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Plant Traits and Reproduction in a Multitrophic System: The Role of Aphids, Parasitoids and Hyperparasitoids

Mitchel E. Bourne^{1,2} | Enric Frago³ | Nina Xiaoning Zhang^{1,4} | Erik H. Poelman¹ | Maximilien A. C. Adam^{1,3}

¹Laboratory of Entomology, Wageningen University & Research, Wageningen, the Netherlands | ²National Centre for Vector Entomology, Institute of Parasitology, Vetsuisse and Medical Faculty, One Health Institute, University of Zürich, Zürich, Switzerland | ³CIRAD, Centre de Biologie pour la Gestion des Populations, Montpellier, France | ⁴Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence: Mitchel E. Bourne (mitchel.bourne@uzh.ch) | Maximilien A. C. Adam (maximilien.adam@cirad.fr)

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ABSTRACT

Hyperparasitoids, which prey on parasitoids developing inside herbivorous hosts, can disrupt top-down control of herbivores in multitrophic food chains. Hyperparasitism releases herbivores from their natural enemies, thereby potentially increasing plant damage and reducing plant performance. However, how hyperparasitoids impact plant fitness under field conditions has not been studied. In this study, we performed a field mesocosm experiment in which we manipulated the presence of aphids *Myzus persicae* Sulzer (Hemiptera: Aphididae), parasitoids *Aphidius colemani* Viereck (Hymenoptera: Braconidae), and hyperparasitoids *Alloxysta victrix* Westwood (previously reported as *Alloxysta fuscicornis* Hartig) (Hymenoptera: Figitidae) on wild mustard plants *Sinapis arvensis* L. (Brassicales: Brassicaceae) to quantify their effects on plant performance and fitness. Parasitoids effectively suppressed aphid populations, but this control was weakened in the presence of hyperparasitoids. In addition, aphid parasitism rates by parasitoids were lower in the presence of hyperparasitoids. However, none of the treatments significantly affected plant traits such as leaf number, flowering time, seed weight and survival. In the absence of natural enemies, aphids marginally reduced seed production compared to plants exposed to aphids, parasitoids and hyperparasitoids, but this effect was weak. Overall, our results suggest that while parasitoids exert strong top-down control on aphids, *S. arvensis* can tolerate aphid herbivory. Consequently, the net fitness benefit of indirect defence via parasitoid recruitment—and its disruption by hyperparasitoids—appears to be limited in this system, potentially due to the rapid life cycle and tolerance strategy of *S. arvensis*.

1 | Introduction

Plants are at the basis of most terrestrial food webs and mediate interactions between herbivores, their natural enemies, and higher-order consumers (Stam et al. 2014). These interactions are determined by a combination of bottom-up effects, regulated by plant traits, and top-down effects, regulated by higher-order consumers (Price et al. 1980; Poelman and Kessler 2016; Tan et al. 2020; Mertens et al. 2021). One of the main challenges for plants is to overcome attacks by insect herbivores, which reduce plant biomass, photosynthetic capacity and ultimately reproductive success (Agrawal 2005; Nabity et al. 2009; Züst

and Agrawal 2017). In response to insect herbivory, plants have evolved an arsenal of defence strategies that aim at maximising their reproductive output, often referred to as direct and indirect plant defences. Direct defences involve morphological traits such as trichomes or chemical compounds that reduce herbivore feeding (Gols 2014; Kant et al. 2015). Indirect defences function by recruiting and/or enhancing the effectiveness of natural enemies of herbivores (Kessler and Heil 2011; Pearse et al. 2020).

Natural enemies help to suppress herbivore populations and thereby reduce herbivore damage to plants. For example, parasitic wasps (parasitoids) lay their eggs inside or on the body of

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their herbivore host, eventually leading to host death when their offspring develops successfully (Godfray 1994; Vinson 1999). Therefore, parasitoids contribute to suppressing herbivore populations, reducing plant damage and potentially increasing plant fitness. However, the final plant fitness outcome of such tritrophic interactions is context-dependent and influenced by multiple ecological factors. For instance, a recent study showed that the effects of parasitoids on plant fitness may vary according to the level of plant competition (Cuny et al. 2025). Consumption of herbivores by natural enemies may even come at a short-term cost for the plant, for example when parasitism causes an increased food intake by herbivores, potentially leading to reduced plant fitness (Xi et al. 2015; Kaiser and Heimpel 2016; Cuny et al. 2021).

