Impact of covert infections with an RNA virus on the susceptibility of Spodoptera exigua to natural enemies

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Abstract Integrated Pest Management (IPM) programs are alternatives to classical pest control through the application of chemical insecticides. IPM goal is to maintain pest populations below economically injurious levels minimizing the use of chemical insecticides. In recent years, research on insect viromes has led to the discovery of many novel viral pathogens in insect pests, some of them producing covert infections that do not cause evident symptoms. However, the impact that these viruses have on the action of natural enemies employed in IPM programs is still unknown. Spodoptera exigua iflavirus 1 (SeIV1) is an RNA virus commonly found in field populations of Spodoptera exigua. In this work, we have analysed the possible impact of those viral infections on the activity of different natural enemies used for the control of this pest. Specifically, we have studied the effects of SeIV1 on susceptibility to entomopathogenic nematodes, fungi, bacteria, and parasitoids. Infections with SeIV1 showed to be compatible with the nematode Steinernema carpocapsae as the infection did not affect the susceptibility or production of new juveniles. Moreover, they are also compatible with the fungus Metarhizium brunneum as the mortality of S. exigua larvae was not affected by the viral infection. Interestingly, infections with SeIV1 increase the susceptibility to Bacillus thuringiensis formulation and increase the mortality caused by the parasitism of Hyposoter didymator. In summary, our results show that the four natural enemies tested are not negatively affected by SeIV1 and their action remains similar or enhanced by the covert infections with this iflavirus.

Keywords Iflavirus · Beet armyworm · Integrated Pest Management · Natural enemies

Introduction

Chemical insecticides have been essential for the growth of agriculture. However, their long persistence in the environment, their toxic effect on non-target organisms, and the quick development of resistance (Ahmad and Arif 2010; Che et al. 2013) have increased the demand for more sustainable pest.
control methods. Integrated Pest Management (IPM) has become a successful alternative to the conventional use of chemical insecticides. The majority of experts define IPM as an approach for controlling plant pests coordinating all available methods taking into account economic and ecological factors, as minimizing chemical insecticides use (Stenberg 2017). Oversimplification of agricultural ecosystems favoured insect pests, yet IPM programs based on natural enemies may recover this loss of ecological balance (Ruiu 2018). Biological control agents are living organisms and viruses used to combat pests for human benefit (Stenberg et al. 2021). These biological control agents are core elements in IPM approaches for pest control.

Traditionally, viruses involved in IPM approaches were discovered after detecting detrimental effects in their hosts. However, recent advances in high throughput sequencing technologies have led to the discovery of a large number of viruses covertly infecting arthropods without producing obvious symptoms on their host and revealed that covert virus infections are ubiquitous (Bonning 2020; Wu et al. 2020). A considerable proportion of these newly discovered viruses are part of the Iflaviridae family, commonly known as iflaviruses. Iflaviruses are positive single-stranded RNA viruses that only infect arthropods (Valles et al. 2017), and they have been detected in field as well as laboratory-reared populations (Dou et al. 2021; Virto et al. 2014). Iflavirus infections can be lethal, like infectious flacherie virus (Aizawa et al. 1964). Nonetheless, most of them do not cause obvious symptoms on the infected individuals (Ryabov 2017). Furthermore, reports on detrimental effects of these viruses are mainly restricted to domesticated insects as silkworms and honeybees (Vootla et al. 2013; Wilfert et al. 2016). Nevertheless, their relative high abundance in natural populations suggest their potential influence on the host ecology by directly influencing on the host physiology or indirectly through their interaction with other trophic levels. Consequently, these viral infections could influence the performance of entomopathogens, and other natural enemies used in IPM programs for the control of their hosts.

Spodoptera exigua iflavirus 1 (SeIV1) was the first iflavirus described in Spodoptera exigua (Millán-Leiva et al. 2012), a worldwide distributed pest that feeds on horticultural crops and ornamental plants causing major loses (Greenberg et al. 2001). Like most iflaviruses, SeIV1 has a genome of about 10Kb with a single open reading frame that is translated into a single polypeptide of about 3200 amino acids which is post-translationally processed into structural and non-structural proteins. SeIV1 has been detected in laboratory-reared colonies but it is commonly found in field individuals (Martínez-Solís et al. 2020; Virto et al. 2014).

