A parasitoid wasp allied with an entomopathogenic virus to control *Tuta absoluta*

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ABSTRACT

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a major threat to worldwide tomato production, and sustainable management methods are needed. *Betabaculovirus phoperculellae* (PhopGV) and the parasitoid wasp *Necremnus tutae* Ribes & Bernardo (Hymenoptera: Eulophidae) were identified as promising biocontrol agents of this pest. Since the virus is most effective against first instar larvae, whereas the parasitoid targets older ones, combining both agents could result in increased pest control. We conducted a replicated semi-field experiment to evaluate the control efficacy of *N. tutae* and PhopGV when used alone and in combination against *T. absoluta* and their compatibility over an entire growth period. Combining the two agents achieved the highest larval (−24 %) and crop damage (−29 %) reduction cumulated over the entire experiment. However, there was no significant additive effect of the two agents. At the end of the trial, which coincided with the emergence of the third generation of *T. absoluta* adults, using the virus alone resulted in the strongest reduction of adult density (−78 %), followed by the virus-parasitoid combination (−59 %). No negative effect of the virus was recorded on the parasitoid population. Our results demonstrate the compatibility and potential of *N. tutae* and PhopGV to reduce population growth and crop damage of *T. absoluta* under semi-field conditions. While more research is needed, our findings provide important insights into an innovative combination of biocontrol agents, thereby contributing to more sustainable agriculture.

1. Introduction

About 190 million tons of tomatoes (*Solanum lycopersicum* L.) are produced yearly on five million hectares worldwide (FAO, 2023). The South American tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), recently reinstated as *Phtorimaea absoluta* (Chang and Metz, 2021), is a major threat to this essential cash crop, relied on by many people (Bergougnoux, 2014). The pest can cause up to 100 % damage in all tomato production systems, ranging from soil grown open-field crops to high-tech greenhouse crops (Biondi et al., 2018; Desneux et al., 2010, 2011). *Tuta absoluta* is native to South America and has, since its arrival in Spain in 2006, spread to nearly 100 countries outside its endemic region (EPPO, 2023). Females lay their eggs individually on leaves, stems, and petioles, preferentially on apical and median plant parts (Cocco et al., 2015b; Torres et al., 2001). The

leaf-mining larvae penetrate the leaves and feed on the mesophyll throughout their four-instar development. Larvae mainly damage leaves and attack fruits when the population density is high (Cocco et al., 2015b; Desneux et al., 2010). Mature larvae usually drop to the soil and pupate on the ground. *Tuta absoluta* has a high reproduction potential and can complete up to 12 generations per year under favourable environmental conditions (Desneux et al., 2010, 2022).

Pest control with synthetic insecticides, largely used to control *T. absoluta* (Desneux et al., 2010, 2022), is unsustainable as the moth rapidly develops high levels of resistance to many chemical compounds (Guedes et al., 2019). Moreover, synthetic insecticides harm key beneficial arthropods, disrupt existing integrated pest management (IPM) programmes and cause environmental and human health concerns (Abbes et al., 2015; Soares et al., 2019; Yadav and Devi, 2017). Therefore, alternative sustainable management methods to control *T. absoluta*

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are required to ensure the economic viability of tomato production in the invaded regions. Biological control methods involving egg parasitoids, mirid predators, and microbial agents have been integrated into pest management programmes with variable success against *T. absoluta* in various regions, such as South America, Europe, Africa, and Asia (Han et al., 2019; Mansour et al., 2018; Zappalà et al., 2013). Using multiple pest control methods, including diverse biological control agents, provides numerous advantages compared to relying solely on one single agent. One benefit is the possibility of enhancing effectiveness, as different control methods have advantages and disadvantages, and combining them can help overcome each method's limitations. For instance, combining various natural enemies may be more effective at controlling a pest population as each species can have distinct foraging behavior or target different pest life stages (Dainese et al., 2017). Regarding *T. absoluta*, most studies on combining different biological control agents focused on combining *Trichogramma* egg parasitoids with other methods (Bueno et al., 2023; Chailleux et al., 2013; Mansour and Biondi, 2021), and the ones testing the efficacy under semi-field conditions are scarce.