In addition, higher order consumers play an important role in shaping the interactions between herbivores and their natural enemies, consequently altering their impacts on plant performance. Parasitoids themselves are targeted by hyperparasitoids, which prey on juvenile stages of parasitoids developing within an herbivorous host (Sullivan and Völkl 1999; Poelman et al. 2022). Hyperparasitoids can also reduce the effectiveness of parasitoids if they trigger avoidance behaviours (Höller et al. 1994; Petersen et al. 2000), which can potentially lead to an increase in herbivore pressure and ultimately reduce plant fitness. For these reasons, hyperparasitoids are generally considered to be detrimental in agricultural settings, particularly in horticulture (Cusumano et al. 2020; Van Neerbos et al. 2024), but their impact in natural ecosystems or on open-field crop production is less clear. Hyperparasitoids may play an important role in the stability of parasitoid populations if they dampen oscillations in herbivore-parasitoid dynamics, thus preventing overexploitation of herbivores and potential collapses of parasitoid populations (Rosenheim 1998; Schooler et al. 2011; Frago 2016).

Trophic food webs comprising aphids and their parasitoids are a good model system to examine complex multitrophic interactions, with strong applied implications for the control of pest aphids, and at the same time providing novel insights for ecological theory (Sanders et al. 2018; Tougeron and Tena 2019). Aphids are among the most destructive pests on crops worldwide, causing damage by extracting valuable plant nutrients through feeding on phloem and the transmission of various plant pathogens. They reproduce rapidly through parthenogenesis, enabling exponential population growth within short time periods. In natural systems such outbreaks dynamics can have dramatic consequences for the fitness of host plants. In agricultural settings, it can quickly lead to severe infestations, causing significant damage and yield losses in crop plants (Guerrieri and Digilio 2008). In many crop production systems, aphid populations are controlled by specialised parasitoid wasps, which colonise croplands naturally, or that are commercialised as biological control agents and mass released (Boivin et al. 2012). The effectiveness of aphid parasitoids in suppressing aphid populations, however, can be challenged by hyperparasitoids. While in natural ecosystems such top-down effects can enable the stability of parasitoid populations as explained above, in croplands hyperparasitoids may cause a complete failure of biological control programmes (Tougeron and Tena 2019; de Boer et al. 2019).

To better understand the cascading effects of hyperparasitoids on plant fitness, field studies in natural insect communities are needed (Cuny et al. 2021). However, most research has only observed the natural occurrence of parasitoids in the field, without experimentally manipulating their presence (Poelman et al. 2022; Segoli et al. 2024). One of the earliest demonstrations of a trophic cascade involving hyperparasitoids comes from Goergen and Neuenschwander (1992), who showed that two hyperparasitoid species slightly reduced cassava plant dry biomass and survival in greenhouse conditions, whereas a single species had no effect.

This study investigated the consequences of parasitoid and hyperparasitoid presence on *Sinapis arvensis* (L., 1753) (Brassicales: Brassicaceae) growth and seed production. In a field mesocosm experiment, we manipulated the presence of insects from the second, third and fourth trophic levels to test the following hypotheses: (i) parasitoids reduce aphid density through parasitism, (ii) reduced aphid density enhances plant growth and fitness traits relative aphid-infested controls without parasitoids, and (iii) the presence of hyperparasitoids counteract this effect by reducing aphid parasitism and increasing aphid density, relative to mesocosms without hyperparasitoids.

2 | Material and Methods

2.1 | Plants and Insects

The focal plant of our study was the charlock mustard *S. arvensis* (L., 1753) (Brassicales: Brassicaceae), a fast-growing annual plant (Figure 1). This plant has been shown to experience fitness consequences under herbivory through reduced seed count, seed weight and seed viability (Gols et al. 2015; Mertens et al. 2024). Seeds to establish the experimental plant population were collected from a natural population near Wageningen, the Netherlands. Germination took place under controlled conditions in a greenhouse (22°C, 60%–70% RH, 16:8 h light:dark photoperiod). Seedlings were then transplanted in peat soil cubes and acclimatized to field conditions under a roofed shelter. Plants along with the peat soil cubes were transplanted to mesocosms (mesh-enclosed box tents of 4.0 m × 4.0 m × 2.5 m with a mesh size of 0.6 mm) when they were 2 weeks old.

The herbivore used was the green peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), a generalist aphid known to colonise a wide array of plants from many families, including Brassicaceae, and *S. arvensis* L. in particular (Mertens et al. 2021). Aphids were obtained from stock cultures maintained at the Laboratory of Entomology, Wageningen University, and reared on Brussels sprouts plants *Brassica oleracea* L. var. *gemmifera* cv. Cyrus (Brassicales: Brassicaceae). The primary parasitoid *Aphidius colemani* (Viereck, 1912) (Hymenoptera: Braconidae), a common biological control agent of *M. persicae*, was obtained as mummies and used as adults for the experiments (Aphipar, Koppert biological systems, The Netherlands). The hyperparasitoid *Alloxysta victrix* (Westwood, 1833) (previously reported as *Alloxysta fuscicornis* [Hartig, 1841]) (Hymenoptera: Figitidae)