In this work, we aim to explore the compatibility of SeIV1 infections with IPM agents used for the control of the S. exigua. For this purpose, we have assessed the impact of SeIV1 infection in the biocontrol capacity of four different types of agents currently used for the biocontrol of this pest: the bacteria Bacillus thuringiensis, the nematode Steinernema carpocapsae, the fungus Metarhizium brunneum, and the parasitoid Hyposoter didymator.

Materials and methods

Insects

The S. exigua population used for all bioassays was originally provided by Andermatt Biocontrol AG (Switzerland) and maintained at the University of Valencia (Spain) for more than 100 generations. Larvae have been reared grouped in plastic boxes on an artificial diet (Elvira et al. 2010) in an incubator that regulates temperature at 25 ± 2 °C, 70 ± 5% RH, and a 16:8 L:D photoperiod. Between three to five larvae were randomly selected in each assay to test for the presence of SeIV1 using qPCR to confirm they were virus-free ahead each experiment as described below.

For the establishment of the SeIV1-infected insects, S. exigua SeIV1-free larvae were infected by the droplet feeding method (Hughes and Wood 1981) with the SeIV1 inoculum as it follows. Three µl drops of a suspension in PBS containing 10% (w/v) Sucrose, 10% (v/v) phenol red and $1 \times 10^9$ genomes of SeIV1 per ml were distributed on a Petri dish forming a circle. One hundred newly moulted second instar larvae were placed inside the Petri dish to feed on the drops containing $1 \times 10^9$ genomes of SeIV1 per ml. As a result of feeding on the drops, the body of the larvae showed red colouring. Larvae were left to feed for 15 min and only red-coloured larvae were selected, ensuring that only larvae that had ingested
the virus were used. Selected larvae were reared also grouped in plastic boxes on an artificial diet (Elvira et al. 2010) at 25 ± 2 °C with 70% relative humidity and a photoperiod of 16/8 h (light/dark). The SeIV1 inoculum used for all the experiments of this work was isolated from a laboratory colony of S. exigua and quantified as described below (Millán-Leiva et al. 2012). Viral infection and absence of viral infection on the control insects was assessed for each infection as described below in randomly selected individuals (n = three to five) for each treatment and condition.

The H. didymator colony was established in 2016 from pupae provided by Dr Anne-Nathalie Volkoff (University of Montpellier, France). H. dydimator were reared on third instar Spodoptera littoralis larvae at 26 ± 1 °C, 70% RH and a 16:8 L:D (Miranda-Fuentes et al. 2020).

Virus detection, and quantification

Routinely tests to confirm the absence of SeIV1 viral infections in controls and the laboratory colony were performed by reverse transcription quantitative PCR (RT-qPCR) as previously described (Jakubowska et al. 2016). Briefly, total RNA was extracted from the samples using Tripure reagent (Merck, USA) and cDNA synthesis was performed on purified RNA using PrimeScript RT reagent kit (Takara Bio Inc., Japan). RT-qPCR was carried out in a StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using HOT FIREPOL EvaGreen qPCR mix Plus (ROX) from Solis BioDyne (Estonia). Specific primers for SeIV1 and S. exigua ATP synthase, as house-keeping gene, were used for quantification of the virus (Martínez-Solís et al. 2020).

Biossays with the entomopathogenic bacteria B. thuringiensis

Two types of bioassays were used to assess the effect of SeIV1 infection on the susceptibility of S. exigua larvae to B. thuringiensis. On the one hand, larvae carrying a SeIV1 covert infection were obtained by infecting larvae by droplet feeding, as described above, on the previous generation used for the bioassay. For these larvae, the infection with B. thuringiensis was generated by droplet feeding on second instar larvae using a suspension in PBS containing 10% (w v⁻¹) sucrose, 10% (v v⁻¹) phenol red, 1 × 10⁹ genomes of SeIV1 per ml and different concentrations of B. thuringiensis. The different dilutions were split and in side-by-side experiments, SeIV1-infected and SeIV1-free larvae were infected with B. thuringiensis.

On the other hand, S. exigua second instar larvae were coinfected by SeIV1 and B. thuringiensis at the same time. For this bioassay, the droplet feeding suspen-sion in PBS contained 10% (w v⁻¹) sucrose, 10% (v v⁻¹) phenol red, 1 × 10⁹ genomes of SeIV1 per ml and different concentrations of B. thuringiensis. In side-by-side experiments, SeIV1-free larvae were infected with the same suspensions devoid the SeIV1.