Betabaculovirus phoperculellae (PhopGV, Baculoviridae: Betabaculovirus) is a granule-shaped virus highly specific to Lepidoptera and infectious to larvae of different Gelechiidae. It was first developed for the biocontrol of the potato moths *Phthorimaea operculella* (Zeller) and *Tecia solanivora* (Povolný), which are closely related to *T. absoluta* (Gómez Valderrama et al., 2018; Mascarin et al., 2010). Infection and replication of baculoviruses occur exclusively in the larval stage. Larvae become infected when they feed on plants contaminated with occlusion bodies (OBs) containing occlusion-derived viruses (ODVs). After ingestion, OBs are dissolved in the insect midgut, releasing ODVs that infect the midgut epithelial cells. Budded viruses (BVs) then disperse and replicate in other susceptible tissues. The larval tegument ruptures upon death, releasing new OBs that contaminate the plant surface (Slack and Arif, 2007; Williams et al., 2017). The PhopGV has a good level of pathogenicity against the first instars of *T. absoluta* larvae and also causes sublethal effects such as retarded larval growth and pupation failure in the older larval instars (Gómez Valderrama et al., 2018; Mascarin et al., 2010).

Necremnus tutae Ribes & Bernardo (Hymenoptera: Eulophidae), previously classified as *Necremnus artynes* (Walker), is native to the Mediterranean region and is the most abundant and widespread larval parasitoid of *T. absoluta* in that area (Desneux et al., 2022; Ferracini et al., 2019). This synovigenic idiobiont ectoparasitoid parasitizes and feeds on second to fourth instar larvae of the pest with a distinct preference for the third instar (Calvo et al., 2013). *Necremnus tutae* causes additional mortality by host-killing (i. e. host-feeding, host-stinging). It is currently not commercially available but occurs spontaneously in tomato production fields in different regions (Abbes et al., 2014; Arnó et al., 2021; Crisol-Martínez and van der Blom, 2019; Desneux et al., 2022).

Augmentative releases of *N. tutae* alone seem insufficient to keep damage caused by *T. absoluta* on tomato plants below the economic threshold (Calvo et al., 2016; Cocco et al., 2015a; Desneux et al., 2022). Regarding PhopGV, high doses (weekly application of the highest recommended concentration) are required to lethally infect *T. absoluta* larvae. In addition, the most damaging older larvae are difficult to reach due to their location inside the mines, so complementary control measures are required (Gonthier et al., 2023a). Combining both biocontrol agents could increase pest control against *T. absoluta*. The virus is more effective on young larvae, while the parasitoid targets the later instars. In addition, no adverse effects of PhopGV were detected on the development of *N. tutae* under controlled conditions, indicating that both agents are compatible for combined use (Gonthier et al., 2023a).

We conducted a replicated semi-field experiment to assess the efficacy of *N. tutae*, and PhopGV used alone and in combination for the biocontrol of *T. absoluta*. More precisely, we addressed the following research questions: Q1 Do *N. tutae* and PhopGV influence the population development of *T. absoluta* when used alone or in combination? Q2 Do *N. tutae* and PhopGV influence the level of crop damage caused by *T. absoluta* when used alone or in combination? Q3 Is the *N. tutae* population influenced by PhopGV under semi-field conditions (effects on population size and sex ratio)?

2. Material and methods

2.1. Study area and greenhouse

The replicated semi-field experiment was conducted from June to August 2021 in the facilities of Agroscope in Conthey (Valais, Switzerland). Twenty-eight walk-in cages (Diatex SAS, France) of 2.6 m^2 surface area and 2.5 m height with thrips-proof mesh (300 × 300 μm^2) were set up in two identical 90 m^2 glass greenhouses. The floor below the cages was covered with a white woven ground cloth (100 g/m^2). Temperature, relative humidity, and irrigation were managed using a remote-control system.

2.2. Biological material

2.2.1. Tomato plants
Ungrafted single-stemmed tomato plantlets cv. Admiro (De Ruiter) 30 cm in height and with five fully expanded leaves with leaflets were repotted in 10-litre pots, fertilized with Osmocote (NPK 15/9/12), a slow release fertilizer providing enough nutrients for four months, and moved into the walk-in cages. No plant protection interventions, other than those detailed in 2.2.2 and 2.2.3, were realised. The average temperature and relative humidity during the experiment were 25.1 °C ± 4.2 SD and 58.8 % ± 15.4 SD. Four plants were placed in each of the 2.6 m^2 walk-in cages. The main stem of each plant was trellised on a string attached to a wire above the cage and passed through an insect-proof hole in the ceiling of the latter. Secondary shoots were removed weekly, and plants were watered daily through drip irrigation.

