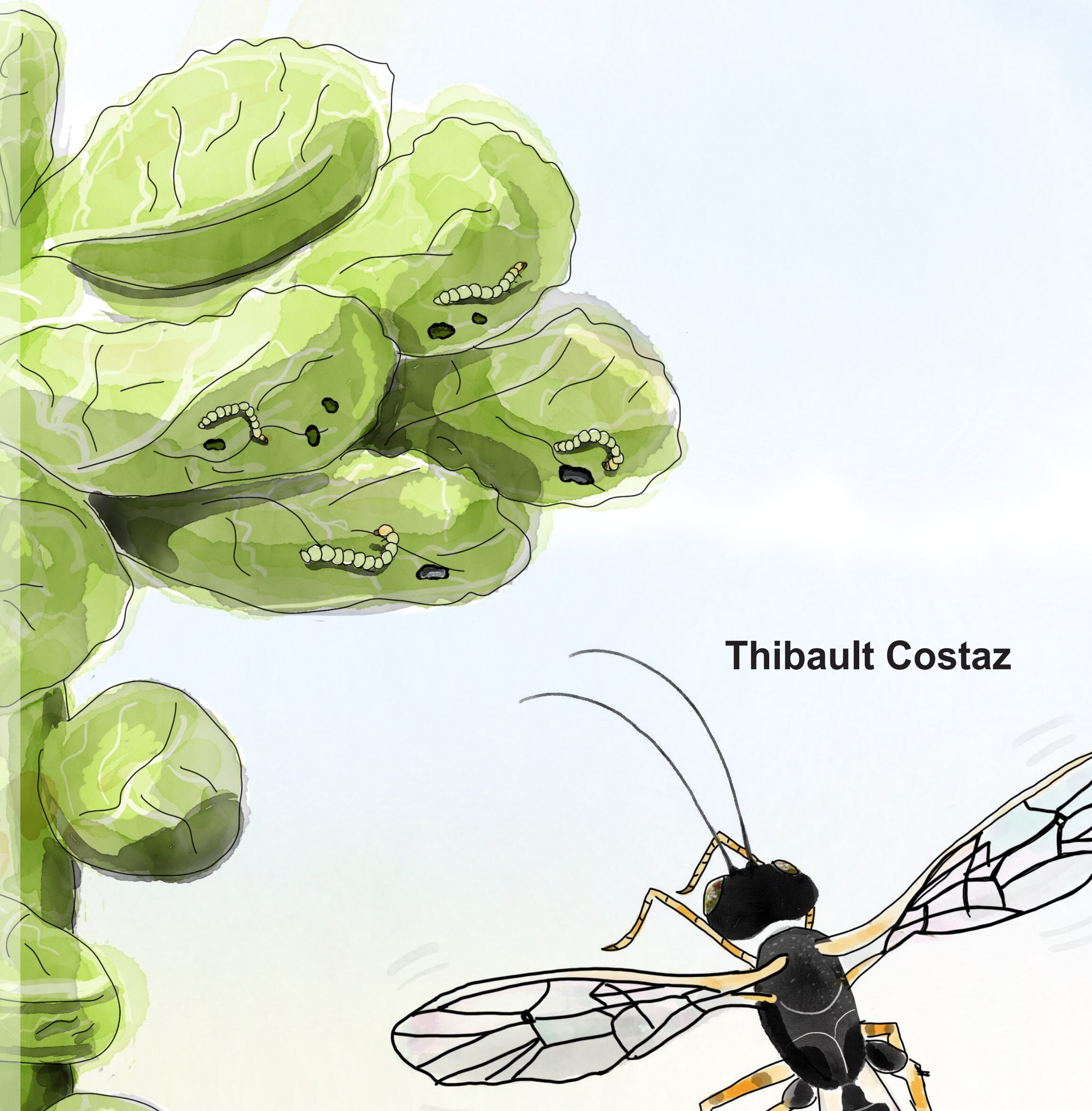
A changing world:

The role of phenotypic plasticity in host-parasitoid interactions facing extreme temperatures



Thibault Costaz

Propositions

1. Extreme temperature events have lasting consequences on host and parasitoid life-history traits.

   (this thesis)

2. Gaining comprehensive insight into plastic responses requires the inclusion of multiple phenotypic traits.

   (this thesis)

3. Effect size is more important than an arbitrary P-value threshold for the significance of scientific results.
4. Abiding by ethical guidelines is essential for all scientists to preserve the moral integrity of academic research.
5. Recognising biological differences between sexes without gender discrimination is essential to achieving equality.
6. Diversity is the only infinite source of wealth known to humanity.

Propositions accompanying the thesis entitled

A changing world: The role of phenotypic plasticity in host-parasitoid interactions facing extreme temperatures.

Thibault Costaz

Wageningen, 14th September 2022

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Thibault P. M. Costaz

Thesis committee

Promotors
Prof. Dr Joop J.A. van Loon
Personal chair at the Laboratory of Entomology
Wageningen University & Research

Prof. Dr Marcel Dicke
Professor of Entomology
Wageningen University & Research

Co-promotor
Dr Peter W. de Jong
Lecturer at the Laboratory of Entomology
Wageningen University & Research

Other members
Prof. Dr Jan Kammenga, Wageningen University & Research
Prof. Dr Jacintha Ellers, Vrije Universiteit Amsterdam
Prof. Dr Marcel Visser, Netherlands Institute for Ecology (NIOO-KNAW), Wageningen
Dr Jetske de Boer, Netherlands Institute for Ecology (NIOO-KNAW), Wageningen

This research was conducted under the auspices of the Graduate School Production Ecology and Resource Conservation

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Thibault P. M. Costaz

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 14 September 2022
at 4 p.m. in the Omnia Auditorium

Thibault P. M. Costaz
A changing world: The role of phenotypic plasticity in host-parasitoid interactions facing extreme temperatures
168 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2022)
With references, with summaries in English and French

ISBN: 978-94-6447-341-4
DOI: *https://doi.org/10.18174/574596*

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Chapter 1.

General introduction

Planet Earth's environmental variability has shaped the evolution of life and may have played a crucial role in forming its primary polymeric compounds (Mattia Bizzarri et al., 2021). The resulting diversity of life forms, physiology and behaviour is, and has always been, the focus of study for ecologists. Cyclical variation in environmental parameters is ubiquitous in natural ecosystems and mediates ecosystem functioning at many organisational levels. Environmental variation is also characterised by stochastic fluctuation, including extreme events such as heatwaves (le Lann et al., 2021). However, rare, extreme events play a non-negligible role in species evolution and ecosystem functioning (Grant et al., 2017; Harvey et al., 2020; Kingsolver and Buckley, 2017; Ma et al., 2015).

To cope with environmental change, organisms have three main options: moving to track suitable environmental conditions (e.g. migration), adapting to the new conditions via genetic evolution, or responding via phenotypic change (Liefting et al., 2009; Reusch & Wood, 2007). These three responses are not mutually exclusive and can concomitantly occur; however, they are subject to specific constraints (Franks et al., 2014). For example, tracking suitable conditions ultimately depends on the capacity of individuals to move across landscapes to find the appropriate set of conditions. Moreover, tracking suitable conditions may rely on specific resources such as plant or animal species (Davis et al., 1998a; Harvey and Malcicka, 2015; Hódar et al., 2021). Adaptation through genetic evolution is dependent on the diversity of the initial gene pools, the rate of environmental change and genetic mutations (Franks et al., 2014; Loeschcke, 1987). At the same time, phenotypic plasticity is constrained by the capacity of the genotype to match its environment (Whitman and Agrawal, 2009).

Nowadays, the ongoing climate change poses unique challenges to natural and agricultural ecosystems, threatening the many ecological services they provide. Climate change alters many en-

Environmental parameters among which temperature and precipitation have been the most stud-ied, leading to an increase in extreme climatic events such as heatwaves, droughts, and floods (Pachauri et al., 2014; Ummenhofer & Meehl, 2017; Vasseur et al., 2014). The speed of these changes is unprecedented in Earth's history, and combined with other anthropogenic stresses, it is thought that most species will be unable to adapt via genetic evolution (Parmesan, 2006). Although evidence exists for evolutionary responses to climate change, phenotypic plasticity is a common response to fast-changing environmental conditions (Hance et al., 2007; Liefting et al., 2009). Understanding the fundamental effects of extreme events on ecosystems and the services they bring to human civilisation is essential to provide the necessary knowledge for conservation and climate-smart farming practices.

Temperature is a well-studied environmental parameter which influences all levels of biological organisation. It especially plays a crucial role for ectothermic organisms such as insects, which cannot regulate their body temperature (Angilletta, 2009; Huey & Berrigan, 2001; Kingsolver & Huey, 2008). Body temperature influences, via various biochemical reactions, the physiological processes of ectothermic species (Brown et al., 2004). In the context of climate change, the ef-fects of a rise in average temperature on ecosystem functioning have been the most documented (Costello et al., 2009). However, insects are exposed to a range of instantaneous ambient tem-peratures. These fluctuating thermal conditions have effects that differ from those exerted by average temperatures caused by the non-linearity in the thermal responses of biochemical rates (Brown et al., 2004; Denny, 2017; Ruel and Ayres, 1999). Moreover, individuals are assembled in species-rich communities characterised by trophic and non-trophic interactions, ultimately influencing the thermal responses of their interacting partners (Davis et al., 1998b).

The aim of this PhD thesis is to evaluate the plastic responses of host-parasitoid interactions to simulated high temperatures. High-thermal extremes are defined by temperature values above the 90*th* or 95*th* percentile of the temperature distribution (Pachauri et al., 2014; Ummen-hofer & Meehl, 2017). Thus, they vary depending on the meso-climatic conditions, convention-ally measured over a 30-year period. To simulate different experimental thermal conditions, I used the temperature records from the Netherlands taken during June-July-August, selecting a non-stressful temperature range (i.e., below the 95*th* percentile) as a reference treatment used in comparison with a heatwave-mimicking treatment derived from literature defining the average characteristics of heatwaves occurring in Europe (de Boeck et al., 2010).

Study system

As a model system, I used a worldwide distributed and economically important pest species, the diamondback moth (*Plutella xylostella* L. Lepidoptera: Plutellidae) and two of its main para-sitoid species *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* Haliday (Hymenoptera: Braconidae), that have been successfully introduced in various coun-tries as biological control agents (Talekar & Shelton, 1993). The diamondback moth is a spe-cialist species, feeding on brassicaceous crops; thus, my thesis includes the domesticated crop Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* cv. Cyrus) as the first trophic level of this system. This specific model system frames this thesis within the context of plant protection and biological control.

Plutella xylostella L. (Lepidoptera: Plutellidae)

The diamondback moth, *P. xylostella*, is a multivoltine micro-lepidopteran species which can reach up to 20 generations per year in tropical countries. Its lifecycle goes from egg, through four larval instars, a prepupal and pupal stage, before the adult moth ecloses. The first instar larva is characterised by a mining phase in which the neonate larvae create galleries within the leaf mesophyll, from which they will emerge during the second-instar phase feeding on the leaf tissue and creating small window-like damage patterns. The adults mate only once before the female starts laying single eggs distributed on suitable host plants of the Brassicaceae family (Talekar & Shelton, 1993). The adult moth locates suitable host plants through volatile and gustatory cues. Brassicaceous plants produce unique secondary compounds called glucosinolates, which are known to act as stimuli for oviposition and feeding of this specialist feeder (Hopkins et al., 2009; Moreira et al., 2016; Thompson and Bidart, 2017; Uefune et al., 2017). The diamondback moth's geographic origin, although not ascertained, may be located in the African or Mediterranean regions and it has colonised tropical and temperate areas worldwide, hence being capable of surviving in a diversity of climatic conditions (Furlong & Zalucki, 2017; Juric et al., 2017). This species can migrate over large distances, and regular outbreaks cause significant agricultural damage (Li et al., 2012; Wei et al., 2013). Moreover, the intensive and systematic use of insecticides has led to the development of resistance to various insecticides (Talekar & Shelton, 1993). The diamondback moth is attacked by a wide array of natural enemies, among which hymenopteran parasitoids contribute significantly to the regulation of its populations (Furlong et al., 2013; Li et al., 2016; Talekar & Shelton, 1993).

Parasitoids are a highly speciose and abundant group of insects, and their unique lifestyle with a parasitic immature phase and a free-living adult phase makes them ideal biological control agents (Godfray, 1994; Waage, 1982). Parasitoids as a group display a large diversity of life-history characteristics. Female wasps lay their eggs directly on (ectoparasitoids) or in (endoparasitoids) their hosts. Parasitoid larvae developing within their hosts can be solitary or gregarious until emergence as adults. An important classification of parasitoids is the idiobiont-koinobiont dichotomy. The former paralyses its hosts while the latter allows its host to continue feeding and growing after parasitism (Brodeur & Boivin, 2004; Godfray, 1994). Some species of koinobiont parasitoids are associated with symbiotic polyDNA viruses that they inject along with their eggs to alter the host physiology to benefit the parasitoid larvae (Dicke et al., 2020; Song et al., 2008).

Diadegma semiclausum Hellén (Hymenoptera: Ichneumonidae)

Diadegma semiclausum is a solitary, koinobiont endo-parasitoid of *P. xylostella*. It is able to successfully attack and develop in all four stages of *P. xylostella* larvae, although mining first instars suffer less from parasitism. Parasitised larvae display numerous changes in their physiology, and the parasitoid can control the growth of its hosts to maximise its fitness gain (Etebari et al., 2013; Huang et al., 2009b). The immature parasitoid exhibits four larval stages feeding on haemolymph and tissue within its host. Ultimately, the immature parasitoid consumes the entire host, except the head capsule, before spinning a white cocoon around the pupa (Huang et al., 2009a; Pourian et al., 2015). *Diadegma semiclausum* is commonly found in Palearctic climates and is a specialist parasitoid, only developing in *P. xylostella* larvae (Furlong et al., 2013).

It is successfully used as a biological control agent against the diamondback moth in many countries, and populations originating from the United Kingdom have been introduced in New Zealand, Australia, Malaysia, Taiwan, Indonesia, India, China, and Kenya, for example (Furlong et al., 2013; Talekar & Shelton, 1993).

Cotesia vestalis Haliday (Hymenoptera: Braconidae)

Cotesia vestalis is a solitary, koinobiont endoparasitoid of *P. xylostella*. Similar to *D. semiclausum*, it is able to successfully attack and develop in all four larval stages of *P. xylostella*, inducing numerous changes in its host's physiology (Bae & Kim, 2004; Barandoc et al., 2010; Song et al., 2008). The immature parasitoid exhibits three larval stages feeding almost exclusively on haemolymph and fat tissue within its host. The third instar exits the host to spin a yellow cocoon; the host will perish one or two days later (Yu et al., 2008). *Cotesia vestalis* is commonly found in Palearctic climates, although it is believed that this species has a larger distribution range than *D. semiclausum*. It was also successfully introduced in many countries and is now found in the European, African, and Oceanic regions (Furlong et al., 2013). It can develop on several species of lepidopteran hosts, thus displaying a larger host range than its counterpart *D. semiclausum* (Hiroyoshi et al., 2017).

Outline of the thesis

Chapter 2 reviews the available literature on the effects of high-extreme temperature on host-parasitoid interactions and their phenotypic responses. This review focuses on the temporal aspects of extreme temperature events. It emphasises individuals' physiological and behavioural responses to these events, including lagged effects occurring after the events themselves. Furthermore, these organismal responses are discussed and integrated within the context of species interactions and community dynamics.

Chapter 3 assesses the effects of prior heat exposure on the subsequent parasitism success of *D. semiclausum* foraging under three constant temperatures, 20°C, 25°C and 33°C. The prior heat exposure followed either the reference or the heatwave-like thermal regimes with both *P. xylostella* larvae and *D. semiclausum* adults exposed to fluctuating temperatures.

High-extreme temperature events can vary in magnitude and duration, differently impacting organisms. Moreover, the timing of these events relative to the individual's ontogenetic stage can have long-lasting effects on individual fitness and population dynamics. Chapter 4 investigates the fitness consequences of high-extreme temperature events occurring at different ontogenetic stages. *Plutella xylostella*, *D. semiclausum* and *C. vestalis* were exposed to the heatwave-like or the reference thermal treatments during their larval and adult stages, leading to four thermal scenarios. I recorded multiple life-history parameters and discuss the ecological aspects of thermal history on individual fitness.

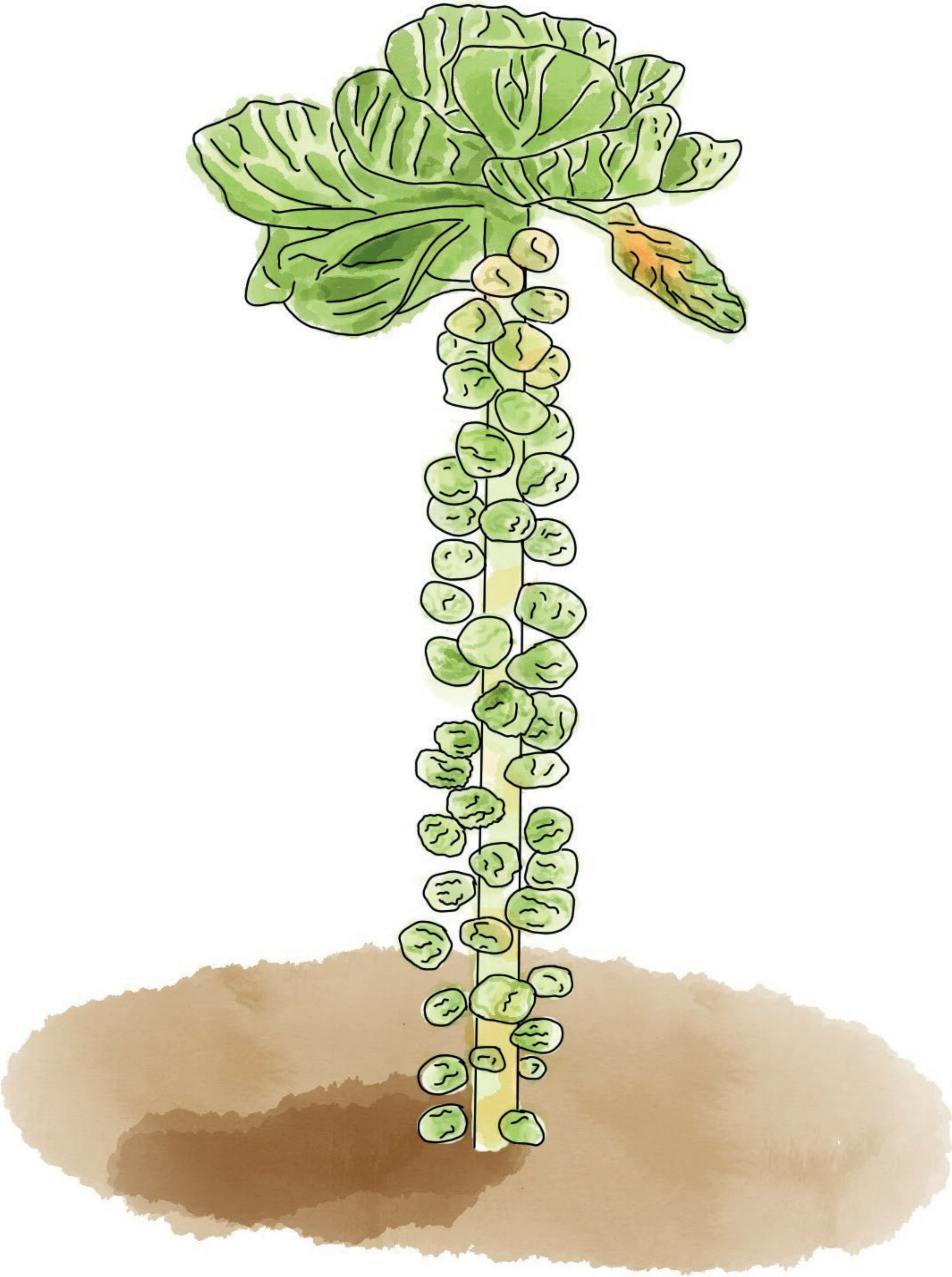
Chapter 5 addresses the effects of extrinsic competition between parasitoid species foraging under three constant temperatures: 22°C, 27°C and 33°C. This chapter aims to assess the distinct role of species composition in mediating the thermal response of a focal species.

Chapter 6 extends the work of chapter 5, and evaluates the interconnection between species at the insect community level. A manipulative field experiment was performed with *Brassica nigra* L. (Brassicaceae) infested with a herbivorous insect community varying in species and feeding-guild richness. We measured the abundance of a focal herbivore species, *P. xylostella*, its parasitism prevalence, the visiting pollinator community and plant fitness-related traits.

The impact of high-extreme temperature events at the community level are discussed in chapter 7. In this chapter, the results of this thesis are integrated and discussed in an ecological context given recently published studies. I highlight the current challenges of studying phenotypic plasticity within their relevant ecological context and promising future research avenues.

Acknowledgements

I thank my promotors, Prof. dr. Joop J.A. van Loon and Prof. dr. Marcel Dicke, and my co-promotor dr. Peter W. de Jong for their valuable comments on this chapter.



Chapter 2.

It is about time: Effects of thermal fluctuations resulting from climate change on host-parasitoid interactions

Thibault P. M. Costaz[1], Peter W. de Jong[1], Joop J. A. van Loon[1], Marcel Dicke[1]

Abstract

Climate change alters many environmental parameters, such as temperature or precipitation. The alterations of these environmental parameters have strong consequences for all levels of ecological interactions, from species interactions to community dynamics. Temperature is crucial in determining ecosystem dynamics, especially for ectothermic species such as plants or insects. Phenotypic plasticity, the capacity of one genotype to produce different phenotypes in response to environmental conditions is a common mechanism by which individuals adapt to changing environments and is observed in multiple traits. In response to temperature plasticity is thermodynamically constrained at the molecular level. The capacity of genotypes to adapt to novel environmental conditions plays a crucial role in structuring ecosystem dynamics and species persistence in adverse conditions. Many studies have assessed plant and insect phenotypic plasticity responses to changing thermal conditions, either alone or with their interactions. It is well recognised that temperature in natural ecosystems fluctuates over multiple time scales (e.g., hour, day, season, year). Moreover, these fluctuations can follow predictable or unpredictable patterns, which have different consequences for phenotypic plasticity and ecosystem dynamics. Understanding how and to what extent phenotypic plasticity can track continuously changing environments and its role in structuring species' ecological niches is of utmost importance in the context of rapid climate change. This review discusses the literature on the role of phenotypic plasticity in fluctuating environments, highlighting the role of temporal dynamics. We focus on host-parasitoid interactions because of their importance in driving herbivorous insect populations and their significant representation in ecosystems. Although we discuss literature on phenotypic plasticity at large, this review emphasises the fundamental effects of extreme temperatures in driving biochemical rates underlying phenotypic plasticity.

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[1] Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

Introduction

Environmental parameters such as temperature, precipitation, and photoperiod vary at different temporal scales (e.g., daily, seasonally), which have strong consequences for species interactions and dynamics of ecological communities (Froy et al., 2003; Park, 2019; Park & Wootton, 2021; Schmidt & Conde, 2006; Welch & Harwood, 2014). Ongoing climate change alters the temporal characteristics of e.g. seasonal rhythms, and the frequency of extreme events, thereby affecting all ecosystem processes (Boeck et al., 2018; Jentsch et al., 2009; Parmesan et al., 2000; Parmesan & Yohe, 2003). Temperature is one of the most prominent environmental parameters. It plays a crucial role in ecological processes, especially for ectothermic organisms such as insects, whose body temperatures are directly affected by ambient thermal conditions (Angilletta, 2009; Huey & Berrigan, 2001; Kingsolver & Huey, 2008). Temperature governs biochemical rates and, thus, physiological processes of ectothermic species (Brown et al., 2004).

Insects have evolved an extensive suite of physiological and behavioural adaptations to cope with thermal constraints (Abram et al., 2017; Angilletta, 2009; Angilletta et al., 2002). Thermal tolerance has a polygenic basis, and underlying mechanisms conferring cellular thermal tolerance range from enhanced stress response inducing the production of protective molecules such as Heat Shock Proteins (HSPs) to cellular tolerance reducing metabolic activity (Stanton-Geddes et al., 2016). The thermal responses at the cellular or gene transcriptional levels depend on the body temperature of a given individual. Although ambient thermal conditions mediate insect body temperature, individuals can modulate it using various thermoregulatory mechanisms (May, 1979). Behavioural thermoregulation relates to the behavioural strategies an individual uses to regulate its body temperature, and relies on the capacity to sense and select appropriate thermal environments (Abram et al., 2017). Moreover, in natural settings, the temperature fluctuates periodically (e.g., daily, seasonally, and yearly) and stochastically (e.g., extreme temperature events), influencing all levels of ecological organisation, from individuals to ecosystem functioning (Bond et al., 2021; le Lann et al., 2021; Park, 2019; Speights & Barton, 2019; Tituskin et al., 2021).

At the individual level, phenotypic plasticity represents the capacity of one genotype to express a suite of phenotypes when exposed to various environments (Agrawal, 2001; Whitman and Agrawal, 2009). Phenotypic plasticity can be subdivided into reversible and irreversible (or developmental) plasticity resulting from the past and present environmental conditions an individual has experienced throughout its ontogeny (Whitman and Agrawal, 2009). The change in phenotype expressed by one species in response to a change in thermal conditions will likely have fitness consequences and may lead to phenotypic changes in its interacting partners. In a fluctuating environment, phenotypic plasticity allows one genotype to track the fitness gradient related to environmental variables(Ghalambor et al., 2007). This creates complex reaction chains, lagged effects, and feedback loops mediating changes in population and community dynamics (Abram et al., 2017; Boukal et al., 2019; Kristensen et al., 2020).

Insect parasitoids and their hosts are an excellent study system to assess the role of phenotypic plasticity in response to fluctuating thermal regimes and high-temperature extremes. Parasitic wasps are a very diverse group of arthropods and contribute significantly to regulating other insect populations (Forbes et al., 2018; Jervis, 2005; Quicke, 2014). Moreover, they are often specialised

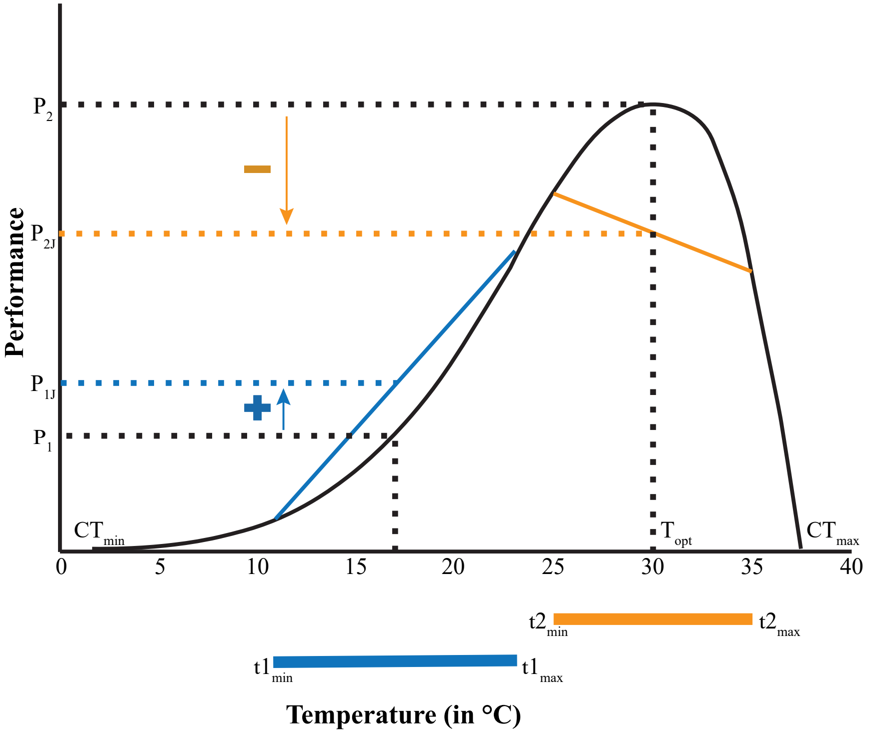


Figure 1: Fictive curve of thermal performance (solid black line) expressed as a function of temperature. The thermal performances for two hypothetical thermal ranges represented by the blue and orange solid lines are plotted on the y-axis. The dotted black lines represent the performance values taken at the average temperature of each thermal range and are denoted P1 and P2. The dotted blue and orange lines represent the thermal performances, P_{1J} and P_{2J}, calculated as averages from performances at the minimal and maximal temperature value of each thermal range $t1_{min}$ and $t1_{max}$ or $t2_{min}$ and $t2_{max}$. The + and–signs emphasise the Jensen's inequality's positive and negative effects, respectively, compared to performances at the mean temperatures.

in attacking one or a few host species, and even specific larval stages within these species. As larvae, parasitoids feed on their host (parasitic stage), which represents a limited source of nutrients; thus, their traits have tightly coevolved with those of their hosts (Godfray, 1994). Here, we review studies on phenotypic plasticity in thermally fluctuating environments, including the effect of high-temperature extremes, highlighting the role of temporal dynamics. We focus on host-parasitoid interactions because of the importance of parasitoids as a functional group and their role in the population dynamics of herbivorous insects (Hance et al., 2007, Jervis and Ferns, 2004, and references therein). First, the kinetic and behavioural responses to thermal fluctuation and extremes are presented at the level of individuals. Then we scale up from individual plastic responses to species interactions and discuss the consequences for population dynamics and community structure. We conclude this review by presenting promising frameworks to analyse and interpret complex multifactorial effects of thermal fluctuations and extremes on species' realised ecological niches.

Box 1: Thermal Performance Curves, Jensen's inequality, and insect thermotolerance

Temperature governs the rates of biochemical reactions and physiological processes. However, each species has a characteristic range of temperatures in which it can sustain biochemical reactions, thus defining its thermal tolerance (Angilletta, 2009; Brown et al., 2004). The effect of temperature on insect metabolism and, more widely, on phenotypic traits is depicted using reaction norms (Angilletta, 2009). The shape of thermal reaction norms varies from species to species and from trait to trait (Berger et al., 2008; Iltis et al., 2019; Romero-Mujalli et al., 2021). When considering the effects of temperature on the fitness of one phenotype, thermal reaction norms are represented as "Thermal Performance Curves" (TPCs)(Angilletta, 2009; Kingsolver et al., 2004; Lande, 2014). TPCs are composed of two critical temperatures (lower and upper thermal limits, delimiting the thermal breadth) between which a given trait exhibits rising values with increasing temperature, up to a maximum at the optimal temperature and declining values as the temperature increases towards the other end of the thermal breadth (Sinclair et al., 2016)(solid black line in Figure 1). TPCs are often constructed based on observations made at constant temperatures but can predict the mean trait value in a fluctuating thermal regime by applying Jensen's inequality (Denny, 2017; Jensen, 1906; Ruel & Ayres, 1999). Due to the non-linearity of the relationship, the mean trait value does not correspond to the trait value at the average temperature but to the average of the trait values at either end of the thermal variation (i.e. maximum and minimum temperature) (Denny, 2017) (depicted by the dotted and solid coloured lines in Figure 1).

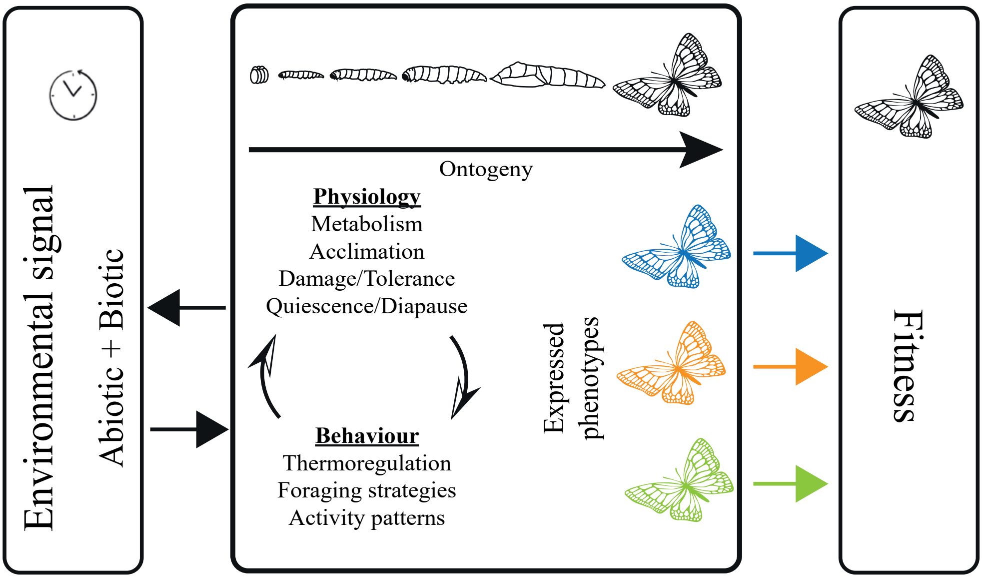


Figure 2: Overview of section 2.1 on the effects of timing of the environmental signal (e.g., temperatures) on an individual's phenotype and its resulting fitness (from left to right). As insect species are engaged in interaction networks with other species, a feedback loop was added from phenotype to biotic environment (see section 2.2).

Physiological and behavioural responses to fluctuating thermal regimes

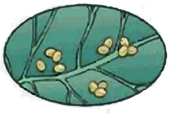
The fundamental role of metabolism in determining life-history traits and fitness at the individual level is central in assessing species distribution and abundance across climatic regimes (Brown et al., 2004). Thermal Performance Curves (TPCs) represent an excellent tool to depict species' thermal tolerance and have been used extensively to predict the impact of climate change on species (Schulte et al., 2011; Sinclair et al., 2016; von Schmalensee et al., 2021). For instance, the climate variability hypothesis (CVH), stating that species living at high latitudes display wider thermal tolerances than species at lower latitudes allowing them to withstand larger thermal fluctuations, was confirmed by Addo-Bediako et al. (2000), who reviewed the thermal tolerance of ectotherms across their latitudinal distribution. Although TPCs are useful in depicting the thermal responses of an individual, they suffer from several shortcomings (Dowd et al., 2015; Sinclair et al., 2016). An essential drawback of TPCs is the lack of temporal dynamics required to accurately measure lagged temperature effects on fitness-related traits throughout ontogeny (Kingsolver & Woods, 2016; Schulte et al., 2011).

Physiological effects of fluctuating thermal regimes

The physiological effects of temperature on insect metabolism are well described and widely used to predict the impact of climate change on the geographical distribution of species (Furlong & Zalucki, 2017; Macfadyen et al., 2018). However, the precision of these models depends on a correct assessment of the non-lethal effects of temperature on physiology as well as on the inclusion of species interactions and their repercussions on the realised ecological niches (Davis et al., 1998a, 1998b; Tylianakis and Binzer, 2014; Woodin et al., 2013). Because temperature fluctuates daily in a predictable way (e.g. day-night) as well as in an unpredictable stochastic way (e.g. extreme events), insects often have to face significant thermal variation during their life cycle. The type of thermal fluctuation encountered by an organism during its life cycle alters its phenotype and strongly influences its fitness outcome (Stoks et al., 2017) (Figure 2).

The thermal environment experienced by an individual during development affects the adult phenotype and thermal tolerance (often referred to as developmental plasticity). Developmental acclimation can lower the resting metabolic rates of individuals reared in warm conditions compared to those reared in milder conditions (Berrigan, 1997; le Lann et al., 2011; Moiroux et al., 2012). However, most of these studies have only tested developmental acclimation of metabolic rates under constant temperature. It can be expected that fluctuating thermal regimes will affect metabolic acclimation differently than stable thermal regimes. Indeed, the thermal tolerance of an individual differs whether it is measured by ramping or plunging assays (Bahar et al., 2013; Nguyen et al., 2014; Terblanche et al., 2011). Ramping assays are performed by gradually increasing temperature and assessing rapid heat hardening, while plunging assays are better suited to determining basal heat tolerance (Bahar et al., 2013; Manenti et al., 2018). Individuals experiencing fluctuating thermal regimes display higher survival and higher critical upper thermal limits than individuals exposed to constant thermal regimes.

Moreover, the thermal tolerance of an individual is not fixed in time but varies with ontogeny, thus modulating the effects of heat stress depending on its timing during the insect life cycle



(Cavieres et al., 2016; Potter et al., 2011; Zhang et al., 2015a). The mechanistic principles behind the variation in thermal tolerance through ontogeny are not entirely elucidated but expected to be linked with allometric scaling in body size. In terrestrial ecosystems, individuals with larger body sizes are usually more thermotolerant than smaller ones (Kingsolver and Buckley, 2020; Klockmann et al., 2017). Moreover, many insects exhibit modular life cycles, meaning that their development is characterised by distinct stages between which partial physiological restructuring occurs. Consequently, they can isolate, to some extent, the adverse effects of thermal stress from one stage to the next (Bowler & Terblanche, 2008; Potter et al., 2011). Interestingly, higher temperatures usually induce faster development and lead to smaller body sizes than achieved in cooler environments (Kingsolver & Huey, 2008). This phenomenon was extensively reviewed by Atkinson (1994) and described as the Temperature Size Rule. The reasons behind the production of smaller body sizes in warmer climatic conditions are thought to involve energetic trade-offs between growth rate, mortality and reproduction. The adaptive value of smaller body sizes lies in more effective thermal exchange (Atkinson, 1994).

