon pisum for the parameter choice. The first one is the Genista tinctoria biotype presented in the table 1. This biotype has the secondary endosymbiont fixed into their natural population [Sochard et al. 2020a], thus we set the model variables $S = 0$, $H = 500$ and $V = 2500$. To select the host survival rate after parasitoid attack we used the experiments from [Sochard et al., 2020a] to obtain $\alpha = 0.549$ and $\theta = 0.676$, more specifically, we used the data from [Sochard et al., 2020b] G. tinctoria biotype. For this task we selected hosts infected with H. defensa plus APSE (“H-Ms2” in the dataset) and uninfected biotype (“Cured” in the dataset) Genista tinctoria (In the data set they are named “G.tinctoria”). During the experiment the hosts were artificially infected with H. defensa strains from another aphid biotype named Medicago sativa (For more details see [Sochard et al., 2020a]). Using $N$ experiments, which briefly consists on offering $M = 15$ aphids to A. ervi female in a glass Petri dish containing a leaf disk of V. faba, we used the number of emerged parasitoids from uninfected hosts $P^*$ to compute $\alpha = -\log \left( \frac{\sum_{n=1}^{N} (M-P^*)}{N} \right)$, and the number of emerged parasitoids from infected hosts $P^v$ to compute $\theta = -\log \left( \frac{\sum_{n=1}^{N} (M-P^v)}{N} \right)$.

The second one is the Medicago sativa biotype presented in table 1. In natural populations H. defensa has intermediate to high frequencies in this biotype, thus we set the model variables $S = 500$, $H = 500$ and $V = 2000$. The survival rates were obtained using the results provided by the first experiment of the work of Dion et al. [2011], more specifically, the results encountered in the section Experiment 1: Aphid clone resistance measurement. Using the equation 2 coupled with the results of $P^* = 143$ parasitoids emerged from $M = 200$ uninfected hosts we obtained $\alpha = 0.285$. We used the equation 3 and the results of $P^v = 33$ parasitoids emerged from $M = 400$ to obtain $\theta = 0.917$. Finally, for both biotypes, we set $\beta = \frac{1}{105}$ and performed a bifurcation analysis to observe the influence of these parameters on the stability of the system.

**Analyses**

**Bifurcation**

To perform the bifurcation analysis we used the parameters obtained from both host biotypes Genista tinctoria and Medicago sativa. For that, we selected each parameter to analyse its effect on the dynamics stability in both scenarios. Using the parameter and variables from table 1, we analyse the following parameters spaces $P = (1, 2, \ldots, 100)$, $\mu = (0, 0.01, \ldots, 1)$, $\tau_H = (0, 0.01, \ldots, 1)$, $\tau_V = (0, 0.01, \ldots, 1)$, $\tau_X = (0, 0.01, \ldots, 1)$, $\alpha = (0, 0.01, \ldots, 1)$, $\theta = (0, 0.01, \ldots, 1)$ and $\beta = (0, 0.01, \ldots, 1)$. For each parameter we computed 5000 model iterations and selected the number of individuals for $S$, $V$ and $H$ in the last iteration (The 5000th iteration). We repeated this process for each value of the the selected parameter space and we also performed this process using the parameters and variable from table 1.
Table 1: Description of parameters and variables used for the host-virus-parasitoid model. The values of each parameter are presented for the species *Medicago sativa* and *Genista tinctoria* hosts biotype. These values were obtain based on the dataset available in the work of Sochard et al. [2020a] and Dion et al. [2011].