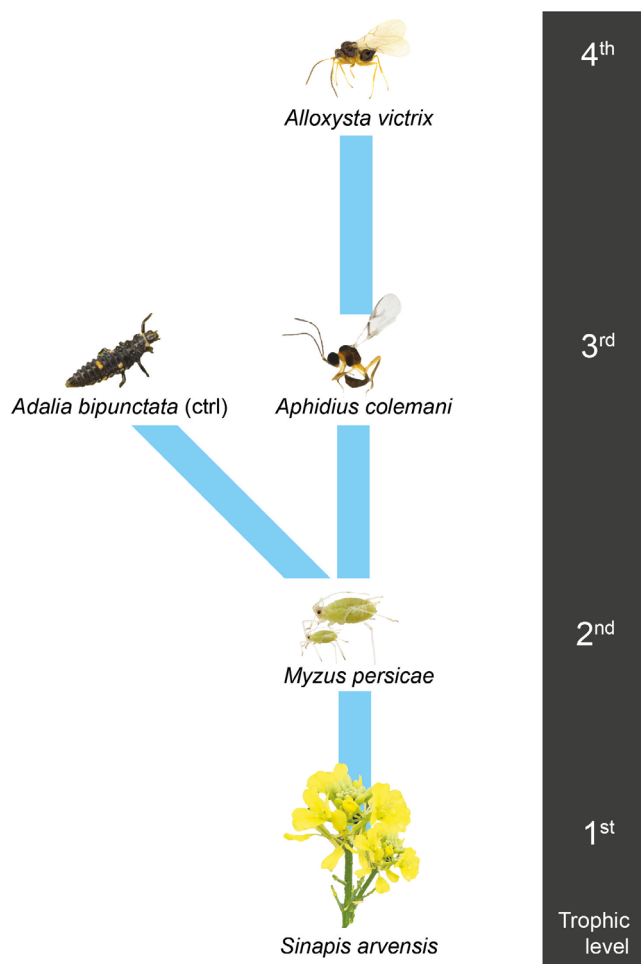


FIGURE 1 | Schematic representation of the multitrophic study system. The plant *Sinapis arvensis* (1st trophic level) is attacked by the aphid *Myzus persicae* (2nd trophic level), which is parasitized by the parasitoid *Aphidius colemani* (3rd trophic level). The parasitoid is in turn attacked by the hyperparasitoid *Alloxysta victrix* (4th trophic level). In control treatments, larvae of the aphid predator *Adalia bipunctata* were released to suppress aphid populations.

was originally collected in 2019 from agricultural fields near Wageningen. Rearing was carried out as follows: a Brussels sprout leaf infested with *M. persicae* aphids was exposed to adult *A. colemani* parasitoids for 2 days. The leaf was then transferred to a rearing cage containing adult *A. victrix* hyperparasitoids, maintained at room temperature under natural light conditions ($22^{\circ}\text{C} \pm 2^{\circ}\text{C}$, 35%–45% RH). Both parasitoids and hyperparasitoids were supplied weekly with a 1:10 honey-water solution and had continuous access to water. Parasitoids and hyperparasitoids were identified by morphology and/or molecular markers as described in Bourne et al. (2026). The hyperparasitoid was previously reported as *Alloxysta fuscicornis* (Hartig, 1841) (Bourne et al. 2026). However, *A. fuscicornis* has recently been synonymized with *Alloxysta consobrina* (Zetterstedt, 1840), which is now considered as *A. victrix* following recent taxonomic revisions (Ferrer-Suay et al. 2025). We therefore refer to this species here as *A. victrix*.

Adalia bipunctata (L., 1758) (Coleoptera: Coccinellidae) ladybird beetle larvae (Aphidalia, Koppert biological systems,

The Netherlands) were released in control mesocosms to prevent unwanted aphid infestations. To ensure pollination, pupae of the common green bottle fly, *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) (Natupol Fly, Koppert biological systems, The Netherlands) were introduced at the appearance of first flowers in mesocosms. For the same purpose, *Bombus terrestris* (L., 1758) (Hymenoptera: Apidae) Bumblebee hives (Natupol Seeds, Koppert biological systems, The Netherlands) consisting of 15 workers, five males, and male brood were also introduced in each mesocosm at the start of Week 3. Throughout the experimental period, plants were provided with a surplus of pollinators to prevent variation in plant reproductive success due to differential pollinator visitation.

2.2 | Experimental Design

To assess the effects of aphids, parasitoids and hyperparasitoids on plant traits under natural conditions, we set up a mesocosm experiment at the experimental field station of Wageningen University. Two-week-old *S. arvensis* seedlings were transplanted into mesocosms that allow for natural environmental conditions while preventing colonisation from other herbivores or natural enemies, and at the same time allowing the augmentative release of natural enemies. Each mesocosm contained 16 *S. arvensis* plants, arranged in a 4×4 grid with a 0.5 m spacing between plants. After transplanting, all plants were watered and left to acclimate to mesocosm conditions for 7 days, after which they were subjected to the different treatments.