After infection, sixteen larvae for each condition and concentration were placed individually in one well (two × two cm) of clear plastic bioassay trays (product no. 9074; Frontier Agricultural Sciences) with a piece of artificial diet, sealed with a microperforated adhesive lid (product no. 9074-L; Frontier Agricultural Sciences) at 25 ± 2 °C and mortality was observed after seven days. Four independent replicates in time and using different batches of insects were performed. We used a commercial formula-tion of B. thuringiensis subsp. aizawai (Xentari™, Kenogard S.A., Spain) at five concentrations (25, 5, 1, 0.2, 0.04 and 0 mg ml⁻¹ in the droplet feeding suspension). A stock suspension of the powder formulate was prepared, aliquoted and stored at -20 °C until they were used.

Bioassay with the entomopathogenic nematode S. carpocapsae

Infective juveniles (IJ) of S. carpocapsae (Capsa-nem from Koppert Biological Systems, Spain) were resuspended in distilled water and quantified with a haemocytometer (Hirschmann Laborgeräte, Germany) counting all the nematodes in 10 µl (n = five). Different dilutions were prepared in distilled water at 0, 50, 100, 200, 400 and 800 IJ ml⁻¹. S. exigua second instar larvae were infected with SeIV1 as previously described and reared until the fourth instar. Groups of sixteen SeIV1-infected and sixteen SeIV1-free newly moulted fourth instar larvae, as they are more suitable for quantification of the nematode reproduction (Acharya et al. 2020; Ebssa and Koppenhöfer 2012), were placed in a Petri dish (54 mm diameter) filled with filter paper previously inoculated with 1 ml of the different concentrations of IJ and provided with fresh diet. Larvae were kept at 25 °C overnight
and then individually placed in bioassay trays at 25 ± 2 °C. Mortality was checked at 48 h post-individualization. Four independent replicates in time and using different batches of insects were performed.

For evaluating the production of new infective juveniles only the 400 IJ ml⁻¹ concentration was used. SeIV1-infected and SeIV1-free S. exigua larvae were inoculated as described above with 400 IJ ml⁻¹. Dead larvae were collected and put in a white trap with distilled water in groups of five dead larvae placed on an inverted Petri dish (54 mm diameter) on which filter paper was placed. Petri dish was then placed inside a larger Petri dish (90 mm diameter) and 15 ml of water were poured to keep moisture on the filter paper that facilitates the transfer of the nematodes from the insect into the water. Infective juveniles were collected after seven and fourteen days and counted with a haemocytometer. Four independent replicates in time and using different batches of insects were performed.

Bioassay with the entomopathogenic fungus M. brunneum

The strain EAMa 01/58-Su of M. brunneum from the culture collection of the Agronomy Department and Unit of Excellence “María de Maeztu” 2020–2023 (DAUCO) of the University of Cordoba (Spain) was used to evaluate the effect of SeIV1 on the activity of entomopathogenic fungi. This strain has been previously used for the control of S. littoralis in multitrophic scenarios (Miranda-Fuentes et al. 2020, 2021). The fungus was cultured onto malt agar (Bio-Cult Laboratories, Spain) in Petri dishes and grown at 25 °C in darkness for 15 days. Conidia were scraped from Petri dishes into a sterile solution of 0.1% Tween 80. The concentration of the resulting conidial suspension was adjusted to 1 × 10⁹ conidia ml⁻¹ with a haemocytometer. Groups of ten SeIV1-infected and ten SeIV1-free second instar S. exigua larvae were inoculated by immersion in the 1 × 10⁹ conidia ml⁻¹ suspension or only 0.1% Tween 80 (control treatment) for 1 min and then kept individually. Mortality was checked after 14 days. To confirm that the death was due to the fungus, everyday dead larvae were removed, surface-sterilized with 1% sodium hypochlorite and rinsed in sterile distilled water for 1 min each. Then, the dead larvae were placed on sterile wet filter paper in sterile Petri dishes, sealed and incubated at 25 °C in darkness for inspecting fungal outgrowth (Quesada-Moraga et al. 2006). Four independent replicates in time were performed.