Molecular tools such as omic techniques can give a complete phenotypic and genotypic picture of an individual at a given time in a given environment, and their use significantly improves the understanding of physiological effects of environmental variables on phenotypic plasticity (Hayward, 2014). Among the different physiological responses to temperature stress that were identified (recently reviewed in González-Tokman et al., (2020), HSPs have received most attention (Feder & Hofmann, 1999; Ju et al., 2011; King & MacRae, 2015; Tian et al., 2021). HSPs are molecular chaperone proteins protecting proteins and cells against the destabilising effect of high temperature, thus playing an important role in insect heat tolerance, rapid hardening, and developmental acclimation (Feder & Hofmann, 1999; Sørensen et al., 2003). The extent to which HSP gene expression is induced in response to heat shocks seems to be lower for individuals maintained in fluctuating thermal regimes than for individuals held in constant thermal regimes (Sørensen et al., 2003). The induction of HSP genes is dependent on the circadian rhythm. Thus, heat protection gained by inducing HSPs varies within the 24 h thermal cycle (de Alba et al., 2021; Manenti et al., 2018). Transcriptomic data of *Drosophila* species under constant or fluctuating thermal regimes showed that a large proportion of the transcriptome was affected by differences in constant temperature. Only a small portion was affected by fluctuating temperatures (Manenti et al., 2018; Sørensen et al., 2016). The large proportion of genes affected by differences in constant temperature probably results from the fundamental effects of temperature on physiological rates. Regarding the proportion of genes whose transcription was affected by fluctuating temperatures, *Drosophila simulans* Sturtevant gene ontology analysis showed that egg-chorion-related genes were downregulated, and those related to cellular heat tolerance were up-regulated (Manenti et al., 2018). These were driven by Turandot genes involved in heat tolerance but with a slower activation rate than HSP genes. Their slower activation rates could prevent maladaptive heat tolerance expression under fluctuating thermal regimes (Feder & Hofmann, 1999; Manenti et al., 2018; Sørensen et al., 2003).

Global warming leads to a faster increase of night temperatures compared to day temperatures. In naturally fluctuating environments, night-time warming is likely to draw species closer to their thermal optimum, thus having beneficial effects on their performance (Speights et al., 2017). On the other hand, night-time warming can reduce recovery time after acute heat exposure during the day (Zhao et al., 2014). Recent work assessing the effects of

alternating consecutive hot days and recovery days on the performance of the grain aphid (*Sitobion avenae* Fabricius) showed that recovery could occur when the insects were gradually exposed to milder thermal conditions (Ma et al., 2018). Additionally, time-specific behaviour and activity patterns could mediate the effect of day versus night warming, especially if we consider diurnal or nocturnal species (Speights et al., 2017; Whitney-Johnson et al., 2005). For example, some species can shift their foraging time to the night, while others may be constrained to diurnal foraging (Speights et al., 2017).

Focusing on seasonal patterns, yearly temperature variations tend to decrease, leading to shorter and milder winter seasons despite a higher probability of extreme weather events such as warm or cold spells (Vasseur et al., 2014; Wang and Dillon, 2014). These changes affect the seasonal ecology of insect parasitoids and their hosts by altering voltinism, diapause, quiescence, and winter survival. The adaptive value of diapause in a novel thermal environment is likely to be species-specific (Tougeron et al., 2020). For example, a decrease in winter severity and duration could lead to a reduction or loss of diapause in some species (Gariepy et al., 2015; Andrade et al., 2016; Tougeron et al., 2017;, 2018), an increase in extreme weather events can favour quiescence (Langer & Hance, 2000) or situations with some individuals undergoing diapause while others remaining active to spread the risk at the population level (le Lann et al., 2021). Overall, the effects of thermal changes on parasitoids are linked to their capacity to maintain synchrony with the seasonal ecology of their hosts (Tougeron et al., 2018, 2020).

Behavioural effects of fluctuating thermal regimes

Insects can sense their environment through visual, gustatory, olfactory, tactile, temperature and humidity cues. Thermoreceptors are present on various body parts depending on the insect species and life stage (e.g., on the antennae or wings). Thermoreceptors sense ambient temperature and transmit the information to the central nervous system that initiates integrated behavioural and physiological responses of the whole organism (reviewed in Abram et al., 2017; González-Tokman et al., 2020). This section will focus on the influence of thermal fluctuations on integrated behavioural responses (Figure 2, left and middle frames).

Behavioural responses to thermal changes range from alteration of diel activity patterns to the modification of foraging strategies. Disentangling behavioural from physiological responses to temperature variations is intrinsically difficult (Augustin et al., 2020). Temperature affects metabolism and cellular integrity, constraining the responses of organisms (Brown et al., 2004). Metabolic activity relates to fitness and, following a TPC curve, increases with temperature up to a maximum and drops as the temperature continues to increase (see Box 1)(Clarke & Fraser, 2004; Gillooly et al., 2001). These temperature-dependent responses on fitness are mediated by body temperatures. Nonetheless, other physiological tolerance-resistance mechanisms allows organisms to partially mitigate the effects of temperature fluctuations on their fitness (see section 2.1). For example, several studies have observed changes in walking speed and attack rates of parasitoids with changes in temperatures, likely driven by metabolic rates (Abram et al., 2017; le Lann et al., 2014a; Moiroux et al., 2016). However, insects can alter their behaviour and regulate body temperature by sensing their thermal environments (González-Tokman et al., 2020).

Behavioural thermoregulation is probably the best known mechanism by which insects regulate their body temperature (Kearney et al., 2009; Kleckova & Klecka, 2016; May, 1979; Nice & Fordyce, 2006; Turlure et al., 2011; Woods et al., 2015). Behavioural thermoregulation can take various forms, such as habitat selection, orientation to solar radiation (basking), temporal shift in activity patterns, or increased metabolism (e.g., flight) (Casey & Knapp, 1987; May, 1979). For example, the social caterpillar *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) optimised its thermal gains to promote growth via shifts between basking in a tight cluster along a thermal gradient and foraging time on the food source (McClure et al., 2011). Behavioural thermoregulation in warm environments allows individuals to avoid harmful temperatures or select optimal foraging locations (Kearney et al., 2009). However, it mainly relies on spatiotemporal thermal heterogeneity of the environment (Caillon et al., 2014; Pincebourde and Woods, 2020; Potter et al., 2013; Woods et al., 2015).

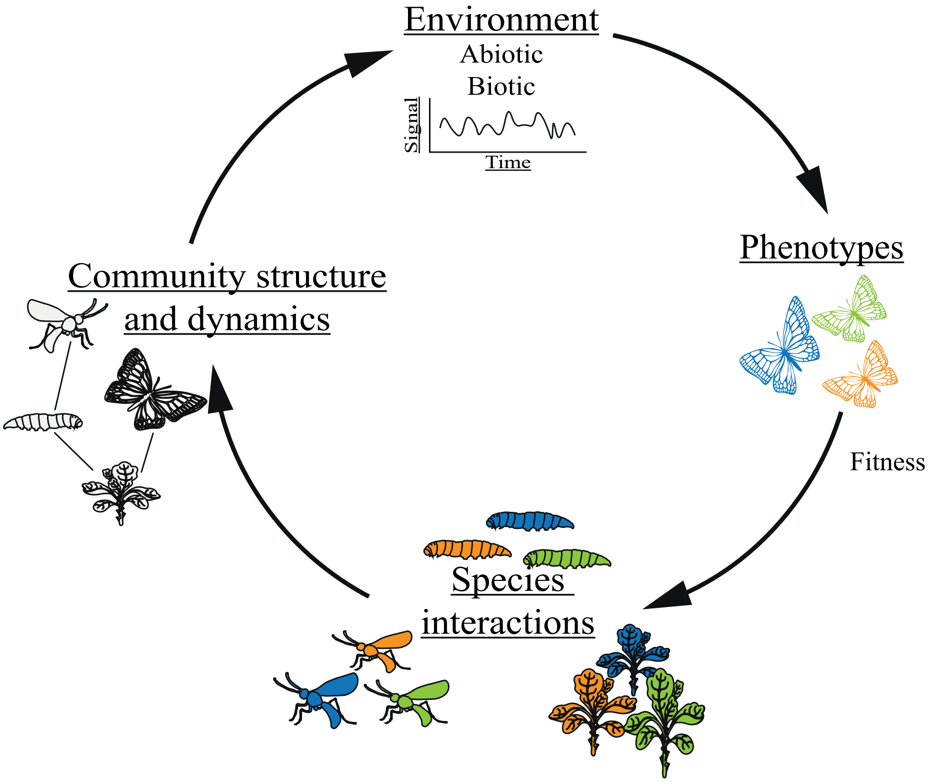
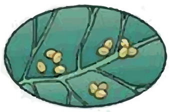


Figure 3: Overview of section 3 with the causal links between dynamic environmental signals and phenotype fitness and consequences for species interactions and community structure and dynamics.

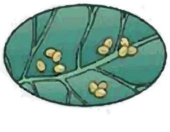
Microclimatic thermal heterogeneity is affected by complex interactions between biotic and abiotic factors (Pincebourde & Woods, 2012). Small insects experience temperatures of the leaf surface (Pincebourde & Woods, 2020). Although abiotic conditions drive leaf temperature, the plant, by regulating its transpiration, can have a cooler or warmer surface than the ambient air temperature (Cook & Dixon, 1964; Leuzinger & Körner, 2007; Pincebourde & Woods, 2012).



Field temperature measurements showed that the diversity in microclimatic conditions was correlated to the degree of landscape complexity, and aphid populations inhabiting these different microclimatic zones differed in thermotolerance (Tougeron et al., 2016). Feeding strategies used by herbivorous insects will impact the stomatal conductance and alter leaf surface temperature. Thus, feeding strategies can affect the microclimate that the herbivore experiences (Pincebourde et al., 2007; Pincebourde & Casas, 2006, 2019). The adaptive nature of thermoregulation and micro-habitat selection needs to be considered in the broader context of species interactions (Pincebourde & Casas, 2019; Woods et al., 2015). Moreover, organisms can shift their diel activity patterns to match favourable thermal conditions (e.g. night feeding, mid-day quiescence) using temporal variation in microclimatic conditions (Scheffers et al., 2017). The benefits of selecting better microclimatic conditions at both spatial and temporal scales need to be considered in terms of species interactions, as antagonistic interactions such as predation, parasitism, or competition can offset the beneficial effects of thermoregulation (Choutt et al., 2011).

Parasitoid foraging strategies affect their fitness since reproductive success relies on the location of suitable hosts by parasitoid females (Godfray, 1994). Parasitoid foraging behaviour depends on physiological status, especially life expectancy and nutritional status. Challenging environmental conditions reducing life expectancy or requiring higher metabolic costs can thus induce risk-prone behaviour (Giraldeau & Boivin, 2008). Risk-prone behaviour increases fitness when an individual in a given environmental context faces low survival odds or intense physiological stress. For example, in response to warmer rearing or foraging conditions, host selection by the aphid parasitoid *Aphidius ervi* Halliday (Hymenoptera: Braconidae) shifted towards hosts of lower quality (i.e., instar selection) (Moiroux et al., 2015). Overall, the foraging strategy adopted to maximise fitness in challenging thermal environments is correlated to habitat quality and, more specifically, linked to the energetic costs of interpatch travel (Denis et al., 2011). However, there is only scarce information on the impact of fluctuating thermal regimes on environmental perception by parasitoids and their hosts (Soravia et al., 2021). Learning and memory can significantly improve host location by parasitoids, but forgetting associations between cues and hosts is equally essential as it integrates the temporal and spatial variation in cue reliability, such as a shift in volatile blend emitted by host-infested plants (Dunlap et al., 2009; Kraemer & Golding, 1997; Soravia et al., 2021). Following a temperature shock, the egg parasitoid *Trissolcus basalis* Wollaston retained its learned cues significantly longer than wasps held in control conditions (Abram et al., 2015). These results support the hypothesis that individuals living in an energetically costly environment retain information longer than those living in less costly environments (Dunlap et al., 2009).

Female hymenopteran parasitoids can control the sex of their offspring as a result of their haplodiploid sex-determination system (Godfray, 1994). Sex allocation of offspring by female parasitoids varies based on environmental conditions such as local mate competition, host quality or temperature (Gols & Harvey, 2009b; Moiroux et al., 2014; Shuker et al., 2007). The egg parasitoid *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae), foraging at high temperature, displayed an intentional male bias in offspring sex ratio, while this was not the case at lower temperatures (Moiroux et al., 2014). The realised sex ratio of *T. euproctidis* at low temperature was also male-biased, most likely caused by other physiological mechanisms such as egg fertilisation or sperm viability (Moiroux et al., 2014; Sales et al., 2018). Alteration



in offspring sex ratio under warm temperatures may have lasting consequences for parasitoid population dynamics by altering competition for mates between males and regulation of host populations.

Thermal regimes and their spatiotemporal variation will drive complex integrated responses at the organismal level. Individuals can alter their thermal tolerance through various mechanisms to cope with their thermal environment. However, the type of strategy used or the adaptive value of these strategies will ultimately alter species interactions (Tituskin et al., 2021). Moreover, the community composition and the thermal responses of the species present will feedback on the ability of a given (or target) species to respond to shifts in thermal regimes (Davis et al., 1998b).

Consequences of fluctuating thermal regimes for species interactions and community structure

Species-specific physiological and behavioural responses to thermal fluctuation and extremes at the organismal level will influence species interactions, both trophic and non-trophic, with repercussions at the community level (Gillespie et al., 2012) (Figure 3).

Consequences for trophic interactions

Consumer-resource interactions are at the heart of food web processes, and shifts in resource physiology and behaviour are likely to induce a change in consumer physiology and behaviour (Rosenblatt & Schmitz, 2016). Temperature can alter an individual's nutritional requirements and nutritional value for its natural enemies by altering the physiological processes of both organisms. For example, higher temperatures may reduce leaf carbohydrate levels leading to an increased tissue consumption by herbivores (i.e. compensatory feeding) to reach their nutritional requirements (Bauerfeind and Fischer, 2013; Jamieson et al., 2015).

Plant phytochemical composition is a strong driver of ecological processes and mediates plant – pollinators–natural enemies interactions via bottom-up processes (Han et al., 2019; Jamieson et al., 2017; Rosenblatt & Schmitz, 2016). Plant phytochemistry fluctuates over time (e.g. year, season, day), allowing plants to optimise their metabolic activity to match their environmental conditions (Doghri et al., 2021; Gols et al., 2007). Mechanical wounding of Lima bean (*Phaseolus lunatus* L.; Fabaceae) leaves as a mimic of herbivore damage during the photo- or scotophase resulted in differential emission of herbivore induced plant volatiles (HIPVs) (Arimura et al., 2008). Similarly, plant metabolite composition varies across time, differentially affecting the fitness of hosts and parasitoids (Liebelt et al., 2019). The composition of secondary metabolites in Brassicaceae crops in response to herbivory by *Mamestra brassicae* L. (Lepidoptera: Noctuidae) peaked at dawn or dusk compared to mid-day, suggesting that plants may anticipate potential threats to match their defences with the timing of a likely attack (Doghri et al., 2021). Recent studies indicate that plants having coevolved with multiple herbivore species may use circadian and seasonal rhythms to anticipate and adapt their defence strategies (Mertens et al., 2021b; Philbin et al., 2021).

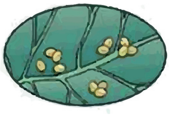
New climatic patterns and increased unpredictability of extreme temperature events will likely disrupt coevolved interactions, thus influencing community dynamics (Liebelt et al., 2019). In

a mesocosm experiment, Barton & Schmitz (2018) studied the spatial location of grasshoppers and spiders in response to day or night warming. In the reference and night warming treatments, grasshopper and spider spatial distributions on the plant overlapped during their diurnal activity, while in the day warming treatment, spiders retreated to lower parts of the plant canopy resulting in a spatial mismatch with their prey. In the night-warming treatment, but not in the reference or day warming treatments, spider and grasshopper distributions on the plant overlapped during nocturnal activity (Barton & Schmitz, 2018).

Besides mediating trophic interactions, plant chemical composition mediates herbivore immune responses against parasitoids and pathogens (Poelman et al., 2014). Although the effect of thermal fluctuations on the immune responses of hosts against their parasitoid has not been studied yet, an increase in rearing temperature led to an increased egg encapsulation of the parasitoid *Tranosema rostrale* Brishke (Hymenoptera: Ichneumonidae) by its larval host *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) (Seehausen et al., 2017). Yet, events of high extreme temperature may not always destabilise consumer-resource interactions due to opposite thermal effects on each species, such as prey encounter rates or walking speed (Bannerman et al., 2011; Boukal et al., 2019; Gvoždík & Boukal, 2021). Additionally, thermal stress can alter parasitoid attack rates and the defensive behavioural responses of hosts (Bannerman et al., 2011; le Lann, Lodi, et al., 2014).

Warmer thermal regimes affect growth rate and usually yield smaller body sizes in many ectothermic species (Kingsolver & Huey, 2008). Body size is a known parameter to mediate prey-predator (or host-parasitoid) interactions. Smaller sized prey may induce a shift in predation rate for bigger alternate prey species, or inversely smaller sized predators cannot attack bigger sized prey (Boukal et al., 2019). The Temperature Size Rule can thus alter the strength of consumer-resource interactions by differentially affecting species growth rates (Atkinson, 1994). The trophic rank hypothesis holds that higher trophic levels are less thermo-tolerant than lower trophic levels; predators are expected to display a more substantial reduction in size than their prey (Furlong & Zalucki, 2017; Voigt et al., 2003). However, its effects on trophic interactions seem more pronounced in aquatic ecosystems than in terrestrial ones (Boukal et al., 2019). Moreover, koinobiont parasitoids can manipulate their host development to optimise growth and development time, unlike idiobiont parasitoids that arrest host development via paralysis (Harvey et al., 2004). It is worth noting that recent studies suggest that under extremely high temperatures, parasitoid larvae failed to control host development leading to increased mortality and abnormal host physiology (Moore et al., 2021; Wang et al., 2021).

Overall, temperature effects on plant metabolites are context-specific as they interact with other abiotic and biotic stresses affecting insect community structure. Over time, variations in plant phytochemistry likely evolved to optimise plant defences against herbivores, either direct defences caused by secondary metabolites and/or indirect defences mediated by volatile emissions (Mertens et al., 2021), but further research is needed to assess the impact of changing climatic conditions on tri-trophic interactions. Research has mainly focused on bottom-up processes mediated by plant chemical profiles, and less is known about the effects of thermal variation on phytochemistry (but see Kask et al., 2016). Top-down effects of herbivory via feeding and foraging strategies influence plant chemical profiles. Due to changes in thermal conditions, changes in these strategies may prevent plants from mounting efficient chemical



defences against herbivores (Chidawanyika et al., 2019; Rosenblatt & Schmitz, 2016). However, thermal alterations in feeding strategies are constrained by other environmental factors, such as predator avoidance, as well (Greeney et al., 2012; Heinrich, 1979).

Consequences for non-trophic interactions

Non-trophic interactions between species are important for population dynamics and community structure (Sentis et al., 2017). The ecological niche a species occupies within a given environment partly depends on the non-consumptive effects that natural enemies have on their resources as well as mutualistic or antagonistic interactions with other species such as competitors (Dajoz, 2006; Laughlin & Messier, 2015).

In a two-year mesocosm experiment, Barton & Schmitz (2018) recorded higher plant diversity in the day-warming treatment and lower in the night-warming treatments compared to the ambient-temperature mesocosm driven by changes in plant-plant competition affected by herbivory. The timing of warming influenced spatial niche exploitation of the spiders, which changed their non-consumptive effects on grasshoppers resulting in higher or lower herbivory pressure on the dominant species in the system, leading to changes in the plant community (Barton & Schmitz, 2018).

Based on the principle of mutual exclusion, two species with a perfectly overlapping ecological niche cannot coexist in the same environment, and one superior competitor dominates the interaction (Dajoz, 2006). Thus individuals and species tend to reduce their niche overlap by attacking different stages of the same host or shifting their time of activity or spatial niche (Hood et al., 2021). However, environmental filtering is expected to reduce the range of an organism's phenotype (i.e. trait values), leading to converging functional traits at the community or guild level. As a result, competitive interactions between individuals and species tend to gain strength (le Lann et al., 2014b). A warming experiment involving two ant species showed that the less thermotolerant species suffered indirectly from warming through antagonistic interaction with a more thermotolerant ant species (Diamond et al., 2017). Thermal variation and extremes are likely to alter temporal and spatial resource partitioning between species, thus changing species' coexistence via thermal asymmetries in competitive interactions (Smith & Amarasekare, 2018).

Despite the high degree of host specialisation, parasitoid species display overlapping niches competing for shared hosts. The plant species' identity or the presence of specific symbionts will reduce competition between competing species creating cryptic niches (Harvey et al., 2013; Poelman et al., 2014). Microorganisms such as bacteria, fungi or viruses interacts with many insects and plants species in various ways (Dicke et al., 2020; Frago et al., 2020). These associations mediate many ecological interactions ranging from protecting the host against a parasitoid (e.g., *Hamiltonella defensa* Moran) via altering host physiology and nutritional properties, to increasing thermotolerance (Corbin et al., 2017; Dicke et al., 2020; Frago et al., 2020). A recent study assessed the impact of day and night warming using fluctuating thermal regimes on the fitness gain provided by the protective symbiont *H. defensa* to the aphid *Acyrtosiphon pisum* Harris in the presence or absence of its endoparasitoid *A. ervi* (Higashi et al., 2020). Carrying *H. defensa* reduced aphid survival under all warming treatments regardless of the timing

of warming. Moreover, *H. defensa* influenced parasitoid mummification success with a higher proportion under the warming treatment (Higashi et al., 2020). Mechanisms through which symbionts alter the thermotolerance are not well known but may involve the higher physiological cost of carrying protective symbionts when the organism faces physiological stress (Corbin et al., 2017). Symbiont-mediated interactions and the effects of thermal fluctuation and temperature extremes on these interactions add another layer of complexity in defining the ecological niche within temporally and spatially dynamic environments.

Concluding remarks and future perspectives

The predictability and unpredictability of environmental cycles, especially temperature regimes, have received increasing attention (le Lann et al., 2021; Liebelt et al., 2019; Park & Wootton, 2021; Welch & Harwood, 2014). Modelling studies assessing the role of periodicity and stochasticity over multiple laboratory generations showed that periodicity plays a prevalent role in driving intra-population variation in life-history traits (Park & Wootton, 2021). Shifts in these environmental cycles are likely to re-organise ecological communities in diverse ways as these communities are linked across multiple time scales (e.g. year, month, day) (Mertens et al., 2021b). The sheer diversity of thermal fluctuations, and community compositions makes identifying broader ecological patterns difficult. However, despite this diversity of species and interaction types, community dynamics are primarily influenced by a subset of key species (Poelman & Kessler, 2016). Focussing on the response of these species to shifts in climatic regimes is essential in identifying the role of temporal and spatial fluctuations in ecosystems.

Although intrinsically dependent on the absolute value of body temperature, thermal variation has distinct effects on the expressed phenotype (Sørensen et al., 2016). The expression of phenotypic plasticity is integrated over multiple traits and mediated by interactions with other biotic and abiotic factors that vary temporally with specific patterns. Temporal thermal variation creates sinusoidal curves that can be expressed in frequencies. Dillon et al., (2016b) proposed to use the Fourier transformation and back transformation to disentangle the effects of thermal variations at various time scales on ectotherm physiology. However, interpreting the impact of temperature time series at different timescales on ectotherm performance using frequencies requires accurately measuring the range of body temperatures that individuals experienced over time. Thus, in the case of small invertebrates such as insects, an accurate measurement of microclimatic conditions is vital (Pincebourde & Woods, 2020). A recent study using data on microclimatic heterogeneity, temporal temperature variations and laboratory-derived TPC (using constant temperatures) managed to predict field development rates of the butterfly *Pieris napi* L. (Lepidoptera: Pieridae) (von Schmalensee et al., 2021). However, TPCs are not constant throughout an individual's lifetime, and future research should further assess the role of thermal history on phenotypic expression and fitness.

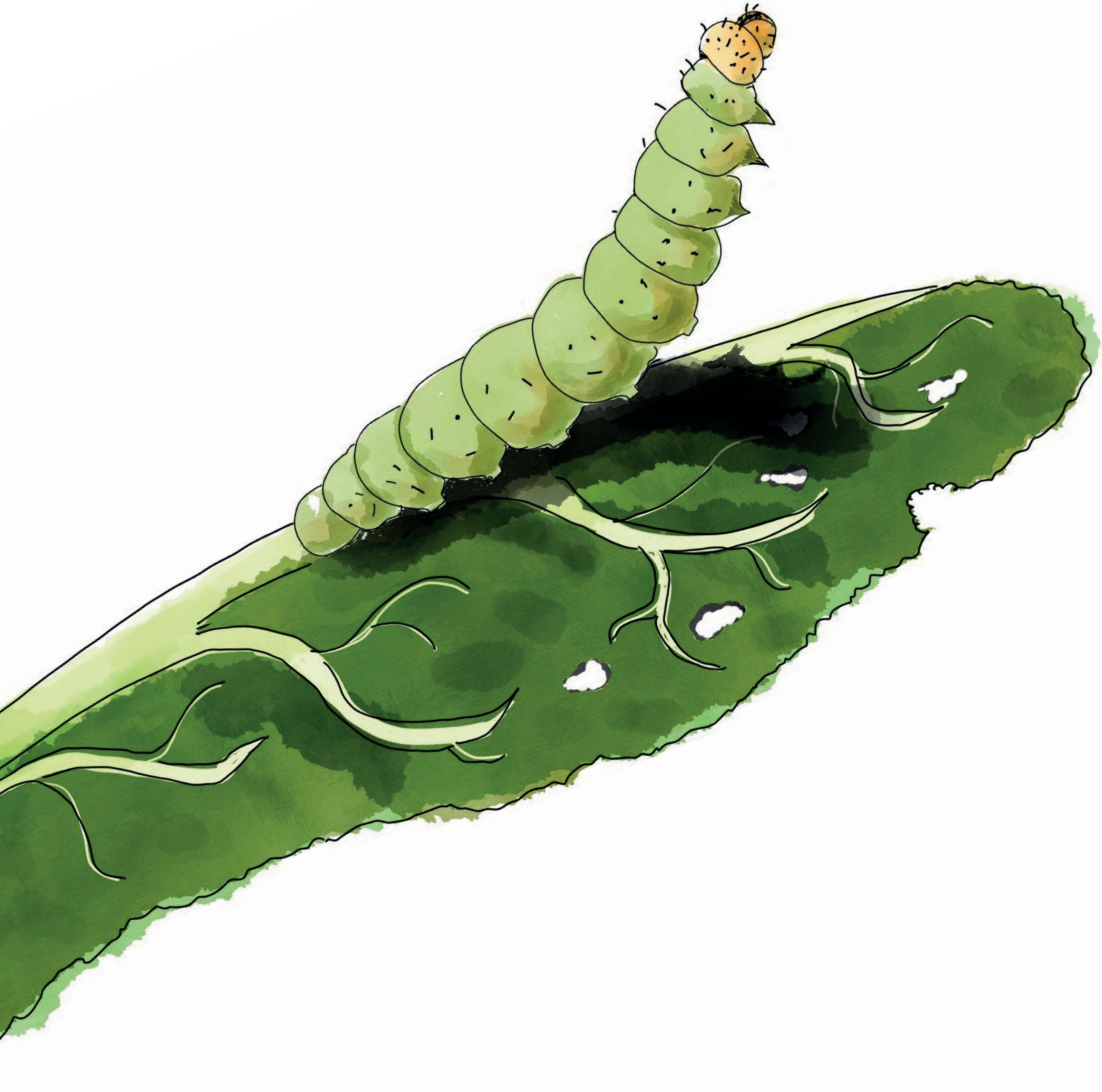
Environmental fluctuation an individual is exposed to throughout its lifecycle induces specific trait plasticity (Whitman and Agrawal, 2009). On the one hand, some traits can continuously adapt to the current environmental conditions, potentially tracking optimal phenotypic values for fitness. On the other hand, other traits are sensitive to environmental conditions at a specific life stage, after which trait values are fixed and cannot be altered by future environmental conditions (Lande, 2014). The continuity between temporal scales at which en-

vironmental variations occur, and the diversity of environmental parameters and phenotypic traits render any predictive exercises extremely challenging. Recent mathematical models and research frameworks used multivariate and dynamic analytical tools to tackle this challenge. Laughlin & Messier (2015) proposed the dynamic adaptive landscape framework to assess the fitness of multidimensional phenotypes along environmental gradients with both interspecific and intraspecific trait covariance. This framework demonstrates the importance of intraspecific trait covariance in driving the potential evolutionary strategies of species but also their ability to track environmental change and, by extension, their geographic range. This approach links the organism's fitness with its phenotypes. However, predicting how population dynamics are impacted by shifts or changes in species phenotypes is not straightforward, as population feedback can influence the effects of environmental change on population dynamics (Brass et al., 2021). Defining ecological niches across ecological gradients is essential to predicting impacts on biological communities under future climatic regimes.

Species live in dynamic environments characterised by periodic and stochastic fluctuations in environmental parameters. Assessing the different types of environmental fluctuations, such as average temperature, thermal fluctuations, with their degree of stochasticity and temporal dynamics, on plasticity they induced at the community level is crucial (Manenti et al., 2014; Speights & Barton, 2019; Valls et al., 2020; Zhao et al., 2019). Future studies on the role of thermal fluctuations across various temporal scales on phenotypes are needed to assess their integrated effects on multiple physiological and behavioural traits. Mathematical and modelling tools such as the Fourier transformation for temperature time series and the adaptive landscape framework can help identify relevant and common characteristics of environmental fluctuations in natural ecosystems. Moreover, they can integrate the multidimensionality of environmental signals on genotypic expression (Dillon et al., 2016a; Laughlin and Messier, 2015). A modelling experiment assessing the role of different types of environmental fluctuations and stochasticity demonstrated that plasticity allowed populations to maintain higher genotypic than phenotypic variance, thus promoting population persistence in stochastic environments (Romero-Mujalli et al., 2021). Recent findings suggests that thermal means, variation, the timing of extreme events and the degree of predictability vs. unpredictability of extreme thermal events induce different plastic responses characterised by experience separate genetic constraints (Kristensen et al., 2020; le Lann et al., 2021; Romero-Mujalli et al., 2021; Sørensen et al., 2016). Further investigations of the impacts of different types or combinations of environmental changes on the evolution of phenotypic plasticity in response to temperature will be especially relevant to predicting ecosystem responses to ongoing climate change.

Acknowledgements

We thank Belén Adiego-Perez for the fruitful help on the figures. The authors declare no conflict of interest linked to this study.



Chapter 3.

Effects of extreme temperature events on the parasitism performance of *Diadegma semiclausum*, an endoparasitoid of *Plutella xylostella*

Thibault P. M. Costaz[1], Rieta Gols[1], Peter W. de Jong[1], Joop J. A. van Loon[1], Marcel Dicke[1]

Abstract

With climate change, the occurrence of extreme weather events such as heatwaves is expected to increase. Extreme temperature events are short periods of high temperature (i.e., >95th percentile of temperature distribution), and their occurrences are difficult to predict. These extreme temperature events pose great challenges to living organisms, especially ectotherms such as insects whose body temperature is greatly influenced by environmental temperature. Temperature is known to play an essential role in driving insect performance as well as inducing behavioural changes. Exposure to extreme temperatures causes physiological damage leading to potential long-lasting effects on insect performance. We aimed at assessing the impact of direct (i.e., current) and indirect (previously experienced) exposure to extreme temperature on the oviposition success of *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae). *Diadegma semiclausum* is a larval endoparasitoid of *Plutella xylostella* L. (Lepidoptera: Plutellidae), a worldwide pest of brassicaceous crops. Oviposition success, measured as the percentage of parasitised *P. xylostella*, was significantly lower at 33°C than at 25°C. However, previous exposure to 33°C did not significantly impact the egg-laying performance of *D. semiclausum*, indicating that, after a heatwave, the egg-laying performance can recover. Neither the direct nor indirect exposure to extreme temperature altered *D. semiclausum* host-instar preference. These results indicate that temperature directly impacts *D. semiclausum* parasitism performance, although more detailed research is needed to disentangle direct physiological mechanisms from behavioural responses. Overall, these study results confirm others, suggesting that control of *P. xylostella* by *D. semiclausum* is likely to be impaired by more frequent heatwaves predicted under climate change scenarios.

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[1] Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

Entomologia Experimentalis et Applicata, 170: 656-665s

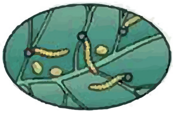
Introduction

In the last decade, there has been a growing interest of the scientific community in understanding the consequences of changing climatic regimes on ecosystems (Sutherland et al., 2013). The overall increase in average temperature creates less stable weather patterns in which extreme climatic events are predicted to be stronger and more frequent (Fischer & Schär, 2010; Pachauri et al., 2014). This is especially relevant for ectotherms, including insects, whose body temperature depends largely on environmental temperature (Vasseur et al., 2014).

The relationship between temperature and insect performance is often described using thermal performance curves (TPCs). A typical TPC displays a performance attribute e.g., the result of physiological processes such as development, growth, or egg production as a function of temperature (Sinclair et al., 2016). With increasing temperature, TPCs typically show a rising part until a maximum is reached (the optimal temperature) before quickly dropping towards zero. Thus, TPCs are a useful tool to describe the effects of thermal variation on insect performance (Denny, 2017). However, TPCs do not include the effects of thermal history on performance (Kingsolver & Woods, 2016; Sinclair et al., 2016; Williams et al., 2016). Thermal history can induce complex physiological responses with lasting consequences on insect performance throughout its life cycle (Bowler and Terblanche, 2008; Harvey et al., 2020).

Extreme temperature events, such as heatwaves, pose a greater challenge for insects than the mere increase in average conditions (Paaijmans et al., 2013). Extreme temperature events (EXT), by definition, are periods (usually lasting several days) during which the maximum temperature falls beyond the 90th percentile of the temperature distribution for a reference period (usually spanning 30 years) (Ummenhofer & Meehl, 2017). Although EXT may last several days, temperature also fluctuates in a diurnal rhythm, with temperatures rising in the morning to peak at midday before decreasing at nightfall, exposing insects only several hours per day to stressful temperatures. Because the incidence of EXT is predicted to increase under conditions of global warming, living organisms are likely to experience increasing thermal fluctuations (Dillon et al., 2016a). These discrete events can affect species performance and species interactions in various ways depending on time-specific mechanisms relative to their occurrence.

Exposure to EXT often results in a decrease in insect performance, although species can exhibit variable levels of thermal tolerance (Colinet et al., 2015; Harvey et al., 2020; Stoks et al., 2017). Prior exposure to EXT may enhance performance under subsequent heat exposure, a phenomenon called rapid heat hardening (Chidawanyika & Terblanche, 2011; Ju et al., 2011). Physiological injuries caused by EXT may be repaired when afterwards being exposed to a milder temperature (Bai et al., 2019; Speights et al., 2017). Heat hardening and cellular repair are linked to heat-shock proteins that help to maintain the cellular machinery during heat stress or help to repair heat injuries once the thermal stress has passed (Bahar et al., 2013; King & MacRae, 2015). Recent studies suggest that the impact of EXT on insect life-history parameters is driven by a complex balance between the frequency of hot versus mild periods both within and between days (Bai et al., 2019; Ma et al., 2018; Zhu et al., 2019). Studies on the effect of



previous exposure to EXT on insect performance are needed to better grasp the consequences of larger thermal fluctuations and more frequent EXT on insect populations.

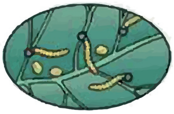
Parasitoids play a key role in herbivore population dynamics and represent a commercially important group of insects often used in integrated pest management (Harvey et al., 2020; Jervis, 2005). Thermal sensitivity increases with increasing trophic level (Voigt et al., 2003). Therefore, parasitoids are expected to be acutely impacted by EXT (Furlong and Zalucki, 2017; Hance et al., 2007). Parasitoid foraging strategies have received a lot of attention, and a vast body of literature is available (Aartsma et al., 2019; Rijk et al., 2013; Wajnberg et al., 2008). Recent studies have reported modifications of parasitoid patch-residence time at different constant temperatures. These changes were mediated by parasitoid walking speed and levels of defensive behaviours exhibited by its host (le Lann et al., 2014a; Moiroux et al., 2016). Moiroux et al. (2015) found that the foraging behaviour of *Aphidius ervi* Haliday (Hymenoptera: Braconidae) on *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae) was significantly altered by the interaction between host instar, temperature during parasitoid development and foraging temperature. They showed that *A. ervi* females having experienced higher temperatures (28°C) during their development or while foraging, attacked lower quality hosts (i.e., first or fourth instars) more frequently than females having experienced lower temperatures (12°C and 20°C). These results were interpreted as risk-prone behaviour caused by a decrease in the expected reproductive success due to reduced expected survival (Wajnberg et al., 2008). *Leptopilina heterotoma* Thomson (Hymenoptera: Eucoilidae) exhibited increased patch residence time and superparasitism (i.e., oviposition in an already parasitised host) in response to a drop in barometric pressure (Roitberg et al., 1993). The direction of change in barometric pressure can be associated with changing weather patterns to which the wasp responded by altering its foraging behaviour (Steinberg et al., 1992). This matches with life-history theory, which predicts that an individual should adapt its behaviour to its physiological status in order to maximise lifetime reproduction (Roitberg et al., 1992, 1993). However, studies assessing the effects of fluctuating temperature on species interactions are rare (but see Schreven et al. (2017); Valls et al. (2020). It is, therefore, essential to assess the effects of these discrete extreme events on host-parasitoid performance attributes in order to better assess the effects of future thermal conditions on trophic interactions (C.-S. Ma et al., 2021; Stoks et al., 2017).

The present study investigates both thermal history and the response to fluctuating temperatures. More specifically, we assessed the direct (i.e., current) and indirect (previously experienced) effects of exposure to extreme temperature on the reproductive output of *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae), a solitary larval endoparasitoid of *Plutella xylostella* L. (Lepidoptera: Plutellidae), which is an important pest in brassicaceous crops (Furlong et al., 2013; Talekar & Shelton, 1993). We hypothesise that reproductive success will be negatively affected when the parasitoid experiences or has previously experienced EXT.

Materials and methods

Insects

Plutella xylostella is a multivoltine moth species, that goes through four instars (L1–L4) before pupating (Furlong et al., 2013). Its development time depends on the temperature and varies



from instar to instar. Personal observations show that instar transition from L2 to L3 lasts two and four days at 25°C and 20°C, respectively. *Diadegma semiclausum* is among the main parasitoid of *P. xylostella* and has been introduced in many countries as a biological control agent (Talekar & Shelton, 1993). It is a solitary endoparasitoid that consumes the host entirely before pupating (Harvey, 2015). This parasitoid species can parasitise all four *P. xylostella* instars (Yang et al., 1993).