The study was conducted by setting up the four following treatments randomly distributed over the twenty available mesocosms, each replicated five times (Figure S1):

- i. Control treatment: No aphids or parasitoids were introduced in these mesocosms. Ladybird beetles were added 3 weeks after plant transfer to prevent aphid colonisation.
- ii. Aphid-only treatment: Seven days after transplanting the plants to the field, each plant in the mesocosm was infested with 10 adult *M. persicae* aphids. If the count of aphids on a plant fell below 10 in the next 2 weeks, the infestation of the plant was ensured by adding adult aphids up to a total of 10.
- iii. Aphid-parasitoid treatment: plants were infested with aphids following the same protocol of the previous treatment. In addition, 100 *A. colemani* mummies (from which adult parasitoids emerged in between 24 and 48 h after) were added to the centre of the mesocosm at 11 days after aphid infestation. This was repeated 3 days later. Such dynamics, with locally high parasitoid densities, are common in aphid–natural enemy systems, where both aphids and their parasitoids develop in dense but ephemeral patches (Dixon 1977).
- iv. Aphid-parasitoid-hyperparasitoid treatment: this treatment followed the same aphid and parasitoid introduction schedule as the previous mesocosm treatment. In addition,

100 *A. viciatrix* hyperparasitoid adults were released in the centre of the mesocosm at 17 days after aphid infestation.

To evaluate the effects of treatments on plant traits and fitness, plants were monitored for 5 weeks after the first aphid infestation. The numbers of aphids and mummies per plant were recorded once a week. The total number of leaves per plant was measured during the third week, as plants began to lose leaves due to senescence in the fourth week. The number of days between aphid infestation and the onset of flowering for each plant was also recorded. Plants were harvested at the onset of senescence but before plant fruiting bodies (known as siliques) began releasing seeds (starting approximately 8 weeks after the first infestation). As proxies for plant fitness, the total number of seeds per plant and the total weight were recorded, allowing calculation of the average seed weight. The number of dead plants was also recorded to measure survival. This experiment was carried out from May to August 2021.

2.3 | Statistical Analysis

Statistical analyses were performed in R version 4.4.1 (R Core Team 2024) using generalised linear mixed models built with the R package 'glmmTMB' (Brooks et al. 2017). Residuals were visually assessed for normality and homoscedasticity using diagnostic plots generated with the DHARMA package in R. When a model yielded a significant *p*-value, pairwise post hoc comparisons were performed using Tukey's honest significant difference (HSD) test.

The number of aphids and the parasitism rate over weeks were analysed using models that included treatment as a fixed categorical factor along with its interaction with week. Week squared was included as a covariate to control for systematic nonlinear trends over time. We also included a correlation structure with the ar1 argument to account for the temporal autocorrelation among repeated measures within mesocosms and plants. An observation-level random factor was included to account for overdispersion. To account for repeated measures within plants and mesocosms, we built a random intercept model by including plants nested within mesocosm identity as a random factor. A Poisson and a binomial distribution were used for the number of aphids and the parasitism rate, respectively.

The number of leaves, number of days until flowering, number of seeds and the weight per seed were analysed using models with the insect treatment as fixed factor and the number of aphids and mummies counted during the previous week as covariates. An observation-level random factor was included to correct for overdispersion. As in the previous models a random intercept model was built by including mesocosm identity as random factors to account for repeated measures within the same mesocosm. A Poisson distribution was used for the number of leaves, days and seeds, and for seed weight a Gamma distribution.

Plant survival (alive or dead at the end of the experiment) was analysed using a generalised linear mixed-effects model with a binomial distribution and logit link. Treatment was included as a fixed factor, while mesocosm identity and observation-level

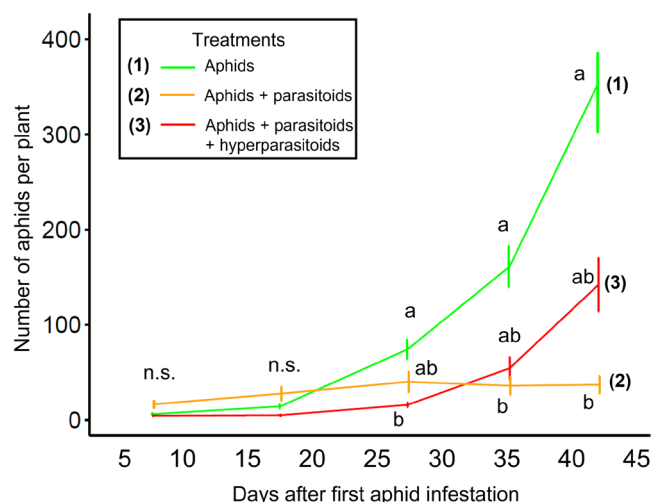


FIGURE 2 | Mean aphid count per *Sinapis arvensis* plant over 43 days after aphid infestation in mesocosms with the following insect treatments: (1) aphids only (*Myzus persicae*), (2) aphids and parasitoids (*Aphidius colemani*) and (3) aphids, parasitoids, and hyperparasitoids (*Alloxysta viciatrix*). Parasitoids and hyperparasitoids were released 11 days and 17 days after aphid infestation, respectively. Different letters indicate significant differences in aphid numbers among treatments within the same measurement ($p < 0.05$), while 'n.s.' indicates no significant differences. Error bars represent standard errors.

factor were included as random effects to account for repeated measures and overdispersion.