2.2.2. Insects
Pupae of *T. absoluta* were provided by Andermatt Biocontrol Switzerland. These were originally collected in Switzerland and reared in the company's facilities, and a sample was checked to ensure a 1:1 sex ratio.

Necremnus tutae parasitoids were collected from commercial tomato fields in El Maresme county, Barcelona, Spain, and a colony was established at Agroscope, Switzerland. Adult parasitoids were kept in cages (50 × 50 × 50 cm) (bug dorm; MegaView Science Co. Ltd., Taiwan) and provided with honey-water (10 % v/v) soaked cotton and tomato plants with *T. absoluta* larvae in their second or third instar for parasitization. After emerging, adult parasitoids were gathered and kept at 12 °C with honey and water. Every ten days, a new generation of parasitoids was started. Only naive, mated (stored with males for at least two days), and less than one-week-old female parasitoids were used in the experiments. Adult parasitoids were briefly cooled in a cold room (4 °C) for counting (sex ratio 1:1). They were then placed in a box for 24 h to feed and mate before being released in the middle of each walk-in cage. Honey was supplied twice a week for one month to the parasitoids to ensure their proper establishment.

2.2.3. Baculovirus
Tutavir®, a biopesticide containing a 2 × 10^{13} OB/l of PhopGV, was produced and provided by Andermatt Biocontrol Switzerland. The product was applied with an electrical backpack sprayer (Fox Motori, Cod. 5-19-180) following the supplier's instructions specific to the trial (see 2.3 Experimental setup). A Teejet nozzle (TXA8001vk) was used and pressure was set at 2.5 bar. Good coverage of the underside of the leaves was achieved by using a sprayer with the nozzle pointing upwards. A plastic sheeting was hung between the cages before each application of PhopGV to avoid cross-contamination.

2.3. Experimental setup

The four following treatments were compared in a completely randomized block design with seven replicates: 1) Control with *T. absoluta* only (CO), 2) *T. absoluta* and *N. tutae* (PA), 3) *T. absoluta* and PhopGV (VI), and 4) *T. absoluta, N. tutae* and PhopGV (VP). To provide *T. absoluta* with food ad libitum, four batches of plants were transplanted in the walk-in cages throughout the trial. A first set of four potted plants was inserted on day one, followed by a second batch on day 45 (Table 1). Ten plants cut in a neighbouring greenhouse were placed in each cage on day 48, and two potted plants were added on day 58. All batches of plants were sprayed with water (CO and PA) or with PhopGV (VI and VP) at standard dosage (0.015 %, $\geq 3 \times 10^9$ OB/l, 0.7 l/plant) on the day of their introduction (Table 1). Additionally, the first batch of plants was sprayed at a standard dosage on days 3 and 10 and at a low dosage (0.0015 %; $\geq 3 \times 10^8$ OB/l) on days 28 and 42. Fifty *T. absoluta* pupae were released in each of the 28 plots on day one. *Necremnus tutae* neither parasitizes nor feeds on first instar *T. absoluta* larvae (Calvo et al., 2013). Thus, the parasitoid was first released on day 16, coinciding with the first availability of the second instar *T. absoluta* larvae (Table 1). At least two studies have conducted semi-field experiments with *T. absoluta* and *N. tutae* previously (Calvo et al., 2016; Campos et al., 2020; Chailleux et al., 2014). We chose a similar setup to the one described by Calvo et al. (2016) since they used similar cages. Thirty couples of adult *N. tutae* were released in each plot of treatments PA and VP. A second release of thirty couples was done on day 41.

2.4. Monitoring

One plant per cage was sampled weekly, starting two weeks after *T. absoluta* release (Table 1). Each week, a different plant was sampled. Two leaflets of leaves 6, 8, 10, 12, and 14 (apex = 1) were collected systematically on each selected plant, resulting in a sample of ten leaflets per cage. The number of healthy *T. absoluta* larvae in each leaflet was counted using a binocular. After counting, leaflets were placed in a transparent sheet and scanned using a standard printer, and the percentage of surface mined by the larvae of *T. absoluta* was assessed using the software ImageJ (Version 1.53j). Total area of leaflets and the damaged area in pixels were measured using different hues, saturation, and brightness of the red threshold colour in the HSB colour space. The percentage of crop damage was calculated by dividing the damaged area by the total area of the leaflets and multiplying the result by 100. Leaflets were put back in their original cage at the latest 36 h after sampling to avoid bias due to the removal of insect individuals.