Plutella xylostella and its parasitoid *D. semiclausum* were collected in fields surrounding Wageningen University (the Netherlands) and reared in the laboratory since 2017 with new individuals added every year. Both, herbivore and parasitoid, were maintained on *Brassica oleracea* L. var. *gemmifera* cv. Cyrus (Brussels sprouts) in separate rooms under constant conditions (22±2°C, 40-50% RH, photophase 16L:8D). Male and female *P. xylostella* moths were placed in a clean cage with 10% sucrose solution and a Brussels sprouts plant as oviposition substrate for 48 h. Then the plant was transferred to a new clean cage and after hatching, larvae were allowed to feed *ad libitum* until pupation. Additional plants were added if necessary. About twenty *D. semiclausum* adult females along with a few males were released in a cage containing a Brussels sprouts plant heavily infested with second instar *P. xylostella* larvae from the stock colony. The parasitised caterpillars were provided *ad libitum* with Brussels sprouts plants until adult parasitoids emerged. The newly emerged adults were collected daily and placed in a clean cage with 10% sucrose solution as a food source. Brussels sprouts plants were also used as food plants for the larvae during the experiments.

Experimental design

Thermal treatments

The foraging temperatures and the two thermal pre-treatments were chosen based on climatic data from the Royal Netherlands Meteorological Institute[1] (KNMI). One of the two thermal pre-treatments emulated "reference" conditions in the Netherlands using daily minimal and maximal temperature for the months of June-July-August of the reference period 1961-1990. The second thermal pre-treatment emulated stressful conditions with minimal and maximal temperatures raised by +3°C and +8°C from the reference conditions, respectively (Table 1). These correspond to an increase in average temperature and accompanying variation mimicking heatwave conditions in Europe (de Boeck et al., 2010). The maximum temperature in the heatwave regime (i.e., 33°C) exceeded the 95th percentile of daily maximal temperature distribution of June-July-August during the reference period of 1961-1990 in the Netherlands (KNMI). The heatwave regime applied temperatures falling within the range of stressful temperatures for *D. semiclausum* (Furlong & Zalucki, 2017; Schreven et al., 2017). The two thermal treatments had a 16L:8D photo:scotophase corresponding to early summer conditions in the Netherlands. The parasitism rate of *D. semiclausum* was then compared at three temperatures (20°C, 25°C, and 33°C) corresponding to temperatures naturally occurring during summer in the Netherlands, with the latter two matching the thermal maximum of each thermal pre-treatments (i.e., 25°C, and 33°C).

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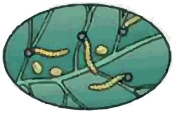
[1] https://www.knmi.nl/klimaat-viewer/kaarten/temperatuur



Experiment 1: Direct effect of temperature

Thirty early second instar (L2) and third instar (L3) *P. xylostella* larvae (15 per instar), taken from the stock colony, were placed on an excised young fully developed Brussels sprouts leaf in a plastic container (115 X 188 mm, volume 770 mL; Gédé Verpakkingen®, the Netherlands; hereafter "container") closed by a mesh lid (Bugdorm®, Taiwan). The leaf petiole was placed in water in an Eppendorf tube and closed with a piece of cotton wool to keep the leaf turgescent throughout the bioassay. Additionally, a piece of moistened filter paper was placed on the bottom of the container to maintain humidity. At the same time, two-to-four-day-old female *D. semiclausum* were kept in a separate cage along with males (sex ratio of 1:1) and provided with honey on the mesh of the cage.

Containers with hosts and cages with parasitoids were placed in each of two climate cabinets (type ECD01E, Snijders Scientific, Tilburg, The netherlands) at 22±1°C. Then the temperature was set at either 20°C, 25°C, or 33°C. After 30 min acclimation to the new conditions, from the cage that was placed in the same cabinet alongside the container with hosts, a single naïve female *D. semiclausum* was released in each container for three hours. Foraging time was set to 3h to avoid complete depletion of the host patch by the parasitoid wasps. A drop of honey was placed on the lid to allow the parasitoid to feed during the bioassay. At the end of the bioassay, the *P. xylostella* larvae were sorted per instar and frozen until dissection. Each of the three temperature treatments was replicated 20 times. The replicates were conducted over several days, allowing to perform all treatments in each of the climate cabinets randomly. The bioassays were performed between 9:00h and 16:00h.

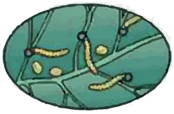
Table 1: Temperature applied for five intervals per day for the two thermal pre-treatments. Reference and heatwave represent normal and heatwave conditions, respectively.

Hour	Reference (Temperature in °C)	Heatwave (Temperature in °C)
0-5	15	18
5-11	20	25
11-15	25	33
15-21	20	25
21-0	15	18

Experiment 2: Indirect effect of temperature

As in experiment 1, the parasitism rate of *D. semiclausum* was compared at three temperatures (20°C, 25°C, and 33°C). However, 24 h prior to the bioassay, the insects were exposed to either of two thermal pre-treatments. This was done to study the effects of previous exposure to high temperatures on parasitoid oviposition performance.

The temperature in the climate cabinets followed stepwise fluctuations to resemble natural daily temperature variations which hosts and parasitoids may encounter in the field (see Table 1



for details of the temperature regimes), with a few hours of high-temperature exposure during midday. Both *P. xylostella* larvae and *D. semiclausum* adults were exposed to either of the two thermal pre-treatments for 24 h. One-to-three-day-old *D. semiclausum* females were kept with males (sex ratio 1:1) and provided with honey on the mesh of the cage. In a separate cage, late L1 and L2 *P. xylostella* were taken from the stock colony and maintained on Brussels sprouts leaves. The following day, 10 early L2 and 10 early L3 *P. xylostella* larvae were selected and placed on a young fully developed Brussels sprouts leaf inside a container (same as in experiment 1). The *P. xylostella* larvae were placed back into the two climate cabinets at either 20°C or 25°C alongside the parasitoid cages (Table 1). Then the temperature was set at either 20°C, 25°C, or 33°C. After 30 minutes of acclimation to the new conditions, a single naïve female *D. semiclausum* was released in each container for three hours. A drop of honey was placed on the lid to allow the parasitoid to feed during the bioassay. At the end of the bioassay, the *P. xylostella* larvae were sorted per instar and frozen until dissection. *Plutella xylostella* larvae and adult female *D. semiclausum* with the same thermal history were tested together, creating a total of six treatments. Each treatment had between 18 to 21 replicates accumulating over time, allowing to switch the thermal pre-treatment and temperature treatments between all climate cabinets. All bioassays were performed between 11:00h and 14:00h.

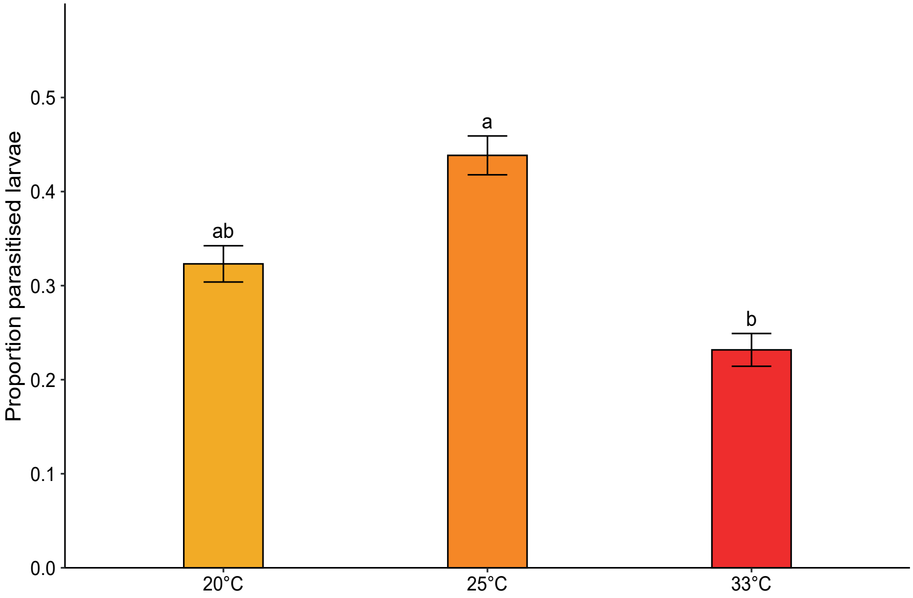
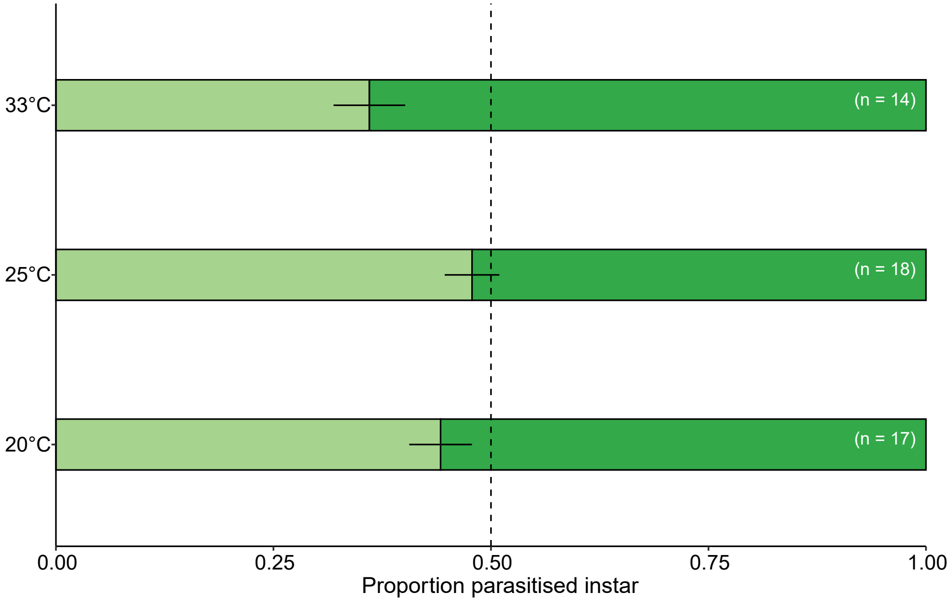


Figure 1: Proportion of parasitised *P. xylostella* larvae by a *D. semiclausum* female at 20°C, 25°C, and 33°C; Error bars represent mean ± SE. Lower case letters represent significant ($P < 0.05$) Tukey-adjusted pairwise comparisons.



Proportion parasitised instars

Figure 2: Proportions of parasitised second (L2) (light green) and third (L3) (dark green) *P. xylostella* instars at 20°C, 25°C, and 33°C. "n" indicates the number of tested parasitoids that each were provided with 15 L2 and 15 L3 host larvae. Females that did not lay eggs were not included in the analysis.

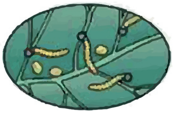
Variables measured

All host larvae were dissected under Zeiss SR stereomicroscope (Oberkochen ,Germany), and the presence/absence of parasitoid eggs was recorded. The occurrence of superparasitism (if more than one egg was found per host) was also recorded.

Statistical analysis

The data were analysed using the same statistical procedure for both experiments. For the first experiment, the response variables were modelled against the temperature treatment (factor with three levels), the climate cabinets (block with two levels) and their interactions as independent variables. For the second experiment, the data were modelled against the temperature treatment (factor with three levels), the thermal pre-treatment (factor with two levels), the climate cabinets used for the thermal pre-treatment (factor with two levels), the climate cabinets used for the temperature treatments (factor with two levels), and their interactions.

We analysed the proportion of parasitised *P. xylostella* larvae using a generalised linear model (GLM) assuming a binomial distribution with a logit link function. As the model suffered from overdispersion, we corrected it by using a quasi-binomial distribution with a logit link



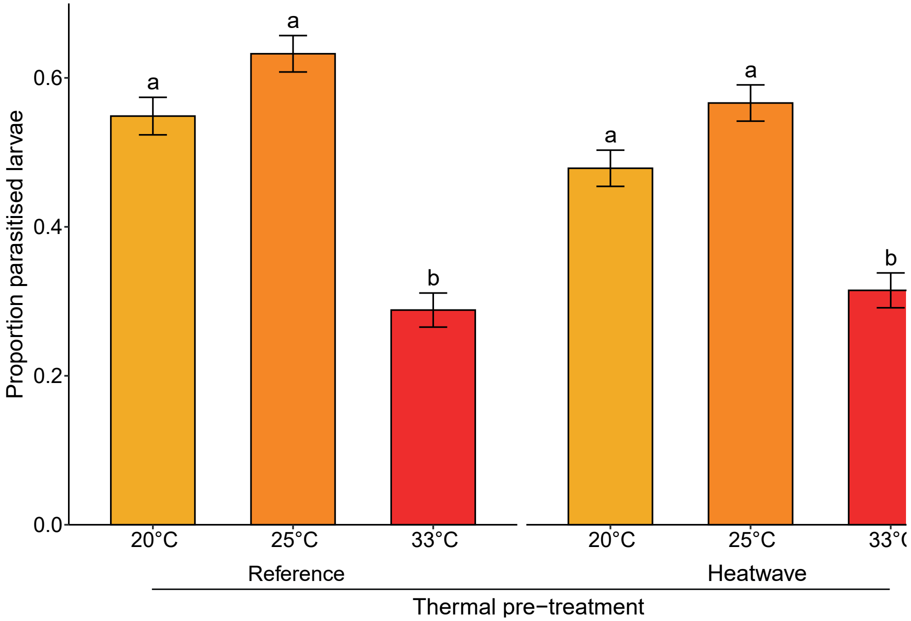


Figure 3: Proportion of *P. xylostella* larvae parasitised by *D. semiclausum* at 20°C, 25°C, and 33°C for both the reference (left side) and heatwave (right side) thermal pre-treatments. Error bars represent mean ± SE. Lower case letters represent significant ($P < 0.05$) Tukey-adjusted pairwise comparisons.

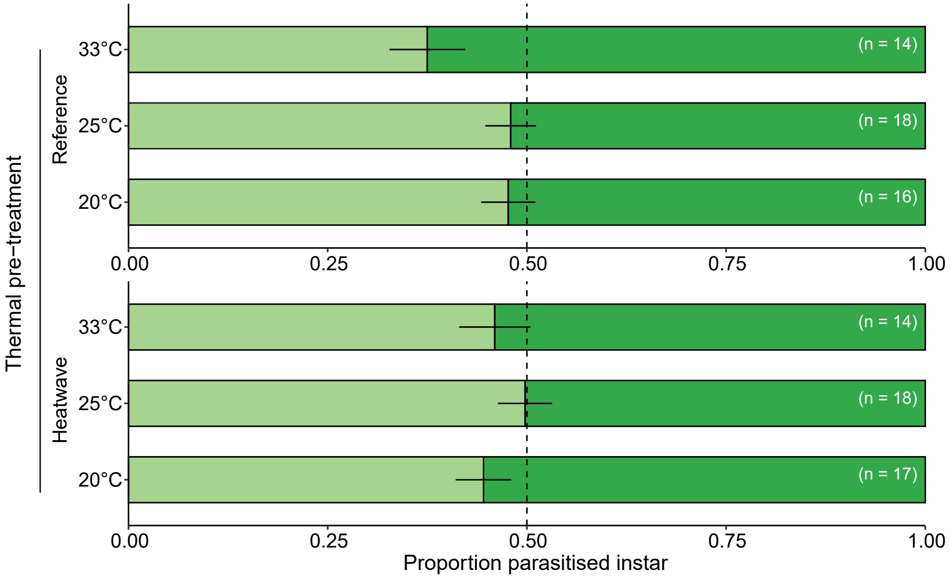
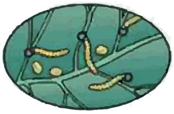


Figure 4: Proportion of parasitised L2 (light green) and L3 (dark green) *P. xylostella* instars at 20°C, 25°C, and 33°C for both the reference (top) and heatwave (bottom) thermal pre-treatments. "n" indicates the number of tested parasitoids; each parasitoid was offered 10 L2 and 10 L3 larvae.



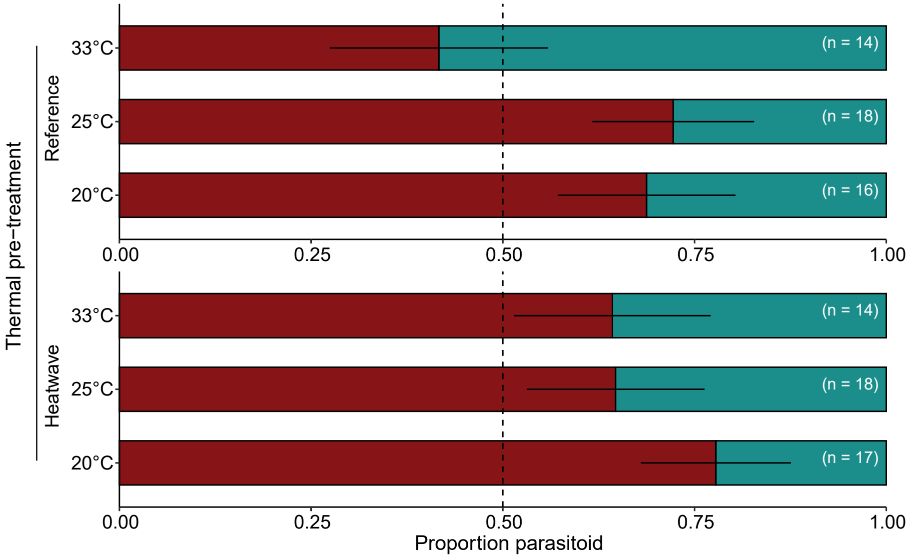


Figure 5: Proportion of *D. semiclausum* parasitoids displaying superparasitism behaviour (blue) versus parasitoids not displaying superparasitism behaviour (dark red) at 20°C, 25°C, and 33°C for both the reference (top) and heatwave (bottom) thermal pre-treatments. "n" indicates the number of tested parasitoids. Females that did not lay eggs were not included in the analysis.

function. Non-significant interaction terms ($P > 0.05$) were excluded from the model. Changes in the proportion of parasitised L2 versus L3 *P. xylostella* instars were analysed using a GLM assuming a binomial error structure with a logit link function. We removed parasitoids that had not oviposited in any host as they do not add information on the respective proportion of parasitised L2 and L3 caterpillars. The model did not suffer from overdispersion. Non-significant interaction terms ($P > 0.05$) were excluded from the model.

The occurrence of self-superparasitism was analysed using a Bernoulli GLM with a logit link function. Parasitoids that exhibited superparasitism behaviour were analysed versus the parasitoids that did not (i.e., 0, 1). In experiment 1, self-superparasitism was only recorded for four individuals (three parasitoids at 33°C and one at 25°C), therefore it was not analysed. In experiment 2, 32 individuals displayed self-superparasitism. One individual from the "reference" thermal pre-treatment tested at 25°C appeared as an outlier with 12 superparasitised hosts for which three to four eggs per larva were recorded and was excluded from the analysis.

All analyses were performed in R version 4.0.2 (R Core Team, 2019). The figures were produced using the "ggplot2" package (Wickham, 2016). The treatments main effects were extracted from the model output using the "car" package (Fox and Weisberg, 2019). When a significant effect of treatment was found, Tukey post-hoc tests were performed for pairwise comparison using the emmeans package (Russell, 2018) and the compact letters were extracted using the "rcompanion" package (Mangiafico, 2021).

Results

Experiment 1: Direct effect of temperature

Temperature influenced *D. semiclausum* oviposition success ($\chi^2 = 6.42$, df = 2, P = 0.040) (Figure 1). The number of parasitised *P. xylostella* larvae across the three tested temperatures resembles the expected shape of thermal performance curves (TPC) with a more parasitised caterpillars at 25°C than at 20°C and 33°C. The latter displays the lowest amount of parasitism, which suggests that 33°C is beyond the optimal parasitism temperature for this species. The difference in proportion of parasitised larvae between 20°C and 33°C is not significant.

The numbers of parasitised L2 and L3 instars did not differ significantly, suggesting that there is no preference for either instar at any of the three tested temperatures ($\chi^2 = 4.74$, df = 2, P = 0.093) (Figure 2).

Experiment 2: Indirect effect of temperature

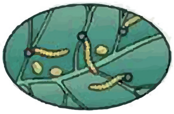
Temperature affected the overall proportion of *P. xylostella* caterpillars parasitised ($\chi^2 = 18.14$, df = 2, $P < 0.001$) whereas the thermal pre-treatment had no effect on parasitism rates ($\chi^2 = 0.37$, df = 1, P = 0.545). The proportion of *P. xylostella* larvae parasitised at the three tested temperatures followed the same pattern as in the previous experiment, with a strong negative effect of high-temperature exposure (i.e., 33°C) on *D. semiclausum* oviposition performance (Figure 3).

No evidence of instar preference was detected for the thermal pre-treatments ($\chi^2 = 0.16$, df = 1, P = 0.687) nor for temperature treatment ($\chi^2 = 2.00$, df = 2, P = 0.367) (Figure 4).

The occurrence of self-superparasitism behaviour was not significantly affected neither by the thermal pre-treatment ($\chi^2 = 0.47$, df = 1, P = 0.495) nor by the temperature during parasitism ($\chi^2 = 2.80$, df = 2, P = 0.246) (Figure 5).

Discussion

The present study aimed at assessing the direct (i.e., current) and indirect (previously experienced) effects of exposure to extreme temperature on *D. semiclausum* parasitism success. As hypothesised, *D. semiclausum* had a lower reproductive success in both experiments when directly exposed to EXT. However, indirect exposure to EXT did not have a significant effect on *D. semiclausum* parasitism rate. These results contrast with the study of Bannerman et al. (2011) who did not find a significant effect of simulated heatwaves on the foraging performance of another parasitoid, *Aphidius matricariae* Haliday (Hymenoptera: Aphidiidae). These discrepancies are likely due to the study design as, in the latter study, female parasitoids could forage for 24h, thus across the whole range of temperatures. The effect of temperature on parasitoid oviposition performance appears to be relatively small for temperatures below the species' optimum. The proportion parasitised *P. xylostella* larvae at 20°C was not significantly different from the proportion parasitised at 25°C in both experiments of this study. Similarly, temperature did not significantly affect the proportion of *Sitobion avenae* Fabricius (Hemiptera: Aphididae) aphids parasitised by *Aphidius rhopalosiphi* De Stefani-Perez (Hymenoptera: Braconidae) exposed to

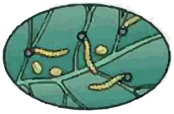


temperatures between 15°C and 25°C (le Lann et al., 2014a). Temperature has a direct kinetic effect on insect metabolism, thus regulating insect performance. Insects are also able to integrate thermal information and adjust their behavioural response accordingly (Abram et al., 2017). Although distinguishing between both effects is not possible in this study, direct exposure to EXT likely has reduced the reproductive performance of *D. semiclausum* via effects on its behaviour. We observed the highest number of individual parasitoids not having oviposited in any host under direct exposure to 33°C. Moreover, a study on the effect of exposure to a heat shock (38°C) on host attack and reproduction by *A. ervi* showed that the effect was largest during actual exposure to this heat shock because the wasps were inactivated (Valls et al., 2020).

Previous exposure to EXT did not significantly alter *D. semiclausum* oviposition performance. This suggests no evidence for rapid heat hardening nor lasting heat injuries of the parasitoid after a pre-exposure to 33°C. Similarly, Valls et al. (2020) did not find a significant effect of previous exposure to high-temperature (38°C) on *A. ervi* parasitism rate when the heat shock (i.e., four hours at 38°C) occurred three days before the foraging assays (Valls et al., 2020). *Aphidius avenae* Haliday (Hymenoptera: Aphidiidae) parasitism success after an exposure of one hour to 36°C followed by 24h recovery at 20 °C led to a significant reduction in the number of parasitised aphids (Roux et al., 2010). These differences may arise from the magnitude of the thermal fluctuation used in the experimental setup. In their study, the temperature provoked heat stupor in the parasitoids whereas in our study the parasitoids were exposed to a lower stress. In contrast, a study looking at the impact of periodic short temperature events (hot and mild days) on life-history traits of *S. avenae* showed a large beneficial effect of intermittent mild temperatures within a period of hot days while an increase in the number of hot days negatively impacted its life-history traits (Zhu et al., 2019). The number of eggs laid by *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) was only significantly reduced by previous exposure to extreme high temperature (>30°C) of more than 24h (Evans et al., 2018). This suggests that fluctuating temperatures may allow for partial recovery but not when the magnitude of EXT exposure reaches near-critical thermal limits or extends for long periods. The effect of developmental temperature on insects usually influences development time and body size, potentially altering the adult's fecundity and longevity. An increase in developmental temperature reduced the number of eggs laid by *A. rhopalosiphi* females (le Lann et al., 2011). Overall, these results suggest that the effect of previous exposure to EXT is complex and future research should aim at assessing its effects on the fitness across insect ontogeny.

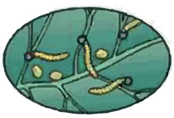
A study measuring the effects of two fluctuating thermal regimes combined with two types of extreme temperature events on *P. xylostella* and *D. semiclausum* developmental parameters (i.e., development time, growth rate, and survival) found variable effects of temperature depending on the intensity of thermal extreme and the species (i.e., host or parasitoid) (Schreven et al., 2017). Overall, their findings are in line with the current paradigm that parasitoids have a higher sensitivity than their hosts when facing extreme temperature events with delayed development time and higher mortality recorded for *D. semiclausum* (Furlong & Zalucki, 2017; Schreven et al., 2017).

Neither direct nor indirect exposure to EXT altered *D. semiclausum* host instar parasitism preference. Direct observations of foraging by *Diadegma mollipla* Holmgren (Hymenoptera: Ichneumonidae) showed that females attacked L2, L3, and L4 *P. xylostella* instars without any pref-



erence despite a lower survival for parasitised fourth instar larvae (Nofemela & Kfir, 2007). Additionally, Gols and Harvey, (2009a) studied the effect of host size at parasitism on *D. semiclausum* development and found lower survival for parasitised L4 *P. xylostella* larvae compared to L2 larvae. Changes in host preference were observed for *A. ervi* females which had developed and were foraging at 28°C compared to females which had developed and were foraging at 12 and 20°C (Moiroux et al., 2015). A shift in host-instar preference is often interpreted as a lower reproductive success due to low fecundity and longevity (Wajnberg et al., 2008). Therefore, according to the risk-prone behaviour theory, to maximise their fitness under stressful conditions, female parasitoids are expected to attack more frequently lower quality hosts (Moiroux et al., 2015; Wajnberg et al., 2008). It is important to note that temperature can also alter host defensive behaviour. Although host defensive behaviour was not quantified in this study, it was reported that higher temperature led to more intense defensive behaviour from *S. avenae* (le Lann et al., 2014a). However, the host's increased defensive display was counterbalanced by the parasitoid's higher attack rate (le Lann et al., 2014a). The differences in results between this study and that by Moiroux et al. (2015b) are likely due to the type of host instar offered in both experiments. In the latter, *A. ervi* females could choose from all four nymphal *S. avenae* instars whereas in our experiment, *D. semiclausum* females were offered L2 and L3 *P. xylostella* instars. When considering the differences in parasitism rate between second and third instar, Moiroux et al. (2015) did not find significant differences. *Diadegma semiclausum* is a koinoibiont parasitoid, thus allowing the host to continue its development after parasitisation. To some extent, they can exert control over host physiology to maximise their fitness Gols and Harvey (2009a). For these parasitoids, host quality does not always correlate with host body size at parasitism, explaining the lack of preference for either instar in this study (Harvey et al., 2004).

Self-superparasitism was only analysed in the second experiment. The proportion of parasitoids expressing this behaviour was not significantly affected by direct or indirect exposure to EXT. It is worth noting that more than half of the female parasitoids from the reference pre-treatment and exposed at 33°C displayed self-superparasitism behaviour. This effect was not significantly different from the other treatments, probably due to the low number of individuals engaging in self-superparasitism (n = 31). Moreover, most wasps engaging in self-superparasitism had enough remaining healthy hosts at their disposal; thus, it is unlikely that this behaviour was caused by a lack of available hosts (Table S1). Self-superparasitism is often considered to be detrimental for solitary parasitoids as only one of their offspring will survive. However, in some cases, self-superparasitism can be beneficial for solitary parasitoids (as reviewed in van Alphen and Visser, 1990). Laying more than one egg into the same host can enhance offspring survival by increasing the odds of overcoming the host immune response (Poelman et al., 2014; van Alphen & Visser, 1990; Wojda, 2017). This scenario is, however, unlikely in the case of *D. semiclausum*, as their eggs do not seem to suffer from encapsulation at any of the tested temperatures (personal observations). A second case in which self-superparasitism



can be advantageous when the density of conspecific parasitoids is high (van Alphen & Visser, 1990). In our study, *D. semiclausum* was foraging alone, and this scenario does not apply. *Leptopilina heterotoma* superparasitised more often after a drop in barometric pressure (associated with reduced life expectancy) (Roitberg et al., 1993). This type of behaviour can be interpreted as risk-prone behaviour (see the paragraph above). In our study, we hypothesized that exposure to high temperature may reduce the life expectancy of *D. semiclausum,* which could, in turn, maximise its fitness outcome by engaging in self-superparasitism. However, this was not confirmed by the data as the percentage of female parasitoid displaying self-superparasitism in the heatwave pre-treatment was similar to the one foraging at 20°C and 25°C from the reference pre-treatment. Yang et al. (1994) showed that female *D. semiclausum* tended to avoid superparasitism and concluded that *D. semiclausum* could differentiate parasitised from unparasitised larvae. Temperature may alter *D. semiclausum*'s capacity to detect parasitised larvae. In this study, rapid heat hardening from previous exposure could explain why this pattern did not appear for the wasp previously exposed to 33°C. Alternatively, this change in behaviour may only occur at higher stress temperatures. Abram et al. (2015) found that previous thermal stress prevented memory loss in the parasitoid *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae). However, for the present study, this interpretation should be taken with care due to the low sample size. More research on temperature stress on (self-)superparasitism is needed to better assess its effect on host patch exploitation.

Overall, this study showed that the reproductive performance in relation to temperature approximates the typical shape of a TPC with an optimal temperature below 33°C. Schreven et al. (2017) found that the development of *D. semiclausum* slowed down when exposed to fluctuating temperatures reaching 35°C whereas this was not the case for its host *P. xylostella*. It is widely accepted that members of higher trophic levels (e.g., parasitoids) are more sensitive to high temperature than organisms at lower trophic levels (e.g., herbivores) (Furlong and Zalucki, 2017; Stoks et al., 2017; Voigt et al., 2003). In addition, temperature-mediated bottom-up effects can alter host-parasitoid interactions further. Indeed, higher temperatures in combination with herbivory are usually associated with an increase in herbivore-induced plant volatile emissions (Jamieson et al., 2017; Truong et al., 2014). Changes in the plant primary and secondary metabolites can, in turn, alter host physiology, affecting parasitoid larvae (Gols et al., 2008). Dosdall et al. (2012) found that at 30°C, *D. semiclausum* could no longer successfully develop in *P. xylostella* larvae feeding on *Brassica napus* L. This was not the case when larvae fed upon *Brassica rapa* L. and *B. oleracea* leaves, suggesting an interaction between plant nutritional quality and temperature on parasitoid fitness. We conclude that our results add to those providing evidence that more frequent extreme temperature events are likely to impair parasitoid-host interactions including the one between *D. semiclausum* and *P. xylostella*. This potentially negatively impacts on the ability of *D. semiclausum* to control *P. xylostella* populations, thus, increasing the risk of pest outbreaks (de Sassi and Tylianakis, 2012; Hance et al., 2007; Ma et al., 2015; Schreven et al., 2017).

Acknowledgements

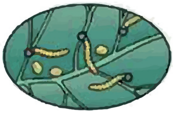
We are grateful to the insect rearing staff of our laboratory: Pieter Rouweler, Andre Gidding, and the late Frans van Aggelen as well as Unifarm for providing the insects and plant materials for

the experiments, respectively. We are thankful to two anonymous reviewers for the constructive comments on the manuscript. The authors declare no conflict of interest linked to this study.

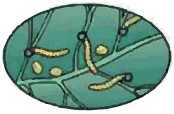
Supplement

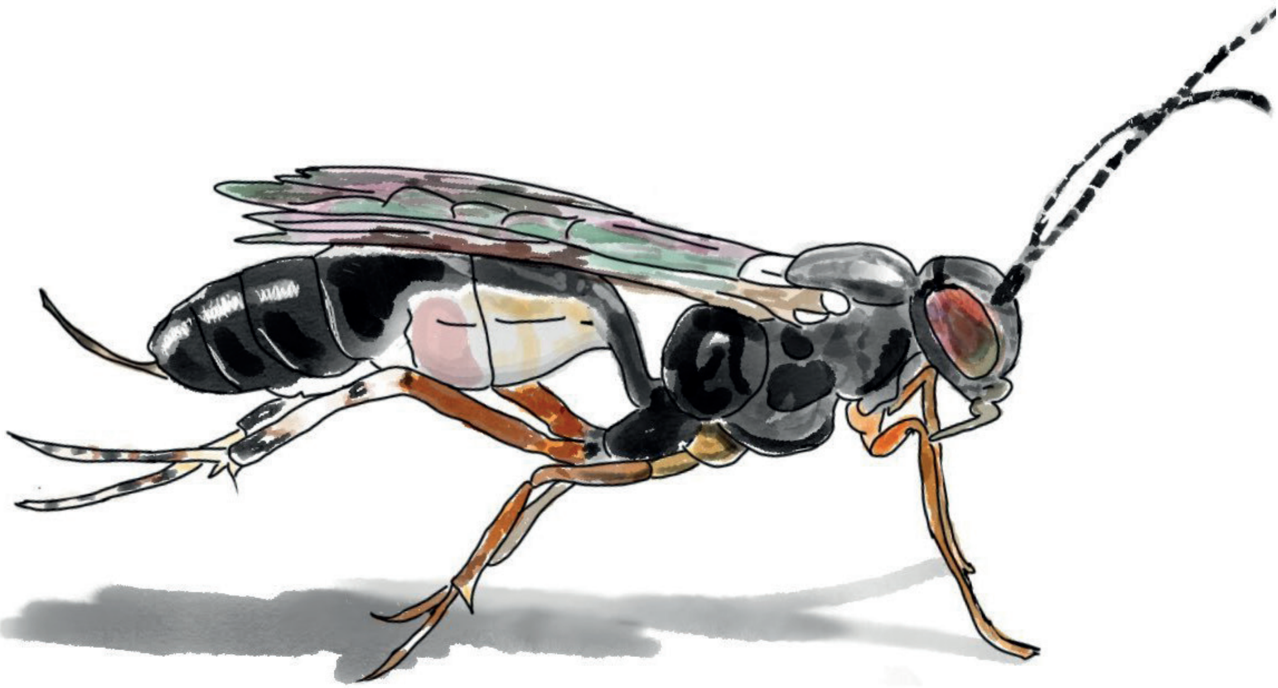
Table S1: Proportion of *P. xylostella* larvae parasitised by *D. semiclausum* displaying self-superparasitism at 20°C, 25°C, and 33°C for both the reference and heatwave thermal pre-treatments. Id represents the unique identifier for each replicate.

Id	Thermal Pre-Treatments	Temperature (in °C)	Proportion	SE
T20CR12	Reference	20	0.65	0.11
T20CR15	Reference	20	0.81	0.09
T20CR16	Reference	20	1.00	0.00
T20CR17	Reference	20	0.85	0.08
T20CR3	Reference	20	0.74	0.10
T25CR1	Reference	25	0.72	0.11
T25CR2	Reference	25	0.94	0.06
T25CR3	Reference	25	0.74	0.10
T25CR5	Reference	25	0.80	0.09
T33CR1	Reference	33	0.58	0.11
T33CR10	Reference	33	0.05	0.05
T33CR20	Reference	33	0.75	0.10
T33CR3	Reference	33	0.58	0.11
T33CR4	Reference	33	0.45	0.11
T33CR5	Reference	33	0.79	0.09
T33CR6	Reference	33	0.55	0.11
T20HWR18	Heatwave	20	0.85	0.08
T20HWR19	Heatwave	20	0.68	0.10
T20HWR21	Heatwave	20	0.63	0.11
T20HWR8	Heatwave	20	0.81	0.09
T25HWR17	Heatwave	25	0.80	0.09
T25HWR18	Heatwave	25	0.55	0.11
T25HWR19	Heatwave	25	0.70	0.10
T25HWR2	Heatwave	25	0.75	0.10
T25HWR20	Heatwave	25	0.80	0.09
T25HWR9	Heatwave	25	0.81	0.09
T33HWR11	Heatwave	33	0.42	0.11
T33HWR15	Heatwave	33	0.75	0.10
T33HWR16	Heatwave	33	0.90	0.06



Id	Thermal Pre-Treatments	Temperature (in °C)	Proportion	SE
T33HWR19	Heatwave	33	0.94	0.06
T33HWR20	Heatwave	33	0.26	0.10





Chapter 4.

Impact of larval and adult exposure to heatwave conditions on host and parasitoid life-history traits

Thibault P. M. Costaz[1], Cristina Alban[1], Peter W. de Jong[1], Marcel Dicke[1], Joop J. A. van Loon[1]

Abstract

Understanding the effects of climatic variation and stochasticity on ecological processes is fundamental for predicting population dynamics under future climatic scenarios. Temperature plays a central role in population dynamics, especially for ectothermic organisms such as insects whose body temperatures and activity are directly dependent on ambient thermal conditions. In natural settings, the thermal conditions can vary substantially throughout an individual life span, and are likely to alter fitness in various ways. Here we assessed the effects of two fluctuating thermal regimes emulating either a non-stressful or a heatwave-like thermal environment on multiple life-history traits of a global insect pest, *Plutella xylostella* (Lepidoptera: Plutellidae) and two of its main larval parasitoids *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* Haliday (Hymenoptera: Braconidae). Exposure of larval and adult stages to different thermal environments had species- and trait-specific effects on life-history traits. Overall, *P. xylostella* showed a higher fecundity when experiencing the heatwave regime during adulthood, while exposure to this treatment in the larval stage had no effects. On the other hand, effects on egg maturation by both parasitoid species showed significant interaction between the thermal regimes experienced during the larval and adult stages. However, initial egg load (i.e., number of mature eggs at emergence) was not affected by the thermal regimes applied during their immature stage, suggesting a stabilising selection on this trait. This study highlights the necessity to include individual thermal history to predict the effects of rapidly changing thermal conditions on ecosystem processes.