3 | Results

The presence of parasitoids and hyperparasitoids significantly affected the global dynamics of aphid populations ($\chi^2 = 6.3211$; $p = 0.042$; Figure 2). The presence of parasitoids significantly reduced the number of aphids, while hyperparasitoids mitigated this effect. We found a significant interaction with the time of measurement ($\chi^2 = 15.308$; $p < 0.001$), as this result was mainly driven by the last three measurements of the field season ($p = 0.043$, 0.009 and 0.014 ; Table S1).

Aphid parasitism was negatively affected by the presence of hyperparasitoids when this data was analysed as the whole dynamics ($\chi^2 = 5.053$; $p = 0.025$; Figure 3), or independently at weekly counts (Table S2), with a significant interaction between treatment and the time of measurement ($\chi^2 = 5.806$; $p = 0.016$).

Plants exposed to different insect treatments did not differ significantly in either the total number of leaves produced ($\chi^2 = 3.121$; $p = 0.373$; Figure 4A) or their flowering time ($\chi^2 = 1.247$; $p = 0.741$; Figure 4B).

The presence of different insects had no significant effect on mean seed weight ($\chi^2 = 2.913$; $p = 0.405$; Figure 5A) or plant survival ($\chi^2 = 5.717$; $p = 0.126$). Plants exposed to aphids, parasitoids and hyperparasitoids produced marginally more seeds than plants with only aphids, with treatments with no aphids or with aphids and parasitoids producing intermediate values of seeds ($\chi^2 = 8.721$; $p = 0.0332$; Figure 5B).

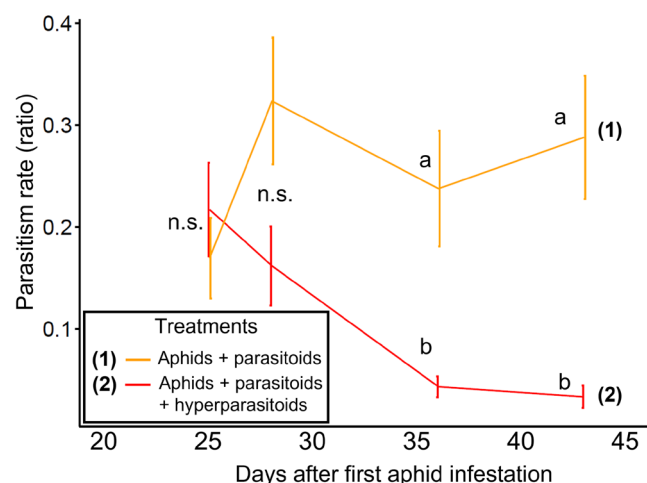


FIGURE 3 | Mean parasitism rates of *Myzus persicae* aphids by the parasitoid *Aphidius colemani* per *Sinapis arvensis* plant in mesocosms without (1) or with (2) *Alloxysta victrix* hyperparasitoids. The first parasitism rate estimation was conducted 25 days after aphid infestation, 14 days after parasitoid release and 7 days after hyperparasitoid release, calculated as the number of mummified aphids divided by the total number of aphids observed on each plant. Different letters indicate significant differences in parasitism rates between treatments within the same measurement ($p < 0.05$), while 'n.s.' indicates no significant differences. Error bars represent standard errors.

4 | Discussion

Parasitoids significantly reduced aphid populations 28 days after aphid infestation, compared to treatments with only aphids, demonstrating their effectiveness in controlling aphids. However, when both parasitoids and hyperparasitoids were present, aphid population size was intermediate between uncontrolled and parasitoid-controlled populations, suggesting that, in agreement with our hypothesis, hyperparasitoids mitigated aphid suppression by parasitoids. Accordingly, the percentage of parasitized aphids was significantly lower in the presence of hyperparasitoids. Despite these marked effects, aphids, parasitoids, and hyperparasitoids had limited effects on plant traits. The presence of insects did not significantly influence the number of leaves, flowering time, seed weight and plant survival. Aphids marginally reduced the total number of seeds produced per plant compared to treatments with aphids, parasitoids, and hyperparasitoids, but no significant differences were found when compared to plants without insects or those with only aphids and parasitoids. Our findings suggest that *S. arvensis* can compensate for aphid feeding in terms of leaf and seed production, and that the presence of parasitoids and hyperparasitoids has only a minor impact on *S. arvensis* fitness. While parasitoids effectively reduced aphid populations and hyperparasitoids mitigated this effect, the resulting top-down trophic cascade from hyperparasitoids to aphids did not extend to the plant level.