Bioassay with the parasitoid H. didymator

H. didymator parasitizes the larval stages of noctuid pests such as species from the Helicoverpa and Spodoptera genera (Hatem et al. 2016). Newly emerged adult female H. didymator (48 h) were individually paired with two male parasitoid adults for 24 h for mating at 25 °C. Ten newly moulted SeIV1-infected and ten SeIV1-free second instar S. exigua larvae were offered to each mated female parasitoid for 24 h. As control, ten SeIV1-infected and ten SeIV1-free larvae were kept without exposure to parasitoids. Then, the S. exigua larvae were kept individually and mortality was checked 14 days after. Four independent replicates in time were performed.

Additionally, different development parameters of the new generation of H. didymator were measured. Parasitised S. exigua larvae were daily checked to quantify the duration of H. didymator larval stage, the number of parasitoid pupae, the size of these pupae, the duration of the pupal stage and the adult H. didymator lifespan.

Statistical analysis

Statistical analysis was performed using R version 3.6.3 (R Core Team 2022). Briefly, the concentrations of B. thuringiensis and S. carpocapsae were log10 transformed, then the calculated concentrations and the proportion of dead larvae were analyzed with Generalized Lineal Models (GLM) with binomial error distributions and a logit link. For calculating the lethal concentrations 50 (LC50) and their SE, using the ecotox package in R (Hlina et al. 2021). Likelihood ratio tests were performed to compare treatment and control regression curves and to test whether the slopes were parallel, and the intercepts were equal. Models with parallel slopes and not equal intercepts were considered statistically different. Nematode production was analysed by calculating the production per larva and comparing the groups using unpaired t-test on GraphPad Prism 8.4.2. Mortality by M. brunneum and H. dydimator was analysed with R version 3.6.3 (R Core Team 2022) also. Exact binomial tests were calculated to compare the different groups.
Impact of covert infections with an RNA virus on the susceptibility of *Spodoptera exigua* to... 

P-values from the multiple exact binomial tests were corrected by Benjamini-Hochberg method, corrected P-values less than 0.05 were considered as statistically significant. Additionally, *M. brunneum* mortality data was used to assess survival analysis using the Kaplan–Meier method and compared using the log-rank tests (Mantel–Cox test) on GraphPad Prism 8.4.2. Biological parameters of the *H. didymator* were compared using unpaired t-tests with GraphPad Prism 8.4.2.

**Results**

SeIV1 infection increases susceptibility to *B. thuringiensis*

Effect of SeIV1 infections on the susceptibility of *S. exigua* larvae to one of the main *B. thuringiensis* products used for its control (Xentari™) was assessed by comparing the dose response curves and LC₅₀ values for SeIV1-free and SeIV1-infected larvae using a droplet feeding assay. Experiments were performed on larvae carrying a covert SeIV1 infection generated after infecting the previous generation of *S. exigua* and also in larvae infected with SeIV1 at the same time that were exposed to *B. thuringiensis*. For both types of assays, the susceptibility to *B. thuringiensis* was significantly increased in the SeIV1-infected larvae when compared to their respective control of SeIV1-free larvae (Table 1). The dose response curve to *B. thuringiensis* of *S. exigua* infected at the same time with SeIV1 and the bacteria was proven as parallel to the dose response curve for SeIV1-free larvae ($\chi^2 = 3.56$, df = 1, $P = 0.06$) but non-equal ($\chi^2 = 18.44$, df = 1, $P < 0.01$) (Supplementary Figure S1a). The dose response curve to *B. thuringiensis* of *S. exigua* carrying the SeIV1 infection from the previous generation was also proven as parallel to the dose response curve for SeIV1-free larvae ($\chi^2 = 0.60$, df = 1, $P = 0.44$) and equal ($\chi^2 = 16.74$, df = 1, $P < 0.01$) (Supplementary Figure S1a). For the two types of bioassays, potency of *B. thuringiensis* against *S. exigua* increased in about twofold when the SeIV1 was present (Table 1).

SeIV1 infection does not affect to *S. carpocapsae* performance or production of new IJ

Impact of SeIV1 covert infections on the susceptibility of *S. exigua* larvae to the entomopathogenic nematode, *S. carpocapsae* was studied by comparing the LC₅₀ of the nematode on SeIV1-free and SeIV1-infected larvae. The mortality caused by *S. carpocapsae* on fourth instar larvae was not affected by the covert infection with SeIV1. LC₅₀ values were of 209 IJ ml⁻¹ and 202 IJ ml⁻¹ for the control and the SeIV1-infected larvae, respectively (Table 2). The dose response curves for the SeIV1-infected insects were proven as parallel to the dose response curve for SeIV1-free insects ($\chi^2 = 0.01$, df = 1, $P = 0.91$) and equal ($\chi^2 = 0.07$, df = 1, $P = 0.79$) (Supplementary Figure S1b). The effect of SeIV1 infection on the production of new IJs from dead larvae was also