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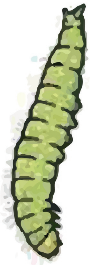
1. Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands

Introduction

Life-history theory predicts that an organism maximises its fitness within a given environment (Flatt and Heyland, 2011; Stearns, 1992). Fitness represents the capacity of an individual to produce viable offspring for the next generation and is the product of multiple life-history components, e.g. development, fecundity and survival (Amarasekare & Savage, 2012; Huey & Berrigan, 2001; Kingsolver & Huey, 2008; Stearns, 1992). Because resources are limited, not all traits can be simultaneously maximised, leading to trade-offs between competing traits (Ellers & van Alphen, 1997; le Lann et al., 2011). For example, an increase in fecundity usually reduces longevity (Berger et al., 2008). Many holometabolous insects have complex life cycles composed of egg, larval, pupal and adult stages and acquire crucial resources during the larval stage that in the adult stage will be allocated to either maintenance or reproduction (Boggs, 2009, 1981; Brown et al., 2004; Ellers and van Alphen, 1997; Jervis et al., 2008, 2005; O'Brien et al., 2002). Thus, such resource-related trade-offs occurring during a specific life-history stage impact future life-history traits (Bowler and Terblanche, 2008; Iltis et al., 2020; Kellermann et al., 2017).

Life-history traits are often plastic, meaning that environmental conditions influence their expression (Agrawal, 2001; Amarasekare and Savage, 2012; Fox et al., 2019; Whitman and Agrawal, 2009). Different environmental conditions will then yield different phenotypes affecting the realised fitness of an individual. Temperature plays a key role in driving the fitness of ectotherms (Angilletta, 2009; Huey & Berrigan, 2001; Kingsolver & Huey, 2008). For example, a well-known effect of temperature on insect phenotypic plasticity is the 'Temperature Size Rule' which states that higher developmental temperatures lead to smaller body sizes (Atkinson, 1994). Body size and development time are considered good fitness predictors as they correlate with the resources available for longevity and fecundity (Berger et al., 2008; Huey & Berrigan, 2001; Kingsolver & Huey, 2008). However, each trait can display a different degree of plasticity in response to temperature, highlighting the importance of measuring multiple life-history traits when assessing phenotypic plasticity (Iltis et al., 2019; Laughlin & Messier, 2015).

The thermal environment often fluctuates in natural settings, having consequences for ectotherm fitness (Bahar et al., 2013; Colinet et al., 2015). Especially in the context of climate change, climatic predictions show an increase in average temperature and larger thermal fluctuations, increasing the frequency of high extreme temperature events (Fischer & Schär, 2010; Pachauri et al., 2014). High extreme temperature events can occur at various stages of an insect life cycle and are likely to have complex consequences for fitness and population dynamics (Clavijo-Baquet et al., 2015; Iltis et al., 2019; Kingsolver and Buckley, 2020; Williams et al., 2016). Species are linked by direct and indirect interactions that structure ecological communities. The plastic response of one species is likely to alter the plastic response of the individuals it interacts with (Berg & Ellers, 2010; Boukal et al., 2019; Tituskin et al., 2021). It is therefore essential to assess the effects of the timing of high extreme temperature events on phenotypic plasticity of ectotherms and on their fitness in order to predict population dynamics under variable thermal environments (C.-S. Ma et al., 2021; Stoks et al., 2017; Williams et al., 2016; Zhu et al., 2019).



Parasitoid–host interactions are very suitable to study the effects of the different timing of high extreme temperature events on phenotypic plasticity. Such interactions are often very specialised as the parasitoid larvae feed in or on their host, representing a limited nutrient source, while the adults are free-living (Godfray, 1994). Competition for shared hosts between parasitoid species is common in ecosystems, and coexisting species often display different life-history strategies and host use (Harvey et al., 2013; Hawkins, 1990; le Lann, Visser, et al., 2014; Ode et al., 2022; Price, 1972). These differences are thought to reduce niche overlaps between competing species allowing their coexistence (le Lann et al., 2012). However, challenging thermal conditions can canalise the phenotypic expression of individuals across species to maximise fitness, reducing the range of ecological strategies available to an individual (Berg and Ellers, 2010; Terblanche et al., 2010). Assessing the temporal effects of fluctuating thermal regimes of different amplitudes on the resulting phenotypic plasticity of hosts and parasitoids can improve our understanding of ecological and evolutionary processes that occur in dynamic environments (Colinet et al., 2015; Hance et al., 2007; le Lann et al., 2021).

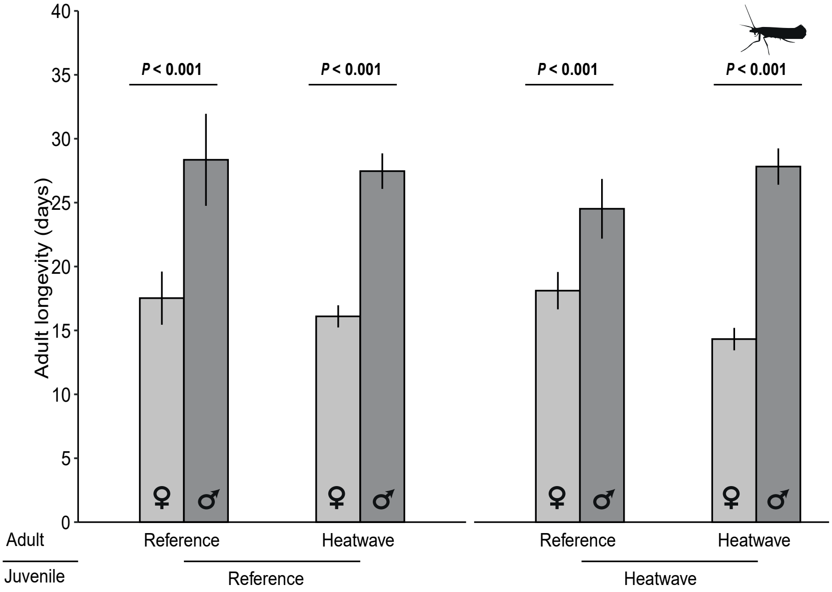
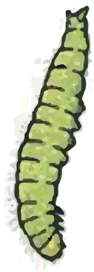


Figure 2: Effects of sex, developmental and adult thermal regime on adult longevity of the moth *Plutella xylostella*. Data represent mean ± standard error of the mean. The light and dark grey colours represent female and male moths, respectively. For details of the thermal regimes see Table 1

This study aimed at investigating the effects of a simulated heatwave occurring in the larval and adult phases on the expression of several life-history traits of a set of species connected by trophic interactions: the herbivore *Plutella xylostella* L. (Lepidoptera: Plutellidae) and two of its parasitoids, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* Haliday (Hymenoptera: Braconidae). Experimental designs measuring the plasticity of life-history traits should employ fluctuating thermal regimes mimicking natural conditions to allow relevant ecological interpretations (Bozinovic et al., 2016; Colinet et al., 2015; Paaijmans et al., 2013; Thompson et al., 2013; Vasseur et al., 2014). Therefore, we exposed the three species to two fluctuating thermal treatments in their larval and adult stages. The first simulated a heatwave, while the other simulated a non-stressful thermal environment. Higher trophic levels



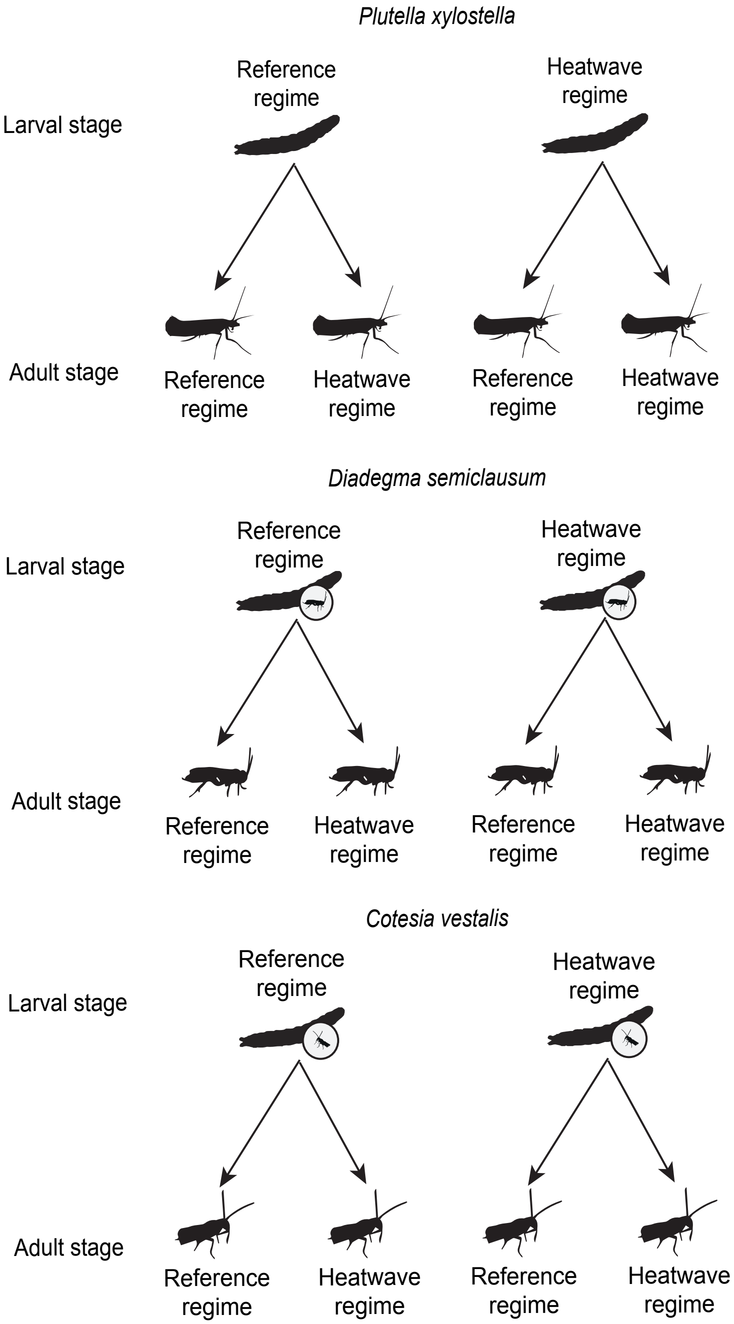
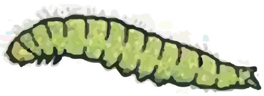


Figure 1: Schematic representation of the experimental design with the four combinations of thermal treatments: reference regime and heatwave regime applied at larval or adult stage for each species: the herbivore *Plutella xylostella*, and the parasitoids *Diadegma semiclausum*, and *Cotesia vestalis*. For details of the thermal regimes (see Table 1).



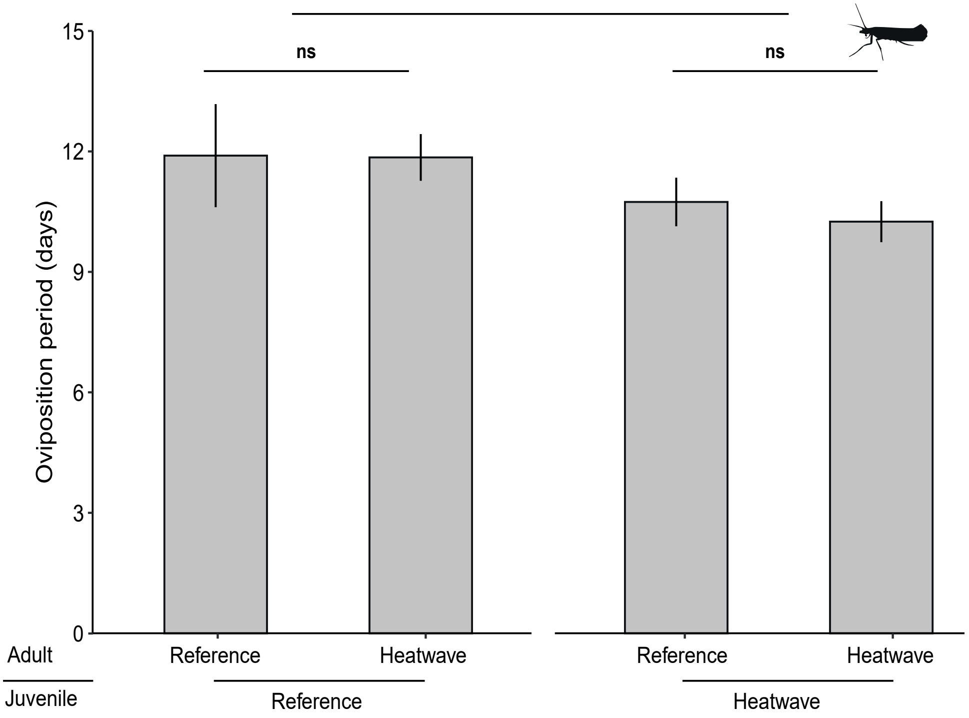


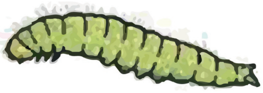
Figure 3: Effect of thermal regimes (reference and heatwave) during larval and adult stage on duration of *P. xylostella* oviposition period. Error bars represent ± standard error of the mean. For details of the thermal regimes see Table 1.

display greater thermal sensitivity (Furlong & Zalucki, 2017; Voigt et al., 2003); thus, a heatwave exposure is expected to have more deleterious effects on the two parasitoids than on their host, *P. xylostella*. According to the Temperature Size Rule, we hypothesised a smaller body size or mass of insects exposed to a heatwave in the larval phase (Atkinson, 1994). Similarly, insects experiencing a heatwave in the larval phase are expected to shift their reproductive efforts towards early in life and display reduced longevity.

Materials and Methods

Insect cultures

The insects used in this experiment, *P. xylostella*, *D. semiclausum*, and *C. vestalis*, originated from the surroundings of Wageningen University (the Netherlands) and were reared in our laboratory since 2017 for *P. xylostella* and *D. semiclausum* and 2018 for *C. vestalis*. New individuals were added every year to the stock colony in order to maintain their genetic diversity. *Plutella xylostella*, *D. semiclausum* and *C. vestalis* were reared on *Brassica oleracea* L. var. *gemmifera* cv. Cyrus (Brussels sprouts) in separate rooms at 22±2°C, 40-50% RH, and photophase 16L:8D.



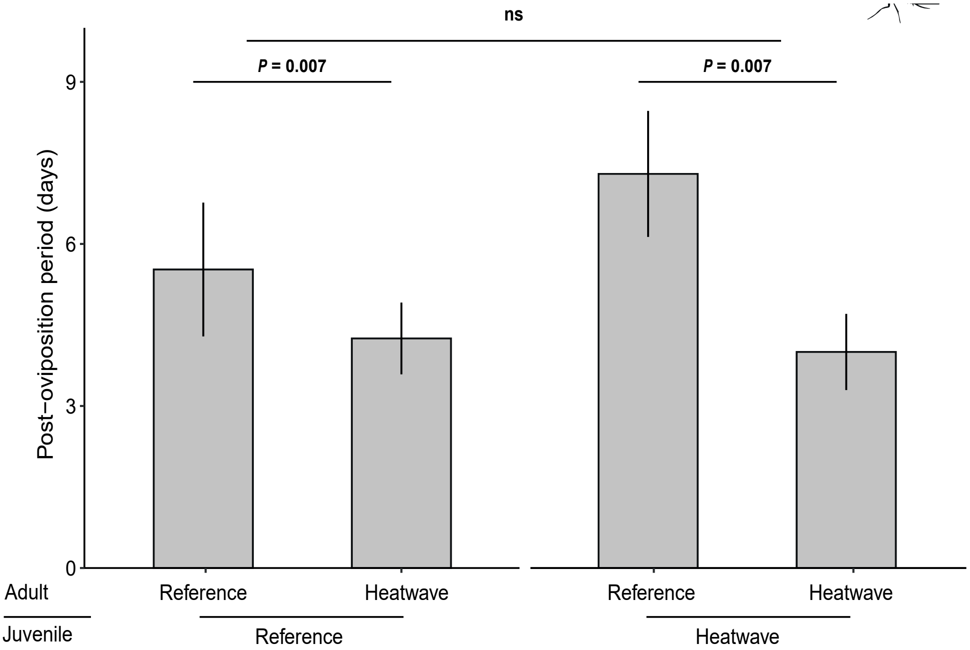
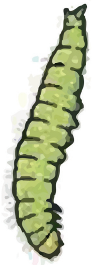


Figure 4: Effect of thermal regimes (reference and heatwave) during larval and adult stage on duration of *P. xylostella* post-oviposition period. Error bars represent ± standard error of the mean. For details of the thermal regimes see Table 1.

Experimental design

We defined two thermal regimes based on climatic data from the Royal Netherlands Meteorological Institute (KNMI). One represented "reference" conditions in the Netherlands using daily temperature variation for the months of June-July-August of the reference period 1961-1990. The second thermal regime simulated heatwave conditions with temperatures 3°C, 5°C and 8°C higher than in the reference treatment, respectively (de Boeck et al., 2010). The two thermal regimes had a 16L:8D photo: scotophase and the temperature in the climate cabinets (Snijders Scientific, type ECD01E) was set to resemble natural daily temperature variation (see Table 1 for the details of the temperature regimes).

Plutella xylostella eggs from the stock colony were collected on a sheet of Parafilm® (3M Company) smeared with Brussels sprouts extract, prepared by crushing Brussels sprouts leaves and adding a few ml of demi-water, after which the sheet was hung in a cage for 24 h. The *P. xylostella* eggs present on the Parafilm® were placed and incubated on Brussels sprouts plants in the reference regime until the larvae reached the second instar (L2). Then, the larvae were randomly assigned to one of three groups. One group was exposed to *D. semiclausum*, another to *C. vestalis* females, both for 8 h, and a third group was kept unparasitised.



The parasitised and unparasitised caterpillars were randomly placed back on Brussels sprouts plants in either the reference or heatwave regimes until pupation of the caterpillar or egression

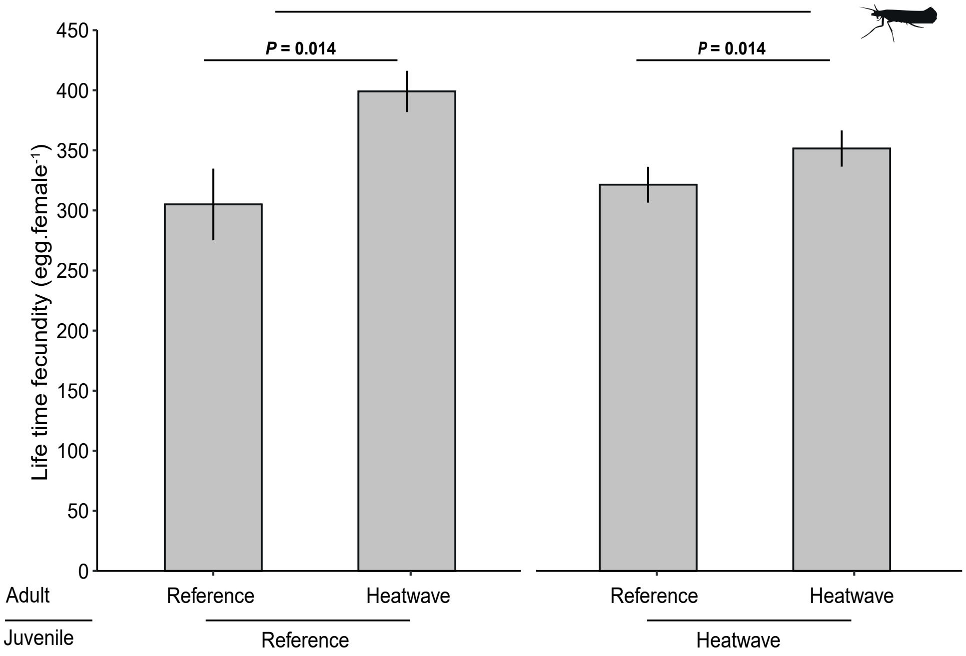


Figure 5: Effect of thermal regimes (reference and heatwave) during larval and adult stage on *P. xylostella* lifetime fecundity. Error bars represent ± standard error of the mean. For details of the thermal regimes see Table 1.

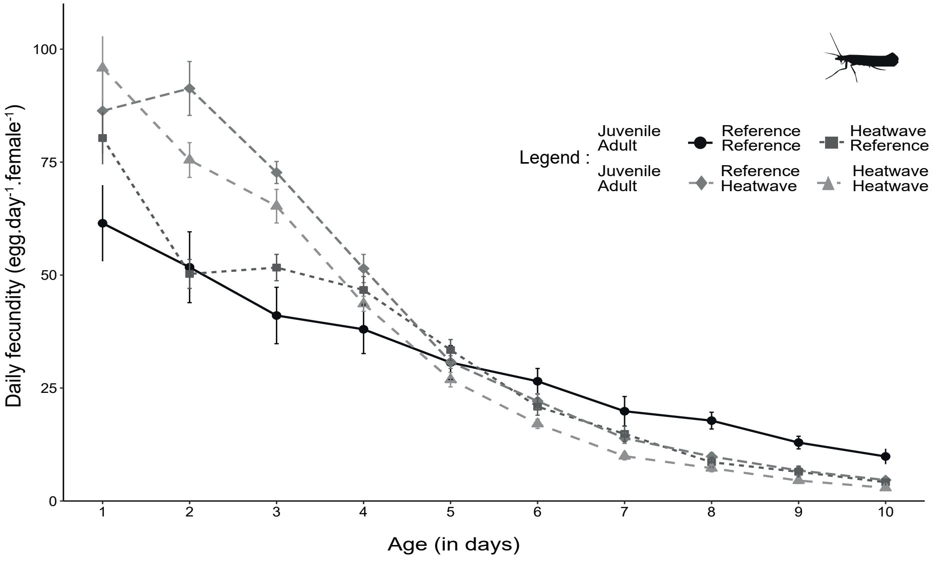
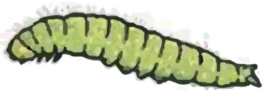


Figure 6: Effect of thermal regimes (reference and heatwave) during larval and adult stage on *Plutella xylostella* average daily fecundity. Error bars represent ± the standard error of the mean. For details of the thermal regimes see Table 1.



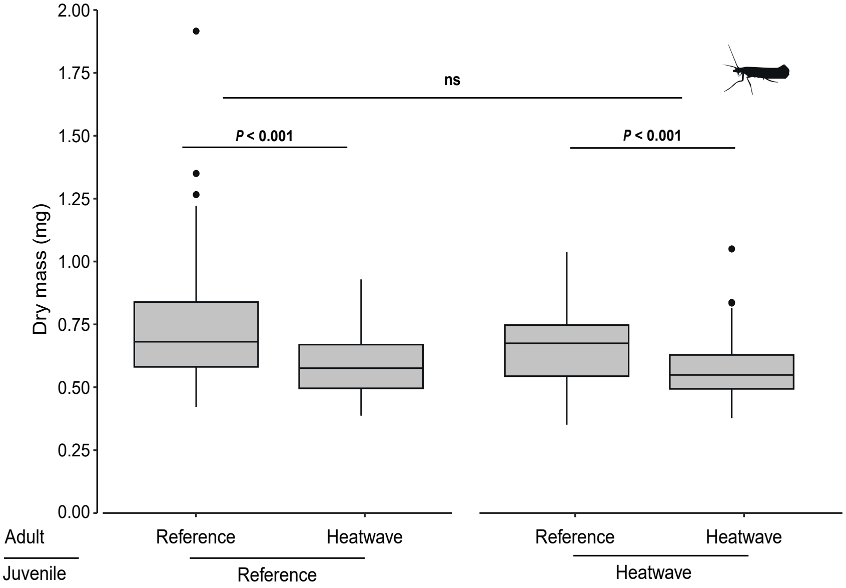


Figure 7: Effect of thermal regimes (reference and heatwave) during larval and adult stage on *P. xylostella* dry mass at death. Sex had no significant effect (P = 0.968), and the data for female and male were merged. For details of the thermal regimes see Table 1.

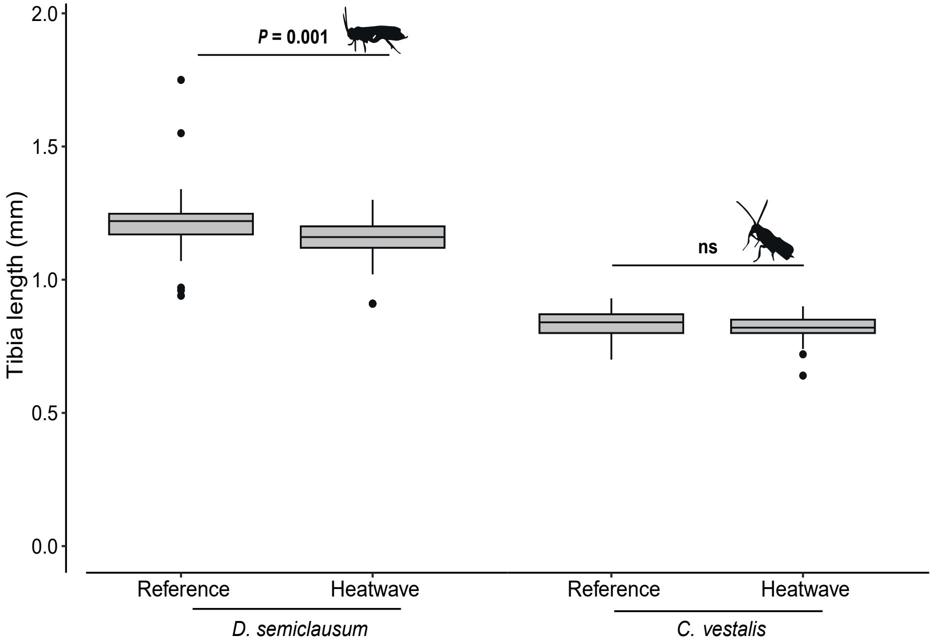
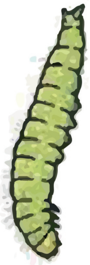


Figure 8: Effect of thermal regimes (reference and heatwave) for the parasitoid species *D. semiclausum* and *C. vestalis* on tibia length as a proxy for parasitoid size. For details of the thermal regimes see Table 1.



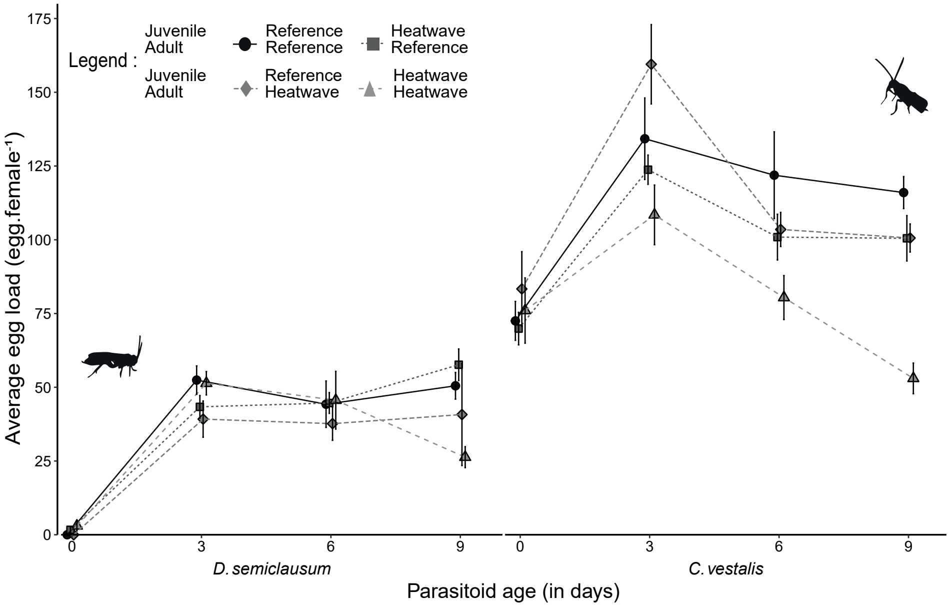


Figure 9: Effect of thermal regimes (reference and heatwave) during larval and adult stage on the average number of mature eggs at 0, 3, 6 and 9 days after emergence for the parasitoids *D. semiclausum* and *C. vestalis*. Error bars represent ± standard error of the mean. For details of the thermal regimes see Table 1.

of parasitoid larvae that subsequently pupated. Then the pupae were collected and individually stored in 24-well plates, covered by a mesh to allow for air exchange. The plates were checked daily, between 8 AM and 9 AM, for adult emergence.

Upon adult emergence, *P. xylostella* moths were paired (1:1 sex ratio) and exposed again to one of the two thermal regimes leading to four treatments (Figure 1). The adults were placed in plastic containers (115 X 107 mm, volume 770 mL; Gédé verpakkingen®, the Netherlands; hereafter “container”) closed by a mesh lid (Bugdorm®). A piece of cotton soaked in 10% honey solution and an egg-laying card wrapped in a sheet of Parafilm® and smeared with cabbage leaf extract was placed in the container. The pairs were monitored daily, between 9 AM and 12 AM, for survival and eggs laid until death. The cotton soaked in 10% honey solution was replaced daily. Because *P. xylostella* females did not only lay eggs on the egg-laying card but on the container and its lid, both were switched every day and the eggs were counted on the entire surface. After death, the moth was dried at 50°C for 24 h, and the dry body weight at death was measured on a microbalance (Sartorius CP2P, Göttingen, Germany). The total number of replicates performed per treatment varied between 19 and 29.

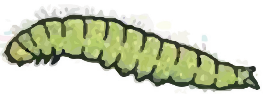


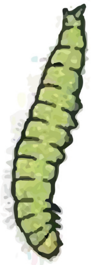
Table 1: Temperatures applied for five periods per day for the two thermal treatments. Reference and heatwave represent normal and heatwave conditions for the Netherlands measured for the years 1961-1990, respectively.

Hour	Reference (Temperature in °C)	Heatwave (Temperature in °C)
0-5	15	18
5-12	20	25
12-16	25	33
16-21	20	25
21-0	15	18

Diadegma semiclausum and *C. vestalis* adult males were frozen upon emergence. The unmated female wasps were split between the two thermal treatments leading to four treatments (Figure 1). The adult wasps were placed in plastic containers (Ø 115 X H 107 mm, volume 770 mL; Gédé verpakkingen®, the Netherlands; hereafter "container") closed by a mesh lid, and fed with 10% honey solution on a cotton ball, replaced when needed. The parasitoids were checked daily for survival. The females were kept for 0, 3, 6, or 9 days (±24 h) before being freeze-killed and dissected to assess egg load. The wasps were dissected in phosphate saline buffer solution under a stereomicroscope (Olympus-SZX2, Tokyo, Japan). Pictures of the mature eggs were taken with a camera mounted on the stereomicroscope (Euromex sCMEX, Arnhem, The Netherlands). The eggs were visually counted with ImageJ®. The length of the left hind tibia was measured as a proxy for parasitoid body size. The number of replicates per treatment varied from combination to combination due to different survival and ranged between 4 and 14. The exact numbers of replicates per combination can be found in the supplementary materials (Table S1). The experiment was repeated over time, switching the thermal treatments between climate cabinets to avoid experimental bias.

Overall, we measured *P. xylostella* adult longevity, end-of-life dry body mass, reproductive and post-reproductive period, daily and lifetime fecundity. For the two parasitoid species, we collected information on egg load at 0, 3, 6, and 9 days and the hind tibia length. The development time for all species was measured as the number of days between the start of the thermal treatments (L2 instar for *P. xylostella*, and eggs for *D. semiclausum* and *C. vestalis*) and adult emergence (Figure S1).

Data analysis



Plutella xylostella adult longevity, reproductive and post-reproductive period, and lifetime fecundity were analysed using a generalised linear model (GLM) assuming a Poisson distribution with a log error link. The larval thermal regime, the adult thermal regime, and the sex of the individuals (only for longevity) were used as explanatory variables. The models were checked for overdispersion and corrected if necessary using a GLM with a negative binomial distribution. The logarithm of *P. xylostella* dry body mass was analysed using a linear model with the developmental thermal regime, the adult thermal regime, and the sex of the individuals as explanatory variables.

To gain more insight into *P. xylostella* fecundity, we analysed its daily fecundity (number of eggs. day^{-1}.female^{-1}) using a generalised mixed-effect model (GLMM) accounting for repeated measures using the female moth ID as a random factor on the intercepts. We assumed a negative binomial distribution and a log link. The fixed variables were larval, adult thermal regimes and moth age. Because the relationship between fecundity and time is not linear, we only selected the moths' early reproductive period (moth age < 7 days and daily egg count > 0) approximating a linear function.

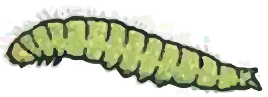
The parasitoid egg loads at 3, 6, and 9 days were analysed separately from the initial egg load at day 0. Both variables were analysed using a GLM assuming a negative binomial distribution (to correct for overdispersion). In the model for egg load at 3, 6 and 9 days, parasitoid species, developmental thermal regime, adult thermal regime and time (in days) were used as explanatory variables. Both parasitoid species were analysed separately. For the initial egg load, *D. semiclausum* showed almost strict syn-ovigeny. Only *C. vestalis* initial egg load was analysed with the developmental thermal regime as the only explanatory variable. Parasitoids that did not reach an age of 3, 6, or 9 days old were not included in the analysis.

All models were validated using graphical displays of model residuals. The non-significant interactions (*P* > 0.05) were removed from the models. All analyses were performed in R version 4.0.2 (R Core Team, 2019). The Poisson and negative binomial GLMs were performed using the "lme4" and "MASS" packages, respectively (Bates et al., 2015; Venables & Ripley, 2002). The GLMM was performed using the "nlme" package (Pinheiro et al., 2020). If significant effects were found, Tukey post hoc tests were performed for pairwise comparison using the "emmeans" or "rcompanion" packages (Mangiafico, 2021; L. Russell, 2018). The figures were produced using the "ggplot2" package (Wickham, 2016).

Results

Plutella xylostella adult longevity was significantly shorter for female moths than for male moths (χ^2= 66.13, df = 1, P < 0.001) but was neither affected by adult thermal regime (χ^2= 0.57, df = 1, P = 0.450) nor by larval thermal regime (χ^2= 0.79, df = 1, P = 0.373) (Figure 2). However, the length of female reproductive period was significantly reduced by exposing the larvae to the heatwave regime (χ^2= 3.91, df = 1, P = 0.048) but not by exposure to the adult thermal regime (χ^2= 0.20, df = 1, P = 0.656) (Figure 3). In contrast, the post-reproductive period was reduced by exposing the adults to the heatwave regime (χ^2= 7.28, df = 1, P = 0.007) but not by the larval thermal regime (χ^2= 0.42, df = 1, P = 0.517) (Figure 4).

Lifetime fecundity of *P. xylostella* was enhanced by exposing the adults but not the larvae to the heatwave regime (adult thermal regime:χ^2= 6.02, df = 1, P = 0.014; larval thermal regime: χ^2= 0.35, df = 1, P = 0.553) (Figure 5). The number of eggs laid daily was significantly affected by moth age (χ^2=618.47, df = 1, P <0.001), adult thermal regime (χ^2= 8.68, df = 1, P = 0.003) and the interactions between larval and adult thermal regimes (χ^2= 5.62, df = 1, P = 0.020), larval thermal regime and moth age (χ^2= 5.87, df = 1, P = 0.015) and adult thermal regime and moth age (χ^2= 31.46, df = 1, P < 0.001) (Figure 6). Overall, the number of eggs laid daily was significantly higher in the adult heatwave regimes but showed a steeper decrease over time than in the reference thermal regimes. Similarly, exposing larvae to the heatwave regime accelerated the number of eggs laid

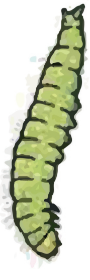


daily. The adult heatwave regime significantly reduced moth dry mass at death (F = 1.45, df = 1, $P < 0.001$) (Figure 7). Parasitoid tibia length, as a proxy for parasitoid size, was significantly affected by the interaction between larval thermal regime and parasitoid species (F = 3.90, df = 1, $P = 0.05$). Exposure to the heatwave regime in the larval phase reduced body size compared to individuals exposed to the reference regime for *D. semiclausum* ($P = 0.001$) but not for *C. vestalis* ($P = 0.374$) (Figure 8). The initial egg load of *D. semiclausum* was consistently zero upon emergence except for five individuals reared in the heatwave regime having between 2 and 9 mature eggs present in their ovaries on the day of emergence. *Cotesia vestalis*, on the other hand, was more pro-ovigenic, with an average of 75 mature eggs present in their ovaries upon emergence. However, the thermal regime applied during the larval phase did not alter *C. vestalis* initial egg load ($\chi^2 = 0.42$, df = 1, P = 0.516) (Figure 9).

In *D. semiclausum*, the mature egg load over time (i.e. from day 3 to 9) was significantly altered by the adult thermal regime ($\chi^2 = 6.06$, df = 1, P = 0.014), the interaction between adult thermal regime and age ($\chi^2 = 9.15$, df = 1, P = 0.002) and the three-way interaction between age, larval and adult thermal regimes ($\chi^2 = 6.45$, df = 1, P = 0.011) (Figure 9). Overall, *D. semiclausum* female parasitoids exposed in the larval phase to the reference thermal regime had a constant egg load across days 3 to 9, irrespective of the adult thermal regime. In contrast, parasitoids exposed to the heatwave regime during the larval phase had a decreasing egg load from day 3 to 9 when exposed to the heatwave thermal regime in the adult stage. When exposed to the reference thermal regime in the adult stage, the egg load increased from day 3 to 9.