We experimentally manipulated the presence or absence of the fourth trophic level under semi-natural field conditions, a context rarely explored despite its ecological relevance (Sanders et al. 2015, 2018; Poelman et al. 2022). We provide experimental evidence that the introduction of hyperparasitoids reduces aphid parasitism rates and weakens top-down control (Boivin et al. 2012). This

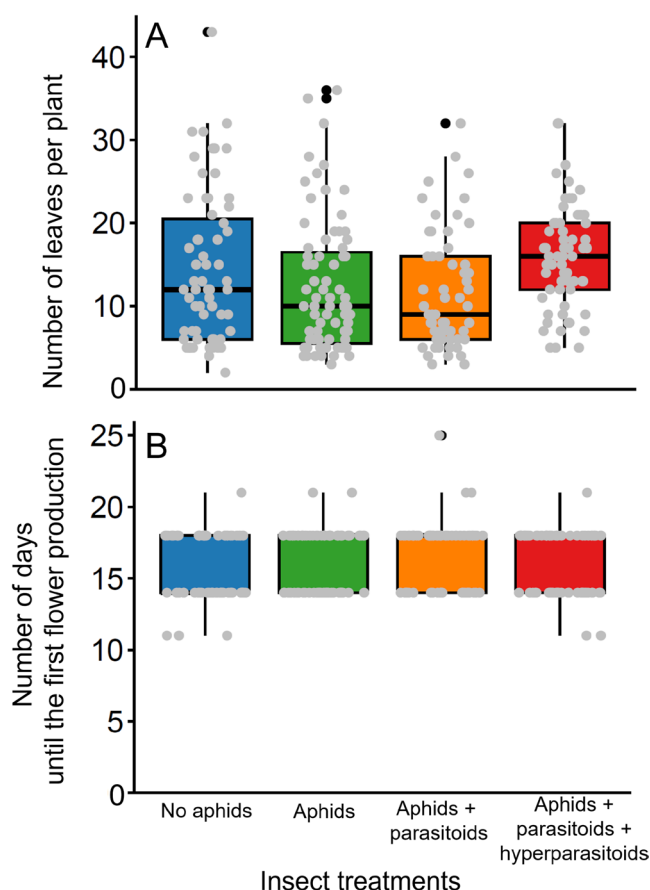


FIGURE 4 | (A) Total number of leaves produced per plant counted during the third week after aphid infestation and (B) number of days between aphid infestation and the production of the first flower per *Sinapis arvensis* plant (most flowers were produced during two observation time points). No significant statistical differences among treatments were found ($p > 0.05$). Jittered grey circles represent the raw data (each circle corresponds to one plant), and black circles are outliers (not excluded from the analyses). The middle line within each boxplot shows the median value of the data, while the box itself covers the range between the first and third quartiles. The whiskers extend from the box to the minimum and maximum values within 1.5 times the interquartile range.

strengthens findings by Goergen and Neuenschwander (1992) who used cages in a greenhouse to test the effect of two hyperparasitoids (*Prochiloneurus insolitus* [Alam, 1961] and *Chartocerus hyalipennis* [Hyat, 1970]) on cassava plants attacked by the cassava mealybug *Phenacoccus manihoti* (Matile-Ferrero, 1977), in the presence of the parasitoid *Epidinocarsis* (= *Apoanagyrus*) *lopezi* (De Santis, 1964). They found that the presence of one hyperparasitoid species did not affect the parasitoid's ability to reduce the negative effects of the cassava mealybug on plants, and the presence of two hyperparasitoid species only had a mild negative impact on plant biomass.

Based on previous literature, hyperparasitoids may have two contrasting top-down effects on plant fitness: (1) they could prevent parasitoids from overexploiting their host and going extinct, thereby maintaining herbivore control and increasing plant fitness, or (2) they could reduce parasitoid pressure on herbivores, leading to herbivore outbreaks and decreased plant

