Journal of Pest Science
https://doi.org/10.1007/s10340-024-01745-6

ORIGINAL PAPER

Better alone than in bad company? Modeling the intra-guild predation and release timing in the biological control of *Pseudococcus viburni*

Margarita C. G. Correa[1] · Juan Pablo Gutiérrez-Jara[1] · Katia Vogt-Geisse[2] · Hugo Benitez[1,6] · Laura M. Pérez[3] · Alexandre Fleisch[4] · Thibaut Malausa[4] · Loïc Queguiner[4] · Sharon Rodríguez[5] · Nicolas Ris[4] · Philippe Kreiter[4]

Received: 13 July 2023 / Revised: 27 December 2023 / Accepted: 8 January 2024

Abstract

The obscure mealybug, *Pseudococcus viburni*, is a serious agricultural pest worldwide. The biological control in commercial fields of *P. viburni* relies on predators and parasitoids, in particular the generalist coccidophagous ladybird *Cryptolaemus montrouzieri* and the specific parasitoid *Acerophagus flavidulus*. However, these two natural enemies can establish an intraguild predation interaction, reducing the efficiency of biological control. *Cryptolaemus montrouzieri* may negatively impact the population dynamics of *A. flavidulus* if it feeds indifferently on healthy and parasitized mealybugs. With the aim of improving the biological control of *P. viburni*, in this work, we studied the feeding behavior of *C. montrouzieri* in the absence or presence of *A. flavidulus* larvae of different age within mealybugs, in laboratory conditions. Subsequently, with the data obtained, we mathematically modeled the dynamics of *P. viburni* to study the impact on *P. viburni* control of different field implementation schedules for the release of ladybird and parasitoid populations. The ladybird fed on parasitized *P. viburni* but reduced its consumption when they were infested by parasitoids aged of 4 days or more. Modeling results suggest that these feeding preferences of predators may have a positive impact on pest control, that releasing predators and parasitoids together is in general more effective than releasing them independently, and that releasing highly effective predators alone could be the best choice. Modeling results also provide information on different release schedules.

Keywords Obscure mealybug · Parasitoid · Predator · Intraguild predation · Biological control release

Communicated by Jay Rosenheim.

✉ Margarita C. G. Correa
mcorreag@ucm.cl

✉ Hugo Benitez
hbenitez@ucm.cl

Juan Pablo Gutiérrez-Jara
jgutierrez@ucm.cl

Katia Vogt-Geisse
katia.vogt@uai.cl

Laura M. Pérez
lperez@uta.cl

Alexandre Fleisch
alexandre.fleisch@fredon-alsace.fr

Thibaut Malausa
thibaut.malausa@inrae.fr

Sharon Rodríguez
sharon.rodriguez@unab.cl

Nicolas Ris
nicolas.ris@inrae.fr

Philippe Kreiter
philippe.kreiter@inrae.fr

[1] Centro de Investigación de Estudios Avanzados del Maule, Universidad Católica del Maule, Av. San Miguel 3605, 3460000 Talca, Chile

[2] Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibáñez, Diagonal Las Torres 2640, Peñalolén 7941169, Santiago, Chile

[3] Departamento de Física, FACI, Universidad de Tarapacá, Casilla 7D, 1000000 Arica, Chile

[4] INRAE, Université Côte d'Azur, CNRS, ISA, 06903 Sophia Antipolis, France

[5] Centro de Biotecnología de Sistemas, Universidad Andrés Bello, Fernandez Concha 700, Las Condes 7550000, Santiago, Chile

[6] Centro de Investigación en Recursos Naturales y Sustentabilidad (CYRENYS), Universidad Bernardo O'Higgins, Santiago, Chile

Introduction

The first successful case of biological control dates from 1888, when a small number of individuals of the ladybird *Rodolia cardinalis* (Mulsant) were released to control the invasive scale *Icerya purchasi* Maskell (De Bach 1965). Since then, biological control of pests has improved, becoming another alternative available to producers to control pests by releasing mass-reared natural enemies (Parra and Coelho 2022), with even more than one natural enemy species commercially available for some pest species. When there is more than a natural enemy available, the question that arises is how many biological control agents do we need to control an insect pest (Myers et al. 1989). Some authors have described an additive effect of more than one natural enemy that tends to favor biological control (Cardinale et al. 2003; Aquilino et al. 2005; Straub and Snyder 2008), while other studies have reported a decreased efficiency of pest control with multiple releases (Rosenheim et al. 1993; Ferguson and Stiling 1996; Takizawa et al. 2000). A meta-analysis of 25 successful projects involving the release of multiple biological control agents release projects against insect pests revealed that only 44 % had two to four species responsible for success, and 56 % of these projects had only one species responsible for success (Denoth et al. 2002). These results depend mainly on interactions between natural enemies, especially a trophic interaction with each other such as parasitism or predation, also called Intraguild Predation (IGP) (Rosenheim et al. 1995; Snyder et al. 2004). One of the most representative intraguild predation interactions that can affect the result of biological control is predators-parasitoids (Weber and Lundgren 2009). Immature parasitoids within their hosts are particularly vulnerable to predation by predators (Snyder et al. 2004) as the latter rarely discriminate between parasitized and unparasitized prey (Colfer and Rosenheim 2001; Bilu and Coll 2007), although this may depend on the age of the parasitoids because pupae or mummies tend to be less preferred than developing host larvae (Chong and Oetting 2007).

Release timing is of crucial interest when agricultural producers choose biological control as the strategy to control pests (Abell et al. 2020). A growing methodology to decide release timing of natural enemies is the use of mathematical models (Pfab et al. 2018). Mathematical models have emerged as a useful tool to predict the output of biological control in integrated pest management programs. Among the studies using mathematical models to gain insight into the biological control of insect pests (Mills and Getz 1996), many are based on the continuous-time framework of the Lotka-Volterra model (Lotka 1925; Volterra 1926) and include different functional responses for parasitism or predation (Royama 1971; Holling 1959; Rogers 1972). For example, a recent article uses a differential equations system to model a three-way pest-parasitoid-predator interaction to control *Tuta absoluta*, a pest of tomato crops from South America, showing that the joint use of parasitoids and predators is the best option to control the pest (Rubio et al. 2022). In (Molter et al. 2023), the authors study the interaction between *Diatraea saccharalis*, key sugarcane pest, and its parasitoids through a system of differential equations, describing the dynamics of the host- parasitoid and implementing different optimal control strategies.

The dynamics of some evolving processes are subject to abrupt changes, involving perturbations of insignificant duration compared with the development of continuous dynamics. Such perturbations are represented by pulses and give rise to the so-called Impulsive Differential Equations (IDE). These types of equations have been used to model problems in physics, ecology, biological systems, epidemiology, and others (Benchohra et al. 2006; Córdova-Lepe et al. 2012; Gutiérrez-Jara et al. 2020, 2023). There are also models of IDE based on Lotka-Volterra systems, such as two models presented in (Tang and Chen 2004) about general pest management. In (Shi et al. 2009), the authors present an IDE model for integrated pest management, with disease in the prey and pulse release of an infective prey population.

Pseudococcus viburni is a mealybug that is widespread throughout the world and feeds on a wide range of hosts (Walton and Pringle 2004; Culik and Gullan 2005; da Silva et al. 2017). Damage caused by this mealybug includes direct damage produced by feeding, economic losses due to a depreciation of fruit commercial value (induced by the development of sooty mold on honeydew secreted by the mealybug), and virus transmission (Golino et al. 2002; Mathulwe et al. 2021). In several countries, biological control is an alternative for integrated pest management of *P. viburni*. The main parasitoid used is the specific gregarious endoparasitoid *Acerophagus flavidulus* (Daane et al. 2008; Pacheco da Silva et al. 2021), and the main predator is the generalist coccidophagous coccinellid, *Cryptolaemus montrouzieri*, that also feeds on several other mealybugs, i.e., *Planococcus citri* Risso, *Maconellicoccus hirsutus* Green, *Ferrisia virgata* Cockerell and *Heliococcus bohemicus* Šulc (Hamid and Michelakis 1994; Kairo et al. 2000; Kreiter et al. 2004; Li et al. 2021).

Even if for decades *A. flavidulus* and *C. montrouzieri* have been promoted and sold as the best biological control agents for *P. viburni*, no mathematical modeling has been used to describe their dynamics and intra-guild predation, and to explore the best release timing for pest control. In this study, our aim is to pave the way for improving the control of *P. viburni* in vineyards using the most widely available biological control agents for this species, *A. flavidulus* and

C. montrouzieri. To do so, we: (i) study and characterize the intraguild predation of these two natural enemies in laboratory conditions by investigating the predation behavior of *C. montrouzieri* on *P. viburni* larvae parasitized by *A. flavidulus* of different age categories, and (ii) we present a mathematical model of impulsive differential equations that describes the dynamics of *P. viburni* populations, taking into account the effects of the interacting predator and parasitoid, to study their impact on the control of *P. viburni* under different release schedules described by pulses.