From day 62 to day 79, batches of two yellow sticky traps (25 × 40 cm^2) (yellow catch-it, Andermatt Biocontrol Switzerland) with a synthetic *T. absoluta* female pheromone dispenser (pheromone dispenser for Delta-trap, Andermatt Biocontrol Switzerland) were hung in each cage to catch all alive adults of *T. absoluta* and *N. tutae*. Once removed from the cages, the traps were wrapped in plastic film, scanned on both sides, and placed in the refrigerator. All scans were sent to Trapview B. V. in the Netherlands, where the numbers of adult *T. absoluta* on the sticky

traps were counted with a faster Rcnn Object Detection Network using the Tensorflow Object Detection API. In addition, the numbers of *N. tutae* males and females were counted visually using a binocular.

2.5. Statistical analysis

Statistical analyses of the dataset (Koller et al., 2023a) were performed with R 4.2.3 (R Core Team, 2023). First, the blocking factor (spatial distribution of cage in the greenhouse) was tested as a fixed effect for all response values, and as there was no significant effect, it was excluded in the further modelling process. To assess whether the biocontrol agents influenced the population development of *T. absoluta* (Q1), two linear models with treatment as a fixed factor and cumulative larvae and adults as response variables, respectively, were fitted using the nlme package (version 3.1–162). To assess whether the biocontrol agents influenced the level of crop damage caused by *T. absoluta* (Q2), the average percentage of crop damage recorded over the trial was fitted as a response value in a linear model with treatment as a fixed effect. To assess whether PhopGV influenced the *N. tutae* population (Q3), the total number of parasitoid adults caught on the yellow traps at the end of the trial were sqrt(x) transformed to normalize distribution before fitting a linear model with treatment as the explanatory variable. To detect potential effects of the virus on the sex ratio of the parasitoid, a generalized linear model with binomial error structure and treatment as a fixed factor was fitted.

Linear models were analysed using two-way ANOVA. To find significant differences between treatment levels, we compared them pairwise using Tukey's HSD test in linear models that were globally significant. Inference from the generalized linear model of parasitoid sex ratio was drawn using a chi-square test. P values lower than 0.05 were considered statistically significant. For all models, assumptions were checked according to the graphical validation procedures recommended by Zuur et al. (2009).

3. Results

Tuta absoluta completed almost three generations during the semi-field experiment (Fig. 1). The first generation lasted from day 1 to day 30, while the second spanned from day 30 to day 60. The third generation started around day 60. The start and end of each generation were determined based on the larval peak populations.

3.1. Effect of the biocontrol agents on the population growth of T. absoluta

PhopGV and *N. tutae* reduced the number of *T. absoluta* larvae cumulated over the trial by 13 % and 14 %, respectively, compared to the control (Fig. 2). Both biocontrol agents applied together decreased the number of larvae by 24 %. However, the differences between the four treatments were non-significant (ANOVA, $F_3 = 1.508$, p = 0.238) (see Fig. 2).

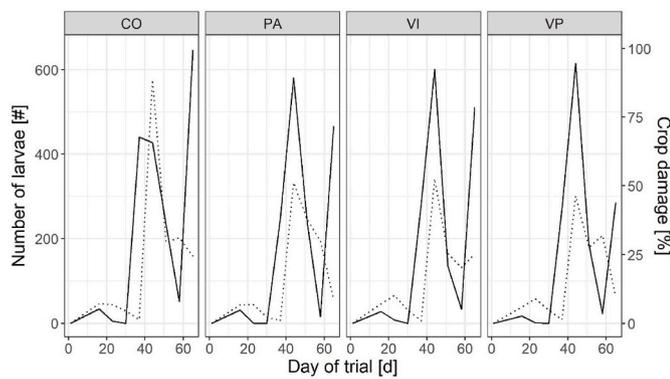Regarding the number of adult *T. absoluta* caught on the sticky

Table 1
Experimental setup of the semi-field experiment. Numbers in the cells below indicate the exact day each action occurred (1 = start of the trial, release of *Tuta absoluta*). [50] = 50 *T. absoluta* pupae, [30] = 30 couples of *Necremnus tutae*, [H] = application of *Betabaculovirus phoperculellae* (PhopGV) at standard dosage (0.015 %, $\geq 3 \times 10^9$ OB/l), [L] = application of PhopGV at low dosage (0.0015 %; $\geq 3 \times 10^8$ OB/l).