| Symbol | Meaning | Values |
|--------|---------|--------|
| **Model variables:** | | |
| $S$ | Uninfected hosts | *Genista tinctoria* | 0 (Initial) | 500 (Initial) |
| | | *Medicago sativa* | 500 (Initial) | 500 (Initial) |
| $H$ | Hosts infected with *Hamiltonela defensa* | 500 (Initial) | 500 (Initial) |
| $V$ | Hosts infected with *Hamiltonela defensa* and APSE | 2500 (Initial) | 2500 (Initial) |
| **Parameters:** | | |
| $b_s$ | Growth rate of population $S$ | 0.4 | 0.4 |
| $b_h$ | Growth rate of population $H$ | 0.2 | 0.2 |
| $b_v$ | Growth rate of population $V$ | 0.3 | 0.3 |
| $\mu$ | Mortality rate | 0.05 | 0.05 |
| $\tau_H$ | Vertical transmission of *H. defensa* by $S$ | 0.995 | 0.995 |
| $\tau_V$ | Vertical transmission of *H. defensa* by $V$ | 0.950 | 0.950 |
| $\tau_X$ | Vertical transmission of APSE | 0.995 | 0.995 |
| $K$ | Carrying capacity | 15,000 | 15,000 |
| $P$ | Parasitoid population | 200 | 200 |
| $\alpha$ | Hosts survival after parasitoid attack | 0.549 | 0.285 |
| $\theta$ | Hosts survival after parasitoid attack | 0.676 | 0.917 |
| $\beta$ | Oviposition ratio | $\frac{1}{105}$ | $\frac{1}{105}$ |

Finally, we performed two dimensional bifurcation analysis with the parameters $(P, \tau_V)$, $(P, \tau_X)$, $(\alpha, \theta)$ and $(c_x, c_1)$. This briefly consists on computing 5000 iterations to obtain $S$, $V$ and $H$. The difference here is that we used a grid $P = (0, 1, \ldots, 1000)$, $\alpha = (0, 0.01, \ldots, 1)$, $\theta = (0, 0.01, \ldots, 1)$, $c_x = (0, 0.01, \ldots, 1)$, $c_1 = (0, 0.01, \ldots, 1)$ and $(0, 0.01, \ldots, 1)$ for $\tau$ parameters. Thus, for each pair of values we obtained $S$, $V$ and $H$ in the last iteration to evaluate the model stability. After that, we transform the density of all populations in a binary vector that represents the presence of all populations ($v = [1, 1, 0]$), only $S$ ($v = [1, 0, 0]$), only $H$ ($v = [0, 1, 0]$), only $V$ ($v = [0, 0, 1]$), $S$ with $H$ ($v = [1, 1, 0]$), $S$ with $V$ ($v = [1, 0, 1]$) and $H$ with $V$ ($v = [0, 1, 1]$). Using these vectors we could represent the presence and absence of the host populations for the grid of parameters. We repeat this process for both host biotypes fixing the other parameters of the model based on table 1 as previously.

Protection evolution

To analyse the time required to extinct the hosts infected with *H. defensa* plus APSE ($V$) from the system with the absence of parasitoids, we obtained the initial values of our model based on the equilibrium point considering the presence of parasitoids in an attempt to guarantee the high frequency of protected hosts. For that, we selected the 5000th densities of $S$, $H$ and $V$. Then, we used the parameters presented in table 1 to calculate the number of model iterations required for $V$ reaches zero. To better observe the influence of the vertical transmission of APSE and the vertical transmission of *H. defensa* from $V$ on the time required to extinct this infected hosts we repeated this process for $\tau_V = (0, 0.01, \ldots, 1)$ and $\tau_X = (0, 0.01, \ldots, 1)$. 

7
3 Results

3.1 Population Dynamics

Considering both biotypes *Genista tinctoria* and *Medicago sativa* we simulate their dynamics based on the model formulation. Figure 2 showed that the parasitoid influences the model differently based on these biotypes analyzed. For the scenario with \( P = 200 \) parasitoids we observed that the *Genista tinctoria* system had an equilibrium point \((S = 12000, H = 0, V = 0)\) and the density of hosts infected with *H. defensa* plus APSE initially increase and after, approximately, 600 model iterations its population reached 0. On the other hand, the *Medicago sativa* system reached the equilibrium point \((S = 1900, H = 0, V = 10000)\) faster than the other biotype showing the importance of the host survival after parasitoid attack \( \alpha \) and \( \theta \) for our model.