Materials and methods

Insect rearing

The mealybug, parasitoid and predator populations used in this study were previously identified with molecular tools. The *P. viburni* population was initially founded with individuals collected from various apple orchards and strawberry greenhouses of Southern France. The mealybugs in the laboratory were reared on sprouted potato tubers placed in hermetic but ventilated plastic boxes placed inside a climatic room at 25 ± 1 °C and 70 ± 10% of relative humidity (hereafter referred to as RH) in the dark. Potato tubers for mealybug rearing were harvested in autumn in southern France and placed in the dark for at least two months at 4± 1 °C. They were regularly moved at 15 ± 1 °C for two up to 3 months to facilitate germination. Finally, they were kept for two to three months at 25 ± 1 °C to obtain long and thick sprouts.

Populations of *A. flavidulus* were obtained from parasitized mealybugs collected in an apple orchard in Manosque (South East of France). Second instar (L2) and third instar (L3) *P. viburni* larvae, the preferred development stage for the *A. flavidulus* egg-laying (Karamaouna and Copland 2000), were used as hosts. They were reared in boxes similar to their hosts, but with slightly different abiotic conditions (25 ± 1 °C, 50 ± 10% RH and a 16 h:8 h light:dark photoperiod). Small honey droplets were deposited on the box lid to ensure the nutrition of adult parasitoids.

Cryptolaemus montrouzieri was obtained in the same apple orchard as *A. flavidulus* and reared on *P. viburni* at INRA Sophia Antipolis (France) since 2001 using *P. viburni* as prey. Abiotic conditions were similar to those of *A. flavidulus* except for the relative humidity (60 ± 10%). Water, honey, and pollen were given to adult ladybirds as complementary resources.

Experimental setup

This experiment aimed to investigate the predation by *C. montrouzieri* on parasitized *P. viburni* by *A. flavidulus* of various age categories. First, we prepared parasitized mealybugs to be used in the experiment by using second or third instar larvae (L2 and L3) of *P. viburni* during 24 h by 2-days-old parasitoids with a mealybugs:parasitoid ratio of 1:5 to 1:10. These ratios of natural enemies: mealybugs were chosen according the biology of the parasitoid, *A. flavidulus* is a gregarious parasitoid that parasitize with a high number of eggs a reduced number of mealybugs (Karamaouna and Copland 2000). Parasitism was controlled through random mealybug dissection to verify parasitism, and 99.7 percent of dissections showed effective parasitism. Under these conditions, the pre-imaginal development time of *A. flavidulus* was approximately 22 days (Karamaouna and Copland 2009). For predators, third instar larvae of *C. montrouzieri*, previously starved for 24 h, were used, as this stage of development is known to be more voracious than adults (Rashid et al. 2012) and less fragile than younger larvae.

After parasitism, parasitoids were left to develop inside mealybugs for 1, 4, 8, 12, or 16 days (hereafter called M1, M4, M8, M12 and M16) before exposing them to predation by the ladybird. A control group, with unparasitized mealybugs, was also prepared (M0). No-choice experiments were carried out in plastic boxes with 15 repetitions each. Each box contained five mealybugs belonging to one of the days group (M0 to M16) and one predator. *Cryptolaemus montrouzieri* was left for 48 h in each box. We counted the number of remaining live mealybugs in each box at five observation times (T): 1.5 h (T1.5), 3 h (T3), 5 h (T5), 7 h (T7), 24 h (T24) and 48 h (T48). Abiotic conditions were maintained at 25 ± 1 °C, 50 ± 10% RH and 16 L: 8D.

To prevent confounding effects between age and time, two experimental designs (hereafter referred to as "design A" and "design B") were used:

- Design A: *P. viburni* larvae were simultaneously exposed to the parasitoid, so the exposure to predator was realized on different days.
- Design B: groups of mealybugs were exposed to parasitism on several dates to provide all the parasitized mealybugs (M0 to M16) to the ladybird the same day.

Statistical analyses

A generalized linear mixed model was used to analyze the proportions of mealybugs preyed upon by *C. montrouzieri* with the LME4 package of R (R Development Core Team, 2011). Our model was > model1< − lmer(cbind(t15, 5-t15) ddpi + (1|modalite), family=binomial, data=crypto). The model was the same at each observation event. The dependent variable was the number of preyed mealybugs (negative binomial distribution). The age mealybug group was considered as a fixed effect, while the "design" (corresponding to a block effect with variations in the physiological states

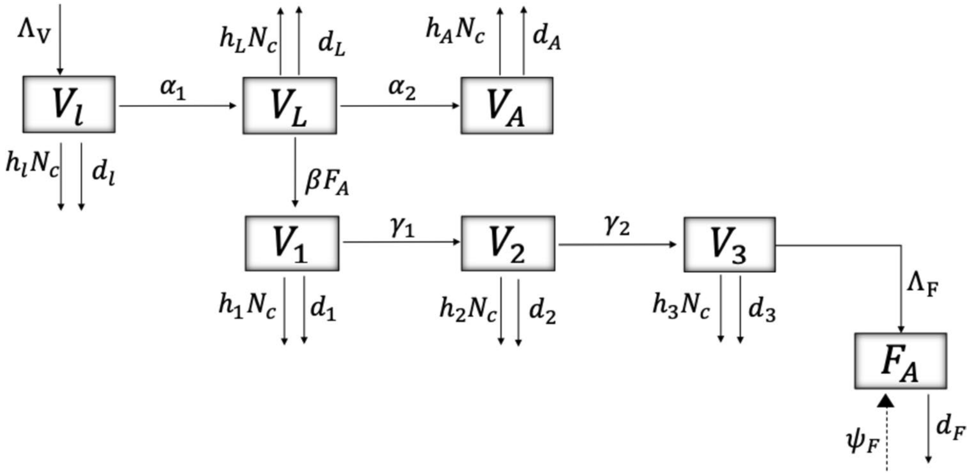


Fig. 1 The schematic shows the flow between the states of parasitized ($V_i, i \in \{1, 2, 3\}$) and non-parasitized (V_l, V_L, V_A) stages of larval (V_l, V_L) and adult (V_A) populations of *P. viburini*, and the population of *A. flavidulus* (F_A). The solid arrows represent transitions between compartments, whereas the dotted arrow represents the pulse release of *A. flavidulus* into the system for the biological control of *P. viburni*

of the mealybug and parasitoid) was considered a random effect. Post-hoc least-square means pairwise differences were examined to check whether the proportions of preyed mealybugs parasitized for 1, 4, 8, 12 and 16 days were different to the proportion of unparasitized mealybugs that were preyed upon by the predator. All analyzes were performed with the LME4 procedure of SAS, with the negative binomial distribution.

Mathematical model

Systems of differential equations have been widely used to model natural phenomena, which, given the possibility of non-experimentation, have served as an essential tool to understand the evolution of the phenomenon. The biological control release of *C. montrouzieri* and *A. flavidulus* happens only from time to time. However, the damage caused by *P. viburni* occurs in continuous time, so it is advisable to model the dynamics between *P. viburni* populations and their predator and parasitoid populations through IDE.

The model described by the schematics in Figs. 1 and 2 is a model of coupled impulsive differential equations given in system (1) with positive initial conditions. The *P. viburni* population is divided into larval stages (parasitized and non-parasitized) and one adult stage. In particular, as shown in

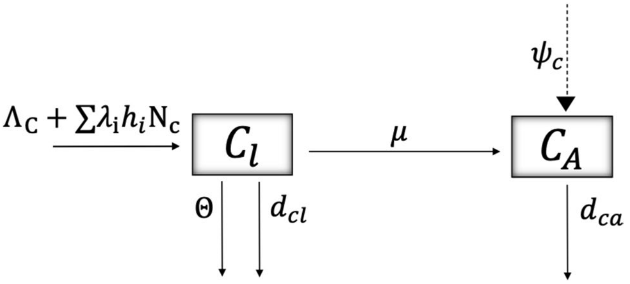


Fig. 2 The schematic shows the flow between the larval stage (C_l) and the adult stage (C_A) of *C. montrouzieri*. The solid arrows represent transitions between compartments, whereas the dotted arrow represents the pulse release of *C. montrouzieri* into the system for the biological control of *P. viburni*

Table 1 Description of variables from the model in system (1)

Variable	Description	Units
P. viburni		
$V_l(t)$	Non-parasitized population at larval stage 1 at time *t*	v
$V_L(t)$	Non-parasitized population at larval stages 2 and 3, at time *t*	v
$V_A(t)$	Non-parasitized population at adult stage, at time *t*	v
$V_1(t)$	Population parasitized for up to 4 days, at time t	v
$V_2(t)$	Population parasitized for between 4 and 12 days, at time *t*	v
$V_3(t)$	Population parasitized for more than 12 days, at time *t*	v
A. flavidulus		
$F_A(t)$	Population at adult stage, at time *t*	f
C. montrouzieri		
$C_l(t)$	Population at larval stage, at time *t*	m
$C_A(t)$	Population at adult stage, at time *t*	m

v:=*P. viburni*, f:=*A. flavidulus*, m:=*C. montrouzieri*

Fig. 1, V_l represents the non-parasitized population in the first larval stage, with recruitment rate Λ_V, which can then transition into non-parasitized larval stages 2 and 3 at a rate α_1, represented by V_L. The latter can subsequently progress to the adult stage, denoted by V_A, at a rate α_2. The average time *P. viburni* remains in the larval stage V_l before transitioning to the next larval stages, and without dying or being parasitized, is denoted by $1/\alpha_1$. Equivalently, $1/\alpha_2$ denotes the average time spent in larval stages 2 and 3 (V_L) before becoming an adult (V_A).