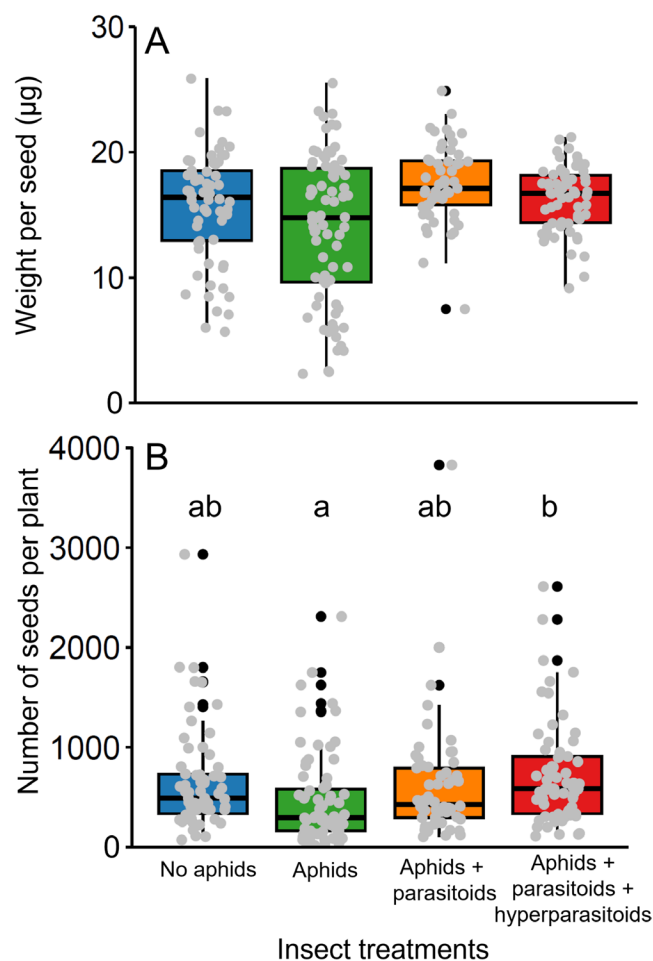


FIGURE 5 | Reproductive output measured as (A) mean seed weight and (B) total number of seeds per *Sinapis arvensis* plant. Different letters above the boxplots indicate significant statistical differences among treatments ($p < 0.05$) according to Tukey's HSD tests. Jittered grey circles represent the raw data (each circle corresponds to one plant), and black circles are outliers (not excluded from the analyses). The middle line within each boxplot shows the median value of the data, while the box itself covers the range between the first and third quartiles. The whiskers extend from the box to the minimum and maximum values within 1.5 times the interquartile range.

fitness (Poelman et al. 2022). Our results suggest a third outcome: despite reducing parasitism, hyperparasitoids did not reduce plant fitness, likely because plants tolerated herbivory. Our results indicate that aphid infestation does not affect seed production, seed weight, flowering time, or plant survival compared to uninfested control plants. This suggests that *S. arvensis* tolerates aphid feeding, at least under the aphid densities they were subjected to in our experiment. Tolerance is defined as a plant defence strategy where physiological compensation, rather than insect suppression or preventing an attack, allows plants to maintain their fitness (Koch et al. 2016; Fernández De Bobadilla et al. 2022). The tolerance to insect herbivory is especially well-known in response against Hemipterans (Koch et al. 2016).

Previous studies highlighted that seed traits of *S. arvensis* are only mildly affected by herbivory by chewing herbivores, with a limited effect of herbivore density (Gols 2025). The fitness costs are largest when *S. arvensis* plants are infested with chewing

herbivores before- or at the onset of flowering (Gols et al. 2015; Gols 2025). In our experiment, aphids colonised the plants from the early rosette stage and remained present throughout the plant's entire lifespan. However, differences in aphid population sizes between treatments only emerged after the plants had flowered, indicating that the lack of effects on plant traits may be due to the fact that the population size of aphids was not large enough at early stages of plant ontogeny. Early-season herbivory by aphids is therefore likely to be easily buffered by plants to prevent any fitness cost. Supporting this, a recent field mesocosm experiment that similarly excluded the natural herbivore community showed that early-season infestations with three aphids per plant did not affect the seed output of *S. arvensis* or three other Brassicaceae species in most cases. This study reports a rare case in which aphid infestation even resulted in an increased seed set (Mertens et al. 2024). A similar effect on seed production was also found in a perennial plant species (Stam et al. 2019). Taken together, these findings suggest that plants with a short lifecycle like *S. arvensis* may be able to escape the negative effects of early herbivory, thereby reducing its dependence on natural enemies like parasitoids to maintain photosynthetic surface and ultimately seed production.

We observed a slight increase in seed production when *S. arvensis* was exposed to aphids, parasitoids and hyperparasitoids compared to plants with only aphids. This occurred despite the treatments having no statistical differences in the size of their aphid populations. We could speculate that the presence of hyperparasitoids modulated aphid behaviour through non-consumptive effects (Abram et al. 2019). As demonstrated in many systems, non-consumptive effects can trigger strong top-down trophic cascades in communities, both terrestrial and aquatic (Werner and Peacor 2003). The mechanisms of these effects are far from being well understood. They can imply the induction of dispersal or the alteration of feeding behaviour that do not reduce population size, as demonstrated, for example, in aphids (Höller et al. 1994; Petersen et al. 2000). Finding that hyperparasitoids reduced parasitism without reducing plant fitness suggests that the ecological costs of hyperparasitism to plants may be lower than expected in systems where herbivore damage is moderate or when plants are tolerant to herbivory. In addition, the relatively short lifespan of *S. arvensis* limited the time window during which parasitoid and hyperparasitoid populations could build up, interact and impact plant fitness. In longer-lived plant species, the extended interactions with herbivores and their natural enemies may allow stronger fitness effects to develop over time. For example, experiments with the longer-lived *Brassica nigra* highlighted more pronounced fitness effects upon early season herbivory with aphids or caterpillars than *S. arvensis* (Mertens et al. 2024).