### Table 1 Effect of SeIV1 infection on *B. thuringiensis* activity

| Treatment                  | LC₅₀ ± SE (mg ml⁻¹) | Relative potency | P **       |
|----------------------------|---------------------|-----------------|------------|
| *B. thuringiensis*         | 2.65 ± 0.49         | 1.00            |            |
| SeIV1 + *B. thuringiensis* | 1.31 ± 0.36         | 2.02            | < 0.01     |
| SeIV1* + *B. thuringiensis*| 1.56 ± 0.25         | 1.70            | < 0.01     |

* Larvae infected with SeIV1 one generation before *B. thuringiensis* exposure

** P for the likelihood ratio testing for the equality of the dose response curves of SeIV1-free and SeIV1-infected *S. exigua*

### Table 2 Effect of SeIV1 infection on *S. carpocapsae* activity

| Treatment                  | LC₅₀ ± SE (IJ ml⁻¹) | Relative potency | P* | Production ± SE (IJ/larvae) | P** |
|----------------------------|---------------------|-----------------|----|----------------------------|-----|
| *S. carpocapsae*           | 209 ± 19            | 1.00            |    | 3841 ± 764                 |     |
| SeIV-1 + *S. carpocapsae*  | 202 ± 18            | 1.03            | 0.79| 3603 ± 625                 | 0.82|

* P for the likelihood ratio testing for the equality of the dose response curves of SeIV1-free and SeIV1-infected *S. exigua*

** P for the unpaired t test testing the production between SeIV1-free and SeIV1-infected *S. exigua*
checked. Our results showed no significant difference on the number of IJ produced per larva between control (3841 IJ larva⁻¹) and SeIV1-infected larvae (3603 IJ larva⁻¹) (t = 0.24, df = 6, P = 0.82) (Table 2).

*M. brunneum* activity is not altered by SeIV1 infection

Effect of SeIV1 infection on the activity of the entomopathogenic fungi *M. brunneum* was analysed by comparing the mortality on SeIV1-free and SeIV1-infected larvae after topical inoculation of the fungal pathogen. Susceptibility of *S. exigua* larvae to *M. brunneum* was not affected by the presence of the virus at the tested concentration and similar mortality due to fungal infection was observed for a giving dose in SeIV1-free larvae (52.5 ± 2.5%) and SeIV1-infected larvae (55.0 ± 5.0%) (χ² = 0.00, df = 1, P = 1.00) (Fig. 1a). The overall mortality was not affected by the viral infection either. Mortality for SeIV1-free (80.0 ± 4.1%) and SeIV1-infected larvae (80.0 ± 9.1%) were similar (χ² = 0.00, df = 1, P = 1.00) (Fig. 1a). Additionally, the survival of the larvae during the infection with the fungus was analysed and no difference was observed between SeIV1-free and SeIV1-infected larvae (χ² = 0.11, df = 1, P = 0.74) (Supplementary Figure S2).

SeIV1 infection influences *H. didymator* parasitism and life cycle

Influence of SeIV1 infections on *H. didymator* parasitism on *S. exigua* larvae was studied by comparing the mortality caused by the parasitoid when SeIV1-free and SeIV1-infected larvae were offered. No difference on the mortality directly caused by the parasitism was observed in either group (χ² = 0.00, df = 1, P = 1.00) (Fig. 1b). However, the overall mortality of the assay was significantly higher in SeIV1-infected larvae (χ² = 5.59, df = 1, P = 0.03) (Fig. 1b). These results reveal certain detrimental effects associated to the combination of covert infection with SeIV1 and the parasitism with *H. didymator*.