Parasitism by *A. flavidulus*, whose population is represented by F_A, occurs mainly in larval stages 2 and 3 (V_L) of *P. viburni*. We assume that parasitism at these stages occurs at a rate β, transitioning to stage V_1, which represents parasitized mealybug larvae that have been parasitized for up to four days. V_2 and V_3 represent the stages of parasitism that have lasted between 4 and 12 days and more than 12 days, respectively. Thus, $1/\gamma_1$ and $1/\gamma_2$ are the average times spent in the stages V_1 and V_2, without dying, before transitioning to the next parasitized stage. It is during state V_3 that *A. flavidulus* (F_A) emerges from its host at a rate Λ_F. Therefore, and since *A. flavidulus* needs *P. viburni* to develop, in our model the population of *A. flavidulus* (F_A) is considered an additional stage of *P. viburni* development, and hence share population units.

In addition, *A. flavidulus* (F_A-compartment) increases at certain times (pulses) (t_n in system (1)) by human release of these wasps into the system, as biological control, at an incremental factor Ψ_F. The natural mortality of *A. flavidulus* is denoted by d_F; similarly, for *P. viburni*, the natural mortality is denoted by d_i, with $i \in \{l, L, A, 1, 2, 3\}$, whose subscript represents the respective compartment. The different stages associated with *P. viburni* are affected by the predation by *C. montrouzieri*, expressed by the Holling's Type II functional response $h_i = p_i(V_i/(k + V_i))$, $i \in \{l, L, A, 1, 2, 3\}$, with p_i representing a predation preference for the i-th stage of *P. viburni* and k a constant. Thus, $h_i N_c$ corresponds to predation force, where N_c corresponds to the total population of *C. montrouzieri*.

Figure 2 explicitly presents the dynamics of *C. montrouzieri*, where C_l and C_A denotes the population of larval and adult stages, respectively ($N_c = C_l + C_A$). Λ_c and $\sum \lambda_i h_i N_c$ are the natural birth rate and the translation of the benefit of predation for *C. montrouzieri*, respectively, both contributing to the larval stage C_l. This state (C_l) is affected by natural death, d_{cl}, and death by cannibalism following larval overpopulation, expressed by $\Theta = \theta[N_C/K]$, where $[\cdot]$ represents the integer part function and K the carrying capacity. The average time spent in the larval stage without dying is given by $1/\mu$, before transitioning to the adult stage. The adult stage, C_A is impaired by natural death, d_{ca}, and its population is augmented by humans' release of *C. montrouzieri* into the system on certain times (pulses) (t_m in system (1)), at an incremental factor Ψ_c.

The positive initial conditions of system (1) are denoted by $V_i(0) = V_{0,i}$, $i \in \{l, L, A, 1, 2, 3\}$, $F_A(0) = F_{0,A}$, $C_l(0) = C_{0,l}$, $C_A(0) = C_{0,A}$, and Tables 1 and 2 describe, respectively, the variables and parameters of the model.

$$\left\{ \begin{array}{l} \left. \begin{array}{l} \dot{V}_l(t) = \Lambda_V - h_l N_C - (\alpha_1 + d_l)V_l \\ \dot{V}_L(t) = \alpha_1 V_l - h_L N_C - (\alpha_2 + d_L)V_L - \beta V_L F_A \\ \dot{V}_A(t) = \alpha_2 V_L - h_A N_C - d_A V_A \\ \dot{V}_1(t) = \beta V_L F_A - h_1 N_C - (\gamma_1 + d_1)V_1 \\ \dot{V}_2(t) = \gamma_1 V_1 - h_2 N_C - (\gamma_2 + d_2)V_2 \\ \dot{V}_3(t) = \gamma_2 V_2 - h_2 N_C - (\Lambda_F + d_3)V_3 \\ \\ \dot{F}_A(t) = \Lambda_F V_3 - d_F F_A \\ \\ \dot{C}_l(t) = \Lambda_C + \sum_{i \in \{l,L,A,1,2,3\}} \lambda_i h_i N_C - (\Theta + \mu + d_{cl})C_l \\ \dot{C}_A(t) = \mu C_l - d_{ca} C_A \end{array} \right\} t \neq \{t_n, t_m\} \\ F_A(t^+) = (1 + \Psi_F)F_A \quad \} t = t_n. \\ C_A(t^+) = (1 + \Psi_C)C_A \quad \} t = t_m. \end{array} \right. \tag{1}$$

Results

Predation behavior of *Cryptolaemus montrouzieri* on parasitized *Pseudococcus viburni*

The parasitism rate of *P. viburni* larvae by *A. flavidulus*, estimated through dissection and direct observation of the parasitoid inside the mealybug, was 99.7% ($n = 290$), confirming that the mealybugs used for the experiments had a parasitism rate close to 100%. The results of the coccinellid predation behavior according to parasitism age (M) are represented by the average number of mealybugs remaining in boxes for each observation time (Fig. 3).

There was no significant difference in consumed mealybugs by the predator between experimental designs. Predation on mealybugs not parasitized (M0) or parasitized for one and four days (M1 and M4, respectively) was complete, with an average of 0 mealybugs remaining after exposition to *C. montrouzieri*. For mealybugs parasitized for 8, 12, or 16 days (M8, M12, and M16), an average of 1, 2, and 4 mealybugs remained (Fig. 3; Design A and Design B), respectively. There was no difference between the proportion of remaining mealybugs when mealybugs were infested by one-day old parasitoids (M1 category) or were not infested (M0), whatever the observation time

Table 2 Description of parameters from the model in system (1)

Parameter	Description	Units	Baseline	Value	Reference
Pseudococcus viburni					
Λ_V	Recruitment rate	vd^{-1}	[1/30, 1/2]	$\sum_i d_i$	Waterworth et al. (2011)
$1/\alpha_1$	Mean larval duration in V_l before transitioning to V_L	d	[2, 4]	3	–
$1/\alpha_2$	Mean larval duration in V_L before transitioning to V_A	d	[4, 8]	6	–
β	Parasitization rate of *P. viburni* by *A. flavidulus*	d^{-1} f^{-1}	[1/10, 1]	0.5	Karamaouna and Copland (2009)
$1/\gamma_1$	Mean larval duration in V_1 before transitioning to V_2	d	[0,4]	4	–
$1/\gamma_2$	Mean larval duration in V_2 before transitioning to V_3	d	[4,12]	8	–
d_i	Natural mortality rate	d^{-1}	[1/22,1/2]	$\begin{array}{c\|cccccc} i & l & L & A & 1 & 2 & 3 \\ & 0.25 & 0.5 & 0.071 & 0.5 & 1 & 1.5 \end{array}$	–
p_i	Predator preference proportion for the *i*th stage of *P. viburni*		[0,1]	$\begin{array}{c\|cccccc} i & l & L & A & 1 & 2 & 3 \\ & 0.23 & 0.23 & 0.23 & 0.23 & 0.07 & 0.01 \end{array}$	
k			[1,∞[	1000	–
Acerophagus flavidulus					
Λ_F	Recruitment rate	d^{-1}	–	$1/(15-(1/\gamma_1+1/\gamma_2))$	Mathulwe et al. (2021)
d_F	Natural mortality rate	d^{-1}	[1/12, 1/8]	0.1	–
Ψ_F	Release incremental factor.	Unitless	–	$\frac{\text{Number released}}{F_A(t_n)}$	Author chosen
Cryptolaemus montrouzieri					
Λ_C	Recruitment rate	cd^{-1}	[1/14, 1/7]	0.1	Mathulwe et al. (2021)
λ_i	Predation conversion rate from *P. viburni* to *C. montrouzieri*	d^{-1}	[0,1]	0.001	–
θ	Cannibalism rate	d^{-1}	[0,1]	1	
$1/\mu$	Mean larval stage duration before becoming adult	d	[20, 30]	25	–
d_{ca}	Natural mortality rate of adults	d^{-1}	[1/50, 1/40]	0.02	–
d_{cl}	Natural mortality rate of larvae	d^{-1}	$0.04 * [1/50, 1/40]$	$0.04 * d_{ca}$	–
Ψ_C	Release incremental factor.	Unitless	–	$\frac{\text{Number released}}{C_A(t_m)}$	Author chosen

$i \in \{l, L, A, 1, 2, 3\}$. d:=day, v:=*P. viburni*, f:=*A. flavidulus*, m:=*C. montrouzieri*

($0.08 < P < 0.17$). Those infested by 4-days old parasitoids (M4) were significantly less consumed than the control group (M0) until observation time T7 ($P < 0.002$ at observation times T1.5, T3, T5, T7; $P > 0.90$ at T24 and T48) (Fig. 3). Those infested by 8-days old or older parasitoids (M8, M12, M16) were consumed less than the control group (M0) at each observation event ($P < 0.0001$ in all cases) (Fig. 3).