The scenario with absence of parasitoids \((P = 0)\) showed that both systems tended for the same equilibrium point \((S = 12500, H = 0, V = 0)\). The initial 150 model iterations showed that the *Genista tinctoria* system presented more hosts infected with *H. defensa* plus APSE than the other biotype. Also, figure 2 c and d showed that \( V \) reached 0 faster for the *Medicago sativa* as compared with *Genista tinctoria* system. Individually, our results showed that the *Genista tinctoria* system had the same behaviour for both scenarios with the presence or absence of parasitoids. Otherwise, we observed that the presence of parasitoids influenced the persistence of the hosts infected with *H. defensa* plus APSE \((V)\), given that these hosts reached 0 faster than the scenario with an absence of parasitoids. On the contrary, the *Medicago sativa* system showed a difference in behaviour with the presence or absence of parasitoids. The hosts infected with *H. defensa* and APSE turned out to be more frequent in the system with parasitoids than the other. The equilibrium point \((S = 1900, H = 0, V = 10000)\) showed in figure 2 d illustrate this behaviour.

3.2 Bifurcation

The bifurcation analysis showed that the parameter \( \alpha \) have a strong influence on *Genista tinctoria* and *Medicago sativa* system equilibrium. As \( \alpha \) increases the equilibrium point change from \((S = 0, H = 0, V = 11900)\) to \((S = 12900, H = 0, V = 0)\) in both systems. However, we observed that this inversion occurred with small values of \( \alpha \) for the *Genista tinctoria*. The figure 3 also shows the influence of the parameter \( \theta \) that, in contrary to the previous parameter, have an expressive difference between both biotypes. As \( \theta \) increases the inversion of equilibrium point from \((S = 10900, H = 0, V = 0)\) to \((S = 12000, H = 0, V = 0)\) only occurred for the *M. sativa* biotype. In the other hand, for the *G. tinctoria* biotype the results showed that changes at the equilibrium point only occurred for \( \theta \geq 0.9 \).

Figures 4 a and c showed that as the death rate \( \mu \) increases for both systems, all populations tend intuitively to zero, but the most frequent population changed between the biotypes, as previously pointed out. As the number of parasitoids \( P \) increases the equilibrium point changed from \((S = 0, H = 0, V = 11900)\) to \((S = 10900, H = 0, V = 0)\) for the *M. sativa* biotype. For the other system, we observed that as the parasitoid increases, this inversion does not occur for these parameter spaces.

Considering the vertical transmission of *H. defensa* by the uninfected hosts \( S \) \((\tau_H)\) the figure 5 showed that for the biotype *G. tinctoria* as this parameter increases the equilibrium point \((S = 12200, H = 0, V = 0)\) remained the same. This behaviour also occurred for the *M. sativa* biotype, but the equilibrium point was \((S = 1000, H = 0, V = 11900)\). As \( \beta \) increases, we observe the inversion of the frequent population only at the *M. sativa* biotype. This inversion occurred from
Figure 2: Population density as a function of time with parasitoid presence $P = 200$ (Figures a and b) and absence $P = 0$ (Figures c and d). The biotype *Genista tinctoria* dynamics is represented by a and c (Parameter used as in table 1) and the biotype *Medicago sativa* dynamics is represented by b and c.

The equilibrium point $(S = 100, H = 0, V = 11900)$ to $(S = 12200, H = 0, V = 0)$. On the contrary, this inversion does not occur at the biotype *G. tinctoria*, given that as $\beta$ increases, the equilibrium with the uninfected hosts $S$ as the most frequent population remains the same.

Considering the vertical transmission of *H. defensa* by hosts infected with *H. defensa* plus APSE ($\tau_V$), for the biotype *M. sativa* (Figure 6) as this parameter increases the equilibrium point change from $(S = 10900, H = 0, V = 0)$ to $(S = 0, H = 0, V = 12000)$. However, for the biotype *G. tinctoria* as $\tau_V$ increases the equilibrium point $(S = 12100, H = 0, V = 0)$ remains the same. The vertical transmission of APSE had similar behaviour for both scenarios, but for the biotype *M. sativa* as $\tau_X$ increases the system presented an equilibrium with all population, for example $(S = 2000, H = 2000, V = 7000)$, showing the possibility of coexistence of these hosts. Finally, for the biotype *G. tinctoria* as $\tau_X$ increases the equilibrium point $(S = 1200, H = 0, V = 0)$ remains constant.