Description	Week Number Year 2021											
	23	24	25	26	27	28	29	30	31	32	33	34
Tomato plants	1						45	48	58			
T. absoluta release	1[50]											
PhopGV application	1[H] 3[H]	10[H]			28[L]		42[L] 45[H]	48[H]	58[H]			
N. tutae release			16[30]				41[30]					
Pest monitoring			16	23	30	37	44	51	58	65		
Yellow sticky traps										62	to	79

Fig. 1. Population dynamic of healthy *Tuta absoluta* larvae and crop damage (percentage of leaf area mined by the larvae) monitored on tomato plants over a three-month semi-field experiment comparing different experimental treatments (CO = control without treatment (only *Tuta absoluta*), PA = parasitoid (N*ecremnus tutae*), VI = virus (PhopGV), VP = virus + parasitoid). The solid line represents the number of larvae and is connected to the y-axis on the left. The dotted line represents the percentage of crop damage and is connected to the y-axis on the right.

yellow traps at the end of the trial, the treatment with the lowest adult density was the virus treatment, followed by the virus-parasitoid combination (Fig. 2). PhopGV alone significantly reduced the third generation of adults by 78 % compared to the control and the parasitoid treatment (ANOVA, $F_3 = 9.545$, p =< 0.001; Tukey's test: VI-CO p = 0.001, VI-PA p = 0.002). Similarly, the virus-parasitoid combination caused a reduction of 59 % and 58 % of adults compared to the control and the parasitoid treatment, respectively (VP–CO p = 0.018, VP-PA p = 0.022). With a reduction of 2 % only, the parasitoid treatment was similar to the control (PA-CO p = 0.999). No significant difference was found between PhopGV used alone and in combination (VI-VP p = 0.713).

3.2. Effect of the biocontrol agents on crop damage caused by T. absoluta

PhopGV and *N. tutae*, both alone and combined, caused a significant and similar damage reduction cumulated over the trial (ANOVA, $F_3 = 8.524$, p < 0.001; Tukey's test: PA-CO p = 0.003, VI-CO p = 0.005, VP-CO p < 0.001, Fig. 2). PhopGV and *N. tutae* reduced crop damage by 24

% and 25 % compared to the control. The combined biocontrol agents reduced crop damage by 29 %. No significant differences were found between the biocontrol agents used alone or in combination (all P values > 0.855).

3.3. Compatibility of the biocontrol agents under semi-field conditions

Necremnus tutae and PhopGV were found to be compatible under semi-field conditions. Similar numbers of parasitoids were recorded at the end of the trial in the parasitoid and the virus-parasitoid treatments (ANOVA, $F_1 = 0.229$ p = 0.641). Similar sex ratios were found in both treatments, indicating no adverse effect of PhopGV on *N. tutae* (Chi-square, $LRT_1 = 0.0202$ p = 0.888, Fig. 3).

4. Discussion

This research demonstrates the potential of the baculovirus PhopGV and the parasitoid *N. tutae* when used alone and combined against *T. absoluta* under semi-field conditions in the greenhouse. Both the virus and the parasitoid have previously displayed potential against this pest when used separately in laboratory and semi-field experiments (Ben Tiba et al., 2019; Calvo et al., 2016; Campos et al., 2020). We found that using both agents in combination resulted in the lowest numbers of pest larvae and the least crop damage cumulated over the entire experiment, yet the additive reduction compared to the use of each agent alone was statistically non-significant. The virus-only treatment, followed by the combined biocontrol agents, resulted in the strongest reduction of adult density recorded on the yellow traps at the end of the experiment (corresponding to the third generation of *T. absoluta* adults).