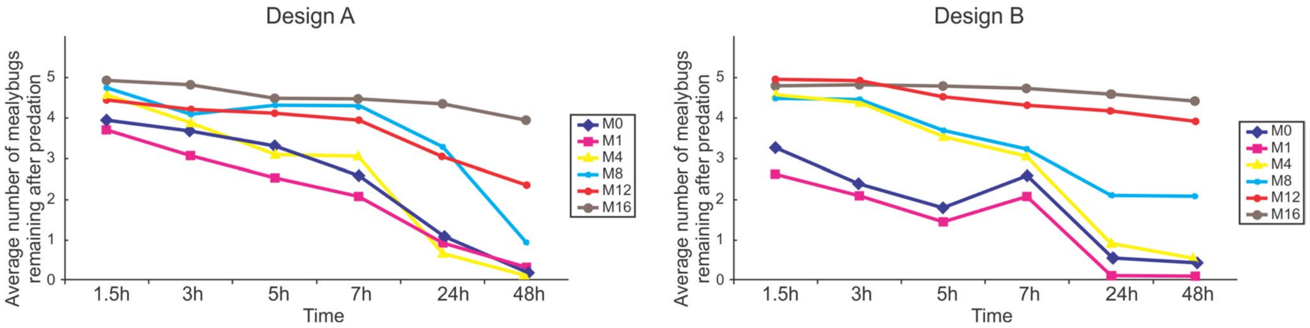


Fig. 3 Average number of mealybugs in groups M0, M1, M4, M8, M12 and M16, remaining after predation of *C. montrouzieri* for experimental design A and B. Parasitoids were left to develop inside mealybugs for 1, 4, 8, 12, or 16 days (groups M1, M2, M4, M8, M12 and M16) before exposing them to predation by the ladybird. M0 refers to the control group with unparasitized mealybugs.

Numerical results of the model: the effect of *Pseudococcus viburni* natural-enemies-releasing-strategies incorporating the intra-guild predation of *Cryptolaemus montrouzieri* and *Acerophagus flavidulus*

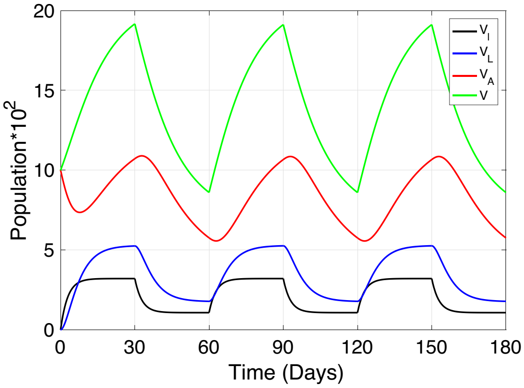
In this section, we present numerical simulations of the model in the mealybug system (1). These were performed using the software (MATLAB version 9.1 (R2016b) 2016). With the aim of presenting the situation before any natural enemy release, we first present the base population dynamic of *P. viburni*: Fig. 4a shows the natural population dynamics of *P. viburni* without the presence of natural enemies, showing three populations curves (red, blue and black) corresponding to the three generations presented by this mealybug species during an agricultural growing season (a total of ~ 6 months), with three clear population peaks at 30, 90 and 150 days, and the green curve representing the total population of unparasitized *P. viburni*, (where $V = V_l + V_L + V_A$). Figure 4b shows the effect of releasing 1000 adult predators and parasitoids at the three population peaks of *P. viburni* (at days 30, 90, and 150), as will occur in the field when producers detect the maxima of *P. viburni* individuals. Figure 4c shows three releases, but occurring at the onset of each *P. viburni* population increase (at days 0, 60, and 120), in this case, producers monitor the pest and release before *P. viburni* population reaches it maximum. We can observe that a larger effect on the reduction of *P. viburni* happens when the release occurs early, before the peak in the pest population.

Figure 5 shows the effect on *P. viburni* population reduction after one, two, or three releases of adult predators and parasitoids at different times and in different amounts. The green curves correspond to the natural dynamics of *P. viburni*, without biological control, while the red and blue curves correspond to releases before and during *P. viburni* population peaks. Figure 5a, c, e, show the *P. viburni* population reduction when releasing 1,000 predators and parasitoids, while Fig. 5b, d, e depicts the case of releasing 2,000 each. Furthermore, Fig. 5a–f, show one, two and three instances of release, respectively. We observe that the higher the frequency and quantity of release is and the sooner since the beginning of the season it occurs (see Fig 5 blue curves vs. red curves), the more significant is the reduction of the *P. viburni* population.

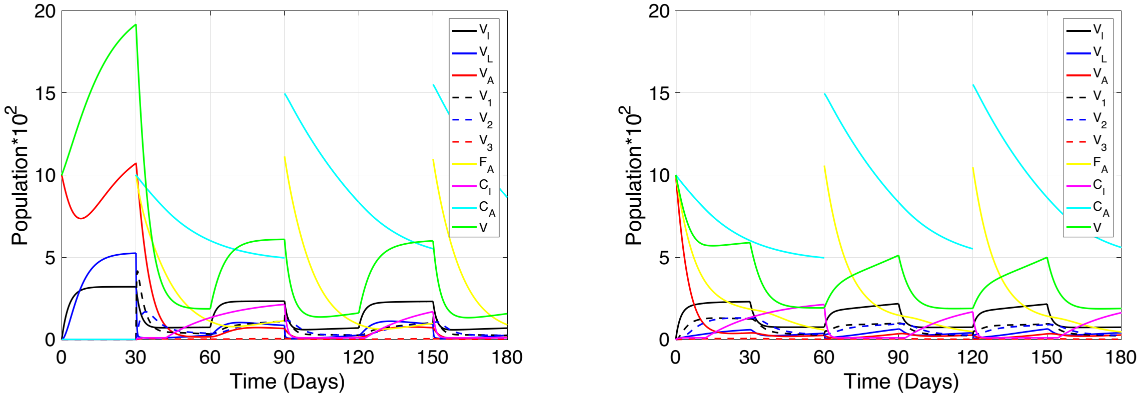
Figure 6 depicts the natural dynamics of *P. viburni* without biological control (green curves) and its population dynamics when releasing on three occasions adult parasitoids or predators or both: at the onset of each wave of *P. viburni* population growth (days 0, 60, and 120).

Figure 6a shows the dynamics when releasing only *A. flavidulus* (red curve) and only *C. montrouzieri* (blue curve) at the beginning of the season (day 0), showing a significant difference in the reduction of the *P. viburni* population between the cases. This difference is even more pronounced for three releases (days 0, 60, and 120) (see Fig. 6c). Figure 6b shows two cases of alternate release between parasitoid and predator, i.e., first, the red curve represents the case where initially (day 0) *A. flavidulus* are released and then (day 60) *C. montrouzieri* are released, which has a minor effect toward the initial decrease in *P. viburni* population compared to the opposite case represented by the blue curve of initially releasing *C. montrouzieri* and then *A. flavidulus*. However, both cases produce the same dynamics and reduction of the *P. viburni* population in time (see curves after day 90).

Figure 7 presents the population dynamics of *P. viburni* under one joint release of predators and parasitoids at the onset of the first population wave of *P. viburni* (day 0), considering different predation preferences of *C. montrouzieri* for parasitoid-infested versus non-infested mealybugs. The blue curve represents predation with a greater preference for non-infested *P. viburni* according to the results presented in Sect. 3.1. In contrast, the blue and black curves represent cases where predation occurs without any preference and with a preference for infested



(a) Dynamics of *P. viburni* without natural enemies releases



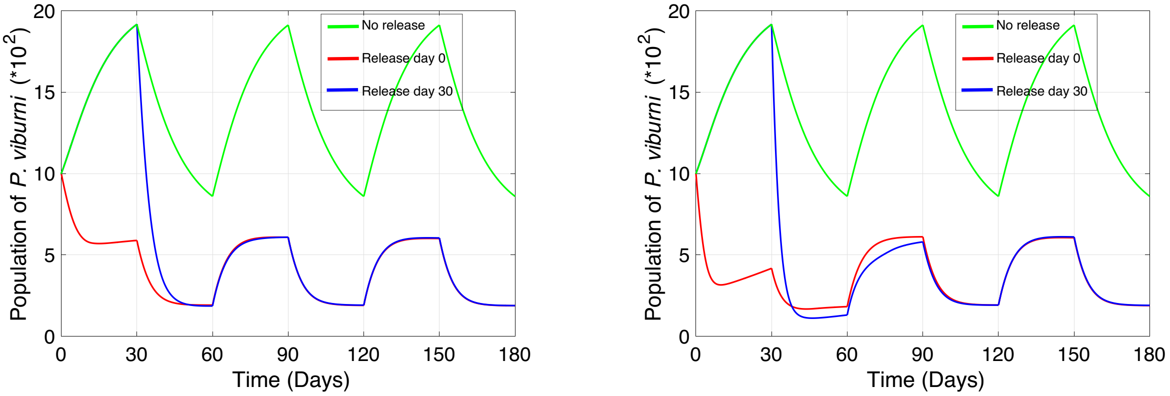
(b) Release of 1000 predators and parasitoids, at days 30, 90, and 150.