For *C. vestalis*, age, larval and adult thermal regimes significantly affected the number of mature eggs (age: $\chi^2 = 30.27$, df = 1, P < 0.001; development: $\chi^2 = 24.97$, df = 1, P < 0.001; and adult: $\chi^2 = 12.05$, df = 1, P < 0.001). Moreover, the interaction between larval and adult thermal regime, and between adult thermal regime and moth age also significantly affected *C. vestalis* egg load (development X adult: $\chi^2 = 6.06$, df = 1, P = 0.014; adult X age: $\chi^2 = 10.19$, df = 1, P = 0.001) (Figure 9). Overall, *C. vestalis* female parasitoids had decreasing egg load from day 3 to day 9. Wasps exposed to heatwave conditions during the larval phase had fewer eggs than wasps reared as larvae in the reference regime. The interactions between larval and adult regimes had a negative effect when the wasps were exposed to the heatwave regime at both stages, but heatwave conditions occurring at either life stage had no effects compared to the wasps exposed to the reference thermal regime during both larval and adult stages. The decrease of *C. vestalis* mature egg load had a steeper slope when exposed to the heatwave regime than to the reference regime during its adult phase.

Discussion



This study assessed the effects of two daily thermal cycles emulating non-stressful or heatwave-like conditions when applied during the larval and/or adult life stages on life-history traits of the herbivore *P. xylostella* and two of its parasitoids, *D. semiclausum* and *C. vestalis*. Our results revealed the complex interplay between the effects of thermal conditions experienced during the larval and adult phases on different life-history traits related to fitness. Daily fecundity and age-specific egg load for the moth and parasitoid respectively, displayed the most striking changes in response to thermal conditions experienced throughout the life cycle. However, each species had its fecundity affected in a specific way. Overall, *P. xylostella* experiencing the heatwave-like thermal conditions during the adult stage displayed higher daily oviposition during their early life and increased its lifetime fecundity. Heatwave-like thermal conditions experienced during the larval phase, on the other hand, most likely accelerated egg maturation allowing female moths to shorten their oviposition period without impacting their lifetime fecundity. Contrary to our expectations, both parasitoid species did not increase their initial egg load when reared in the heatwave-like thermal conditions suggesting that other evolutionary constraints influence parasitoid ovigeny index (i.e., the initial egg load divided by the potential lifetime fecundity). Both species displayed different degrees of syn-ovigeny, and the thermal conditions experienced during the larval and adult stages resulted in age-specific egg loads. These results suggest that the thermal history experienced by an individual throughout its life cycle has important consequences on its fitness and are species-specific.

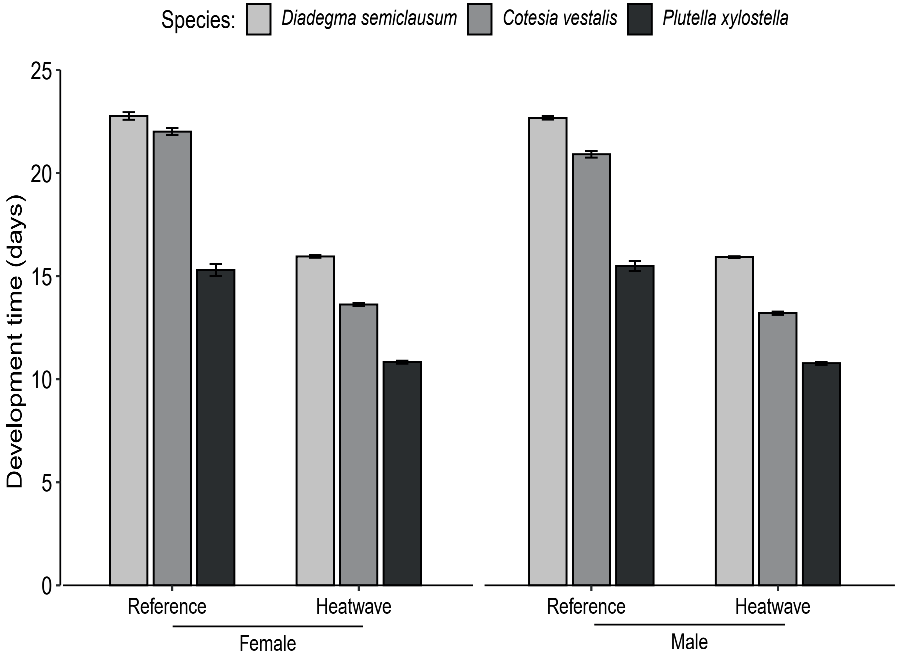
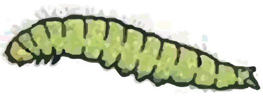


Figure S1: Development time (in days) observed in the two thermal regimes from egg to adult for D. semiclausum (light grey), C. vestalis (medium grey), and from 2nd instar to adult for P. xylostella (dark grey), respectively. Error bars represent ± standard error of the mean.

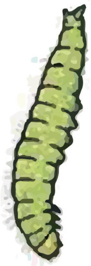
Thermal variation directly affects physiological rates, with each species and trait having its own thermal optimum and -tolerance (Iltis et al., 2019). The correlation between temperature and growth rates usually has an asymmetrical curvilinear shape (Atkinson, 1994; Brown et al., 2004; Colinet et al., 2015). Thus, higher fluctuations and mean temperature can either bring individuals or traits closer to their thermal optimum or to their critical maximum relative to their thermal tolerance (Colinet et al., 2015; le Lann et al., 2011; Moore et al., 2021). In the latter case, cellular damage caused by the destruction of cell membranes and protein denaturation can occur. The induction of physiological repairs and biosynthesis of chaperone molecules is expected to increase trade-offs between maintenance and reproduction. However, insects may



mitigate the effects of environmental stress experienced in the larval stage to the adult stage via physiological re-organisation during metamorphosis (Potter et al., 2011; Xing et al., 2014). Based on our results, we argue that both processes are at play and plasticity differences reflect trade-offs between life-history traits relative to the species-specific ecological strategies.

Previous studies on the thermal plasticity of parasitoids in terms of body size have found contrasting results with thermal fluctuation inducing smaller or bigger body sizes (Colinet et al., 2007; Schreven et al., 2017). *Diadegma semiclausum* larvae feed on host haemolymph and tissues, ultimately consuming the entire host except its head capsule, while *C. vestalis* only feeds upon host haemolymph and exits the host before pupating (Godfray, 1994). Parasitoids have evolved to optimise host resource utilisation and trade between extended development time to grow bigger and lower survival probability due to predation (Brodeur & Boivin, 2004). Differences in host utilisation could explain the reduction in body size displayed by *D. semiclausum* but not by *C. vestalis*. *Cotesia vestalis* was found to prioritise its body size and developmental time (Harvey et al., 2014; Harvey & Strand, 2002). In addition, *D. semiclausum* is considered to be less thermotolerant than *C. vestalis*; thus, warmer conditions can have more stressful impact on this species (Ngowi et al., 2019; Talekar & Yang, 1991). *Plutella xylostella*'s end-of-life dry body mass was significantly reduced by exposure to the heatwave regime experienced during adulthood, suggesting that the moths used more stored energetic reserves than adults experiencing the reference thermal regime (Colinet et al., 2007).

Both parasitoid species display very different reproductive strategies. *Diadegma semiclausum* is strictly synovigenic and produces large anhydropic (yolk-rich) eggs. In contrast, *C. vestalis* is partially pro-ovigenic and produces small hydropic (yolk-poor) eggs (Jervis et al., 2008). This results in a slow egg maturation for *D. semiclausum* and a more rapid egg maturation for *C. vestalis* (Harvey et al., 2014). Our results align with this, as *C. vestalis* had a higher egg load than *D. semiclausum* across all time points. The number of mature eggs present at the three time points was significantly affected by different larval and adult thermal regime combinations for both parasitoid species. For *C. vestalis,* the larval heatwave regime reduced its egg load, while for *D. semiclausum*, it either increased or decreased egg load depending on the type of thermal regime occurring in the adult stage. Most parasitoid species lack lipogenesis in the adult stage, and all lipidic resources required for egg maturation must be acquired during the larval stage (Jervis et al., 2008; Visser & Ellers, 2008). However, synovigenic parasitoids can re-absorb mature eggs and thus control the number of mature eggs present in their ovaries dependent on their physiological status (e.g. age, lipidic reserves) and host availability (Ellers & Jervis, 2003). In this study, both moths and parasitoids had *ad libitum* access to a sugar source during adulthood, likely reducing the energetic trade-offs between maintenance and egg maturation (Jervis et al., 2008). Intriguingly, the initial egg load of both parasitoid species was unaffected by the larval thermal regime. The lack of plasticity in this trait contrasts with the shift towards earlier reproduction observed in *Aphidius ervi* Haliday (Hymenoptera: Braconidae) developing under warmer conditions (Moiroux et al., 2018). However, it may be adaptive for the female parasitoid to match egg maturation with environmental quality, such as host availability (Berger et al., 2008; le Lann et al., 2021). Therefore, the lack of plasticity in the initial egg load may result from eco-evolutionary processes. More detailed studies are needed to assess the combined effects of multiple environmental conditions (e.g., host patch, host density, temperature, flower nectars) on the evolution of parasitoid life-history strategies such as ovigeny. *Plutella xylostella*'s lifetime fecun-

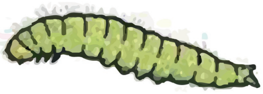


dity was significantly larger under adult heatwave conditions than under reference conditions but was not affected by the thermal regime applied during the larval phase. These findings align with previous results on the cross-stage effects of heat stress on *P. xylostella* life-history traits showing diminishing temperature effects as heat stress occurred further away from the adult stage (Zhang et al., 2015a, 2015b). However, the reproductive period was shortened by exposure to a heatwave during the larval phase, most likely resulting from faster egg maturation rates (Xing et al., 2019). This is further highlighted by the significant interactions between the larval thermal regime and moth age. Adult exposure to the heatwave regime enhanced *P. xylostella* fecundity (this study), which was likely caused by an accelerated egg maturation rate and oviposition rate as suggested by other studies (Berger et al., 2008; Colinet et al., 2015). Moreover, adult feeding may lead to reduced energetic trade-offs between adult maintenance and reproduction, allowing a larger lifetime fecundity (Jervis et al., 2005; O'Brien et al., 2000, 2002, 2004).

In combination with recent literature, our study confirms earlier predictions on the vulnerability of parasitoids, in comparison to their herbivorous hosts, to extreme temperature events such as heatwaves (Furlong & Zalucki, 2017; Voigt et al., 2003). The host *P. xylostella* displayed better capabilities to mitigate the effects of thermal conditions experienced during its larval or adult stages to maximise its fecundity. In contrast, parasitoid initial egg load was not altered by the thermal conditions experienced during larval stages. Parasitoids have limited access to resources as they are confined within their host and are limited in their capacity to mitigate the effects of exposure to extreme temperature events during their larval stage. It is important to note that interaction between thermal regime and host-plant species likely plays a crucial role in driving host and parasitoid fitness through bottom-up effects affected by plant chemistry (Gillespie et al., 2012; Gols and Harvey, 2009b; Jamieson et al., 2017; Zhaozhi et al., 2017). Previous work demonstrated the inability of *D. semiclausum* to pupate from *P. xylostella* larvae feeding on *Brassica napus* (Brassicaceae) at 35°C (Dosdall et al., 2012). Bottom-up effects from plant chemistry are therefore likely to modulate life-history plasticity in response to thermal conditions and have repercussions on host-parasitoid interactions (Gillespie et al., 2012; Gols and Harvey, 2009b; Jamieson et al., 2017). Placing these results in the context of climate change, the increase in magnitude and frequency of extreme temperature events is likely to drive complex responses in an insect community via multiple and contrasting combinations of effects on species as well as cascading effects from trophic and non-trophic species interactions, thus profoundly altering ecological community networks (C. Chen et al., 2019a; le Lann et al., 2021; C.-S. Ma et al., 2021).

Acknowledgements

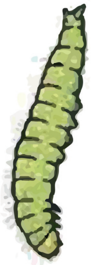
We thank the rearing staff of our laboratory, Pieter Rouweler, André Gidding, and the late Frans van Aggelen for maintaining the insect colonies used in this study. We would also like to thank the personnel of Unifarm for providing the plant materials needed in this study.

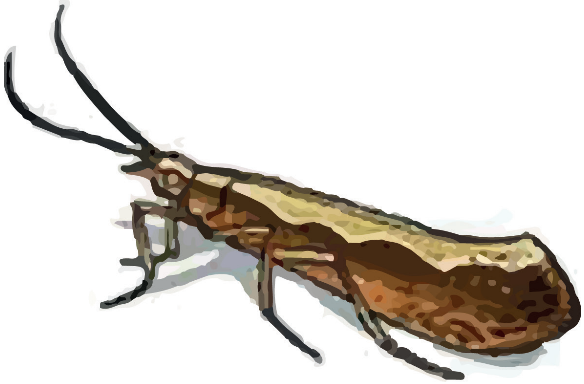


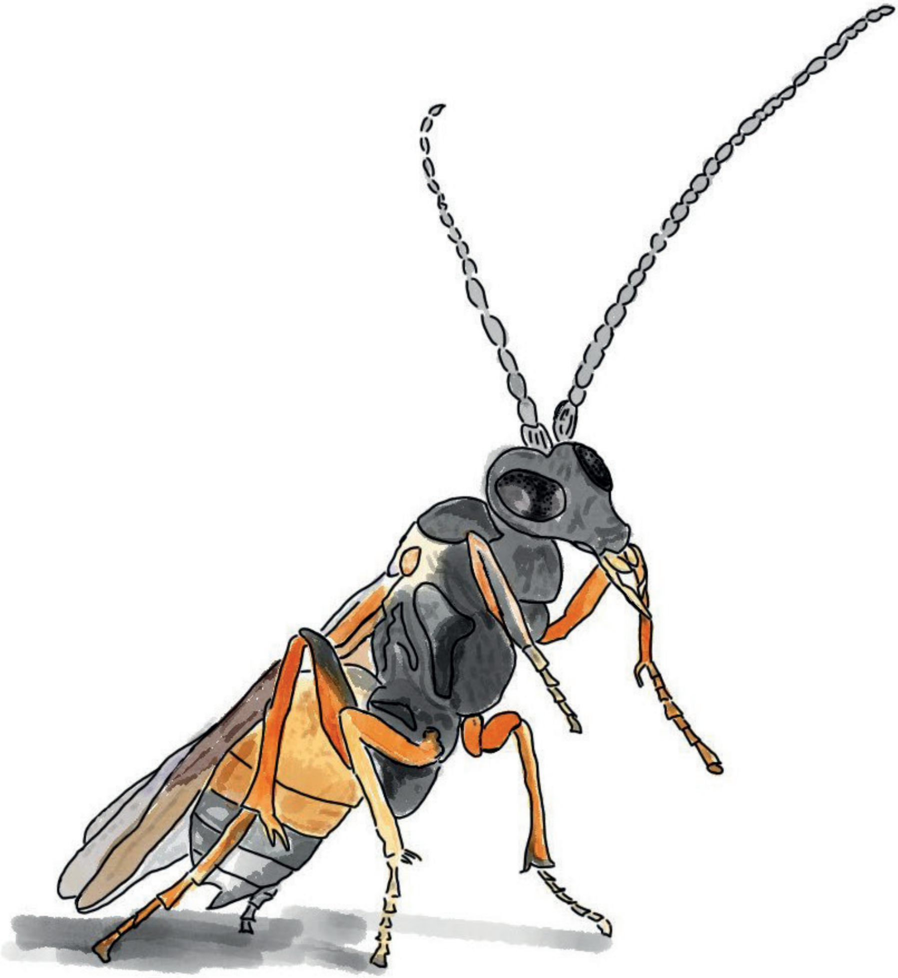
Supplement

Table S1: Number of replicates included in the parasitoid egg load maturation analysis. Species represent the two parasitoid species studied. Larval treatment and adult treatment are the thermal regimes applied during developmental and adult stages, respectively. Age is the parasitoid adult age in days and n represents the number of replicates included in the analysis.

Species	Larval treatment	Adult treatment	Age	n
D. semiclausum	Reference	Reference	3	7
D. semiclausum	Reference	Heatwave	3	9
D. semiclausum	Reference	Reference	6	8
D. semiclausum	Reference	Heatwave	6	9
D. semiclausum	Reference	Reference	9	8
D. semiclausum	Reference	Heatwave	9	4
D. semiclausum	Heatwave	Reference	3	14
D. semiclausum	Heatwave	Heatwave	3	14
D. semiclausum	Heatwave	Reference	6	12
D. semiclausum	Heatwave	Heatwave	6	5
D. semiclausum	Heatwave	Reference	9	11
D. semiclausum	Heatwave	Heatwave	9	10
C. vestalis	Reference	Reference	3	8
C. vestalis	Reference	Heatwave	3	6
C. vestalis	Reference	Reference	6	9
C. vestalis	Reference	Heatwave	6	8
C. vestalis	Reference	Reference	9	8
C. vestalis	Reference	Heatwave	9	5
C. vestalis	Heatwave	Reference	3	8
C. vestalis	Heatwave	Heatwave	3	9
C. vestalis	Heatwave	Reference	6	10
C. vestalis	Heatwave	Heatwave	6	10
C. vestalis	Heatwave	Reference	9	8
C. vestalis	Heatwave	Heatwave	9	6







Chapter 5.

Temperature and density interact in affecting the competition outcomes between two sympatric endoparasitoids

Thibault P. M. Costaz[1], Peter W. de Jong[1], Jeffrey A. Harvey[2], Joop J. A. van Loon[1], Marcel Dicke[1], Rieta Gols[1]

Abstract

Under anthropogenic climate change, the frequency and magnitude of climatic extremes, such as extreme temperature events (ETEs), are predicted to increase. This will impose stress on ectothermic organisms, such as insects, at both physiological and behavioral levels. Via species-specific effects ETEs are likely to cause disruption of interactions between organisms and may give rise to novel communities. Examining the effects of ETEs within and between different trophic levels is crucial for understanding their broader implications on community and ecosystem-level processes. Here, we studied extrinsic competition under variable temperatures, emulating ETE conditions, in two endoparasitoid species with overlapping host niches. Two combined experiments were performed to study the effect of parasitoid density, when foraging at 22°C, 27°C or 33°C, on parasitism rate. We measured parasitism rate of two sympatric endoparasitoid species, *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* (Hymenoptera: Braconidae), in their shared host, caterpillars of *Plutella xylostella* (Lepidoptera: Plutellidae), on *Brassica oleracea* (Brassicaceae) plants. When each species foraged alone, the rate of parasitism increased with increasing parasitoid density. When both species foraged simultaneously, parasitism rate by *D. semiclausum* was not affected at 22°C but decreased at 27°C and 33°C. Parasitism rate by *C. vestalis* was reduced in the presence of *D. semiclausum* at all temperatures. Parasitism rates by each species tended to converge at higher temperatures. These results suggest that *D. semiclausum* is an extrinsically stronger competitor than *C. vestalis*, but the latter appears to exhibit more thermal resilience, reducing competitive differences at higher temperatures. Increases in the magnitude and frequency of ETE under climate change are likely to have differential effects on species involved in intimate interactions, depending on the community species composition.

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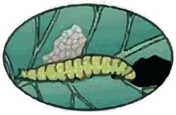
1. Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands
2. Department of Terrestrial Ecology, Netherlands Institute of Ecology, Droevendaalsesteeg 10, 6708PB, Wageningen, The Netherlands

Introduction

Anthropogenic climate change and associated processes such as extreme climatic events (e.g. heat waves, droughts, rain downpours and fire) strongly impact biodiversity as well as on a range of ecological interactions (Boeck et al., 2018; Dee et al., 2020; Dillon, Woods, Pincebourde, et al., 2016; Garcia et al., 2014; Greenville et al., 2018; Parmesan et al., 2000). In response to seasonal shifts in temperatures, many species have extended their ranges pole-wards or to higher elevations, sometimes resulting in the formation of novel ecological interactions (Parmesan & Yohe, 2003; Robinet & Roques, 2010). If disruption of ecological interactions occurs, it will propagate throughout the ecological community assembly having repercussions on ecosystem functioning (Thébault & Loreau, 2005; Valiente-Banuet et al., 2015). Because of their unpredictability, extreme temperature events (ETEs) pose even greater challenges. Short-term exposure to extremely high temperatures, can negatively affect species performance via physiological and behavioral mechanisms, thus altering interaction strengths between species (Abram et al., 2017; Amarasekare, 2019; Angilletta, 2009). Because the range of temperature tolerance is species-specific, the effects of exposure to ETEs on species interactions are highly variable and still poorly understood (Harvey et al., 2020; Rosenblatt et al., 2019; Stoks et al., 2017). Studies examining the impacts of ETEs on species interactions are therefore urgently needed to better understand the broader impacts of climate change on communities and ecosystems.

Among the countless number of species interactions, those between hosts and parasitoids have received considerable attention due to their significant role in regulating insect herbivore populations (Godfray, 1994; Stireman et al., 2005; Waage, 1982). Adult parasitoids are free-living organisms, while their offspring are intimately associated with a single host (Godfray, 1994). In nature, it is not rare to find several parasitoid species attacking the same host species (Hawkins, 1990). When parasitoid densities are high, there may be strong intra- or interspecific competition among female parasitoids for access to hosts (extrinsic competition) (Cusumano et al., 2016; Wajnberg, 2006). Similarly, parasitoid larvae may compete with one another for the same host resources (intrinsic competition) (Godfray, 1994; Harvey et al., 2013). The outcome of competition between parasitoids depends on many factors linked to the environmental context and species traits in which the competition occurs. Species traits, such as host foraging efficiency, host specificity, egg load and parasitoid age can influence how female parasitoids interact. In addition, abiotic and biotic environmental factors, habitat characteristics, and temperature can modulate the outcome of competition (Cusumano et al., 2016; Harvey et al., 2013; Poelman et al., 2014; Vayssade et al., 2012).

Optimal foraging models predict that female parasitoids should try to maximize fitness gain while foraging for hosts that are often patchily distributed (Hubbard & Cook, 1978; Wajnberg, 2006; Wajnberg et al., 2008). Parasitoid foraging behavior is affected by a range of cues including host density, the presence of other foraging parasitoids and the parasitism status of the host (Godfray, 1994; Vet, 2001; Wajnberg et al., 2008). The marginal value theorem projects that patch time allocation by foraging parasitoids should increase with increasing host density (Charnov, 1976; McNair, 1982; Nonacs, 2001; Wajnberg et al., 2000). Parasitoid aggregation (i.e., increased parasitoid density) can lead to antagonistic encounters among different individuals leading to a decrease in *per capita* parasitism success (Hassell, 1971; Hassell & Varley, 1969; Mohamad et al., 2015). Interference competition in parasitoid wasps is thought to play a



significant role in driving the evolution of behavioral, physiological, and morphological traits in parasitoids, as well as in structuring parasitoid communities (Bonsall et al., 2002; Hood et al., 2021; Ode et al., 2022; Price, 1972; van Alphen & Visser, 1990).

Parasitoids, like all insects, are ectotherms. Thus, temperature plays a vitally important role in their physiology and performance (Colinet et al., 2015; Hance et al., 2007). Temperature alters parasitoid foraging efficiency, walking speed, attack rates, and host age preference (Augustin et al., 2020; le Lann, Lodi, et al., 2014; Moiroux et al., 2015, 2016). Parasitoid thermal tolerance is mostly species-specific, although other factors, such as the geographic origin or the thermal history of an individual can modify its thermal tolerance (Bowler and Terblanche, 2008; Diamond et al., 2017; Tougeron et al., 2020). Divergent thermal tolerance between sympatric parasitoid species was found to allow their coexistence *in situ* (le Lann et al., 2011; Mutamiswa et al., 2018). Moreover, many studies report diverging thermal tolerance between hosts and their associated parasitoids, with in most cases the parasitoid having a lower thermal tolerance than its hosts (Agosta et al., 2018; Furlong & Zalucki, 2017; Moore et al., 2021; Schreven et al., 2017). For example, species whose native geographical ranges are located at lower latitude may be better adapted to warmer and more frequent ETE than species whose distributions are located at higher latitudes (Carbonell & Stoks, 2020; le Lann et al., 2021). Studies assessing the effects of temperature on parasitoid competition are rare (but see (C. Chen et al., 2019b) and references therein). Increased exposure to high temperatures under climate warming might alter the interaction strength in host-parasitoid networks, potentially resulting into new community assemblies followed by local species extinction and periodic herbivore outbreaks (Harvey et al., 2020; Stoks et al., 2017; Thierry et al., 2019). Understanding the impacts of extremely high temperature events on species interactions is of utmost importance to better predict the effects of climate change on communities and ecosystems (Berg and Ellers, 2010; C.-S. Ma et al., 2021).

The aim of this study is to assess the effects of adult female parasitoid density and temperature, including simulated temperature extremes, on the outcome of extrinsic intra- and interspecific competition between the solitary endoparasitoids, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* Haliday (Hymenoptera: Braconidae) foraging for caterpillars of their shared host, the diamondback moth *P. xylostella* L. (Lepidoptera: Plutellidae). Both parasitoid species attack L2-L4 instars of *P. xylostella* and are among its most important natural enemies in the field, where it is a major pest of brassicaceous crops worldwide (Furlong et al., 2013; Sarfraz et al., 2005). They have also been successfully introduced in many temperate and tropical countries as biological control agents of *P. xylostella* (Furlong et al., 2013; Talekar & Shelton, 1993). It has been reported that *C. vestalis* has a broader host range than *D. semiclausum* and has a more southerly distribution in the Palearctic realm, although local distributions of these two species do overlap in many areas (Afiunizadeh & Karimzadeh, 2015; Ngowi et al., 2019; Sarfraz et al., 2005; Talekar & Shelton, 1993). An initial experiment was conducted to assess the effects of parasitoid density on both intra- and interspecific competition under a 'normal' summer temperature in the Netherlands (T_{max} = 22°C, average for 1991-2020)[2]. A second experiment focused on the impact of increased temperature on the outcome of intra- and interspecific competition (at a fixed parasitoid density). We used a set of three temperatures (22°C, 27°C and 33°C) with the latter two corresponding to temperatures exceeding the 95th percentile

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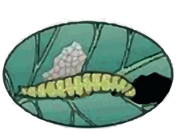
[2] https://www.knmi.nl/klimaat-viewer/kaarten/temperatuur



of temperature distribution in the Netherlands1. We expected that under 'normal' temperature conditions, *D. semiclausum* to be a superior extrinsic competitor to *C. vestalis*, although contrasting field prevalence has been reported for both species (Afiunizadeh & Karimzadeh, 2015; Ngowi et al., 2019). We also hypothesized that *D. semiclausum* but not *C. vestalis* parasitism rate will be negatively impacted by temperature above 30°C as the former is less thermotolerant than the latter (Furlong & Zalucki, 2017; Sarfraz et al., 2005). We expected that as the parasitoid density increases, the number of parasitized hosts should quickly reach an asymptote due to increasing levels of mutual interference between the two species (Hassell, 1971). We discuss the importance of stochastic temperature extremes on competitive interactions among parasitoids and implications of this for species coexistence, host dynamics and community-level processes.

Materials & Methods

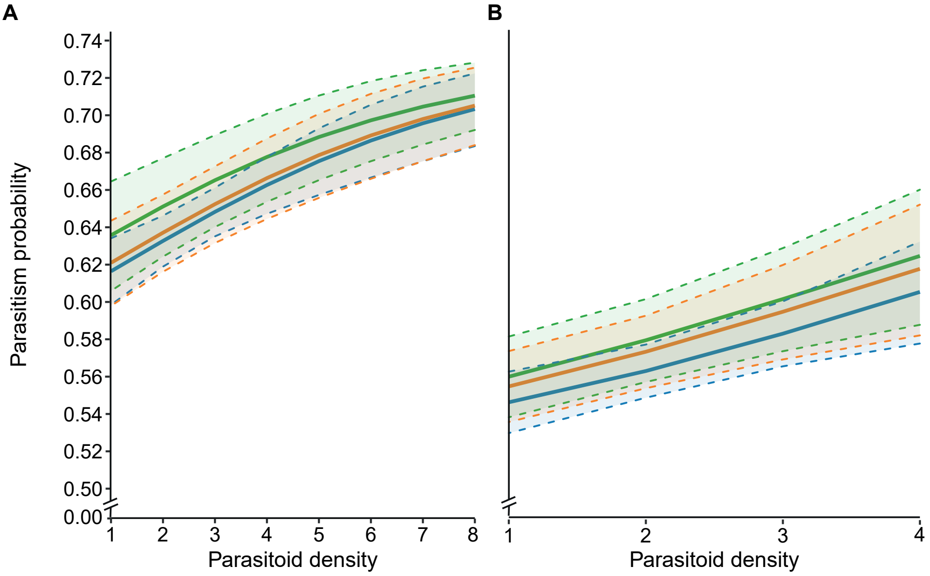
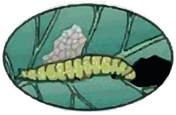


Figure 1: Parasitism probability (i.e., proportion of parasitoid pupae) by parasitoid density of foraging A. *D. semiclausum* and B. *Cotesia vestalis*. The blue, green, and orange solid lines represent the three temperatures 22°C, 27°C and 33°C, respectively. The three dotted lines demarcate the 95% confidence interval as calculated by the quasibinomial generalized linear model. The Y-axis is plotted at the same scale in both panels.

Insect cultures

Plutella xylostella and its two parasitoids, *D. semiclausum* and *C. vestalis*, were collected in fields adjacent to Wageningen University campus, the Netherlands. *Plutella xylostella* and *D. semiclausum* have been reared at the Laboratory of Entomology since 2017 and *C. vestalis* since 2018. New individuals were added every year to maintain genetic diversity.

Both the herbivore and its parasitoids were maintained on *Brassica oleracea* L. var. *gemmifera* cv. Cyrus (hereafter Brussels sprouts) in separate rooms under constant conditions (22±2°C,



40-50% R.H., photo:scotophase 16:8). Male and female *P. xylostella* moths were placed in a clean cage with 10% sugar solution and a Brussels sprouts plant for a 48 h oviposition period. After 48 h, the Brussels sprouts plant was transferred to a clean cage. Newly hatched *P. xylostella* larvae were allowed to feed *ad libitum* until pupation with additional plants added, if necessary. *Diadegma semiclausum* and *C. vestalis* were reared using a similar protocol. About 20 adult females along with a few males were released in a cage containing a Brussels sprouts plant heavily infested with second to third instar *P. xylostella* larvae from the stock colony. The host caterpillars were provided *ad libitum* with Brussels sprouts plants until their pupation. The newly emerged adult parasitoids were collected daily and placed in a clean cage with 10% sugar solution as a food source and used for rearing or experiments.

Brassica oleracea L. var. *gemmifera* cv. Cyrus plants were used as a food source for the larvae in both experiments and were 4 to 6 weeks old. Experimental females were between 2 and 5 days old. To this date, no specific ethical approvals are required for research studies on arthropods.

Experiment 1: Parasitoid density

Thirty *P. xylostella* larvae (L2 and L3) were placed on a 6-week-old Brussels sprouts plant and allowed to settle and feed on the plant for approximatively 12 h. The plant-host complex was placed inside a mesh cage (40 x 40 x 60 cm, Vermandel®, the Netherlands) in a greenhouse compartment (22 ±2 °C, 50-70% R.H., photo:scotophase 16:8).

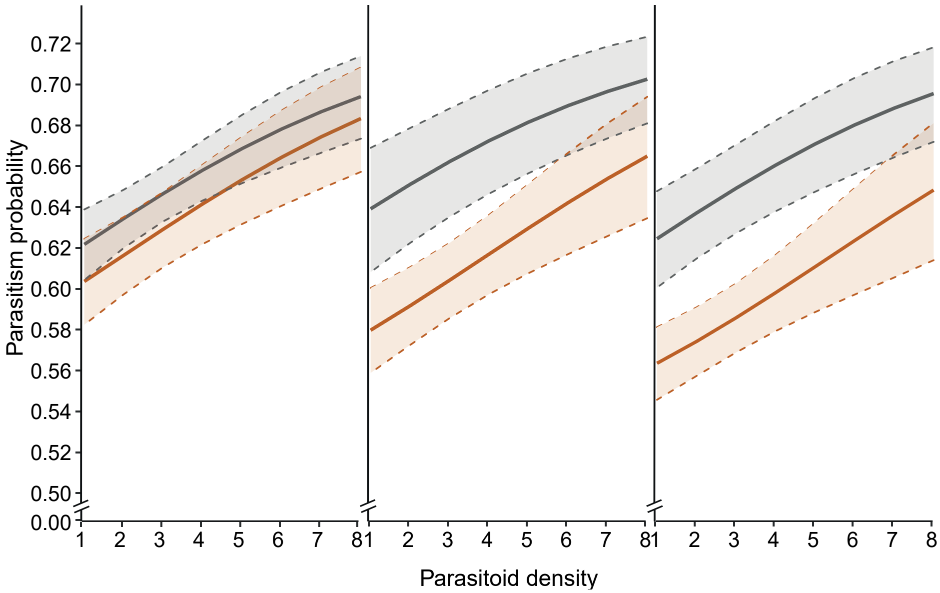
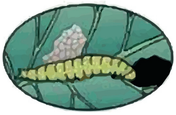


Figure 2: *Diadegma semiclausum* parasitism (i.e., proportion of parasitoid pupae) by density of foraging *D. semiclausum* at 22°C, 27°C and 33°C, respectively. The solid lines represent *D. semiclausum* foraging alone (grey) or together with *C. vestalis* (orange). The two dotted lines are 95% confidence interval as calculated by the quasibinomial generalized linear model.



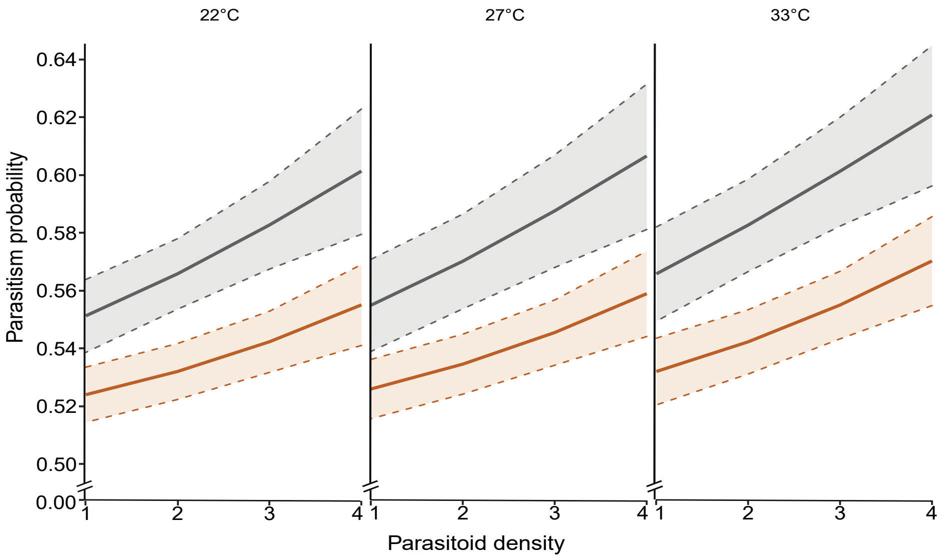
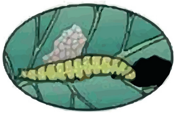


Figure 3: *Cotesia vestalis* parasitism (i.e., proportion of parasitoid pupae) by density of foraging *C. vestalis* at 22°C, 27°C and 33°C, respectively. The solid lines represent *C. vestalis* foraging alone (grey) or together with *D. semiclausum* (orange). The two dotted lines are 95% confidence interval as calculated by the quasibinomial generalized linear model.

Parasitoid females were released in the cages with a single host-infested plant either alone or with other females of the same or the other species and allowed to forage freely for three hours. We tested the following nine parasitoid combinations varying both parasitoid density and species interaction:

1. One *D. semiclausum* female,
2. Two *D. semiclausum* females,
3. Four *D. semiclausum* females,
4. Eight *D. semiclausum* females,
5. One *C. vestalis* female,
6. Two *C. vestalis* females,
7. Four *C. vestalis* females,
8. One *D. semiclausum* and one *C. vestalis* female,
9. Two *D. semiclausum* and two *C. vestalis* females.

After the foraging period, all parasitoids were removed from the mesh cages and the *P. xylostella* larvae were kept on the same plant within the same greenhouse compartment until pupation. If necessary, extra Brussels sprouts plants were added to the cage. After pupation or parasitoid egression, pupae of *P. xylostella*, *D. semiclausum*, and *C. vestalis* were counted. Between 11 and 26 replicates per treatment were completed over four completely randomized temporal blocks, and all the bioassays were performed between 10 AM and 2 PM. Additionally, plants infested with thirty *P. xylostella* larvae were left without parasitoids, and larval survival was recorded (i.e., the number of caterpillars that were recovered as pupae) as a control for “naturally” occurring mortality. The average percentages of insect pupae recovered per treatment are presented in the supplementary materials (Table S1).



Experiment 2: Temperature

A similar protocol as in Experiment 1 was used to study the effect of temperature (22, 27 or 33 °C) on the outcome of intra- and interspecific extrinsic competition. We used simulated 'realistic' high temperature levels in the Netherlands recorded in the period 1991-20201. To emulate these temperature regimes, cages were placed in climate-controlled cabinets (Hettcube 600, Hettich Benelux B.V.) during pre-treatment and the three-hour foraging period. We compared parasitoid combinations ii, vi, viii and ix from Experiment 1 at 27 and 37 °C, respectively. To compare the results with Experiment 1, we also repeated the assay with two *D. semiclausum* females at 22°C. As in the previous experiment, 30 *P. xylostella* larvae were placed on a Brussels sprouts plant in a mesh cage, which could settle for approximately 12 h at 22°C and 16:8 photo:scotophase in a climate cabinet. Prior to the foraging assay, a temperature acclimation phase was added to prevent unwanted thermal stress on the parasitoid and the host. Female *D. semiclausum* and *C. vestalis* were collected in glass vials which were closed with cotton wool before they were placed inside the mesh cages to prevent them from foraging on the hosts during this acclimation phase. The temperature was either left at 22°C or gradually increased from 22°C to 27° or to 33°C. After one hour acclimation, the parasitoids were released in the mesh cage and allowed to forage for three hours before being recaptured.