Both biocontrol agents, alone and combined, significantly reduced crop damage compared to the control. Larval density in the presence of *N. tutae* displayed a cumulative reduction of 14 % compared to the control, yet this effect was non-significant. It must be noted that the number of larvae recorded in the control on day 44, at the larval peak of the second generation, may have been unrepresentative of the number of larvae present in the cages (Fig. 1). The percentage of crop damage measured that day was nearly 100 %, and the larvae may have already left the leaflets sampled during the monitoring to seek food elsewhere in the cages. In addition, larval numbers displayed high variance within treatments. According to Cocco et al. (2015b), counting mines on the median proportion of the tomato canopy is best suited to provide
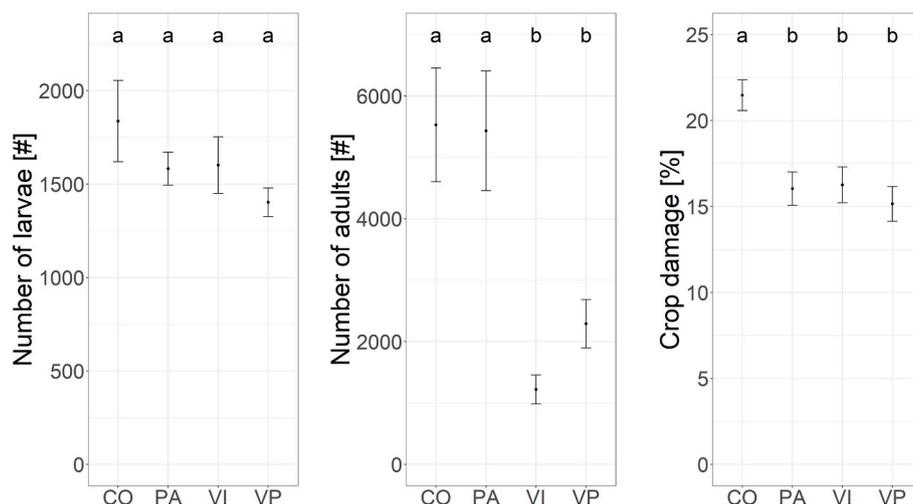

Fig. 2. Cumulated number of healthy *Tuta absoluta* larvae (left), total number of adult *T. absoluta* (middle), and average percentage of crop damage (leaf area mined by the larvae) (right) monitored over a three-month semi-field experiment comparing different experimental treatments (CO = control without treatment, PA = parasitoid (*Necremnus tutae*), VI = virus (PhopGV), VP = virus + parasitoid). Larvae and crop damage were monitored weekly on tomato plants, and third-generation adults were caught on sticky yellow traps at the end of the trial. Error bars represent the standard error. Different lowercase letters indicate significant differences between the experimental treatments according to Tukey's HSD.
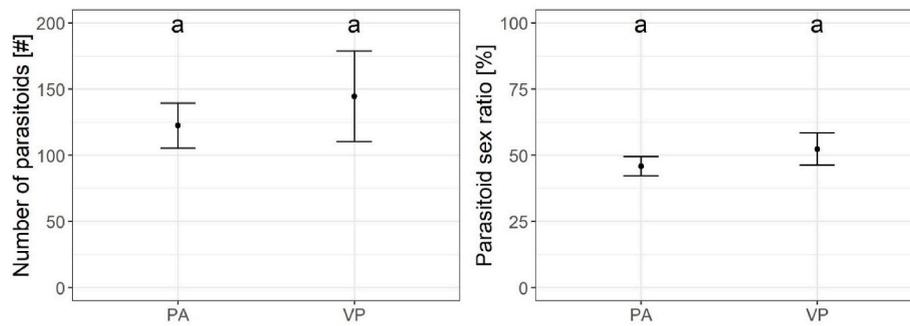
Fig. 3. *Necremnus tutae* adults (left) and mean percentage of *N. tutae* females (right) recorded on sticky yellow traps at the end of a three-month semi-field experiment including different experimental treatments (CO = control without treatment, PA = parasitoid (*N tutae*), VI = virus (PhopGV), VP = virus + parasitoid). Error bars represent the standard error. Different lowercase letters indicate significant differences between the experimental treatments according to Tukey's HSD (adults) and chi-square test (sex ratio).

reliable estimates of the total infestation. We monitored specifically this part of the plant; however, it is possible that high levels of infestation may have caused an altered distribution of larvae on the plant and thus masked differences that would be visible otherwise.

Two similar semi-field studies using the same parasitoid found a significant reduction in larval density by the end of the experiment (Calvo et al., 2016; Campos et al., 2020). As in these studies, we used a parasitoid-pest ratio of 1.2 but applied a different release sequence. Calvo et al. (2016) progressively released 48 adult *T. absoluta* per week over three consecutive weeks and 24 adult *N. tutae* per week over seven consecutive weeks starting two weeks after the first pest release. Campos et al. (2020) made two releases, each of 40 adult *T. absoluta,* with two weeks between releases. This was followed two weeks later by a release of 50 adult *N. tutae*. We released 50 adult *T. absoluta* on the first day, 30 adult *N. tutae* on day 16 and, again 30 adult *N. tutae* on day 41 (Table 1). According to Bodino et al. (2019), a single *N. tutae* individual is able to attack a maximum of 10 host larvae per day. Given the high adult density of the pest recorded in the parasitoid-only treatment at the end of the trial, it appears that *N. tutae* was outnumbered and, therefore, unable to keep *T. absoluta* under control. Releasing a sufficient number of parasitoids is crucial at the beginning of the pest infestation. Moreover, progressive and frequent parasitoid releases are recommended to ensure good establishment of *N. tutae* in a more realistic setup with continuous pest infestation.