Biological parameters of the *H. didymator* developing in *S. exigua* were also measured (Table 3). The duration of the larval stage (12 days) and the size of the pupae (6 mm) were the same for both groups (duration of larval stage, t = 0.00, df = 14, P > 0.99) (size of the pupa, t = 0.00, df = 14, P > 0.99). However, parasitoids developed on the SeIV1-free larvae for parasitisation. Mortality not directly related with successful parasitism is included as other (white bar). The error bars represent the SE of four replicates. Asterisk indicates significant difference in the overall mortality between SeIV1-free and SeIV1-infected larvae (P < 0.05).
had a shorter duration of the pupal stage (five days) when compared to the ones derived from the SeIV1-infected larvae (seven days) \( (t = 7.07, \text{df} = 14, P < 0.01) \). In addition, the adult longevity was significantly longer for the wasps emerged from SeIV1-infected larvae (39 days) when compared to the ones derived from the SeIV1-free larvae (16 days) \( (t = 2.78, \text{df} = 14, P = 0.01) \).

**Discussion**

High throughput sequencing has revealed the widespread presence of RNA viruses producing covert infections in reared as well as field insects (Bonning 2020). However, no information about their impact on the activity of other biological agents used for the control of these pests is available. In this study, we have observed that covert infections with SeIV1 in *S. exigua* do not have a negative impact on the activity of four different types of natural enemies used for the control of this pest. Natural populations of *S. exigua* can carry covert infections with SeIV1 in *S. exigua* do not have a negative impact on the activity of four different types of natural enemies used for the control of this pest. Natural populations of *S. exigua* can carry covert infections with SeIV1 in *S. exigua* (Martínez-Solís et al. 2020; Virto et al. 2014). Previous reports revealed that covert infection with SeIV1, as well as co-infection with SeIV1, increases susceptibility to the baculovirus Spodoptera exigua Multiple Nucleopolyhedrovirus (SeMNPV) (Carballo et al. 2017, 2020). Our results, and previous studies, reveal the compatibility of the entomopathogenic virus (SeMNPV), bacteria (*B. thuringiensis*), nematode (*S. carpocapsae*), fungus (*M. brunneum*), and parasitoid (*H. didymator*) for the control of *S. exigua* when covertly infected with SeIV1.

Compatibility between *B. thuringiensis* and entomopathogenic viruses has been previously tested in few other pathosystems. When *B. thuringiensis* is coinoculated with Panolis flammea nucleopolyhedrovirus for control of *Mammestra brassicae*, the viral mortality and primary transmission are improved, although the secondary transmission is negatively affected (Hesketh and Hails 2015). Nonetheless, when *B. thuringiensis* is coinoculated with the Heliothis virescens ascovirus, the bacteria competes with the virus and depending on their concentrations one or the other reaches an effective infection (Yu et al. 2021). Our results have demonstrated that the susceptibility to *B. thuringiensis* increases when *S. exigua* larvae are infected by SeIV1. This effect could be due to the sublethal effects caused by the SeIV1 (Carballo et al. 2020) which would allow a better performance of *B. thuringiensis* and increase the susceptibility of the pest.

Currently, *B. thuringiensis* is used in two different ways: as sprayable mixtures of its spores and parasporal crystals (as Xentari™ used in this work) or expressing its insecticidal genes in transgenic crops (Pinos et al., 2021). Due to its extensive use, some cases of laboratory and field resistance to *B. thuringiensis* have been reported for different pests (Jurat-Fuentes et al. 2021). Multiple studies have focused in finding a way to manage this resistance acquisition by trying to combine *B. thuringiensis* with another biocontrol agent. For instance, nucleopolyhedroviruses and entomopathogenic nematodes can increase the fitness cost of the resistance to Bt products (Gassmann et al. 2006; Raymond et al. 2006; Sarfraz et al. 2010) contributing to the management of the resistance. In the case of *S. exigua*, resistance to *B. thuringiensis* can be generated in laboratory by artificial selection (Hernández-Martínez et al. 2010). As SeIV1 infections increase susceptibility to *B. thuringiensis* on *S. exigua*, it would be interesting to test the effect of the SeIV1 infections on resistance acquisition and the stability of the resistance when the selective pressure is removed.

Entomopathogenic nematodes and fungi are also important agents for insect pest control. They