(c) Release of 1000 predators and parasitoids, at days 0, 60, and 120.

Fig. 4 **a** the natural population dynamics of *P. viburni* without the presence of predators and parasitoids; in **b** the population dynamics of once releasing 1,000 predators (turquoise line) and parasitoids (yellow line) at the three population peaks of *P. viburni*, and in **c**, once releasing 1000 predators (turquoise line) and parasitoids (yellow line) at the onset of each pest population increase. The parameters used in the simulations are as in Table 2 and the initial conditions: $V_{0A} = 1000$, $V_{0,i} = F_{0,A} = C_{0,l} = C_{0,A} = 0$, $i \in \{l, L, 1, 2, 3\}$. The variables referred to in the legend are defined in Table 1 and V represents the total number of unparasitized mealybugs, i.e. $V = \sum_j V_j$, $j \in \{l, L, A\}$.

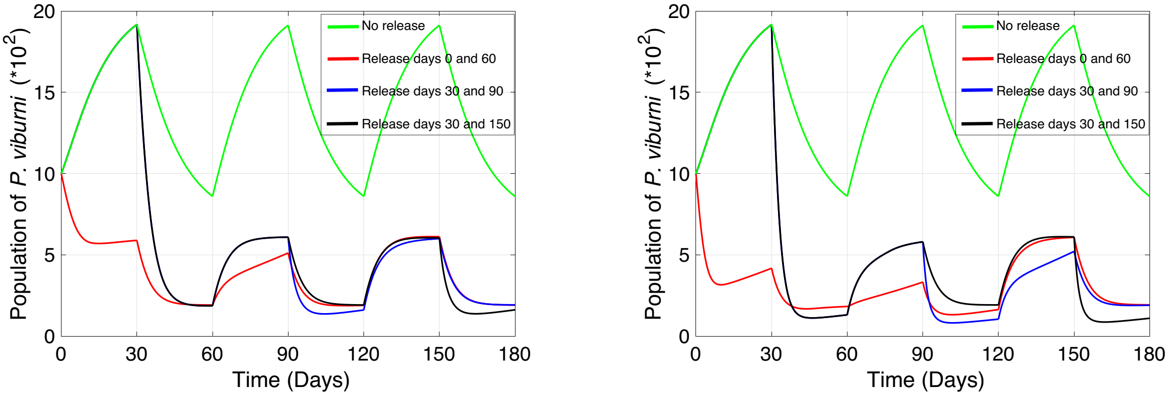
mealybugs, respectively, in order to illustrate the difference between all three scenarios.

Finally, Fig. 8 shows the dynamics of *P. viburni* without biological control (green curve), when predators and parasitoids are both released on days 0, 60 and 120 (red curve) and when only predators are released on those days. Each of the scenarios described above is depicted for different values of the constant k of the model in system (1). More specifically, the figure aims to illustrate the variable effect that predation of *C. montrouzieri* on *P. viburni* may have due to uncertainty in natural factors that can alter it, as reflected in that constant k. In fact, in the proposed model, predation on each *P. viburni* stage occurs at a rate $h_i = p_i[V_i/(k + V_i)]$, which is predetermined by predation preferences (p_i), the population to be consumed (V_i) and the aforementioned constant k, whose values are variable and scarce in the literature. This constant affects the intensity of predation on the *P. viburni* population (see Fig. 8), indeed, from Fig. 8, it is explicitly observed that there is high predation for a small value for k (see e.g. $k = 1$), in which case the incorporation of another biological control agent does not produce a significant additional impact (see dashed blue and red curves, and observe that, in fact, they overlap). However, this is far from reality, since other external factors may decrease predation. If this constant increases, predation decreases, and the positive effect of including parasitoids starts to become evident (solid red and blue curves, and dash-dot-dashed red and blue curves). Therefore, the level of predation of *C. montrouzieri* on *P. viburni* may be determinant in order to decide on a biological control mechanism.



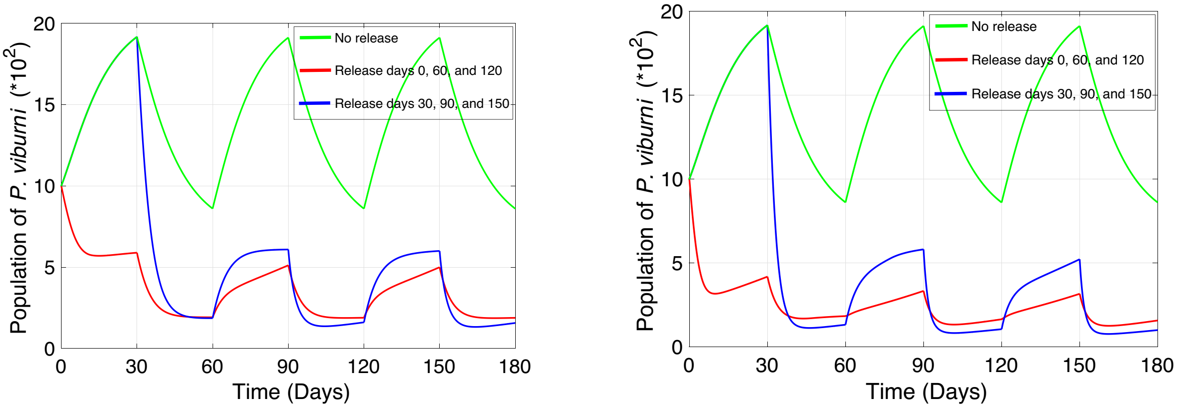
(a) Release of 1,000 predators and parasitoids.

(b) Release of 2,000 predators and parasitoids.



(c) Release of 1,000 predators and parasitoids.

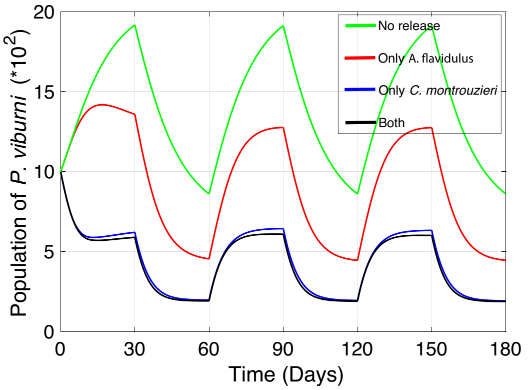
(d) Release of 2,000 predators and parasitoids.



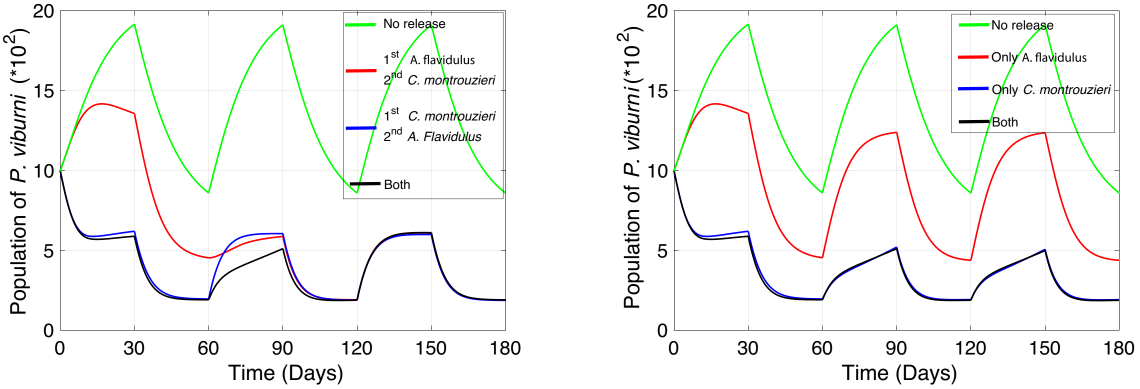
(e) Release of 1,000 predators and parasitoids.

(f) Release of 2,000 predators and parasitoids.