Based on the results of the population persistence in figure 7, the grids performed with vertical transmission of APSE ($\tau_X$), vertical transmission of *H. defensa* by $V$ ($\tau_V$) and parasitoid density $P$.
Figure 3: Results of the Bifurcation analysis for the parameters $\theta$ and $\alpha$. The results are presented for both biotypes *Genista tinctoria* (Letters a and c) and *Medicago sativa* (Letters b and d). Here we selected the point $(S,H,V)$ after 5000 model iterations for each parameters value of $\alpha$ and $\theta$.

did not change the *Genista tinctoria* system equilibrium, given that the uninfected hosts $S$ persisted as the unique population regardless of the parameter’s values. On the contrary, we observed that the *Medicago sativa* system varies its composition according to the grid of these parameters. The second column of figure 7 shows that the grids resulted in the following population combinations:

1. Uninfected hosts $(S)$
2. Uninfected hosts $(S)$ and infected hosts with *H. defensa* plus APSE $(V)$
3. Uninfected hosts $(S)$, infected hosts with *H. defensa* and infected hosts with *H. defensa* plus APSE $(V)$,

Figure 7 b and f showed that according to the grid $(\tau_X, P)$ and $(\tau_Y, P)$ the uninfected hosts $(S)$, infected hosts with *H. defensa* $(H)$ and infected hosts with *H. defensa* plus APSE $(V)$ are more frequent than the grid of $\tau_X$ and $\tau_Y$ presented in figure d.
Figure 4: Results of the Bifurcation analysis for the parameters $\mu$ and $P$. The results are presented for both biotypes *Genista tinctoria* (Letters a and c) and *Medicago sativa* (Letters b and d). Here we selected the point $(S, H, V)$ after 5000 models iterations for each parameters value of $\mu$ and $P$.

### 3.3 Protection evolution

Figure 10 shows that after 5000 model iterations with the presence of $P = 200$ parasitoids, the *G. tinctoria* system persisted with only uninfected hosts. Thus, according to the population density, bifurcation analysis and this figure, we observed that the selected parameter represents the *G. tinctoria* system stabilize with $(S > 0, H = 0, V = 0)$ regardless of the presence or absence of parasitoids. Given this result, we only considered the biotype *M. sativa* for this analysis. Finally, the figure 11 shows that the transmission of APSE ($\tau_X$) and the transmission of *H. defensa* by $V$ ($\tau_V$) are proportional to the time that hosts infected with *H. defensa* plus APSE reaches 0. This time vary from 0 to 700 model iterations for the parameter $\tau_V$ and a variation from 0 to 500 for the parameter $\tau_X$, where 0 indicates that the presence of parasitoids did not influence the increase of the infected hosts $V$. We observed that the time only increased when $\tau_V \geq 0.6$ and $\tau_X > 0.6$. 
4 Discussion

The influence of microbial symbionts on animal protection against natural enemies is an important open question in ecology [Kwiatkowski and Vorburger, 2012, Vorburger et al., 2017, Leybourne et al., 2020]. We introduced a new mathematical model based on the bacteriophage effect on parasitism resistance. We have shown that different combinations of host survival after the parasitoid attack for unprotected hosts $S$ plus $H$ ($\alpha$) and protected hosts $V$ ($\theta$) resulted in four possible outcomes: coexistence of all hosts ($S$, $H$ and $V$), extinction of all hosts, the unique presence of uninfected hosts $S$ and infected hosts with $H. \text{defensa}$ plus APSE $V$. The analysis of biotype $G. \text{tinctoria}$ also illustrated these results, given that our model’s unique presence of infected hosts $S$ was a common outcome. This outcome indicates that $H. \text{defensa}$ can be excluded of $G. \text{tinctoria}$ biotype population. This finding expanded the results provided by [Sochard et al., 2020b] given that our model provides additional support for the fact that $G. \text{tinctoria}$ which received $H. \text{defensa}$ strains of $\text{Medicago sativa}$ biotype provides no protection against parasitoids.

Besides, this result also shows the effect of the costs involved in harbouring endosymbionts.
added the constitutive cost \( c_c = \frac{b_s - b_h}{b_h} \) and the induced cost \( c_i = \frac{b_s - b_v}{b_h} \) considering \( b_s \geq b_v \geq b_h \) (i.e Growth rates). Figure 8 indicates that the coexistence of all populations also mainly depends on the induced cost (See figure 8), which supports previous results provided by Kwiatkowski and Vorburger [2012], where they identified employing a mathematical model that the induced cost as a potential driver of coexistence. Kwiatkowski and Vorburger [2012] also indicates that the vertical transmission of \( H. \) defensa can be a potential driver of coexistence, and here we substantiated this finding and complemented their results by showing that the vertical transmission of APSE, \( H. \) defensa by uninfected \( S \) and infected hosts \( V \), hosts’ survival after parasitoid attack for unprotected hosts \( S \) plus \( H \) (\( \alpha \)) and protected hosts \( V \) (\( \theta \)) can be potential drivers of coexistence.