### Table 3: Effect of SeIV1 infection on *H. didymator* biological parameters

| Parameter                        | Treatment                      | \( P^* \) |
|----------------------------------|--------------------------------|-----------|
| Duration of larval stage (days)  | SeIV1-free (± SE)              | 12.0±0.0  |
| Size of pupae (mm)               |                                | 6.0±0.1   |
| Duration of pupal stage (days)   | SeIV1-infected (± SE)          | 12.0±0.3  |
| Adult lifespan (days)            |                                | 6.0±0.1   |
|                                  |                                | 5.0±0.2   |
|                                  |                                | 7.0±0.2   |
|                                  |                                | <0.01     |
|                                  |                                | 16.0±3.3  |
|                                  |                                | 39.0±7.6  |
|                                  |                                | 0.01      |
have been proven useful for the biocontrol of many insects (Kamali et al. 2018; Katiski da Costa Stuart et al. 2020; Malan et al. 2011; Miranda-Fuentes et al. 2021), including S. exigua (An et al. 2016; Han et al. 2014; Kaya and Grieve 1982). In this work, no effect of the SeIV1 infection on the performances of the species of nematode and fungus tested was observed. Although the outcome could be different using other species of nematodes and fungi or other concentrations of the fungus, as one concentration was tested, our results suggest that the SeIV1 infection did not negatively affect their biocontrol activity. Therefore, S. carpopcapsae and M. brunneum can be used to control SeIV1-infected S. exigua larvae as effectively as SeIV1-free insects.

Endoparasitoids are parasitoids that deposit eggs inside the body of the host. After oviposition, parasitoid’s larvae develop by feeding on the host which eventually dies due to the parasitism (Moreau and Asgari 2015). In our work, we studied the compatibility of SeIV1 infections with H. didymator to control S. exigua. We prove that biocontrol by H. didymator is compatible with SeIV1 infection of S. exigua as the successful parasitism is not affected by the presence of the virus. The relatively low values of successful parasitism observed could be explained by the use of second instar S. exigua larvae for the bioassays as this instar has been shown suboptimal in a close relative species, S. littoralis (Miranda-Fuentes et al. 2020). Interestingly, we detected substantial levels of mortality not directly caused by the development of the parasite in SeIV1-infected hosts. Incomplete parasitism by H. dydimator has already been described in S. littoralis (Hatem et al. 2016). The fact that we only observed this additional mortality in SeIV1-infected insects could be associated to the effect of SeIV1 infection in the host quality (Harvey 2005). H. didymator, when ovopositing into the larval body, also injects other components such as venom, calyx fluid or symbiotic viruses (Hyposoter didymator Ichnovirus, HdIV) to guarantee successful parasitism. Another hypothesis is that the presence of SeIV1 would influence the effect of the other components injected, as the HdIV, which could lead to the death of the host. Nevertheless, this increase in mortality suggests that SeIV1-infected insects are overall more affected by H. didymator than the SeIV1-free insects.

Our data has additionally shown a longer duration of the pupal stage and adult longevity of the parasitoids in SeIV1-infected S. exigua larvae. This delay in the pupal development is not a desirable effect because it increases the time that the parasitoid takes to kill the pest, therefore the insect cause damage to the crop during more time. However, the extended adult longevity observed could provide an advantage on the biological control, since extended longevity of the adult could increase the chances of parasitism in the field. Similarly, extended longevity was observed for the parasitoid Habrobracon hebetor when it parasitizes Helicoverpa armigera larvae exposed to sublethal doses of B. thuringiensis (Sedarian et al. 2014).

Viral replication of iflaviruses during coinfections has not been extensively investigated. Enhanced replication of a picorna-like virus when the host is coinfected with the fungus Beauveria bassiana has been found in Riptortus pedestris (Yang et al. 2016). This enhanced viral replication suggests that the fungal infection is shifting the viral infection from a covert to an overt infection. From a pest control point of view, this finding is important because the manipulation of this shift, in this and other viruses, could lead to a better management of viral infections in the field. Infections by SeMNPV associated to SeIV1 in S. exigua have shown no effect on the replication of the baculovirus but an enhancement for the SeIV1 replication (Jakubowska et al. 2016). However, other entomopathogens have been reported to have little or no effect on viral infections, at least for baculoviruses (Deschodt and Cory 2022; Hesketh and Hails 2015). Further experiments focusing on the replication of SeIV1 during coinfections with the natural enemies used in this study would provide new insights on the impact of other pathogens in the ecology of covertly infecting RNA viruses.

In conclusion, our results show that SeIV1 infections do not negatively affect the biocontrol efficacy of S. exigua. Natural enemies’ performance was similar or even enhanced (B. thuringiensis or H. dydimator) when the insects were infected with SeIV1. These covert infections have been described in many different insect pests. Therefore, these results highlight the importance of research on these viruses as they could be playing an important role in the field.

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Impact of covert infections with an RNA virus on the susceptibility of Spodoptera exigua to…

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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