Fig. 5 The figure shows in **a**, **c**, and **e** the dynamics of the *P. viburni* population without biological control (green curves), when releasing 1000 adult predators and parasitoids at the onset of the *P. viburni* population increase before a peak (red curves) and during a population peak (blue curves). Specifically, **a** shows the case of one release before (red curve) and at (blue curve) the first peak; **c** shows the case of two releases: before the first two peaks (red curve), at the first two peaks (blue curve) and at the first and third peaks (black curve); **e** shows three releases before each peak (red curve) and at each peak (blue curve). **b**, **d**, and **f**, depict the same release dynamics as shown in **a**, **c**, and **e**, respectively, but increasing the adult predator and parasitoid population to 2000 each. The parameters used in the simulations are as in Table 2 and the initial conditions: $V_{0A} = 1000$, $V_{0,i} = F_{0,A} = C_{0,l} = C_{0,A} = 0, i \in \{l, L, 1, 2, 3\}$



(a) Release 1,000 predators or parasitoids at day 0.



(b) Release of 1,000 predators or, parasitoids, at days 0 and 60.

(c) Release of 1,000 predators or, parasitoids at days 0, 60, and 120.

Fig. 6 The figure shows the dynamics of *P. viburni* population without biological control (green curves) and when releasing parasitoids and predators each time jointly (black curves) or individually (red and blue curves). **a** shows the initial release (at day 0) of only *F. flavidulus* (red curve) and of only *C. montrouzieri* (blue curve). **b** depicts an alternate release of predators and parasitoids: the red curve represents the initial release (day 0) of only *F. flavidulus* and, after 60 days, the release of only *C. montrouzieri*; and the blue curve represents the same release but first releasing predators and then parasitoids. **c** shows the dynamics of *P. viburni* under three *F. flavidulus* releases (red curve) and three *C. montrouzieri* releases (blue curve), each at days 0, 60 and 120. The parameters used in the simulations are as in Table 2 and the initial conditions: $V_{0A} = 1000$, $V_{0,i} = F_{0,A} = C_{0,l} = C_{0,A} = 0, i \in \{l, L, 1, 2, 3\}$

Discussion

In this work, we presented clear evidence of an interaction of the type of intraguild predation (Rosenheim et al. 1995; Colfer and Rosenheim 2001) between the mealybug predator *C. montrouzieri* and the specific parasitoid *A. flavidulus* of *P. viburni*. The ladybird consumed healthy and parasitized mealybugs and was able to even differentiate *P. viburni* parasitized for different lengths of time by *A. flavidulus*. In fact, *C. montrouzieri* differentiated between mealybugs parasitized by *A. flavidulus* aged of more than 4 days. Although this ability to discriminate between healthy and parasitized mealybugs of *C. montrouzieri* have been previously documented for other mealybug-parasitoid species (Chong and Oetting 2007; Muştu et al. 2008), here this detection ability occurs very early in the development of the parasitoid (4 days) than in the mummification stage. However, it is impossible to determine whether the particularly early detection capacity observed in our study is due to a difference in the experimental protocol or to differences in the species and populations used.

The differences in feeding behavior may be due to the ability of *C. montrouzieri* to avoid unhealthy or dead mealybugs or to detect the presence of the parasitoid. Our results cannot distinguish between these two hypotheses, but strongly support at least the first one. Indeed, *C. montrouzieri* clearly avoids mealybugs parasitized for 8 days and more. This corresponds to the beginning of the mummification process induced by the development of *A. flavidulus* larvae. Probably mealybugs starting the mummification process constitute “alternative" prey for the predator. Indeed, most of the Coccinellidae family are predaceous insects able to

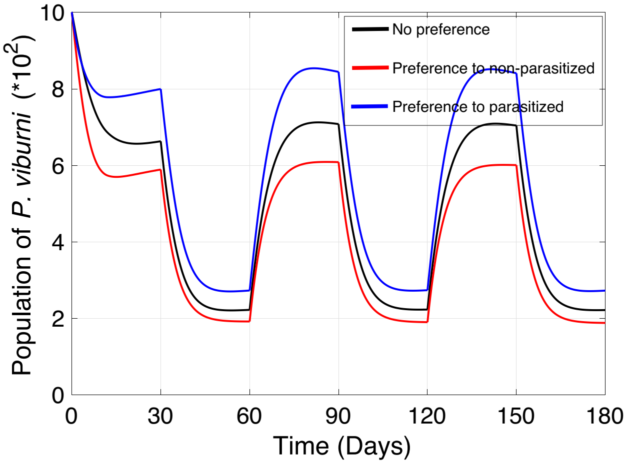


Fig. 7 The figure shows the population dynamics of *P. viburni* after the release of 1000 parasitoids and predators each on day 0, considering no predation preference of *C. montrouzieri* (black curve), and predation preference for infested (blue curve) versus non-infested (red curve) mealybugs. The parameters used in the simulations are: No preference (black curve): $p_l = 0.17, p_L = 0.166, p_A = 0.166, p_1 = 0.166, p_2 = 0.166, p_3 = 0.166$ Preference to non-parasitized (red curve): $p_l = 0.23, p_L = 0.23, p_A = 0.23, p_1 = 0.23, p_2 = 0.07, p_3 = 0.01$. Preference to parasitized (blue curve): $p_l = 0.05, p_L = 0.1, p_A = 0.2, p_1 = 0.15, p_2 = 0.2, p_3 = 0.3$ and the remaining as in Table 2. The initial conditions were chosen as: $V_{0A} = 1000, V_{0,i} = F_{0,A} = C_{0,l} = C_{0,A} = 0, i \in \{l, L, 1, 2, 3\}$

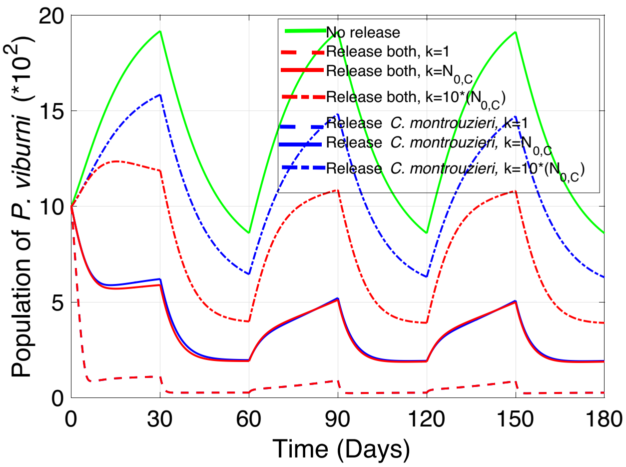


Fig. 8 The figure shows the dynamics of the *P. viburni* population without biological control (green curves), when releasing 1000 adults of *A. flavidulus* and *C. montrouzieri* each, on days 0, 60 and 120 (red curves), and when releasing on those days only *C. montrouzieri* (blue curves). These curves are depicted for different values of the constant k, appearing in the expression $h_i = p_i(V_i/(k + V_i)),\ i \in \{l, L, A, 1, 2, 3\}$, from the model in system (1). The blue and red curves for $k = 1$ overlap. The parameters used in the simulations are: $p_l = 0.23, p_L = 0.23, p_A = 0.23, p_1 = 0.23, p_2 = 0.07, p_3 = 0.01$; $N_{0,C} = 1000$ and the remaining parameters as in Table 2. The initial conditions were chosen as: $V_{0A} = 1000, V_{0,i} = F_{0,A} = C_{0,l} = C_{0,A} = 0$, $i \in \{l, L, 1, 2, 3\}$

differentiate their prey as essential (main prey), alternative (they survive predating them, but growing and reproductive traits are affected), and clearly rejected prey (Bhupendra Kumar 2023).

In scale insects, preference for parasitized and non-parasitized hosts is not always the case, i.e., the interaction between the parasitoid *Aphytis lingnanensis* and the predator *Chilocorus circumdatus*, where the ladybird did not show differences in predation between healthy and parasitized hosts (Vanaclocha et al. 2013). Extending the comparison to other taxonomic groups reveals that the detection capacity displayed by *C. montrouzieri* does not seem to be a general rule in tri-trophic systems predator - parasitoids - phytophagous insects. Indeed, other studies with other species of coccinellid prey have documented a range of different situations. In two cases, such a detection capacity was observed: (i) *Serangium parcesetosum* (Coleoptera: Coccinellidae) avoided *Bemisia tabaci* (Aleyrodidae) parasitized by *Encarsia formosa* (Hymenoptera: Aphelinidae) (Al-Zyoud and Sengonca 2004; ii) likewise, various development stages of coccinellid *Delphastus catalinae* rejected *B. tabaci* when parasitized by *E. sophia* (Zang and Liu 2007). On the contrary, with aphids as prey, *Coccinella undecimpunctata* showed no preference for parasitized versus non-parasitized aphids when allowed to choose between two types of prey (Bilu and Coll 2007). Cases where parasitized preys were even preferred by coccinellids were also documented, for example, by Meyhöfer and Klug (2002) who showed that *Coccinella septempuncata* consumed more aphids parasitized by *Lysiphlebus fabarum* (Hymenoptera: Aphidiidae) than healthy aphids. Whether this parasitized versus non-parasitized discrimination behavior of a predator depends on the predator or parasitoid species characteristics remains an open question, and before proposing a biological control program using a predator and a parasitoid, intensive laboratory experiments should be performed (Bhupendra Kumar 2023).