The triggering mechanisms of the trophic relationships above mentioned have, besides from ecological relevance for trophic systems, a high degree of complexity and are often determinants of success or failure between parasitoid controlling hosts, with significant importance for the biological control of agricultural pests, scenarios especially relevant when the participating microorganisms can confer resistance to the hosts against parasitoids Vorburger [2018]. The use of natural enemies for biological control and integrated pest management in agriculture has been consolidated over time, mainly through the use of microorganisms, such as entomopathogenic fungi and macro-control

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**Figure 6**: Results of the Bifurcation analysis for the parameters \( \tau_X \) and \( \tau_V \). The results are presented for both biotypes *Genista tinctoria* (Letters a and c) and *Medicago sativa* (Letters b and d). Here we selected the point \((S,H,V)\) after 5000 models iterations for each parameters value of \( \tau_X \) and \( \tau_V \).
agents, such as predatory arthropods and mainly parasitoids van Lenteren et al. [2018]. In the specific case of parasitoids, the results have been quite promising, especially in the control of pests of critical crops Chailleux et al. 2012, Veiga et al. 2013, van Lenteren et al. 2018.

Our results provided by the protection evolution analysis are also applied to the biological control context. Our findings suggest that the vertical transmission of APSE and H. defensa by V can be potential drivers of the time to the extinction of infected hosts V. We observed that these parameters are proportional to when V reaches extinction in a scenario with parasitoids’ absence and a high density of protected hosts V. This indicates that the host population will become entirely susceptible to parasitoid attack again. Thus, interrupting the release of parasitoids to apply different strategies, such as the release of predators Parra and Coelho, 2019, has the potential to maintain the high efficiency of biological control with parasitoids. Finally, genetic and genomic techniques are required to observe such details of microbial interference in the host-symbiont-parasitoid system.

Also, new standardised methodologies should be developed to optimise the identification of these microorganisms Vorburger, 2018 and facilitate the detection for extensive surveys. Finally, as discussed by Leung et al. 2020, integrating genetics and genomics tend to be the next generation of biological control yielding the procedure optimisation, so our finding is in good agreement with this interdisciplinary field.

To summarise, we have investigated the bacteriophage effect on parasitism resistance by introducing a new mathematical model that considers the microorganism interaction in the host-parasitoid system. We analysed two hosts biotypes by using parameters obtained from literature sources. It allow us to identify that the vertical transmission of APSE, H. defensa by uninfected (S) and infected hosts (V), hosts’ survival after parasitoid attack for unprotected hosts S plus H (α) and protected hosts V (θ) as possible drivers of coexistence. Also, we have shown that vertical transmission of the bacteriophage APSE and the vertical transmission of the H. defensa by V can be potential drivers of the time to the extinction of infected hosts V. Finally, we intend to continue our work to expand the model to consider spatial interactions and add new components such as migration.

Acknowledgements

GRP would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (proc. no. 2014/16609-7 and proc. no. 17/19984-1) for financial support.

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Figure 7: Population persistence for a range of parasitism ($P$), vertical transmission of the bacteriophage ($\tau_X$) and vertical transmission of $H. defensa$ by $V$ considering the *Genista tinctoria* (a, c and e) and *Medicago sativa* biotype (b, d and f).
Figure 8: a) Population persistence for a range of parasitoid attack survival $\alpha$ and $\theta$ considering the *Medicago sativa* biotype. b) Population persistence for a range of $c_c$ and $c_i$ considering the *Medicago sativa* biotype.

Figure 9: Time until the population loses resistance as a function of $\tau_V$ and $\tau_X$ considering the biotype *M. sativa*. When the number of iterations reaches 0 it means that the stability with the presence of parasitoid presented $V = 0$. 