We did not find differences in plant survival between treatments, consistent with previous studies (Goergen and Neuenschwander 1992). This result may reflect the high tolerance of *S. arvensis* to aphid infestation under the resource-rich conditions of our experiment. In natural conditions, plants typically grow under competition, which reduces resource availability and limits their ability to tolerate herbivory. A recent field experiment showed that parasitoids increased *B. nigra* plant fitness only under strong competition (Cuny et al. 2025). However, species-specific responses should be considered, as high herbivore density has a stronger negative effect on *B. nigra* fitness than on *S. arvensis* (Gols 2025).

The biotic and abiotic conditions within the mesocosms may have further contributed to the weak differences in plant traits. Microhabitat variation within mesocosms, differences in soils (and soil legacy), vegetation or even wind-flow and temperature, may also cause differences in plant or insect performance, potentially increasing the variation in our study and reducing the statistical power to detect differences. This is shown by the relatively high variation explained by the 'mesocosm' factor in the models. Whilst we included random effects to control for this variation, future studies could benefit from integrating more fine-scale monitoring within mesocosm parameters to quantify these dynamics. In this study, we only quantified seed output and weight; future studies could also measure seed germination or viability. These traits are important for the realised fitness of the plant and could reveal cross-generational costs of herbivory (Gols et al. 2015; Campbell et al. 2017; Gols 2025).

Our study supports recent calls for more studies under natural conditions to assess the full impact of natural enemies and adds to ongoing discussions questioning their benefit to plants when herbivores do not reduce plant fitness (Pearse et al. 2020; Cuny et al. 2021). Longer-term or multi-season studies where plant recruitment dynamics are measured are necessary to show how the impact of natural enemies and higher trophic levels persists over time and/or generations (Stam et al. 2019). Climate change, through rising temperatures and more frequent heat waves, is expected to alter interactions among herbivores, parasitoids, and hyperparasitoids, further complicating the interpretation of our results in natural ecosystems and for biological control efforts (Tougeron and Tena 2019; Segoli et al. 2024). Given the sensitivity of these multitrophic interactions to both climate and habitat changes, it is important to study hyperparasitoid management in both greenhouse and open-field cropping systems to maintain the effectiveness of biological control. Previous studies have shown that hyperparasitoids can hamper classical biological control of aphids by parasitoids. In addition, native hyperparasitoids may expand their host range to include introduced biocontrol agents, thereby interfering with aphid suppression (Broadley et al. 2018). This risk is especially pronounced in closed greenhouse systems, where hyperparasitoids are particularly efficient at reducing parasitoid populations (Cusumano et al. 2020).

In summary, while parasitoids exerted strong top-down control on aphid populations and hyperparasitoids disrupted this control, both had limited effects on plant traits and fitness. We hypothesise that *S. arvensis* can tolerate or escape aphid infestations due to its rapid lifecycle. Future studies using longer-lived plant species will be essential to identify top-down fitness effects mediated by higher-order consumers, as longer interactions may allow such effects to accumulate over time.

Author Contributions

Enric Frago: writing – review and editing, formal analysis. **Erik H. Poelman:** conceptualization, funding acquisition, writing – review and editing, methodology, project administration, supervision. **Nina Xiaoning Zhang:** writing – review and editing. **Mitchel E. Bourne:** conceptualization, investigation, writing – original draft, methodology, writing – review and editing, supervision, visualization, data curation, project administration. **Maximilien A. C. Adam:** conceptualization,

investigation, writing – original draft, writing – review and editing, visualization, methodology, formal analysis, data curation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in figshare at <https://doi.org/10.6084/m9.figshare.30128269>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Visual representation of the experimental design, illustrating the layout of the field mesocosms (i.e., tents) and the assigned treatments. Mesocosms ($4.0 \times 4.0 \times 2.5$ m) are colour coded per treatment ($n = 5$ mesocosms per treatment). Each mesocosm contained 16 *Sinapis arvensis* plants. The experiment was carried out at an experimental field with the coordinates: $51^{\circ}59'24.6''$ N $5^{\circ}39'48.9''$ E. **Table S1:** Summary of statistical results from the general model analyzing aphid numbers across all measurements, as well as from separate models run for each individual time point (measurements 1–5). **Table S2:** Summary of statistical results from the general model analyzing parasitism rates across all measurements, as well as from separate models run for each individual time point (measurements 1–4).