To support decision-making regarding biocontrol, mathematical modeling may play a crucial role. In fact, when we take into account the laboratory experimental results to nourish the model presented here, we may be able to conclude that the mealybug predator alone could– under certain conditions– be the best choice when releasing natural enemies to control *P. viburni*. The results from the mathematical model, through its hybrid effect, simulate biocontrol field experimentation, while including predator preferences obtained in the laboratory experiments in the model structure. The results show the effect of different release strategies of *A. flavidulus* and *C. montrouzieri* on the reduction of the *P. viburni* population, with and without considering the preference for predation of *C. montrouzieri* (Figs. 4, 5, 6, 7). We observe that biocontrol most effectively reduces pest population—and hence the economic burden it conveys– when implemented at the beginning of each *P. viburni* population wave (see red vs. blue curves in Fig. 5e, f versus. a–d, which represent less frequent release schedules). This points to the necessity to perform field tests to identify the onset of mealybug population growth. The results also show that a larger amount of parasitoids and predators released produce a larger effect on the reduction of pests (see Fig. 5a–d, e vs. b–f)).

Furthermore, the results indicate that the preference of predators for nonparasitized mealybugs (as described by the experimental results in Sect. 3.1) produce an important positive impact on pest control. In fact, when the model parameters consider such preference, the *P. viburni* population is reduced further than otherwise (red vs. blue and black curves in Fig. 7). This highlights the importance of our experimental results, which when complemented with a mathematical model, can provide important insight for biocontrol.

Moreover, the predation rate (force of predation) of *C. montrouzieri* is a determining factor for decision making for biological control, determined in the model by the parameter k (Fig. 8), since it impacts model recommendations; and therefore needs to be further understood by experimental studies on site. We observe that at certain levels of *C. montrouzieri*'s force of predation ($k = 1000$), ladybird's sole effect on *P. viburni* population reduction (blue curves in Fig. 6) is greater than the effect on pest reduction of solely *A. flavidulus* release (red curves in Fig. 6). Furthermore, we can observe that– under the same predation rate of *C. montrouzieri*– the contribution of *A. flavidulus* on *P. viburni* control when jointly released with *C. montrouzieri* may be marginal (black vs. blue curve in Fig. 6a, c). Additionally, alternating the release of only predators and only parasitoids is most effective when releasing first predators (blue vs. red curve in Fig. 6b), however, observe that this release strategy is slightly less effective than releasing both (predators and parasitoids) at each scheduled time (black vs. blue curve in Fig. 6b).

Also, taking into account in the proposed model the predation preference of ladybirds toward unparasitized mealybugs has led to a decrease in the population of *P. viburni* available to be parasitized by *A. flavidulus*, reducing their impact on biological control. Thus, increasing the value of the model's parasitization rate (β) is not a determining factor for control as long as predators are present. Additionally, it is worth mentioning that information on the values associated with parasitization and predation rates is scarce, and that in this article we have considered what was found in the literature. Hence, future work must be conducted through laboratory studies to more precisely determine the values of the rates indicated. This way, the proposed model could be better nourished and provide more concrete conclusions, which can serve as input for decision making. For the moment, we are evaluating the impact, through a novel mathematical model, that the release of these biological controllers may have in a scenario of predation preferences of ladybirds toward the unparasitized mealybugs, using as input the results from the laboratory study we present in this article.

Finally, it is important to note that the number of natural individuals will depend on environmental conditions, terrain, economic factors, and other variables, depending on each orchard; the same applies to the timing and recurrence of release. However, the model proposes possible effects that different biological control strategies may have under ideal conditions, highlighting that predation preferences of *C. montrouzieri* for parasitized and unparasitized *P. viburni* are a factor that alters the population dynamics of *P. viburni*. Hence, this factor should be taken into account for this type of study.

Acknowledgements The authors would like to thank their different laboratories. The EME lab UCM and the "Agro-Math Epidemiological joint laboratory" UCM-UAI.

Funding Authors receive funding for this study by the ANID, Chile grant FOVI220194, the French grant ANR-10-JCJC-1708 'BICORAMICS' and the European Union FP7 grant Marie Curie IRSES 'IPRABIO' 269196. They also received funding from the UAI TRL-s Level Up program.

Declarations

Conflict of interest There is no conflict of interest.

References

Abell KJ, Duan JJ, Shrewsbury PM (2020) Determining Optimal Parasitoid Release Timing for the Biological Control of Emerald Ash Borer (Coleoptera: Buprestidae). Florida Entomologist 102(4):691–694. https://doi.org/10.1653/024.102.0403

Al-Zyoud F, Sengonca C (2004) Prey consumption preferences of *Serangium parcesetosum* sicard (col., coccinelidae) for different prey stages, species and parasitized prey. J Pest Sci 77:197–204. https://doi.org/10.1007/s10340-004-0054-5

Aquilino KM, Cardinale BJ, Ives AR (2005) Reciprocal effects of host plant and natural enemy diversity on herbivore suppression: an empirical study of a model tritrophic system. Oikos 108(2):275–282. https://doi.org/10.1111/j.0030-1299.2005.13418.x

Benchohra M, Henderson J, Ntouyas S (2006) Impulsive differential equations and inclusions. Contemporary mathematics and its applications. Hindawi Publishing Corporation

Bhupendra Kumar O (2023) Insect Predators in Pest Management. Taylor and Francis Group

Bilu E, Coll M (2007) The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. Biocontrol 52:753–763. https://doi.org/10.1007/s10526-007-9071-7

Cardinale BJ, Harvey CT, Gross K, Ives AR (2003) Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. Ecol Lett 6(9):857–865. https://doi.org/10.1046/j.1461-0248.2003.00508.x

Chong J-H, Oetting RD (2007) Intraguild predation and interference by the mealybug predator Cryptolaemus montrouzieri on the parasitoid Leptomastix dactylopii. Biocontrol Sci Tech 17(9):933–944. https://doi.org/10.1080/09583150701596305

Colfer R, Rosenheim J (2001) Predation on immature parasitoids and its impact on aphid suppression. Oecologia 126:292–304. https://doi.org/10.1007/s004420000510

Colfer RG, Rosenheim JA (2001) Predation on immature parasitoids and its impact on aphid suppression. Oecologia 126(2):292–304. https://doi.org/10.1007/s004420000510

Córdova-Lepe F, Pinto M, González-Olivares E (2012) A new class of differential equations with impulses at instants dependent on preceding pulses. applications to management of renewable resources. Nonlinear Analysis: Real World Applications 13(5):2313–2322. https://doi.org/10.1016/j.nonrwa.2012.01.026

Culik MP, Gullan PJ (2005) A new pest of tomato and other records of mealybugs (hemiptera: Pseudococcidae) from espirito santo, brazil. Zootaxa 964:1–8. https://doi.org/10.11646/zootaxa.964.1.1

Daane KM, Cooper ML, Triapitsyn SV, Andrews JW Jr, Ripa R (2008) Parasitoids of obscure mealybug, *Pseudococcus viburni* (hem.: Pseudococcidae) in California: establishment of *Pseudaphycus flavidulus* (hym.: Encyrtidae) and discussion of related parasitoid species. Biocontrol Sci Technol 18(1):43–57. https://doi.org/10.1080/09583150701729906

De Bach P (1965) Book review by FDB biological control of insect pests and weeds. Tropical Agriculture

Denoth M, Frid L, Myers JH (2002) Multiple agents in biological control: Improving the odds? Biol Control 24(1):20–30. https://doi.org/10.1016/S1049-9644(02)00002-6

Ferguson KI, Stiling P (1996) Non-additive effects of multiple natural enemies on aphid populations. Oecologia 108:375–379. https://doi.org/10.1007/BF00334664

Golino D, Sim S, Gill R, Rowhani A et al (2002) California mealybugs can spread grapevine leafroll disease. Calif Agric 56(6):196–201. https://doi.org/10.3733/ca.v056n06p196

Gutiérrez-Jara JP, Córdova-Lepe F, Muñoz-Quezada MT, Chowell G (2020) Pesticide application, educational treatment and infectious respiratory diseases: a mechanistic model with two impulsive controls. PLOS One. https://doi.org/10.1371/journal.pone.0243048

Gutiérrez-Jara JP, Vogt-Geisse K, Correa MC, Vilches-Ponce K, Pérez LM, Chowell G (2023) Modeling the impact of agricultural mitigation measures on the spread of sharka disease in sweet cherry orchards. Plants 12(19):3442. https://doi.org/10.3390/plants12193442

Hamid H, Michelakis S (1994) The importance of *Cryptolaemus montrouzieri* mulsant (col., coccinellidae) in the control of the citrus mealybug *Planococcus citri* (risso)(hom., coccoidea) under specific conditions. J Appl Entomol 118(1–5):17–22. https://doi.org/10.1111/j.1439-0418.1994.tb00773.x