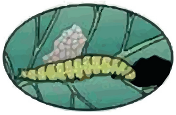
Following the foraging period, the plant-host complexes were transferred to a greenhouse compartment (22°C±2°C, 50-70% R.H., photo:scotophase 16:8) and reared until pupation. If necessary, extra Brussels sprouts plants were added to the mesh cage. After pupation, *P. xylostella*, *D. semiclausum*, and *C. vestalis* pupae were counted. Each treatment was replicated between 16 and 21 times. Temperature regimes and species combinations were randomly swapped daily among the two cabinets. All bioassays were performed between 10 AM and 2 PM.

In addition, unparasitized *P. xylostella* caterpillars were exposed to the temperature treatments, and their survival was recorded. The average percentages of insect pupae recovered per treatment are presented in the supplementary materials (Table S1).

Statistical analysis

The data from Experiment 1 and 2 were combined and analyzed together as both experiments had overlapping treatments (see above). We assumed that the differences of foraging environment (i.e., greenhouse and climate cabinets) did not alter the main effects of temperature and density on the competitive interactions between foraging parasitoids (intra- and inter-specific).

The intraspecific interaction (i.e., parasitoid density) and the interspecific interaction (i.e., the presence or absence of an interspecific competitor) were analyzed with generalized linear models assuming a binomial distribution with a logit link. The proportion of parasitoid pupae for each species was modelled against the parasitoid density (as a continuous variable), the temperature (as a discrete variable) and the presence or absence of an interspecific competitor (as a discrete variable) (only for the interspecific interaction model). Models were checked for overdispersion and were corrected using a quasi-binomial distribution with a logit link.



To further analyze changes in the interspecific competition strength between *D. semiclausum* and *C. vestalis*, we compared the number of *D. semiclausum* pupae versus the number of *C. vestalis* pupae using a GLM assuming a binomial distribution with a logit link. The species-specific parasitoid density (continuous variable), and temperature (discrete variable) were considered as independent variables. The model suffered from overdispersion and was corrected by applying a quasi-binomial distribution with a logit link. For this model, the replicates for which no parasitoid pupae were recovered, were removed from the analysis (a total of three, and one replicate at 22°C and 33°C for *D. semiclausum*, respectively, four, one, and two replicates at 22°C, 27°C and 33°C, respectively for *C. vestalis*, and one replicate at 33°C for the combination of both species).

All the model assumptions and quality were assessed using graphical displays of Pearson residuals. The non-significant interactions ($P > 0.05$) were removed from the models. If a significant ($P < 0.05$) effect of treatment was found, post hoc tests with Tukey's adjustments were performed for pairwise comparison between factorial treatments.

The analysis was performed in R using version 4.0.2 (R Core Team, 2020), using the "lme4" package (Bates et al., 2015). Post hoc tests were completed with the "emmeans" package (Lenth et al., 2018), and the figures were produced using the "ggplot2" package (Wickham, 2016).

Results

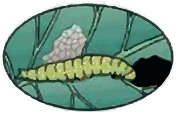
Intraspecific competition

The proportion of caterpillars that were parasitized increased with parasitoid density for both species, while temperature did not have a significant effect on this parameter (Figure 1, Table 1). For *D. semiclausum*, the proportion of parasitized caterpillars increased with parasitoid density, at a decelerating rate when densities were higher than four individuals ($\chi^2 = 19.61$, df = 1, p < 0.001). For *C. vestalis*, the proportion of parasitoid pupae increased linearly with increasing parasitoid density, indicating that interference among foraging parasitoids did not impede individual foraging efficiency ($\chi^2 = 11.41$, df = 1, p < 0.001). At a given density, parasitism rates were generally higher for *D. semiclausum* than for *C. vestalis* (Figure S1).

Table 1: Results of the generalised linear model on foraging parasitoid intraspecific competition for A. *Diadegma semiclausum* and B. *Cotesia vestalis*. Bold statistics highlight significant effects (p < 0.05).

A. *Diadegma semiclausum*	χ^2	**df**	**p**
Density	**19.61**	**1**	**<0.001**
Temperature	1.42	2	0.491

B. *Cotesia vestalis*	χ^2	**df**	**p**
Density	**11.41**	**1**	**<0.01**
Temperature	1.74	2	0.420



Interspecific competition

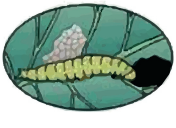
The presence of an interspecific competitor significantly reduced the proportion of parasitoid pupae produced by both parasitoid species (Figure 2, Figure 3, and Table S2). For *D. semiclausum*, the interaction between temperature and the presence/absence of *C. vestalis* in the foraging arena was significant ($\chi^2 = 7.24$, df = 2, p = 0.027). At 27 and 33°C, in the presence of *C. vestalis*, the proportion of *D. semiclausum* pupae was significantly lower than when foraging alone (Tukey's adjustment *post hoc* test, $p = 0.649$, $p = 0.016$, and $p < 0.001$ at 22°C, 27°C and 33°C, respectively). Parasitoid density had a similar effect as described in the intraspecific competition section, with an increasing proportion of *D. semiclausum* pupae as parasitoid density increased ($\chi^2 = 16.82$, df = 1, p < 0.001). For *C. vestalis*, the presence of *D. semiclausum* significantly reduced its parasitism success ($\chi^2 = 29.76$, df = 1, p < 0.001) while the temperature did not have a significant effect on the proportion of *C. vestalis* pupae ($\chi^2 = 3.72$, df = 2, p = 0.156). Parasitoid density also had a similar effect as the one described in the intraspecific competition section, with an increase in the proportion of *C. vestalis* pupae as parasitoid density increased ($\chi^2 = 18.07$, df = 1, p < 0.001).

When comparing the proportion of *D. semiclausum* versus the proportion of *C. vestalis* pupae, *D. semiclausum* parasitism success relative to *C. vestalis* parasitism success was reduced at higher temperatures (Figure 4; $\chi^2 = 7.53$, df = 2, p = 0.023). The effect of parasitoid density was not significant ($\chi^2 = 1.50$, df = 1, p = 0.221).

Discussion

Our results revealed that parasitoid density and temperature affected foraging and both intra- and interspecific competition in variable ways. We initially hypothesized that parasitism would quickly reach an asymptote as the density of foraging parasitoids increased and that the effect of ETEs would be species-specific with a negative impact on *D. semiclausum* but not *C. vestalis* due to differences in thermal tolerance. In partial support of our hypothesis, parasitism success in *D. semiclausum* (but not in *C. vestalis*) increased with parasitoid density but levelled off as density continued to increase. Extreme temperature (in this study 33°C) had a negative impact on the parasitism success of *D. semiclausum* only in the presence of its interspecific competitor *C. vestalis*. In contrast, in the latter species, parasitism success was not affected by increasing temperature but was reduced in the presence of *D. semiclausum*. Overall, these results highlight the importance of the insect community context in determining the response of the focal species to ETEs.

Diadegma semiclausum exploited host caterpillars per unit of time more efficiently than *C. vestalis*, and parasitized up to 80% of recovered hosts, compared with a maximum of 45% for *C. vestalis*. Furthermore, this pattern was consistent across all three tested foraging temperatures. This result suggests that, at the parasitoid densities tested here, parasitism success of both species was only marginally affected under intraspecific competition. Interference competition between foraging parasitoids usually decreases the *per capita* parasitism rate (Saini & Sharma, 2018; Yang et al., 1994). Sentis et al., (2017) has investigated the effects of warming on trophic and non-trophic interactions and showed a weakening effect of temperature on the strength of non-trophic interactions between predators at all prey densities and, independently, a strengthening effect of temperature on trophic interactions. Species density or abundance

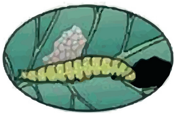


play a determining role in community structure and functioning (Boukal et al., 2019; Kankaanpää et al., 2020). ETEs alter parasitoid foraging performances, thus potentially altering species trophic and non-trophic interactions via shifts in ecological niche exploitation (Rosenblatt et al., 2019; Smith & Amarasekare, 2018).

Under intra-specific competition, temperature did not significantly affect parasitism success in either *D. semiclausum* or *C. vestalis*. Given its more northerly range in the Palearctic, these results partly contrast with our expectations of *D. semiclausum* being more negatively affected by temperatures above 30°C than *C. vestalis* (Furlong & Zalucki, 2017; Sarfraz et al., 2005). As discussed above, parasitism success increased with increasing *D. semiclausum* density. An observational study by (Ngowi et al., 2019) reported a shift in *P. xylostella* parasitoid assembly with a predominance of *C. vestalis* in the warmer lowlands to a predominance of *D. semiclausum* in the cooler highlands. Although temperature is not the only parameter that can explain these differences in population and parasitism success, it undoubtedly contributed to this pattern (Talekar and Yang, 1991). Together, these findings suggest interactive effects of community composition (species assembly) and temperature on spatial niche partitioning among competing parasitoids (Rosenblatt et al., 2019).

The presence of an interspecific competitor reduced the parasitism success of both species. *Diadegma semiclausum* was a superior competitor to *C. vestalis* at all parasitoid densities tested, but its extrinsic superiority decreased at higher temperatures. This pattern was highlighted by a significant interaction between *D. semiclausum* foraging temperature and interspecific competition. By contrast, *C. vestalis* parasitism success was not affected by the foraging temperature. These results showed that the relation between *D. semiclausum* parasitism success and the foraging temperature depends on the presence of *C. vestalis* in its environment. Similarly, the effect of warming and thermal tolerance of competing ant species resulted in a decrease in the abundance of the thermosensitive species when a competitor with higher thermal tolerance was present (Diamond et al., 2017). Temperature has species-specific kinetic effects on metabolism (e.g. translating to faster walking speed, shorter host handling time) and behavior (e.g., activity versus quiescence) (Abram et al., 2015; Augustin et al., 2020; Boukal et al., 2019; Moiroux et al., 2016). Species-specific differences in thermal tolerance will yield more complex outcomes at community than at individual level (Boukal et al., 2019; Gilman et al., 2010; Gvoždík & Boukal, 2021; Outreman et al., 2018). In an exemplary experiment, Davis et al. (1998b) showed that the competitive outcome between three *Drosophila* species was driven by temperature, but that the addition of a competing species altered the focal species' thermal optima. To our knowledge, there is a lack of understanding of the underlying mechanisms at the basis of inter-dependence between the community structure and individual species thermal performances, and we encourage future research to tackle the inter-dependence between community structure and species thermal performances.

It is essential to mention that despite the increase in *C. vestalis* competitive strength as the temperature increases, it does not necessarily imply that *D. semiclausum* will be displaced as environmental heterogeneity will favor coexistence of both species (Bonsall et al., 2002; Gols et al., 2005; Hood et al., 2021; Outreman et al., 2018; Poelman et al., 2014; Price, 1972). The study of the effects of ETEs on parasitoid assembly requires a high level of integration across temporal scales (e.g., seasonal), spatial scale (e.g., landscape and patch connectivity), occurrence and



magnitude of extremes, and food web structure (e.g., bottom-up and top-down forces) creating multiple possible scenarios (Han et al., 2019; Rosenblatt & Schmitz, 2016; Thierry et al., 2019; Tougeron et al., 2016).

Overall, this study highlights the importance of community structure and composition in the realized thermal performance of a focal species. Furthermore, this study showed a reduction in the competitive strength of *D. semiclausum* to *C. vestalis* as the temperature increases. These results imply a convergence in niche exploitation by the two parasitoids. With the predicted increase in frequency and magnitude of ETEs, this convergence will likely become predominant, having significant consequences for community structure, function and diversity (le Lann et al., 2014b; Outreman et al., 2018; Sentis et al., 2017). It is crucial to understand their effects at a community-wide level. However, the tremendous richness of community types (i.e., structure and composition) and the lack of mechanistic understanding of the effect of temperature variability across the multiple layers of biological organization render this task extremely challenging to ecologists (le Lann et al., 2021; C.-S. Ma et al., 2021). This study contributes to this goal by demonstrating short term change in non-trophic interactions induced by ETEs. We especially encourage future research into the impacts of ETEs over longer temporal scales (Harvey et al., 2020; Kankaanpää et al., 2020; Koussoroplis et al., 2017; le Lann et al., 2021).

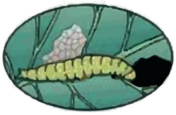
Acknowledgements

We thank the insect rearing staff of our laboratory: Pieter Rouweler, André Gidding, and the late Frans van Aggelen as well as Unifarm for providing the insects and plant materials for the experiments, respectively. The authors declare no conflict of interest.

Supplement

Table S1: Average number *P. xylostella* larvae recovered from the 30 caterpillars that were initially introduced onto the plant with the standard error around the mean (SE) and the number of replicates (n) per treatment (Species, Density and Temperature). The control, *C. vestalis*, *D. semiclausum*, and Mix are treatments were *P. xylostella* larvae were not exposed, exposed to either parasitoid independently, or exposed to both parasitoid simultaneously, respectively.

Species	Density	Temperature (in °C)	Average	SE	n
Control	0	22	21.29	1.16	14
Control	0	27	22.33	2.42	6
Control	0	33	25.00	1.34	6
Cotesia vestalis	1	22	18.65	1.78	17
Cotesia vestalis	2	22	16.75	1.67	16
Cotesia vestalis	2	27	21.38	1.14	16
Cotesia vestalis	2	33	21.55	1.29	20
Cotesia vestalis	4	22	15.13	1.51	15
Diadegma semiclausum	1	22	18.60	1.85	15



Species	**Density**	**Temperature (in °C)**	**Average**	**SE**	**n**
Diadegma semiclausum	2	22	18.33	0.94	27
Diadegma semiclausum	2	27	18.00	1.18	13
Diadegma semiclausum	2	33	18.82	1.24	22
Diadegma semiclausum	4	22	18.08	1.59	13
Diadegma semiclausum	8	22	9.83	0.89	12
Mix	2	22	16.50	1.10	26
Mix	2	27	18.19	1.14	16
Mix	2	33	19.37	0.96	19
Mix	4	22	18.18	1.58	11
Mix	4	27	18.21	1.16	19
Mix	4	33	17.00	0.96	21

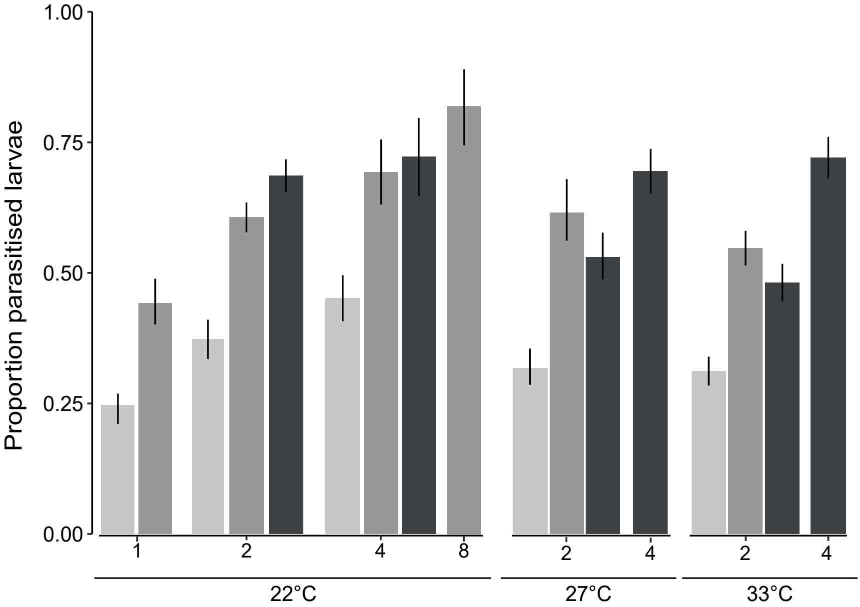


Figure S1: Mean proportion of parasitized *P. xylostella* hosts by *C. vestalis*, *D. semiclausum*, and both species together from light to dark grey, respectively, for each tested female density at 22°C, 27°C, and 33°C. The error bars represent ± the standard error around the proportion.

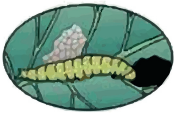
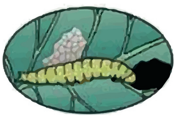
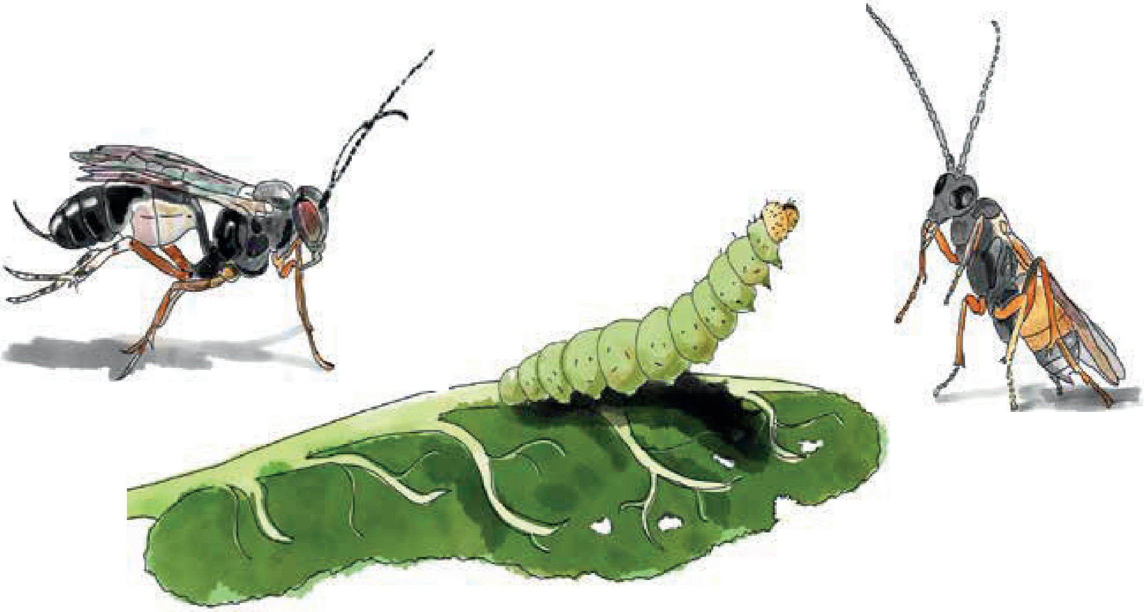


Table S2: Results of the generalised linear model on parasitism under conditions of parasitoid inter-specific competition for A. *Diadegma semiclausum* and B. *Cotesia vestalis*. The model parameters are foraging parasitoid density, temperature, and species (i.e., presence or absence of the other species) on the focal species parasitism rate. Bold statistics highlight significant effects ($p < 0.05$).

Diadegma semiclausum	χ^2	**df**	**p**
Density	**16.82**	**1**	**<0.001**
Temperature	4.99	2	0.083
Species	**23.33**	**1**	**<0.001**
Temperature X Species	**7.24**	**2**	**0.027**

Cotesia vestalis	χ^2	**df**	**p**
Density	**18.07**	**1**	**<0.001**
Temperature	3.72	2	0.156
Species	**29.76**	**1**	**<0.001**





Chapter 6.

Herbivore species richness influences plant interactions with an herbivorous insect, its natural enemies, and plant reproduction

Maite Fernández de Bobadilla[1*]; **Quint Rusman**[1,2*]; **Thibault Costaz**[1*]; **Cristina Alban**[1];
Kalliopi Arkoumanea[1]; **Daan Mertens**[1,3]; **Erik H. Poelman**[1]

Abstract

A main challenge in ecology is to assess how loss of species and consequently species interactions affect ecosystem stability. This knowledge is especially relevant within the context of insect decline that planet Earth faces. Here, we studied how insect herbivore species richness and trait composition of these species affect plant interactions with other insect community members (i.e. a herbivore, its parasitoids and pollinators) and whether these interactions affect plant fitness. We show that herbivore richness, its composition of functional traits and specific herbivore species affected the abundance of *Plutella xylostella*, the parasitism rate of *P. xylostella* and pollinator visitation to *Brassica nigra* plants. The composition of the introduced herbivore community affected plant fitness directly via reducing plant biomass that predicted resource investment into reproductive organs and these effects were stronger than fitness consequences of herbivore-induced plant-mediated interactions with other community members. Fitness effects of indirect interactions often included a network of multiple organisms and could be mediated by plant traits. All of these interactions involved effects of herbivores on pollinator visitation that closely correlated with the total number of seeds produced by a plant. For a species-rich community of phloem feeders as well as for the leaf chewer rich community seed set was reduced via a chain of interactions that reduced pollinator visitation. The effects of herbivore richness and functional traits in herbivore communities on plant interactions with subsequent herbivores, parasitoids and pollinators show how complex interaction networks on individual plants may determine plant fitness.

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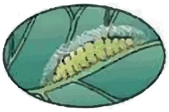
* These authors contributed equally to this article

1. Laboratory of Entomology, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands
2. Department of Systematic and Evolutionary Botany, University of Zürich, Zollikerstrasse 107, 8008 Zürich,Switzerland
3. Section of Evolution and Ecology, University of California, Davis, CA 95616, USA

Introduction

Planet Earth is facing rapid changes in ecosystem dynamics and species interactions. Important drivers of these changes are human overpopulation, climate change, habitat loss and species extinction (Tylianakis et al., 2006, 2008). The loss of integral parts of ecosystems, such as the worldwide insect decline, has caused rising concerns (Althaus et al., 2021; Hallmann et al., 2017; Halsch et al., 2021; Harvey et al., 2020; Wagner et al., 2021; Warren et al., 2021). An inherent consequence of species loss is a reduction in the number of ecological interactions which may result in reduced ecosystem stability and secondary extinctions (Soliveres et al., 2016; Tylianakis et al., 2008). Experimental and modelling studies have identified that loss of individual species in insect communities not only has cascading effects on other community members (Sanders et al., 2013, 2018), but also affects plant reproductive success (Lankau & Strauss, 2008; Soper Gorden & Adler, 2018). The cascading effects of interaction loss on plant fitness signals evolutionary consequences of species loss for plant trait selection (Zeng & Wiens, 2021). To understand and predict changes in selection regimes on plant traits by declines of organisms of higher trophic levels, a major challenge is identifying which insect groups and interactions more strongly affect plant fitness. This includes exploring direct and indirect interactions among antagonistic and mutualistic community members that may further modulate fitness outcomes of plant interactions with their insect community (Ando et al., 2016; Poelman and Kessler, 2016; Rusman et al., 2019a).

In terrestrial ecosystems, plants are at the basis of many food chains being the food source for a species-rich community of insect herbivores (Schoonhoven et al., 2005) with 85% of plants also providing pollen and nectar to a diversity of flower-visiting insects (Ollerton et al., 2011). Consequently, plants are central in a network of species that interact directly or indirectly via the plant (Ohgushi, 2005). The high diversity of organisms interacting with a plant creates a playground of interacting selective agents on the plant (e.g., herbivores, parasitoids or pollinators, abiotic stress and competition for resources with other plants). Due to the complexity of the plant-associated network, prediction of selection on plant traits is not straightforward (Strauss, 2014). Indeed, diffuse evolution of plant traits is often observed, where selection on traits is not only explained by one-to-one species interactions, but rather is the effect of the network of interactions (Poelman & Kessler, 2016; Strauss, 2014). Important selective agents on plant traits are herbivores, natural enemies of the herbivores and pollinators. Herbivores can directly affect plant fitness by consuming photosynthetic plant tissues, flowers and/or seeds and indirectly by altering the attraction of other herbivores, natural enemies of the herbivores and by altering pollinator visitation (Grass et al., 2018; Rusman et al., 2020). Natural enemies of the herbivores, such as parasitoids and predators, can have positive direct effects on plant fitness by reducing herbivore pressure (Bustos-Segura et al., 2020) and indirect effects by altering herbivore, omnivore and pollinator behaviour. Interactions with pollinators strongly affect plant fitness directly, because pollinators mediate plant reproduction. The increase in seed number may in turn attract seed-feeding herbivores (Pérez-Barrales et al., 2013). Thus, direct and indirect interactions between herbivores and other community members affect the insect community associated with a plant, including other herbivores (Poelman et al., 2008), parasitoids (Blubaugh et al., 2018; Bukovinszky et al., 2012), pollinators (Chrétien et al., 2021; Rusman et al., 2018), and can affect plant fitness (Cozzolino et al., 2015; Soper Gorden & Adler, 2018). However, it is poorly understood how plants maximise fitness when defending against



attack by diverse assemblages of herbivores while maintaining the attraction of parasitoids/ predators and pollinators.

Here, we study how herbivore species richness and its trait composition affect plant interactions with its insect community and whether these interactions affect plant fitness. We con-

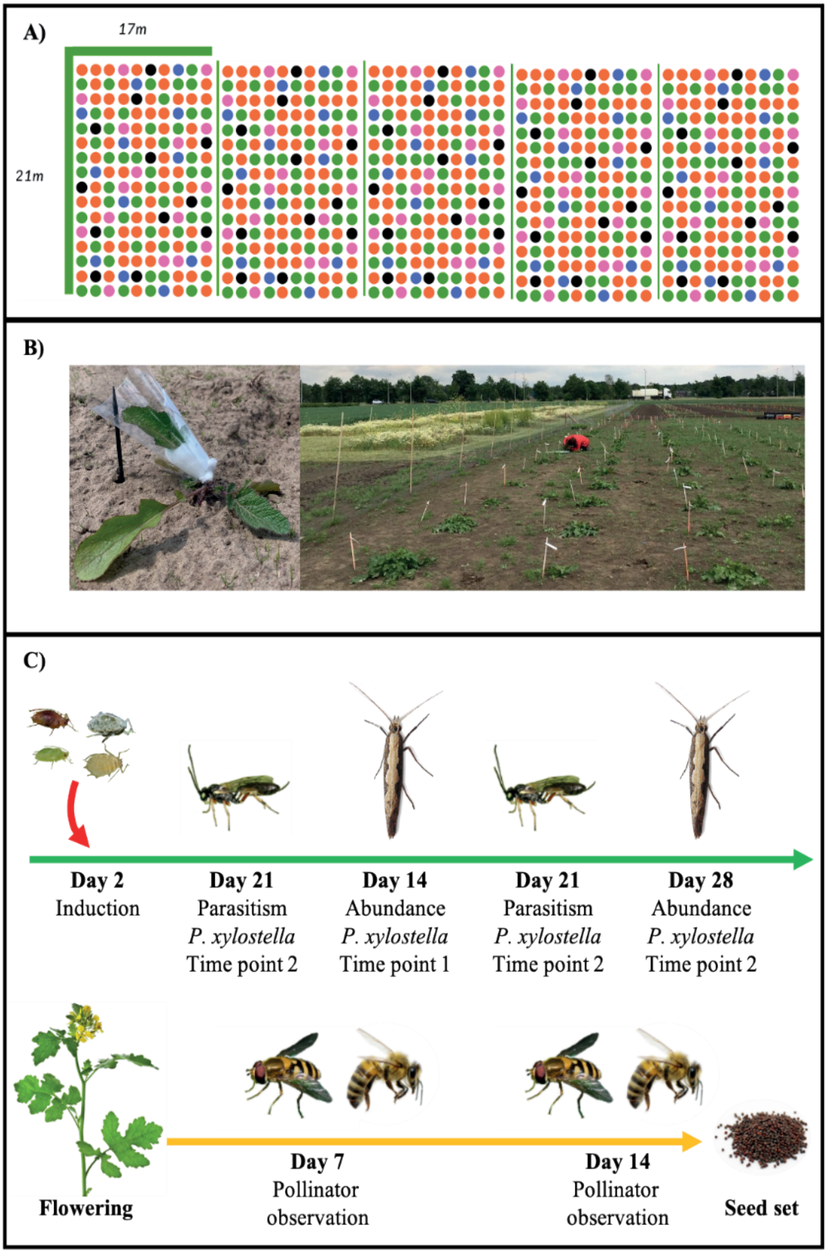
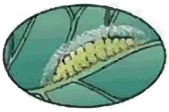


Figure 1: Experimental setup. A. Field setup: 5 blocks, each colour represents one herbivore treatment. B. Experiment time line of the measurements



ducted a manipulative field study where we subjected *Brassica nigra* plants to attack by an insect herbivore community with increasing species richness from one and/or two feeding guilds (i.e. phloem feeders, leaf chewers or a mix of both). Recent studies identified that *B. nigra* plants respond differentially to attack by different herbivore feeding guilds as well as the number of species attacking the plant (Fernández de Bobadilla et al., 2021), and that indirect interactions due to herbivore-induced plant responses affect plant reproductive success (Pashalidou et al., 2015; Rusman et al., 2018, 2020). We specifically studied how the composition of the introduced herbivore community affected a) plant colonisation by the herbivore *Plutella xylostella*, one of the most prevalent herbivores attacking *B. nigra* plants (Mertens et al., 2021a); b) top-down control of this herbivore population by parasitoids; c) visitation of flowers by pollinators; and d) how these interactions collectively affect plant fitness in terms of seed production. We examined the relations between the different community members and effects of varying levels of herbivore richness and feeding guild by applying structural equation modelling (SEM). This allowed us to partition the direct and indirect effects of different community members on plant fitness, and how early-season herbivore attack alters these relationships. We consider as direct effects those where initial herbivory induces plant phenotypic changes that result in effects on plant fitness, whereas indirect effects are herbivore-induced effects on other recorded community members that have fitness consequences. By assessing the direct and indirect effects of herbivore community composition on important members of a plant-associated insect community this work sheds light on which interactions more strongly affect plant fitness, and potentially impose selection on plant traits.

Materials and Methods

Plants and insects

Seeds of black mustard (*B. nigra*, Brassicales: Brassicaceae) were obtained from a natural population in the vicinity of Wageningen, The Netherlands (51° 57' 32" N, 5° 40' 23" E). Seeds were sown on peat soil (Lentse Potgrond) and germinated in a greenhouse at 22 ± 2 °C, 60-70 % RH and 16:8 h L:D. One week after germination, the plants were transplanted into peat soil cubes and planted in the field when three weeks old. One week before planting in the field, the plants were acclimatized to field conditions in an open greenhouse (i.e. without climate control).

Eight insect species that commonly attack *B. nigra* were used to manipulate the composition of the starting herbivore community on individual plants (Table 1). We used four chewing herbivore species: the turnip sawfly, *Athalia rosae* (*Ar*) (Hymenoptera: Tenthredinidae); the mustard leaf beetle, *Phaedon cochleariae* (*Pc*) (Coleoptera: Chrysomelidae); the cabbage moth, *Mamestra brassicae* (*Mb*) (Lepidoptera: Noctuidae); and the large cabbage white, *Pieris brassicae* (*Pb*) (Lepidoptera: Pieridae). We used four phloem-feeding herbivore species: the cabbage aphid, *Brevicoryne brassicae* (*Bb*)*;* the mustard aphid, *Lipaphis erysimi* (*Le*); the green peach aphid, *Myzus persicae* (*Mp*); and the tobacco aphid, *Myzus persicae* subsp. *nicotianae* (*Mpn*) (all Hemiptera: Aphididae) (Table 1). *Mamestra brassicae*, *P. brassicae*, *B. brassicae* and *P. xylostella* were reared on Brussels sprouts plants (*Brassica oleracea* L. var. *gemmifera* cv. Cyrus). *Myzus persicae*, *M. persicae* subsp. *nicotianae*, *L. erysimi*, *A. rosae* and *P. cochleariae* were reared on radish (*Raphanus sativus*). All insects were obtained from the stock rearing of the Laboratory of Entomology, Wageningen University kept under greenhouse conditions (22 ± 2 °C, 60–70 % RH and 16:8 h L:D).

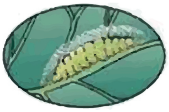


Table 1: Insects used for the experiment as inducers, in brackets abbreviations used throughout the document.

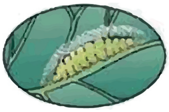
Species (Abbreviation)	**Picture**	**Order**	**Feeding guild**
Athalia rosae (Ar)		Hymenoptera	Leaf chewer
Mamestra brassicae (Mb)		Lepidoptera	Leaf chewer
Phaedon cochleariae (Pc)		Coleoptera	Leaf chewer
Pieris brassicae (Pb)		Lepidoptera	Leaf chewer
Brevicoryne brassicae (Bb)		Hemiptera	Phloem feeder
Lipaphis erysimi (Le)		Hemiptera	Phloem feeder
Myzus persicae (Mp)		Hemiptera	Phloem feeder
Myzus persicae subsp nicotianae (Mpn)		Hemiptera	Phloem feeder

Plant treatments

The effect of species richness and trait composition and trait richness of the herbivore community on plant interactions with herbivores, parasitoids and pollinators and on plant seed production were studied in the Droevendaal experimental fields of Wageningen University (51° 59’ 26.5” N, 5° 39’ 50.5” E) during the summer of 2019. We planted 800 plants divided over 5 blocks: each block was a field of 21x17m with 160 plants planted on the same day at 1.5m distance from each other. Blocks were planted separately every two weeks between the 6th of May and 1st of July 2019 (Figure 1).

Two days after planting in the field, we infested plants with different herbivore communities by varying species richness of herbivores (one, two or four herbivore species) and their trait composition in terms of the feeding guilds they belong to (leaf-chewing, phloem-feeding, or a mix of both). This resulted in 13 treatments: control plants without herbivore treatment (Con-0), four different treatments with one chewing herbivore species (Chew-1), one treatment with the four chewing herbivore species combined (Chew-4), four different treatments with one phloem-feeding herbivore species (Phlo-1), one treatment with the four phloem-feeding herbivore species combined (Phlo-4), mixture of one chewing and one phloem-feeding herbivore species (Mix-2), mixture of two chewing and two phloem-feeding herbivore species (Mix-4). We excluded species richness of three species, because of the imbalance that these treatments would have in numbers of leaf-chewer and phloem-feeder species. In each block, we included 12 replicates per herbivore combination for the chewer and phloem feeder treatments (Chew-1, Chew-4, Phlo-1 Phlo-4). For the Mix-2 and Mix-4 treatments, we included one replicate for each herbivore combination: Mix-2 resulted in 16 insect combinations, while for Mix-4 a selection of 12 of the 36 species combinations were included. Combinations were selected to have the largest differences between species combinations, while each herbivore is present the same number of times (6 times) (Fernández de Bobadilla et al., 2021). For a complete list of all species combinations see Table 2. All treatments were randomised per block.

In all herbivore communities the starting density consisted of four individuals for leaf chewer treatments and eight individuals for phloem feeder treatments, divided over one or four species. For example, Chew-1 included four individuals of the same leaf chewer species, while Chew-4 included one individual of each leaf chewer species. For the mixed treatments, we used two leaf-chewer individuals plus four phloem-feeder individuals, divided over two or four spe-



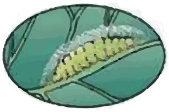
cies. For chewing herbivores, first-instar larvae were placed on the youngest fully expanded leaf of the plant, while for phloem-feeding herbivores we used wingless adults. Insects could freely move and feed from their preferred feeding sites on the plant. The number of species and densities of inducers were based on field observations of insect communities of *B. nigra* plants in the same area (Mertens et al., 2021a).

Table 2: Overview of insect combinations for the mixed species richness treatments. For Mix-2 we did all the possible insect combinations. For Mix-4 we selected 12 out of the 36 possible combinations (underlined and bold). Bb= *Brevicoryne brassicae,* Mp= *Myzus persicae,* Mpn= *Myzus persicae* subsp. *nicotianae,* Le= *Lipaphis erysismi,* Ar= *Athalia rosae,* Pb= *Pieris brassicae*, Pc= *Phaedon cochleariae*, Mb=*Mamestra brassicae*.

Mix-2 16 treatments		Mix-4 12 treatments	
		Bb-Mp-Mb-Ar	Mp-Mpn-Mb-Ar
Bb-Mb	*Mpn-Mb*	Bb-Mp-Mb-Pb	***Mp-Mpn-Mb-Pb***
Bb-Ar	*Mpn-Ar*	Bb-Mp-Mb-Pc	Mp-Mpn-Mb-Pc
Bb-Pb	*Mpn-Pb*	Bb-Mp-Ar-Pb	Mp-Mpn-Ar-Pb
Bb-Pc	*Mpn-Pc*	Bb-Mp-Ar-Pc	***Mp-Mpn-Ar-Pc***
		Bb-Mp-Pb-Pc	Mp-Mpn-Pb-Pc
		Bb-Mpn-Mb-Ar	Mp-Le-Mb-Ar
Mp-Mb	*Le-Mb*	Bb-Mpn-Mb-Pb	Mp-Le-Mb-Pb
Mp-Ar	*Le-Ar*	***Bb-Mpn-Mb-Pc***	***Mp-Le-Mb-Pc***
Mp-Pb	*Le-Pb*	***Bb-Mpn-Ar-Pb***	***Mp-Le-Ar-Pb***
Mp-Pc	*Le-Pc*	Bb-Mpn-Ar-Pc	Mp-Le-Ar-Pc
		Bb-Mpn-Pb-Pc	Mp-Le-Pb-Pc
		Bb-Le-Mb-Ar	***Mpn-Le-Mb-Ar***
		Bb-Le-Mb-Pb	Mpn-Le-Mb-Pb
		Bb-Le-Mb-Pc	Mpn-Le-Mb-Pc
		Bb-Le-Ar-Pb	Mpn-Le-Ar-Pb
		Bb-Le-Ar-Pc	Mpn-Le-Ar-Pc
		Bb-Le-Pb-Pc	***Mpn-Le-Pb-Pc***

Natural colonisation of *P. xylostella*

To investigate the effects of trait composition and species richness of the herbivore community on subsequent interactions with the herbivore community, we monitored natural colonisation of the herbivore *P. xylostella*. *Plutella xylostella* is one of the dominant herbivores colonising brassicaceous plants in the vegetative state. We recorded the number of larvae of *P. xylostella* that naturally colonised individual plants of each of the 13 herbivore community treatments including plants that were not experimentally infested with herbivores. Plants were checked for caterpillar presence at two time points, two and four weeks after planting them in the field. We did so by carefully checking each leaf for the presence of caterpillars of *P. xylostella* (L2-L4). To further support the field observations on caterpillar presence, we tested moth oviposition preference for plants infested with the different herbivore communities in a greenhouse setup (Figure S1, Tables S1 and S3).