While PhopGV doubled the natural mortality of *T. absoluta* larvae in the laboratory (Gonthier et al., 2023a), no significant effect of the baculovirus on pest larval density was visible during the eight weeks of the semi-field experiment. Weekly application of the high dose (0.015 %, $\geq 3 \times 10^9$ OB/l) is usually recommended for controlling *T. absoluta* (Andermatt Biocontrol, personal communication). In this trial, we assessed the efficacy of PhopGV sprayed at high and low doses (0.0015 %) to unveil the potential long-term effects of the virus. Even at low doses, baculoviruses can have a long-term impact on the population, possibly via covert infection (Burden et al., 2003) or sublethal infection, such as reduced feeding activity (Larem et al., 2019). The effect might be visible only later in the pest population or plant damage reduction. Following this, we found a strong reduction in crop damage in the virus treatments. Sublethally affected larvae might have produced fewer and smaller mines than healthy ones. Adult *T. absoluta* caught on the yellow sticky traps at the end of the trial represented the third pest generation. Larvae of this generation fed and developed on plants sprayed with the high dosage of PhopGV on days 45, 48, and 58 (Table 1). The inconsistent results between the larval and adult densities suggest that these larvae suffered no direct mortality from the virus, but rather failed to pupate later in their development. As a result, adult density was significantly reduced by 78 % and 59 % in the virus-only and virus-parasitoid treatments, respectively, highlighting the virus's potency when sprayed repetitively at high doses.

Our semi-field trial showed that combining both agents tended to result in the strongest pest and damage reduction cumulated over the entire experiment; however, compared to using both agents alone, the effect was insufficient to be significant. PhopGV showed its full potential in the third generation of pest adults after plants were sprayed at the recommended high dose. So far, few studies have addressed the combination of entomopathogenic viruses with parasitoids beyond the laboratory scale. In a greenhouse trial, damage to tomato plants by *Lacanobia oleracea* (Lepidoptera: Noctuidae) was reduced by the *Betabaculovirus lacoleraceae* (LaolGV) and the parasitoid *Meteorus gyrator* (Hymenoptera: Braconidae). Similar to our study, combining the two agents further reduced damage, but the difference was not significant (Matthews et al., 2004). When *Alphabaculovirus spexiguae* (SpexNPV) and *Microplitis pallidipes* (Hymenoptera: Braconidae) were used on cabbage grown in commercial greenhouses, the reduction in the *Spodoptera exigua* (Lepidoptera: Noctuidae) population was greater when using *M. pallidipes*-carrying SeNPV than with parasitoids without virus (Jiang et al., 2011). In this case, the parasitoid could transmit the virus, for example, when emerging from an infected host.

Timing and sequence are crucial parameters for successfully combining entomopathogens with parasitoids (Koller et al., 2023b). Parasitization after virus application was detrimental for *Euplectrus plathypenae* (Hymenoptera: Eulophidae), whereas infesting *S. exigua* with SpexNPV two days post parasitism had no significant effects on the parasitoid (Stoianova et al., 2012). Likewise, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) survival increased with a greater delay between parasitism and the infection with the *Alphabaculovirus spofrugiperdae* (SfMNPV) of the host (Escribano et al., 2000). Since the PhopGV becomes less effective with the increasing age of *T. absoluta* larvae (Larem et al., 2019) and *N. tutae* targets older larvae (Chailleux et al., 2014), both agents would best complement each other for pest control when the virus is applied several days before the parasitoid. Although in this scenario some indirect effects on the parasitoid can be expected due to reduced host quality from virus infection, laboratory assays have shown those effects to be of minor importance for *N. tutae* (Gonthier et al., 2023a). Previous research has shown that prior parasitism can reduce the pathogenicity of baculoviruses in hosts (Abbes et al., 2014; Cossentine, 2009). We are unsure whether this played a role in the limited positive effect of the combination in our study, and further investigation is needed to explore this aspect of the interaction. However, since the number and sex ratio of parasitoids found in the PhopGV-treated plots was similar to that in the plots without the virus at the end of the trial, we can confirm that infection with PhopGV after parasitization has no negative impact on the population of *N. tutae* (Gonthier et al., 2023a). Consequently, PhopGV should also be compatible with naturally occurring populations of *N. tutae*, which is a significant advantage for its use in conservation biocontrol due to the wide distribution of this parasitoid.