Holling CS (1959) Some characteristics of simple types of predation and parasitism1. Can Entomol 91(7):385–398. https://doi.org/10.4039/Ent91385-7

Kairo MT, Pollard GV, Peterkin DD, Lopez VF (2000) Biological control of the hibiscus mealybug, *Maconellicoccus hirsutus* green (hemiptera: Pseudococcidae) in the caribbean. Integr Pest Manag Rev 5(4):241–254. https://doi.org/10.1023/A:1012997619132

Karamaouna F, Copland MJ (2000) Oviposition behaviour, influence of experience on host size selection, and niche overlap of the solitary *Leptomastix epona* and the gregarious *Pseudaphycus flavidulus*, two endoparasitoids of the mealybug *Pseudococcus viburni*. Entomol Exp Appl 97(3):301–308. https://doi.org/10.1046/j.1570-7458.2000.00743.x

Karamaouna F, Copland MJ (2009) Fitness and life history parameters of *Leptomastix epona* and *Pseudaphycus flavidulus*, two parasitoids of the obscure mealybug *Pseudococcus viburni*. Biocontrol 54:65–76. https://doi.org/10.1007/s10526-008-9169-6

Kreiter P, Graille G, Thaon M, Lanza R, Tamonte M, Germain C, Germain J-F, Hantzberg H et al (2004) Biological control against two mealybugs, newly present on strawberries in southern france. Phytoma 568:38–40. https://api.semanticscholar.org/CorpusID:133492092

Li H-S, Huang Y-H, Chen M-L, Ren Z, Qiu B-Y, De Clercq P, Heckel G, Pang H (2021) Genomic insight into diet adaptation in the biological control agent *Cryptolaemus montrouzieri*. BMC Genomics 22:135. https://doi.org/10.1186/s12864-021-07442-3

Lotka AJ (1925) Elements of physical biology. Williams and Wilkins, Baltimore

Mathulwe LL, Malan AP, Stokwe NF (2021) A review of the biology and control of the obscure mealybug, *Pseudococcus viburni* (hemiptera: Pseudococcidae), with special reference to biological control using entomopathogenic fungi and nematodes. Afr Entomol 29(1):1–16. https://doi.org/10.4001/003.029.0001

MATLAB version 9.1 (R2016b) (2016) The Mathworks, Inc., Natick, Massachusetts. The Mathworks, Inc

Meyhöfer R, Klug T (2002) Intraguild predation on the aphid parasitoid *Lysiphlebus fabarum* (marshall)(hymenoptera: Aphidiidae): mortality risks and behavioral decisions made under the threats of predation. Biol Control 25(3):239–248. https://doi.org/10.1016/S1049-9644(02)00104-4

Mills NJ, Getz WM (1996) Modelling the biological control of insect pests: a review of host-parasitoid models. Ecol Model 92(2–3):121–143. https://doi.org/10.1016/0304-3800(95)00177-8

Molter A, Bezerra JI, Rafikova E, Nava DE, Rafikov M (2023) Dynamics and biological control of the sugarcane borer with two parasitoids. Ecol Model 481:110371. https://doi.org/10.1016/j.ecolmodel.2023.110371

Muştu M, Kilinçer N, Ülgentürk S, Kaydan M (2008) Feeding behavior of *Cryptolaemus montrouzieri* on mealybugs parasitized by *Anagyrus pseudococci*. Phytoparasitica 36:360–367. https://doi.org/10.1007/BF02980815

Myers JH, Higgins C, Kovacs E (1989) How many insect species are necessary for the biological control of insects? Environ Entomol 18(4):541–547. https://doi.org/10.1093/ee/18.4.541

Parra JRP, Coelho A (2022) Insect rearing techniques for biological control programs, a component of sustainable agriculture in brazil. Insects 13(1):105. https://doi.org/10.3390/insects13010105

Pfab F, Stacconi MVR, Anfora G, Grassi A, Walton V, Pugliese A (2018) Optimized timing of parasitoid release: a mathematical model for biological control of Drosophila suzukii. Thyroid Res 111(4):489–501. https://doi.org/10.1007/s12080-018-0382-3

Rashid M, Khattak M, Abdullah K, Amir M, Tariq M, Nawaz S et al (2012) Feeding potential of *Chrysoperla carnea* and *Cryptolaemus montrouzieri* on cotton mealybug, *Phenacoccus solenopsis*. J Anim Plant Sci 22(3):639–664

Rogers D (1972) Random search and insect population models. J Anim Ecol. https://doi.org/10.2307/3474

Rosenheim JA, Wilhoit LR, Armer CA (1993) Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. Oecologia 96:439–449. https://doi.org/10.1007/BF00317517

Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA (1995) Intraguild predation among biological-control agents: theory and evidence. Biol Control 5(3):303–335. https://doi.org/10.1006/bcon.1995.1038

Royama T (1971) A comparative study of models for predation and parasitism. Popul Ecol 13:1–91. https://doi.org/10.1007/BF02511547

Rubio FA, Montes FC, Alpízar-Brenes G, Parra JRP, Jamielniak JA, Junior LPL, Vilches TN (2022) A predator-parasitoid mathematical model to describe the biological control of the tomato leafminer Tuta absoluta. Ecol Complex 50:100995. https://doi.org/10.1016/j.ecocom.2022.100995

Shi R, Jiang X, Chen L (2009) A predator-prey model with disease in the prey and two impulses for integrated pest management. Appl Math Model 33(5):2248–2256. https://doi.org/10.1016/j.apm.2008.06.001

Silva VCP, Nondillo A, Galzer ECW, Garcia MS, Botton M (2017) Effect of Host Plants on the Development, Survivorship, and Reproduction of Pseudococcus viburni (Hemiptera: Pseudococcidae). Florida Entomologist 100(4):718–724. https://doi.org/10.1653/024.100.0418

Silva VC, Aquino DA, Crochard D, Malausa T, Botton M, Palero F (2021) Parasitoids (hymenoptera) of mealybug pests (hemiptera: Pseudococcidae) from southern Brazil: molecular and morphological characterization. Neotrop Entomol 50:759–766. https://doi.org/10.1007/s13744-020-00841-3

Snyder WE, Ballard SN, Yang S, Clevenger GM, Miller TD, Ahn JJ, Hatten TD, Berryman AA (2004) Complementary biocontrol of aphids by the ladybird beetle Harmonia axyridis and the parasitoid *Aphelinus asychis* on greenhouse roses. Biol Control 30(2):229–235. https://doi.org/10.1016/j.biocontrol.2004.01.012

Straub CS, Snyder WE (2008) Increasing enemy biodiversity strengthens herbivore suppression on two plant species. Ecology 89(6):1605–1615. https://doi.org/10.1890/07-0657.1

Takizawa T, Yasuda H, Agarwala BK (2000) Effects of parasitized aphids (homoptera: Aphididae) as food on larval performance of three predatory ladybirds (coleoptera: Coccinellidae). Appl Entomol Zool 35(4):467–472. https://doi.org/10.1303/aez.2000.467

Tang S, Chen L et al (2004) Modelling and analysis of integrated pest management strategy. Discrete and Continuous Dynamical Systems Series B 4:759–768. https://doi.org/10.3934/dcdsb.2004.4.759

Vanaclocha P, Papacek D, Monzó C, Verdú MJ, Urbaneja A (2013) Intra-guild interactions between the parasitoid *Aphytis lingnanensis* and the predator *Chilocorus circumdatus*: implications for the biological control of armoured scales. Biol Control 65(2):169–175. https://doi.org/10.1016/j.biocontrol.2013.02.008

Volterra V (1926) Fluctuations in the abundance of a species considered mathematically. Nature 118(2972):558–560. https://doi.org/10.1038/118558a0

Walton V, Pringle K (2004) A survey of mealybugs and associated natural enemies in vineyards in the western cape province, south africa. S Afr J Enol Vitic 25(1):23–25. https://doi.org/10.21548/25-1-2134

Waterworth RA, Wright IM, Millar JG (2011) Reproductive biology of three cosmopolitan mealybug (Hemiptera: Pseudococcidae) species, *Pseudococcus longispinus*, *Pseudococcus viburni*, and *Planococcus ficus*. Ann Entomol Soc Am 104(2):249–260. https://doi.org/10.1603/AN10139

Weber DC, Lundgren JG (2009) Assessing the trophic ecology of the coccinellidae: their roles as predators and as prey. Biol Control 51(2):199–214. https://doi.org/10.1016/j.biocontrol.2009.05.013

Zang L-S, Liu T-X (2007) Intraguild interactions between an oligophagous predator, *Delphastus catalinae* (coleoptera: Coccinellidae), and a parasitoid, *Encarsia sophia* (hymenoptera: Aphelinidae), of *Bemisia tabaci* (homoptera: Aleyrodidae). Biol Control 41(1):142–150. https://doi.org/10.1016/j.biocontrol.2007.01.009