Parasitism of P. xylostella

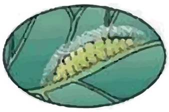
To investigate the effects of trait composition and species richness of the herbivore community on the top-down control of *P. xylostella* by parasitoids, we monitored natural parasitism of caterpillars of *P. xylostella* on plants infested with each of the 13 herbivore community treatments including plants that were not infested with herbivores. We placed 15 L2-L3 *P. xylostella* larvae on a young leaf of each plant, nine and eighteen days after the initial induction. The leaf petiole was covered with some cotton and surrounded with a transparent plastic sheet shaped as a funnel to prevent the caterpillars from escaping (Figure 1). The caterpillars were placed five days before assessing the abundance of *P. xylostella* (see previous section) to ensure that, in the rare case of a caterpillar escaping, these would have pupated and would not be counted as natural colonisation of *P. xylostella*. The caterpillars were placed at 10 a.m. and, two days later, at 5 p.m., caterpillars were recollected, transferred to Petri dishes and stored in a refrigerator (5 °C) or in a freezer (-20 °C) for longer storage until dissection. The caterpillars were dissected and checked for the presence of parasitoid eggs using a stereomicroscope (Olympus SZ51 KL-300 LED). The parasitoid eggs or larvae were not identified to the species level. However, field studies show that the most common parasitoids of *P. xylostella* caterpillars in The Netherlands are *Diadegma spp* (Bukovinszky et al., 2004).

Pollinator visitation

To investigate effects of trait composition and species richness of the herbivore community on pollinator visitation, we recorded pollinator visitation on plants infested with each of the 13 herbivore community treatments including plants that were not infested with herbivores. Recordings were done at two time points: a) between 7 and 9 days and b) between 14 and 16 days after plants had started flowering. Pollinator visitation to a plant was monitored for 10 min. When a pollinator arrived to a plant and had contact with a flower, the identity of the pollinator and the number of flowers visited were recorded (Rusman et al., 2018, 2020). The identity of other pollinators that visited the plant during this observation was recorded as well. If the same pollinator individual returned to the plant under observation after having visited a different plant, we scored that visit as a new visit (Rusman et al., 2018, 2020). Recordings were done during the day (between 9 a.m. and 1 p.m., or 2 p.m. and 5 p.m.) only when weather conditions were favourable for pollinator activity (15–30 °C and wind speed ≤6 m/s (Rusman et al., 2018, 2020), using a handheld computer (Psion Workabout Protm 3) programmed with The Observer XT software (version 10, Noldus Information Technology, Wageningen, The Netherlands).

Plant phenotype and seed production

To investigate the effects of herbivore-induced changes on plant phenotype and assess whether these changes affect plant interactions with the insect community, we measured multiple plant parameters: the number of leaves, plant height, plant diameter and percentage of leaf damage. Measurements were taken after assessing the abundance of *P. xylostella* (see previous section), using a tape measure. The percentage of leaf damage on a full plant scale was visually estimated on a percentage scale (0-100%). Plants were monitored every 2-3 days for the start and end of the flowering period. Plants were considered flowering when the first flower opened and considered finished with flowering when all buds and flowers were gone, with only developing and ripe siliques remaining on flower stalks (Rusman et al., 2020). We calculated the duration of

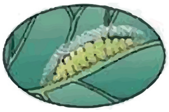


flowering by subtracting the number of days needed to start flowering from the number of days to the termination of flowering (Rusman et al., 2020).

Life-time seed production was assessed by seed number and weight. First harvesting date for each plant was selected before the first siliques would lose their seeds (Rusman et al., 2018, 2020). At first harvest, we collected all ripe siliques and left immature siliques and flowers on the plant. Then, plants were checked weekly and all siliques were harvested when ripe. Siliques were stored in paper bags in a dry storage room until seeds were manually extracted from the siliques. We calculated the total number of seeds per plant by weighing 100 seeds, and the total weight of seeds harvested per plant (Rusman et al., 2018, 2020). We estimated the total number of seeds by dividing total seed weight by the weight of 100 seeds and multiplied the result by 100. The weight of one seed was estimated by dividing the weight of 100 seeds by 100.

Statistical analysis

The effects of species richness and trait composition of the herbivore community were first analysed separately for plant interactions with *P. xylostella*, parasitoids, pollinators, and plant traits (flowering traits, seed production) using generalized linear mixed modelling, (GLMM). For count data such as number of insects (e.g., number of herbivores, parasitoid eggs, or pollinators), flowers visited, days (from infestation, since flowering), and seed numbers, we used GLMM with a Poisson distribution and a log link function, or negative binomial distribution with a log link function to correct for overdispersion when necessary. For continuous data such as seed weight, we used linear (mixed) models with a Gaussian distribution and identity link function or a Gamma distribution with a log link function if the data did not follow a normal distribution. Two models were fitted to the data: the first model included herbivore treatment and time point as fixed factors. The second model included herbivore species richness (treatments assigned to 0, 1, 2 or 4 species), herbivore feeding guild (treatments assigned to control, chewing, phloem-feeding, or combination), and time point as fixed factors. Non-significant interactions ($P > 0.05$) were excluded from the model. Random factors were selected using a backward approach: all random factors such as block, day (only for pollinators), time (morning or afternoon; only for pollinators) were added to the models and removed if they explained <3% of the variation or were statistically non-significant ($P > 0.05$). Model assumptions were assessed graphically using the Pearson residuals against the fitted value by the models as well as against all the included covariates (i.e. fixed factors). Pairwise comparisons were evaluated using Tukey's *post hoc* test for significant variables ($P < 0.05$). For comparisons within treatment and combined herbivore species richness * feeding guild we used non-adjusted p values to cope with the high number of treatments. For comparisons within herbivore species richness and feeding guild we used adjusted p values. For number of parasitized *P. xylostella* recaptured, an offset with the natural logarithm of the total number of recaptured *P. xylostella* larvae was added to the model to account for differences in the total number of larvae recaptured per plant. Plants for which no *P. xylostella* larvae were recovered were not included in the analysis as they do not provide information on the prevalence of parasitism of *P. xylostella* larvae. Unfortunately, larvae from the fifth block were lost due to unexpected storage failure by malfunction of the freezer and could not be included in the analysis. For plant seed production, plants that produced ≤2,500 or >40,000 seeds were considered artifacts. Removing these outliers affected all treatments equally (no overall effect of herbivory; $P = 0.431$ for plants with 0 to 2,500 seeds).



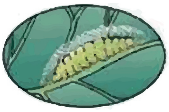
In addition, plants from block 5 were excluded due to extremely high mortality (almost 80%). We used the lme4 (Bates et al., 2015), multcomp (Hothorn et al., 2008), lsmeans (Lenth, 2016), lmtest (Zeileis & Hothorn, 2002), emmeans (L. Russell, 2018), ggplot2 (Wickham, 2016) packages for these analyses.

We performed structural equation modelling (SEM) to examine the effects of the herbivore treatment on relations between plant traits and plant interactions with *P. xylostella* and their natural enemies, pollinators, and plant fitness. We built three models: one including each herbivore species treatment, one including herbivore species richness, and one including herbivore feeding guild. We did this to identify effects of individual herbivore species, as well as broader patterns based on herbivore richness or herbivore feeding guild. All included variables are displayed in figure 14. The variables height and radius were used as independent variables instead of dependent variables because of model identification problems. For parasitism rate and number of pollinators and flowers visited we only used data from time point 1, because time point 2 contained too few observations. We only used total pollinator visits and total flowers visited because including individual pollinator species/groups reduced the number of observations. All variables were checked for normality and homogeneity of variance, and if violated, variables were log-transformed. Since transformation did not solve assumption violations in all cases, we used the "Yuan-Bentler" test statistic. Good model fit was verified by inspecting the robust Test statistic (P >0.05), CFI (>0.9), RMSEA (<0.08), RMSEA Low (<0.01), RMSEA High (<0.1), SRMR (<0.08). We used the lavaan package for these analyses (Rosseel, 2012). All analyses were performed in R version 4.0.2 (R Core Team, 2020).

Results

Natural colonisation of *Plutella xylostella* is affected by herbivore richness

The abundance of *P. xylostella* differed among plants induced by herbivore communities that differed in richness and species composition ($df = 1, \chi^2 = 4.67, P = 0.031$, Table 3). These effects were restricted to P. xylostella abundance two weeks after induction of B. nigra plants and not observed within one week after induction (Figure. 2). We found fewer *P. xylostella* caterpillars on plants that did not receive initial herbivory and on plants attacked by four leaf chewers compared with the other induction treatments. Irrespective of species richness, phloem-feeder induction made the plants more attractive to *P. xylostella* compared to untreated plants. In contrast, in the case of leaf chewers the richness of attackers was important, as plants attacked by one chewer species had a larger number of *P. xylostella* than plants attacked by four species of leaf chewers (Figure 2). When plants were attacked by the highest species richness of herbivores (i.e. four species) the abundance of *P. xylostella* differed depending on the guild of the attacker. Plants attacked by four species of phloem feeders had the largest number of *P. xylostella*, followed by plants attacked by chewers and phloem feeders at the same time (i.e. Mix-4) and plants attacked by four species of chewers had the smallest number of *P. xylostella*. Likewise, in a greenhouse study, the oviposition preference of *P. xylostella* changed depending on the guild of the herbivores that had previously attacked the plant. *Plutella xylostella* laid more eggs on plants attacked by four species of phloem feeders compared with plants attacked by a mix of two phloem feeders and two chewers (i.e. Mix-4) ($P < 0.001$; Wilcoxon signed rank test, Figure S2). In contrast to the overall influence of trait composition and species richness of the attacker



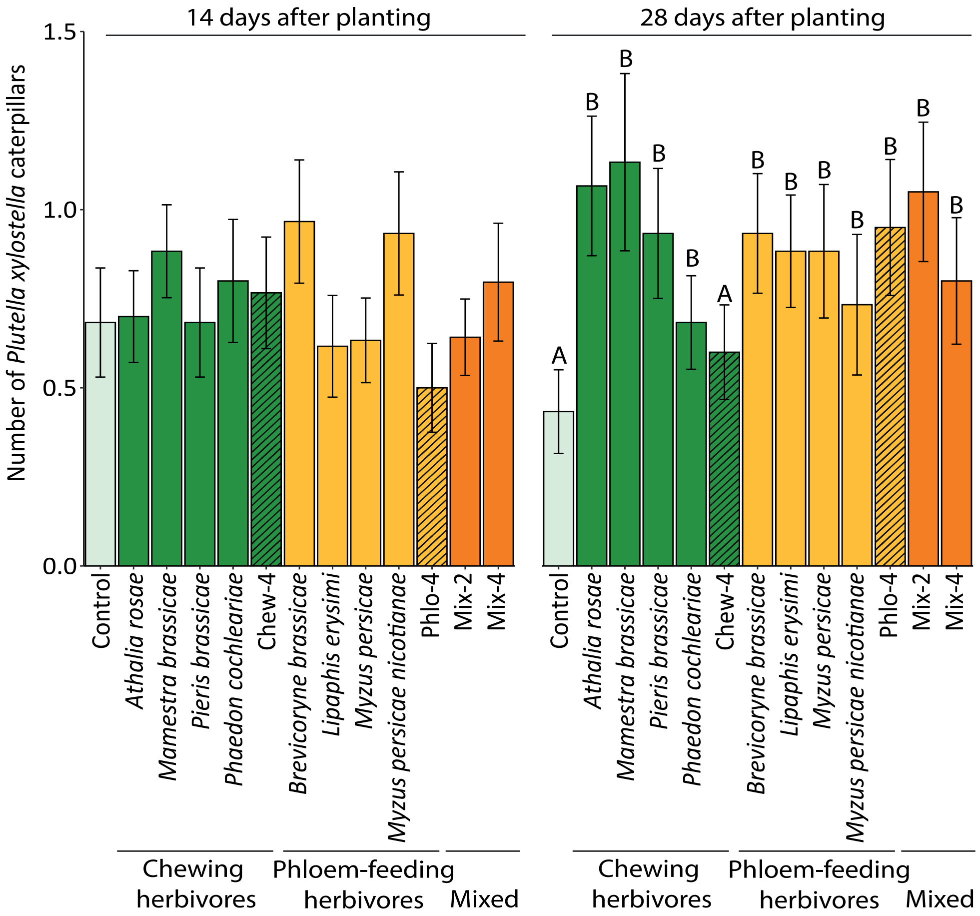
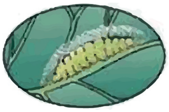


Figure 2: Abundance of Plutella xylostella larvae 14 and 28 days after planting on herbivore-treated- or on untreated plants. Bars represent the mean ± the standard error of the mean. Capital letters above bars indicate significant differences ($P \leq 0.05$) between herbivore treatments within a time point based on non-adjusted Tukey's post hoc tests.

community, the abundance of *P. xylostella* in the field was not affected by specific herbivores (Table 4). However, in a greenhouse study, chewer identity affected the oviposition preference of *P. xylostella*. The moths laid fewer eggs on plants attacked by *M. brassicae* and more eggs on plants attacked by *P. cochleariae* compared to plants attacked by the four chewer species ($P < 0.05$, Wilcoxon signed rank test, Figure S3). The oviposition preference of *P. xylostella* was not affected by the identity of phloem feeders nor by the richness of species in a mix with chewers and phloem feeders (Figures S4, S5).

Parasitism of *Plutella xylostella* in the field is affected by the guild of initial attackers

The parasitism of *P. xylostella* was significantly different across feeding guild and time point (Feeding guild: $df = 3, \chi^2 = 16.18, P < 0.001$; time point: $df=1\ \chi^2 = 67.99, P < 0.001$; Figure 3, Table



3). The number of parasitised larvae was significantly higher on plants induced by a mixture of chewers and phloem feeders (i.e. Mix-4) than on plants attacked by one feeding guild alone (Figure 3). Between leaf chewer and phloem feeder communities there was no difference in the number of parasitised *P. xylostella* larvae (Figure 3). The species richness of herbivores attacking the plant marginally affected the parasitism of *P. xylostella* (df = 2, $\chi^2 = 5.47$, P = 0.065; Figure 3 and Table 3). In addition to feeding guild and richness of the attacker community, specific herbivores affected the parasitism rate of *P. xylostella* (df = 12, $\chi^2 = 23.26$, P = 0.026; Figure 4 and Table 3). The degree of *P. xylostella* parasitism was higher on plants attacked by *P. brassicae*, *M. persicae*, *M. persicae* subsp. *nicotianae* and by a mix of two phloem feeders and two chewers (i.e. Mix-4) compared with untreated plants (Figure 4). The parasitism on Mix-4 plants was higher than on plants induced by the four species of phloem feeders, and plants induced by the mix of the four species of leaf chewers. In addition to the herbivore treatments, the time of measurement also affected the parasitism rate of *P. xylostella* larvae which was higher nine days after initial induction, compared to 18 days after initial induction (df = 1, $\chi^2 = 67.55$, P = 0.001; Figures 3 and 4, Tables 3 and 4).

Table 3. Part A: Output of generalized linear (mixed) models showing the effects of different fixed factors (species richness, feeding guild, time-point) on interactions of *Brassica nigra* plants with *Plutella xylostella* and its natural enemies, attraction and visitation by pollinators, flowering traits, and plant seed production. All random factors were initially included in the model, and factors which explained less than 3 percent variation or with a P > 0.05 were excluded from the model. Bold values indicate results where P ≤ 0.05. Italic values indicate results where P ≤ 0.1.

	Species richness (SR)			**Feeding guild (FG)**			**Time point (TP)**			**SR*FG**			**SR*TP**		
	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*
Plutella xylostella abundance	2	2.90	0.235	3	2.27	0.518	1	1.39	0.239	1	0.11	0.744	2	1.93	0.381
Plutella xylostella parasitism	2	5.47	0.065	3	16.18	**0.001**	1	68.00	**<0.001**	-	-	-	-	-	-
Number of pollinators	3	3.31	0.346	2	8.35	**0.015**	1	0.08	0.774	1	0.10	0.750	3	4.75	0.191
Flowers visited by pollinators	3	1.83	0.608	2	4.79	*0.091*	1	1.02	0.313	1	3.74	0.053	3	3.90	0.272
Time to flower	3	1.86	0.601	2	3.93	0.140	-	-	-	1	0.13	0.714	-	-	-
Flowering time	3	2.00	0.573	2	1.85	0.397	-	-	-	1	0.00	0.984	-	-	-
Plant seed production	3	6.34	*0.096*	2	4.04	0.133	-	-	-	1	0.00	0.967	-	-	-
Seed weight	3	11.40	**0.010**	2	6.89	**0.032**	-	-	-	1	4.18	**0.041**	-	-	-

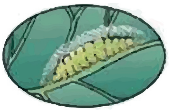
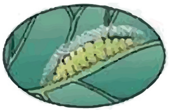


Table 3. Part B: Output of generalized linear (mixed) models showing the effects of different fixed factors (species richness, feeding guild, time-point) on interactions of *Brassica nigra* plants with *Plutella xylostella* and its natural enemies, attraction and visitation by pollinators, flowering traits, and plant seed production. All random factors were initially included in the model, and factors which explained less than 3 percent variation or with a P > 0.05 were excluded from the model. Bold values indicate results where P ≤ 0.05. Italic values indicate results where P ≤ 0.1.

	FG*TP			**SR*FG*TP**			**Block / Day (pollinators)**		
	df	χ^2	*P*	df	χ^2	*P*	df	χ^2	*P*
Plutella xylostella abundance	3	2.41	0.491	1	4.67	0.031	1	31.47	**<0.001**
Plutella xylostella parasitism	-	-	-	-	-	-	1	28.57	**<0.001**
Number of pollinators	2	5.15	0.076	1	0.98	0.323	1	51.32	**<0.001**
Flowers visited by pollinators	2	0.76	0.684	1	0.01	0.937	1	24.25	**<0.001**
Time to flower	-	-	-	-	-	-	1	610.60	**<0.001**
Flowering time	-	-	-	-	-	-	-	-	-
Plant seed production	-	-	-	-	-	-	1	17.19	**<0.001**
Seed weight	-	-	-	-	-	-	1	154.70	**<0.001**

Table 4: Output of generalized linear (mixed) models showing the effects of different fixed factors (herbivore species, time-point) on interactions of *Brassica nigra* plants with *Plutella xylostella* and its natural enemies, attraction and visitation by pollinators, flowering traits, and plant seed production. All random factors were initially included in the model, and factors which explained less than 3 percent variation or with a *P* > 0.05 were excluded from the model. Bold values indicate results where *P* ≤ 0.05. Italic values indicate results where *P* ≤ 0.1.

	Herbivore treatment (T)			**Time point (TP)**			**T*TP**			**Block**			**Day**		
	df	χ^2	*P*	**df**	χ^2	*P*	**df**	χ^2	*P*	**df**	χ^2	*P*	**df**	χ^2	*P*
Plutella xylostella abundance	12	11.99	0.446	1	1.44	0.231	12	14.99	0.242	1	32.25	**<0.001**	-	-	-
Plutella xylostella parasitism	12	23.26	0.026	1	67.55	<0.001	-	-	-	1	28.25	**<0.001**	-	-	-
Number of pollinators	12	23.58	0.023	1	0.11	0.744	12	19.31	*0.081*	-	-	-	1	42.72	<0.001
Flowers visited by pollinators	12	17.22	0.141	1	1.49	0.222	12	14.71	0.258	1	25.03	**<0.001**	-	-	-
Time to flower	12	11.26	0.507	-	-	-	-	-	-	1	610.24	**<0.001**	-	-	-



	Herbivore treatment (T)			Time point (TP)			T*TP			Block			Day		
	df	χ²	*P*	df	χ²	*P*	df	χ²	*P*	df	χ²	*P*	df	χ²	*P*
Flowering time	12	28.40	0.005	-	-	-	-	-	-	-	-	-	-	-	-
Number of seeds	12	21.48	0.044	-	-	-	-	-	-	1	14.31	<0.001	-	-	-
Seed weight	12	44.09	<0.001	-	-	-	-	-	-	1	158.06	<0.001	-	-	-

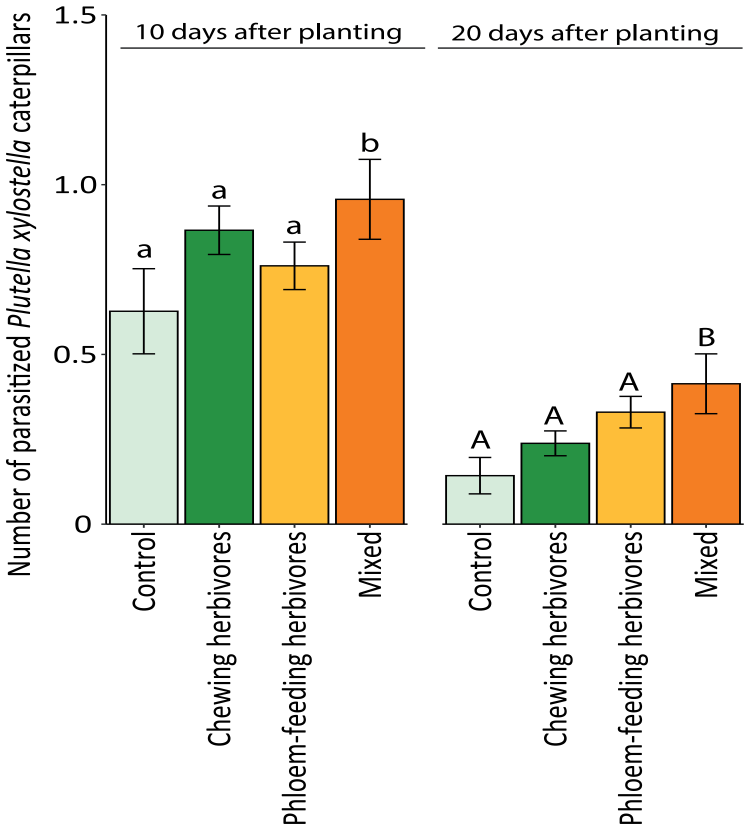
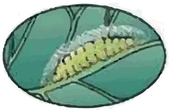


Figure 3: Number of parasitized *Plutella xylostella* larvae on *Brassica nigra* plants treated with different herbivores grouped by feeding guild and species richness. Plants were treated 2 days after planting or were left untreated at the two time points measured. Bars represent the mean ± the standard error around the mean. Letters above bars indicate significant differences ($P \leq 0.05$) between herbivore treatments within a time point based on adjusted Tukey's post hoc tests, small and capital letters were used for different time points.

Pollinator visitation in the field is affected by richness of initial attackers

We observed 4077 flowering plant visits by at least 21 insect groups/species (Table 5). Almost 80 percent of the visits were by two dominant species: *Eristalis tenax* and *Apis mellifera*. For the remaining visits we observed more than seven syrphid fly species, more than four bumblebee species, and several solitary bee, fly, butterfly and wasp species.



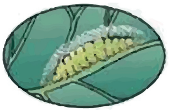
Overall pollinator visitation was affected by the feeding guild of the herbivores infesting the plants (Tables 3 and 4). The effect depended marginally on time point and was more apparent two weeks after flowering than one week after flowering (Tables 3 and 4). Two weeks after flowering, plants attacked by phloem feeders received fewer pollinator visits compared to undamaged plants, to plants attacked by chewers and to plants attacked by a mix of phloem feeders and chewers (i.e. Mixed) (Figure 5). In addition, two weeks after flowering the overall pollinator visitation was affected by specific herbivores (Figure 6). Plants attacked by the phloem feeders *L. erysimi* or *M. persicae* subsp. *nicotianae* were visited by fewer pollinators than plants that did not receive herbivory, than plants that were attacked by *Athalia rosae*, by *M. brassicae*, by *Pieris brassicae*, or by a mix of one chewer and one phloem feeder (Figure 6). The overall flower visitation by pollinators was marginally affected by the interaction between herbivore richness and feeding guild (Table 3). Overall flower visitation was not affected by specific herbivores (Figure 7). Compared to control plants, the number of flowers visited by pollinators was not different on plants attacked by one or four species of phloem feeders (i.e. Phlo-1 or Phlo-4), by four species of chewers (i.e. Chew-4) or by two or four species of a mix of chewers and phloem feeders (i.e. Mix-2, Mix-4) (Figure 8). In contrast, the number of flowers visited by pollinators was higher on plants attacked by one leaf-chewer species than on control plants (Figure 8).

Plant phenotypic traits are affected by herbivore richness and functional traits

Herbivore communities introduced early in the season affected flowering time, but not the onset of flowering of *B. nigra* plants, and effects depended on herbivore identity rather than trait composition or richness of the herbivore community (Figures. 9 and 10). *M. persicae* subsp. *nicotianae* induced shorter flowering compared to any other treatment (Figure 10). Interestingly, none of the other phloem feeders, nor the combination of the four phloem feeders, reduced flowering time compared to control plants. On the contrary, plants that were attacked by *B. brassicae* flowered longer than plants attacked by the four chewers or than plants attacked by *P. brassicae* (Figure 10).

Table 5: Pollinator groups/species observed visiting flowers of *Brassica nigra*.

Pollinator groups	Species	Total number observed	Percentage of total visitors
Honeybees	*Apis mellifera*	1583	38.8
Syprhid flies	*Eristalis tenax*	1594	39.1
	Eupeodes corollae	218	5.3
	Sphaerophoria scripta	198	4.9
	Episyrphus balteatus	35	0.9
	Scaeva pyrastri	29	0.7
	Helophilus pendulus	18	0.4
	Syrphus spp.	7	0.2
	Elves (*Meliscaeva* spp., *Meligramma* spp., *Melangyna* spp., *Fagisyrphus* spp., *Epistrophella* spp.)	1	0.0
	other Syrphidae	110	2.7



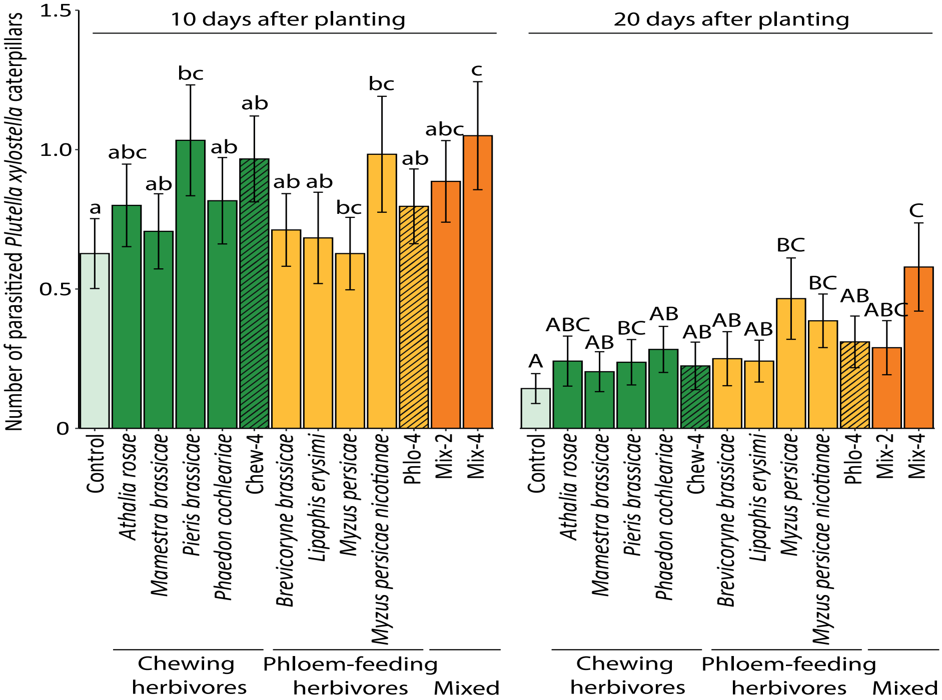
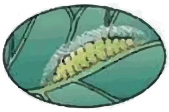


Figure 4: Number of parasitized *Plutella xylostella* larvae 10 or 20 days after planting on plants that received herbivore treatments or were left untreated. Bars represent the mean ± the standard error around the mean. Letters above bars indicate significant differences (P ≤ 0.05) between herbivore treatments within a time point based on non-adjusted Tukey's post hoc tests, small and capital letters were used for different time points.

Pollinator groups	Species	Total number observed	Percentage of total visitors
Solitary bees	*Andrena* spp. *Lasioglossum* spp.	92	2.3
Flies	*Calyptrata* spp	91	2.2
Bumblebees	*Bombus lapidarius*	18	0.4
	B. pascuorum	5	0.1
	B. terrestris	2	0.0
	B. hypnorum	1	0.0
	other *Bombus* spp.	4	0.1
Butterflies	*Pieris* spp.	10	0.2
	other Lepidoptera	6	0.1
Wasps	*Vespula* spp., *Polistes* spp.	16	0.4
Other insects	Unidentified	31	0.8
	Total	4077	100



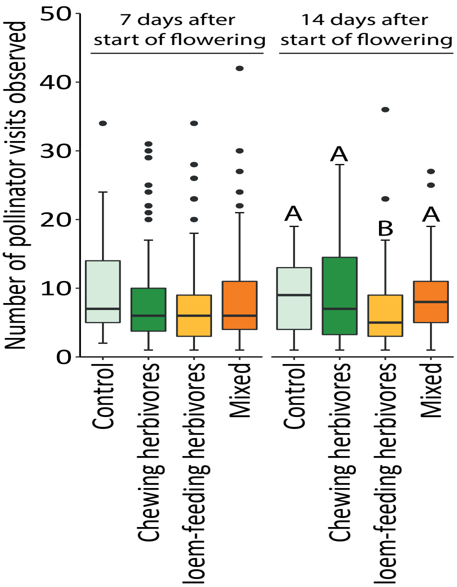


Figure 5: Number of pollinator visits observed on flowers of *Brassica nigra* plants treated with different herbivores grouped by feeding guild or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Observations lasted for 10 minutes and were made at two time points: between 7 and 9 days, and 14 and 16 days after plots had started flowering. Letters above bars indicate significant differences (P ≤ 0.05) between herbivore treatments within a time point based on adjusted Tukey's post hoc tests, capital letters were used for different time points.

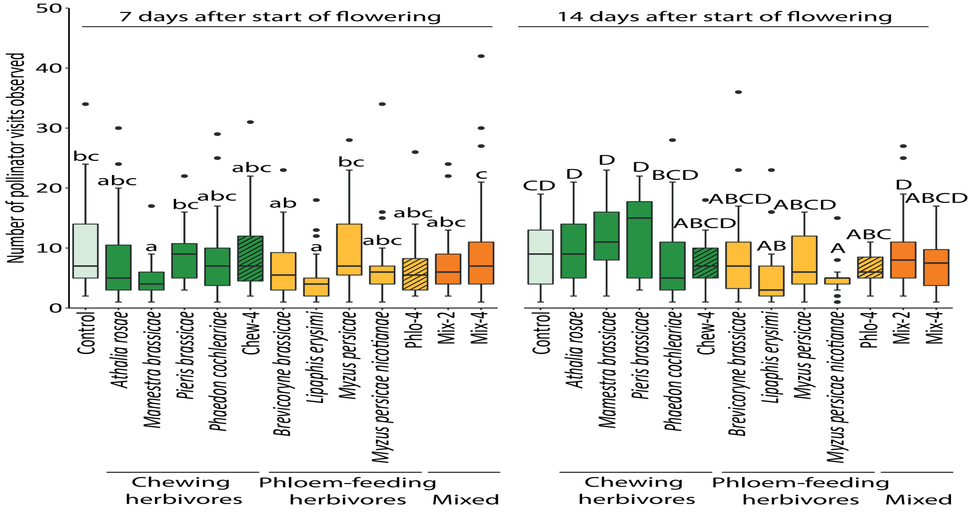
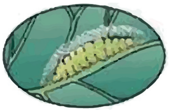


Figure 6: Number of pollinator visits observed on flowers of *Brassica nigra* plants treated with different herbivores or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Observations lasted for 10 minutes and were made at two time points: between 7 and 9 days, and 14 and 16 days after plots had started flowering. Letters above bars indicate significant differences (P ≤ 0.05) between herbivore treatments within a time point based on non-adjusted Tukey's post hoc tests, small and capital letters were used for different time points.



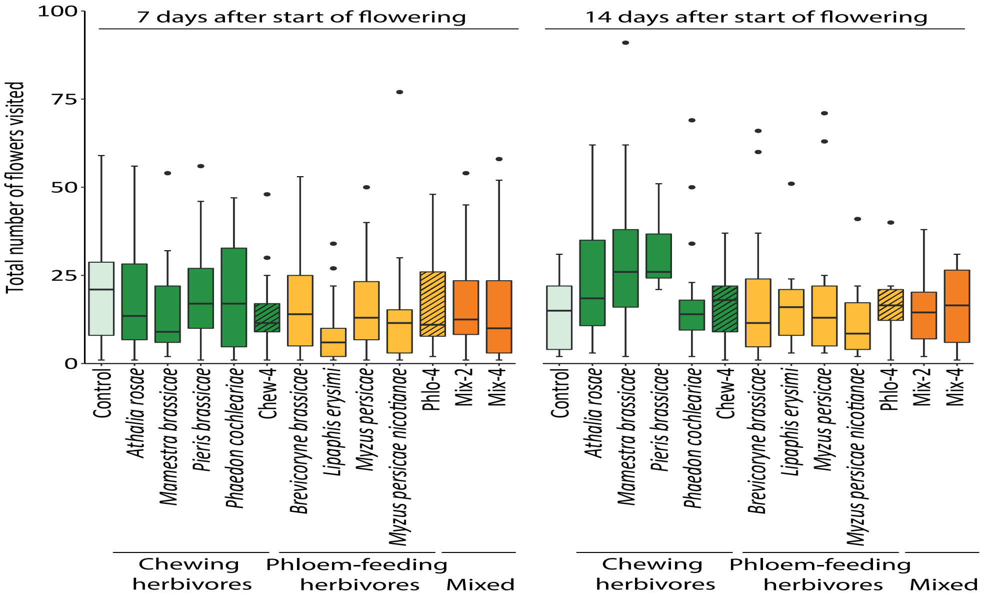


Figure 7: Number of flowers visited by all pollinators on *Brassica nigra* plants treated with different herbivores or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Observations lasted for 10 minutes and were made at two time points: between 7 and 9 days, and 14 and 16 days after plots had started flowering. Letters above bars indicate significant differences (P ≤ 0.05) between herbivore treatments within a time point based on non-adjusted Tukey's post hoc tests, small and capital letters were used for different time points.

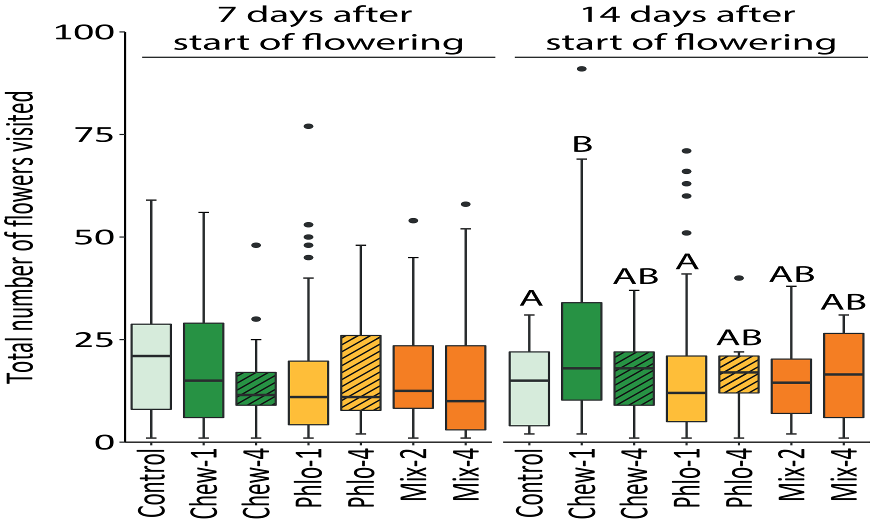
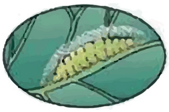


Figure 8: Number of flowers visited by all pollinators on *Brassica nigra* plants treated with different herbivores grouped by feeding guild and species richness or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Observations lasted for 10 minutes and were made at two time points: between 7 and 9 days, and 14 and 16 days after plots had started flowering. Letters above bars indicate significant differences (P ≤ 0.05) between herbivore treatments within a time point based on Tukey's post hoc tests, small and capital letters were used for different time points.



Plant seed production

The number of seeds produced by *B. nigra* plants was not directly affected by herbivore species richness (df = 3, χ2 = 6.34, P = 0.096) nor by herbivore feeding guild (df = 2, χ2 = 4.04, P = 0.133) . However, specific herbivore species reduced the number of seeds produced (df = 12, χ2 = 21.48, P = 0.044) . Plants attacked by *A. rosae*, by *Myzus persicae* subsp. *nicotianae*, and by the highest richness of chewers and phloem feeders (i.e. Chew-4 and Phlo-4 respectively) produced fewer seeds compared to plants that did not receive herbivory (Figure 11). Seed weight was affected by herbivore species richness (df = 3, χ2 = 11.40, P = 0.010), herbivore feeding guild (df = 2, χ^2 = 6.89, P = 0.032), and by their interaction (df = 1, χ2 = 6.34, P = 0.041) (Figure 12). The richness of attackers only affected seed weight on mixed-induced plants. Plants attacked by the lowest richness of the mixture of phloem feeders and chewers (i.e. Mix-2) produced fewer seeds than plants attacked by the higher mixed species richness (i.e. Mix-4) (Figure 12). The seed weight produced by Mix-2-induced plants was lower than plants that did not receive initial herbivore treatment or than plants that were induced by one species of phloem feeder (Phlo-1) (Figure 12). In addition to trait composition and richness of the attacker community, specific herbivores affected the seed weight produced by *B. nigra* plants (df = 12, χ2 = 44.09, P = 0.001). *Athalia rosae*-treated and Mix-2-induced plants produced lighter seeds whereas *B. brassicae*-treated and Mix-4-induced plants produced heavier seeds (Figure 13).