The semi-field setup of our study allowed for precise application, dosage, and release timing. Yet such a controlled environment cannot perfectly represent field conditions. Factors such as temperature, low aeration, and lack of shading screens can significantly impact plant growth and the biological cycle of the pest and the parasitoid. Additionally, environmental factors such as UV radiation, heat, and desiccation may increase the degradation of PhopGV, and inferior leaf coverage due to spraying larger surfaces may reduce the effectiveness of the virus, further affecting the interaction outcome. The infestation was set artificially high in our experiment and conducted in a single day. Such a high pest density may have led to an underestimation of the efficacy of *N. tutae* and PhopGV. Real-world populations of *T. absoluta* are more complex, with individuals immigrating and emigrating, aggregated spatial distribution, and multiple developmental stages present simultaneously (Cocco et al., 2015b). Under those conditions, where precise control may be challenging, combining the two biocontrol agents can offer greater flexibility in the timing and dosage of application. Predictive models show that biocontrol intervention timing and intensity are crucial for success and cost efficiency (Gonthier et al., 2023b). Further research should be conducted to better understand the effectiveness of biocontrol agent combinations in real-world settings and determine whether and how parasitoids can assist in spreading or accelerating the spread of the virus within the pest population (Jiang et al., 2011).

The difficulties linked with the mass rearing of *N. tutae* may challenge the widespread implementation of this strategy (Desneux et al., 2022). Combining PhopGV with the larval parasitoid *Dolichogenidea gelechiidivoris* (Marsh) (Hymenoptera: Braconidae), which is also compatible with PhopGV (Gonthier et al., 2023a), could be an interesting alternative. The parasitoid has the potential to reach an 86 % parasitism rate on *T. absoluta* and is easy to mass rear (Morales et al., 2013). Moreover, its combined use with the entomopathogenic *Metarhizium anisopliae* (Metschnikoff) (Hypocreales: Clavicipitaceae) in the laboratory resulted in additive control effects despite the reduction of parasitoid fitness by the fungus (Mama Sambo et al., 2022).

5. Conclusions

Our results demonstrate the compatibility and the potential of *N. tutae* and PhopGV to reduce population growth and crop damage of *T. absoluta* under semi-field conditions. *Necremnus tutae* significantly restrained population growth and crop damage cumulated over the entire experiment but appeared to be outnumbered at the end of the trial. Intensity and timing of parasitoid releases are crucial and must be adjusted precisely according to the time and level of pest infestation to ensure effective biological control under field conditions. PhopGV reduced crop damage to the same extent as *N. tutae* but strongly reduced adult density in the third pest generation, whereas the parasitoid did not. Under the conditions of our semi-field experiment, the combined use of PhopGV and *N. tutae* had no additive effect compared to each biocontrol agent used alone. However, the interaction outcome could be affected by altered population dynamics and environmental factors in real-world settings, as well as by reduced leaf coverage during application of the virus under field conditions. Further investigations are warranted to optimize the timing of application and dosage. The system should be studied under various environmental conditions to advance this research area further, fully understand its potential, and estimate possible benefits under real-world field or greenhouse conditions. Additionally, a cost-benefit assessment of this control strategy could provide valuable information for smallholders, organic growers, and large-scale productions. It would also be essential to study the seasonal and infestation level variations in the efficacy of this control strategy. In summary, while more research is needed to fully evaluate the potential of combining PhopGV and *N. tutae* for *T. absoluta* control, our findings provide interesting insight into an innovative combination of biological control agents.

CRediT authorship contribution statement

Janique Koller: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Jérémy Gonthier:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Lindsey Norgrove:** Supervision, Writing – review & editing. **Judit Arnó:** Methodology, Writing – review & editing. **Louis Sutter:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Jana Collatz:** Conceptualization, Methodology, Project administration, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data are stored in a public repository (https://doi.org/10.5061/dryad.cfxpnvx9x).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2024.106617.

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