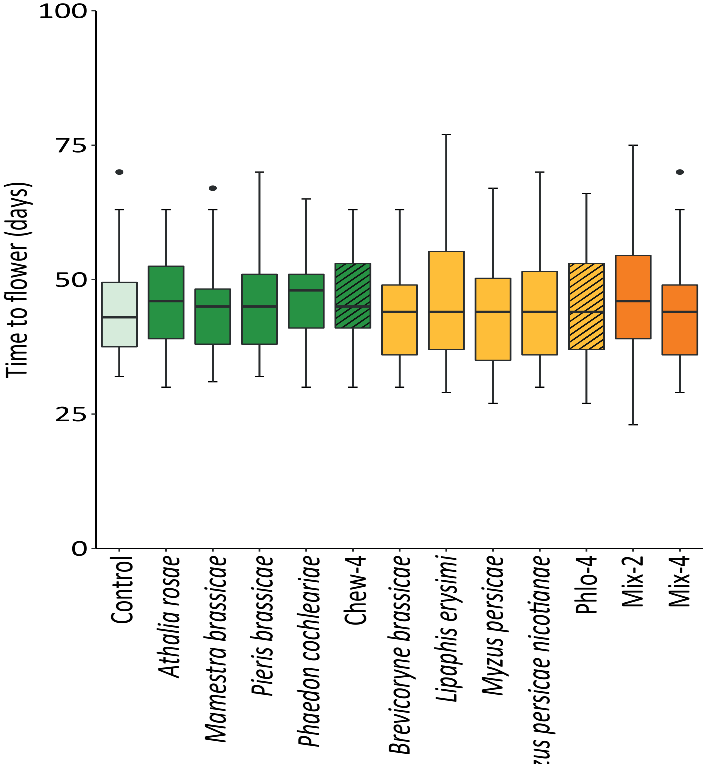


Figure 9: Time needed for *Brassica nigra* plants to start flowering after being treated with different herbivores or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles.

Direct and indirect effects of community members on plant fitness

Structural equation modelling (SEM) shows that the introduced herbivore communities directly and indirectly altered interactions between community members and that this change in interactions has consequences for plant fitness (Figure 14). Plant performance parameters were strong indicators of plant reproductive success. Plants with more leaves produced more but lighter seeds (i.e. positive correlation of number of leaves with seed number but negative correlation with seed weight) (Figure 14). Bigger plants (i.e. bigger plant radius) produced heavier seeds (Figure 14). Plants with a longer duration of flowering produced more and heavier seeds (Figure 14). The introduced herbivore communities affected plant fitness frequently by altering plant performance traits. *Myzus persicae* was the only herbivore with a direct positive connection with seed weight (Figure 14). *Myzus persicae*-induced and *B. brassicae*-induced plants produced heavier seeds explained by a reduction in the number of leaves of the plant (Figure 14). In contrast, *A. rosae* and a mix of one phloem feeder and one chewer (i.e. Mix-2) had a negative effect on seed weight by reducing the duration of flowering (Figure 14). The amount of leaf damage positively affected seed weight by increasing the duration of flowering (Figure 14). Although the introduced herbivore communities affected abundance of *P. xylostella*, its parasitism rate and plant visitation by pollinators (Figure 14), these effects were weakly connected to plant fitness parameters. The strongest connections were made through multiple interactions among organisms and were often via plant traits (Figure 14). In itself, the abundance of *P. xylostella* did not correlate with plant fitness (number of seeds and seed weight). However, the parasitism rate of *P. xylostella* one week after induction negatively correlated with the number of seeds produced by the plants (Figure 14). Pollinators were the strongest predictors for plant fitness (Figure 14). The number of pollinators visiting the plant had a positive effect on the number of seeds produced by increasing the number of flowers visited per plant (Figure 14). Flowering time had a negative effect on the numbers of seeds produced because it reduced the numbers of flowers visited by pollinators (Figure 14). By interaction with pollinators, the abundance of *P. xylostella* and its parasitism rate affected plant fitness. Abundance of *P. xylostella* had an indirect positive effect on the number of seeds produced by increasing the number of flowers visited by pollinators (Figure 14). The parasitism rate of *P. xylostella* positively correlated with the number of flower visitors, that enhanced the number of flowers visited and positively correlated with seed set. These interactions were differentially influenced by the composition of the herbivore community introduced early in the season. Introduction of a species rich phloem feeder community (Phlo-4) negatively correlated with plant damage, that corresponded with higher parasitism rates, cascading to increased number of flower visitors and visitations that enhanced seed number (Figure 14). Thus, introduction of the phloem feeder community eventually resulted in lower plant fitness (see also Figure 11). A similar negative effect on seed number could be deduced for the specific phloem feeder *L. erysimi* that reduced the number of flowers visited by pollinators (Figure 14). Also, the species-rich leaf chewer community (Chew-4) reduced the number of flowers visited by pollinators that positively correlated with seed number, thus yielding overall reduced fitness. A mix of herbivores (Mix-2, Mix-4), three out of four leaf chewer species (*A. rosae*, *P. cochleariae*, *P. brassicae*) and three out of four phloem feeder species (*Myzus persicae*, *Myzus persicae nicotianae*, *B. brassicae*) positively affected abundance of *P. xylostella* that in turn positively correlated with the number of flowers visited by pollinators, which resulted in increased seed set.

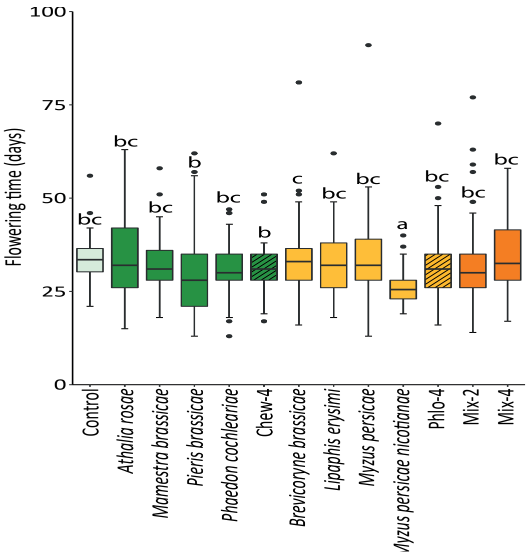


Figure 10.:Flowering time of *Brassica nigra* plants after being treated with different herbivores or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Letters above bars indicate significant differences ($P \leq 0.05$) between herbivore treatments based on non-adjusted Tukey's post hoc tests.

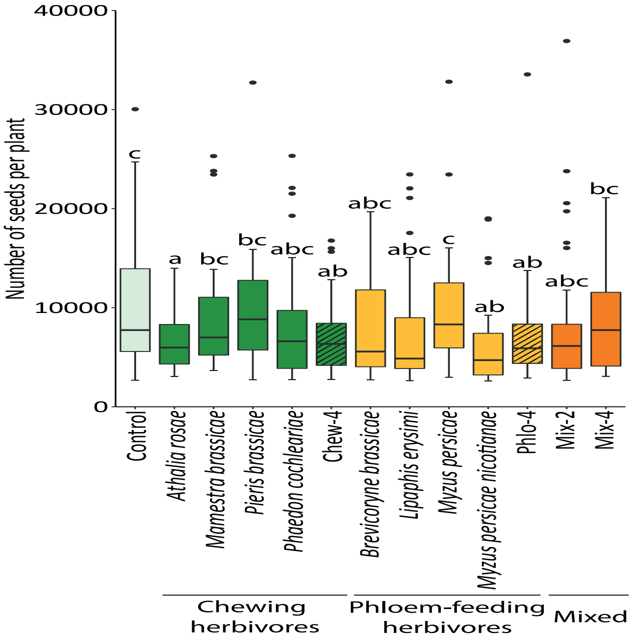
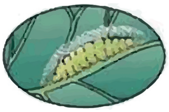


Figure 11: Number of seeds produced by *Brassica nigra* plants after being treated with different herbivores or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Letters above bars indicate significant differences ($P \leq 0.05$) between herbivore treatments based on non-adjusted Tukey's post hoc tests.



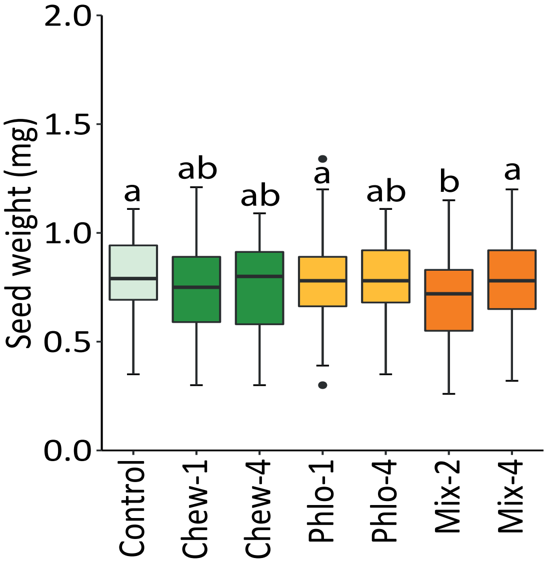
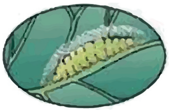


Figure 12: Weight of individual seeds produced by *Brassica nigra* plants after receiving different herbivore treatments grouped by species richness or feeding guild or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Letters above bars indicate significant differences ($P \leq 0.05$) between herbivore treatments based on adjusted Tukey's post hoc tests.

Discussion

Herbivore richness, its combination of functional traits and specific herbivore species affected the abundance of *P. xylostella*, the parasitism rate of *P. xylostella* and pollinator visitation to *B. nigra* plants. The composition of the initial herbivore community affected plant fitness predominantly through its effects on plant performance such as growth, flowering time, and amount of leaf damage that strongly correlated with number and weight of seeds produced. In addition, species-rich communities of a single feeding guild (either only leaf chewers or only phloem feeders) negatively affected plant fitness indirectly through a chain of interactions involving reduced pollinator visitation rates that positively correlated with seed number. In contrast, plants induced with a mix of phloem feeders and leaf chewers, as well as plants induced by individual herbivore species, promoted the presence of *P. xylostella* that corresponded with enhanced flower visitation and increased seed production. These indirect interactions that resulted in positive effects on seed production did in most cases not compensate for the negative effect of herbivores on fitness via effects on plant performance.

Herbivore richness, herbivore functional traits and herbivore identity affected the interaction network of *B. nigra*. In general, *P. xylostella* was more abundant on plants that received herbivory early in the season regardless of the richness and the functional traits, compared with control plants. Likewise, in a field study *P. xylostella* was more abundant on *Brassica oleracea* plants attacked by *Pieris rapae*, compared with undamaged plants (Poelman et al., 2008). In our study, not only *P. xylostella* was affected by herbivory, but also its parasitoids. The parasitism rate of *P. xylostella* larvae was affected by specific herbivores attacking the plant, and by their



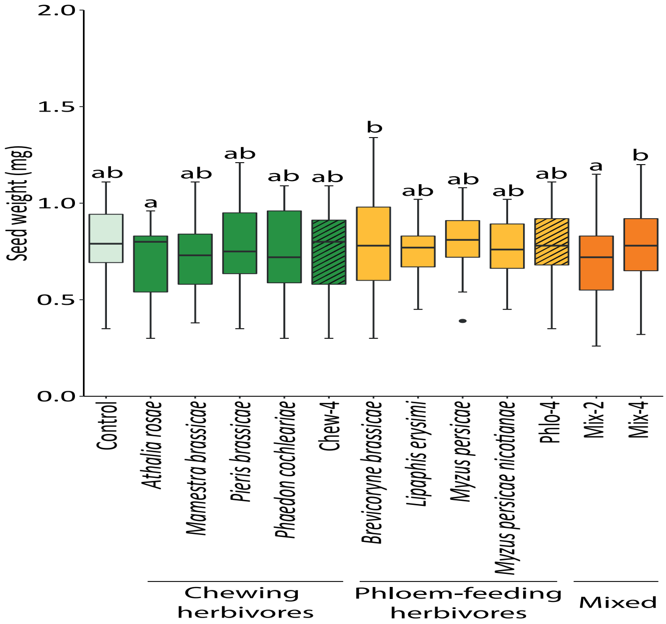
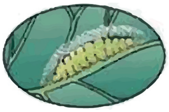


Figure 13: Weight of individual seeds produced by *Brassica nigra* plants after receiving the herbivore treatments or left untreated. Boxplots show median (line), first and third quartiles, minimum and maximum. Outliers (1.5 times the interquartile range below the first or above the third quartile) are represented by circles. Letters above bars indicate significant differences ($P \leq 0.05$) between herbivore treatments based on non-adjusted Tukey's post hoc tests.

feeding guild. Plants attacked by a mix of two chewers and two phloem feeders (i.e. Mix-4) had higher rates of parasitism than parasitism rates on control plants. A recent study showed that compared to the performance on undamaged plants, *P. xylostella* grew less on plants induced by chewers, while on mixed-induced plants the performance was not reduced (Fernández de Bobadilla et al., 2021). Parasitoids may be more attracted to mixed-induced plants because of the higher detectability and the association of the mixed-induced volatiles with a higher quality of the host (Aartsma et al., 2019). Additionally, in a choice assay in the greenhouse, *P. xylostella* laid fewer eggs on plants attacked by a mix of two chewers and two phloem feeders than on plants attacked by phloem feeders. The reduced preference of *P. xylostella* towards the plants that in the field received the highest parasitism may be indicative of an adaptation of *P. xylostella* to avoid herbivore communities that predict high risk of parasitism.

In our study, *P. xylostella* abundance did not have direct effects on plant fitness. Thus herbivore-herbivore interactions between the species we studied did not explain fitness. However, *P. xylostella* abundance connected with an increase in the number of flowers visited by pollinators, which was positively correlated with plant fitness. This suggests that interactions from early-season herbivory to a later arriving herbivore that affects flower visitation by pollinators imposes selection on plant traits (Mertens et al., 2021a; Rusman et al., 2019a). Similarly, simulated florivory in *Impatiens capensis* plants, reduced pollinator and pollen thieve visits, and the alterations in interactions affected plant fitness (Soper Gorden and Adler, 2018, 2016). Plants attacked by the phloem feeder *L. erysimi* and by the four chewers simultaneously (i.e. Chew-4) received fewer visits by pollinators. Likewise, a recent study shows that herbivory by aphids



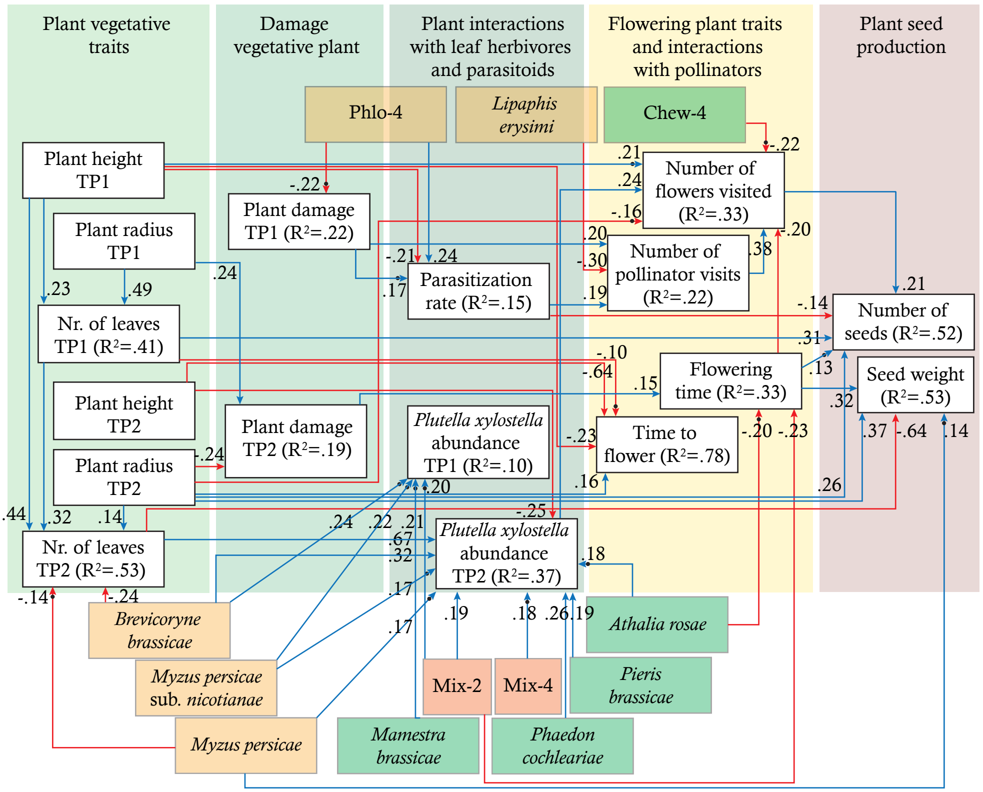
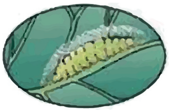


Figure 14: Structural Equation Modelling showing variables directly affected by the herbivore treatments, Blue arrows represent positive links whereas, red negative. A dot in front of the arrows represents correlations where $0.05 < P < 0.10$. TP1 and TP2 refer to measurements made at the first and second time point, respectively. Chew-4, and Phlo-4 are plants that received four species of chewers and phloem feeders, respectively. Mix-2 and Mix-4 are plants that respectively received four or two species of a mix of chewers and phloem feeders.

caused nectar shortage which reduced pollination visitation and led to a reduction in plant fitness (Devegili & Chalcoff, 2020). The herbivore-induced effects on plant interactions with herbivores, parasitoids and pollinators could be attributed to a change in important cues for insect foraging, such as the volatile blend or visual cues (Kessler and Chautá, 2020; Moreira et al., 2019; Rusman et al., 2019b).

Plant fitness was affected by the richness of herbivores attacking the plant, by their feeding guild, and by their interaction. Attack by the highest richness of chewers and phloem feeders (i.e. Chew-4 and Phlo-4 respectively) as well as by *A. rosae*, or by *M. persicae* subsp. *nicotianae* negatively affected plant fitness in terms of seed numbers. The negative effect on plant fitness was connected with a reduction in pollinator visitation. Additionally, when feeding individually, the two most common aphids attacking *B. nigra* (i.e. *M. persicae* and *B. brassicae*) (Mertens et al., 2021a) had a positive effect on seed weight by inducing a reduction in the number of leaves. The negative correlation between number of leaves and seed production could be explained by the plant reallocating the energy used for leaves to other parts, such as the roots, increasing the plant nutrient uptake (Ruan et al., 2013). Additionally, a reduction in numbers of leaves



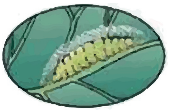
could make the plant less attractive to herbivores not monitored in this study that have strong fitness consequences. However, we did not find any correlation between seed weight and leaf damage. Therefore, the herbivore reducing plant fitness on plants with fewer leaves should be a species that does not cause notable leaf damage to the plant at the time measured (e.g., a root- or phloem-feeding herbivore). *Delia radicum* larvae feed on Brassicaceae roots, causing high fitness costs being unnoticed for weeks (Soler et al., 2005, 2007). Alternatively, the estimated leaf damage of a full plant might not be a good predictor of plant fitness (de Vries et al., 2017, 2018, 2019). In contrast to the positive effect of some individual phloem feeder species on plant fitness, when phloem feeders attacked the plant in combination with chewers (i.e. Mix) the collective effect on plant fitness was negative. Induction of plants with only leaf chewers also reduced plant fitness. Plants attacked by the chewer *A. rosae* and a by mix of one chewer and one phloem feeder (i.e. Mix-2) produced lighter seeds through reduced flowering time. The reduction in seed weight linked to shorter flowering is potentially explained by a reduction in pollinator visitation or by lower quality pollination of individual flowers.

We show that herbivore richness, functional trait diversity and herbivore identity affect plant fitness and plant interactions with *P. xylostella*, parasitoids and pollinators. Herbivory strongly affected plant fitness through reducing plant performance and flowering time. Indirect interactions between subsequent herbivores, parasitoids and pollinators affected plant fitness mediated by plant traits. All of these interactions involved effects of inducing herbivores on pollinators that closely connected with the total number of seeds produced by a plant. Under climate change and insect decline, where herbivore communities are predicted to be poorer in species richness, we may expect effects on plant trait evolution. One of the groups that is already suffering a worrying decline is pollinators (Althaus et al., 2021). If the pollinator decline situation continues, pollinator limitation will likely magnify the fitness consequences of indirect interactions observed in our study, as all the routes affecting plant fitness connected to pollinators. Additionally, the natural biological control of herbivores by parasitoids is expected to be disrupted due to climate change (Chidawanyika et al., 2019). Under climate change, predictions pose that plants will suffer more stress and the extensive effects of herbivore richness, functional trait and identity on the interaction network will likely add to this impact on plant fitness (Wagner et al., 2021).

Our study has uncovered the tip of the iceberg of how herbivore richness, functional trait and identity alters direct and indirect interactions with other insects and has fitness effects on plants. Our work allows us to predict that the current situation of insect decline and climate change will have dramatic consequences on the structure of the community interacting with a plant. However, due to the complexity and high connectivity of the interactions network it is very difficult to make predictions on directions of effects for plant trait selection.

Acknowledgements

The authors thank Sean Zending for helping in data collection of the plant flowering traits and pollinator visitation, and Karin Winkler for her kind help with the design of the device used to measure *P. xylostella* field parasitism. We thank Marcel Dicke for the fruitful comments on earlier version of this manuscript. We are thankful to the staff of Unifarm for setting up and maintaining the experimental fields.



Supplement

Materials and methods for experiments studying the effect of herbivore richness and guild on the oviposition preference of *Plutella xylostella* on *Brassica nigra* plants

Plants and insects

3-4 weeks old black mustard, *Brassica nigra* (Brassicales: Brassicaceae) were used for the experiments. The plants were grown and maintained in a greenhouse at 22 ± 2 °C, 60-70 % RH and 16:8 h L:D photo regime. Insects used as inducers were the same species and reared in the same conditions as the ones used the field experiment (see main text).

Plant induction and Plutella xylostella oviposition preference

Four leaves of each plant were induced with the herbivore treatments, which were the same treatments that were used in the field study (Con-0, Phlo-1, Phlo-4, Chew-1, Chew-4, Mix-2, Mix-4). Each of the four leaves received the total number of herbivores applied on the whole plant in the field. After herbivore infestation, each leaf was enclosed in a mesh bag, to prevent the movement of the herbivores to different leaves. The induction lasted for five days. Con-0 plants functioned as control, these plants did not receive any inducing herbivore, but were treated in a similar way as plants receiving herbivores. Plants that received the different treatments were placed in the greenhouse in a randomized block design.

We prepared plastic oviposition cages, which had as base a large Petri dish (145/20 mm) on which plastic sheets (210x297 mm) were installed to create the walls of the cages (as described in Poelman et al. 2008) (Fig S1). Two 5 ml glass vials were glued to the bottom of the oviposition cages and were filled with water. Right before the oviposition preference assays, one leaf from each of the two plants to test was detached from the plant with scissors, and the leaf petiole was immediately submerged in one of the two vials filled with water, to avoid dehydration, and the herbivores were removed with a brush. To avoid differences in oviposition preference caused by leaf age, pairs of leaves of similar age were chosen for the two-choice set-up. The leaves were placed in a way that prevented them from touching each other and were randomly assigned to one of two positions in the plastic cage (Fig S1).

The oviposition preference of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) between leaves of plants that received different treatments was studied. Oviposition preference of *P. xylostella* moths is mostly driven by post alighting gustatory perception of non-volatile glucosinolates, leaf surface waxes, chemosensory stimulation and abiotic factors (Justus and Mitchell, 1996; Zhang et al., 2012). One female *P. xylostella* moth was released in each oviposition cage together with one adult male, to ensure mating. The exact age of the moths was unknown, and younger and older butterflies were mixed to randomize our design. Since it is known that *P. xylostella* prefers to oviposit at dusk, the oviposition preference was measured twenty-four hours after the release of the couples. This was done by carefully inspecting and counting the number of eggs found on each leaf. The number of eggs present on the walls of the oviposition cage was also recorded. The leaves of each choice set-up and the *P. xylostella* moths were used only once. Each oviposition cage was considered as one replicate.

Oviposition preference comparisons

To test if the oviposition preference of *P. xylostella* moths is affected by the herbivore treatments, we conducted two main experiments, performed one after each other in the same greenhouse compartment. The first experiment was designed to evaluate the effect of herbivory and of herbivore guild on the oviposition preference of *P. xylostella*. To test the effect of herbivory, *P. xylostella* moths were allowed to choose oviposition site between a leaf from an undamaged plant (i.e. Con-0) or from a plant induced with the highest richness of phloem feeders, leaf chewers or a mix of both (i.e. Phlo-4, Chew-4 or Mix-4). To test the effect of guild of the attacker, the moths were allowed to choose oviposition site between leaves from the highest herbivore richness of phloem feeders, chewers or a mix (Table S1). The experiment was divided in four blocks over time, performed one after each other. Each choice was repeated 12 times in each of the blocks, making a total of 48 replicates per treatment comparison.

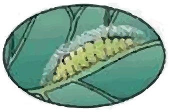
In a second experiment we studied the effect of herbivore richness within feeding guild on the oviposition preference of *P. xylostella*. The moths were allowed to choose between leaves induced with the lowest or highest richness of herbivores within each guild (Table S2). This experiment was also divided in four blocks over time, performed one after each other. For the effect of phloem-feeder and chewer richness, each combination was repeated eight times in each of the blocks, making a total of 32 replicates per treatment comparison. For the mixed richness, two species of Mix-2 were compared with the same two species of Mix-2 plus two more species. We selected treatments, so that each combination of one phloem feeder and one chewer species of Mix-2 was tested against three different combinations of Mix-4, making a total of 48 different comparisons (Table S2). Due to the high number of choice combinations, and space and time limitations, each comparison was repeated only once in each of the four blocks.

Statistical analysis

In some cases, the moths did not lay any egg and these replicates were not included in the analysis (Table S3). In other cases, *P. xylostella* laid most eggs on the walls of the oviposition cage (Table S3). When the percentage of eggs found on the walls of the cage exceeded 50%, we excluded the replicate from further analysis (Table S3). Oviposition preference data was analysed using two-sided paired t-tests if normally distributed, or by Wilcoxon signed-rank tests in case the data did not follow a normal distribution. The normality was visually examined by making histograms of the difference of the eggs that were laid in the 1st choice leaf and the 2nd choice leaf in each dual oviposition preference assay (in each oviposition cage). All the statistical analyses were performed using RStudio version 1.1.463.

Results from experiments studying the effect of herbivore richness and guild on the oviposition preference of *Plutella xylostella* on *Brassica nigra* plants

The oviposition preference of *P. xylostella* was not affected by herbivory. The preference of *P. xylostella* did not differ between undamaged leaves and leaves that were induced with the maximum richness of phloem feeders, chewers and a mix of both (Con-0 vs Phlo-4: P = 0.1935; Con-0 vs Chew-4: P = 0.8736; paired t-test; Con-0 vs Mix-4: *P* = 0.1842; Wilcoxon signed rank test Figure S2A-C). *Plutella xylostella* female moths also did not discriminate between leaves induced with highest richness of phloem feeders and chewers, nor between leaves induced with the highest richness of chewers and mix (Phlo-4 vs Chew-4: *P* = 0.87, paired t-test; Chew-4 vs



Mix-4: $P = 0.34$; Wilcoxon signed rank test; Figure S2 D, E). In contrast, the moths preferred to lay eggs on leaves induced with the highest richness of phloem feeders compared to leaves induced with the highest richness of the mix (Phlo-4—Mix-4: $P < 0.001$; Wilcoxon signed rank test, Figure S2F).

The richness of phloem feeders did not affect the oviposition preference of *P. xylostella* and the number of eggs laid by the moths on leaves induced by one species or by four species of phloem feeders was not different (Phlo-1 vs Phlo-4: $P = 0.57$, paired t-test; Figure S3). No significant differences were found in the oviposition preference on leaves induced with *Brevicoryne brassicae*, *Lipaphis erysimi*, *Myzus persicae* and *Myzus persicae* subsp. *nicotianae* compared with leaves induced with all phloem-feeding species simultaneously (*B. brassicae* vs Phlo-4: Wilcoxon signed rank test $P = 0.72$, *L. erysismi* vs Phlo-4: Wilcoxon signed rank test $P = 0.22$; *M. persicae* vs Phlo-4: Wilcoxon signed rank test $P = 0.17$; *M. persicae* subsp. *nicotianae* vs Phlo-4: paired t-test $P = 0.10$.; Figure S3A-D). In line with the lack of phloem-feeder richness on the oviposition preference of *P. xylostella*, the moths did not discriminate between leaves induced with one or with four chewer species (Chew-1—Chew-4: $P = 0.72$, paired t-test). However, some specific herbivores affected *P. xylostella* preference. Moths laid more eggs on *Phaedon cochleariae*- induced leaves than on the maximum chewer diversity (*P. cochleariae* vs Chew-4: Wilcoxon signed rank test $P < 0.05$; Figure S4C), while they preferred to oviposit on leaves induced by the highest chewer richness than on *Mamestra brassicae*-induced leaves (*M. brassicae* vs Chew-4: Wilcoxon signed rank test $P < 0.01$; Figure S4B). In contrast, *P. xylostella* did not discriminate between leaves induced with the highest richness of chewers and *Pieris brassicae*-induced or *Athalia rosae*-induced leaves (*P. brassicae*—Chew-4: paired t-test $P = 0.46$; *A. rosae* – Chew-4: Wilcoxon signed rank test $P = 0.54$; Figure S4 A, D). The oviposition preference of *P. xylostella* was also not affected by the richness of herbivores on a mix of phloem feeders and chewers (Mix-2 vs Mix-4: paired t-test $P = 0.58$; Figure S5).

Figures for experiments studying the effect of herbivore richness and guild on the oviposition preference of *Plutella xylostella* on *Brassica nigra* plants

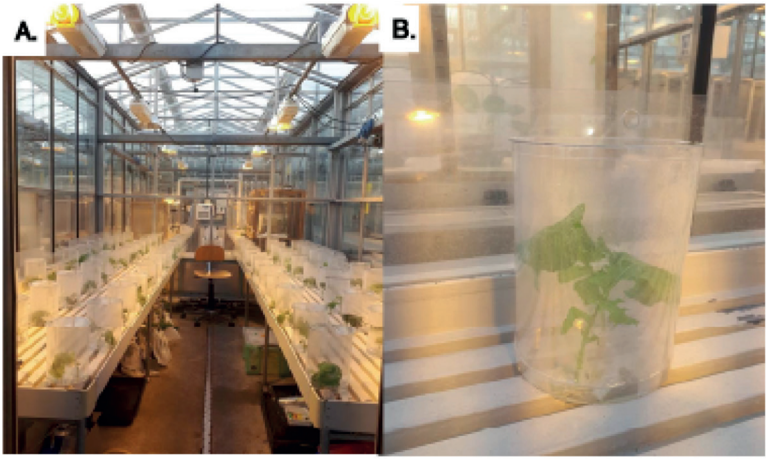
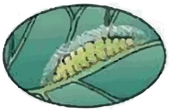


Figure S1: (A) Greenhouse compartment with the cages in which we tested the oviposition preference of *Plutella xylostella* in one block. (B) Closer look of one oviposition cage, with two leaves coming from two different induction treatments with their petioles in the two glass vials.



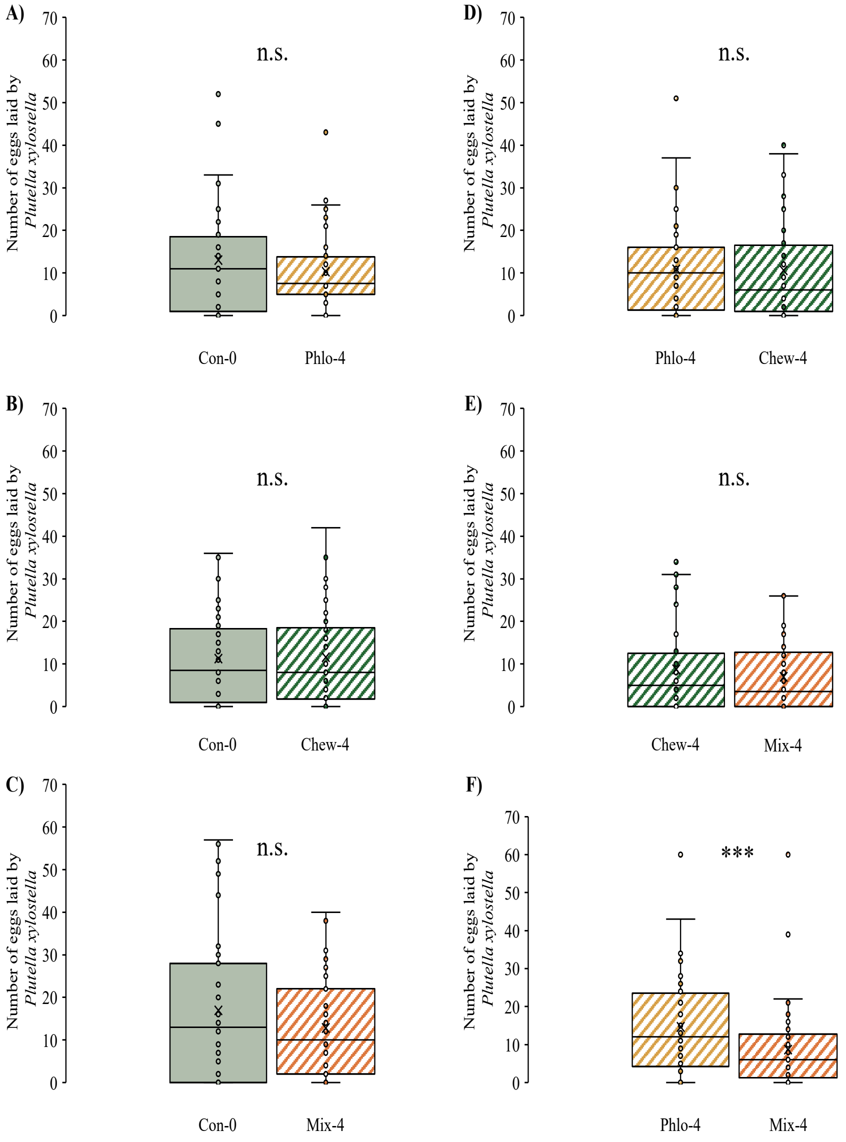
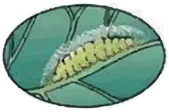


Figure S2: Number of eggs laid by *Plutella xylostella* moths on each *Brassica nigra* leaf of the two-choice assay between A. Untreated plants (Con-0) and plants treated with the highest species richness of phloem feeders (Phlo-4); B. Untreated plants (Con-0) and plants treated with the highest species richness of chewers (Chew-4); C. Untreated plants (Con-0) and plants treated with the highest species richness of a mix of phloem feeders and chewers (Mix-4); D. Plants treated with the highest species richness of phloem feeders (Phlo-4) and plants treated with the highest species richness of chewers (Chew-4); E. Plants treated with the highest species richness of chewers (Chew-4) and plants treated with the highest species richness of a mix of phloem feeders and chewers (Mix-4); F. Plants treated with the highest species richness of phloem feeders (Phlo-4) and plants treated with the highest species richness of a mix of phloem feeders and chewers (Mix-4). Boxplot height corresponds to the first and third quartiles (Q1 and Q3), and the middle line to the median. Asterisks above the boxplots show significant differences (***$P < 0.001$; Wilcoxon signed rank test).n.s.=non-significant differences ($P > 0.05$; Wilcoxon signed rank test).



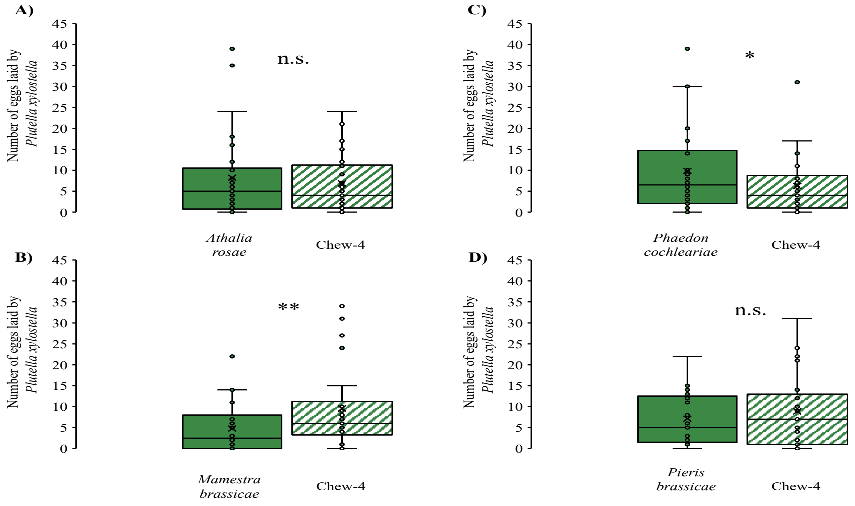


Figure S3: Number of eggs laid by *Plutella xylostella* moths on each *Brassica nigra* leaf of the two-choice assay between A. Plants treated with the chewer *Athalia rosae* and plants treated with the highest species richness of chewers (Chew-4); B. Plants treated with the chewer *Mamestra brassicae* and plants treated with the highest species richness of chewers (Chew-4); C. Plants treated with the chewer *Phaedon cochleariae* and plants treated with the highest species richness of chewers (Chew-4); D. Plants treated with the chewer *Pieris brassicae* and plants treated with the highest species richness of chewers (Chew-4). Boxplot height corresponds to the first and third quartiles (Q1 and Q3), and the middle line to the median. Asterisks above the boxplots show significant differences (**P* < 0.05; ***P* < 0.01; Wilcoxon signed rank test). n.s.=non-significant differences (*P* > 0.05; Wilcoxon signed rank test).

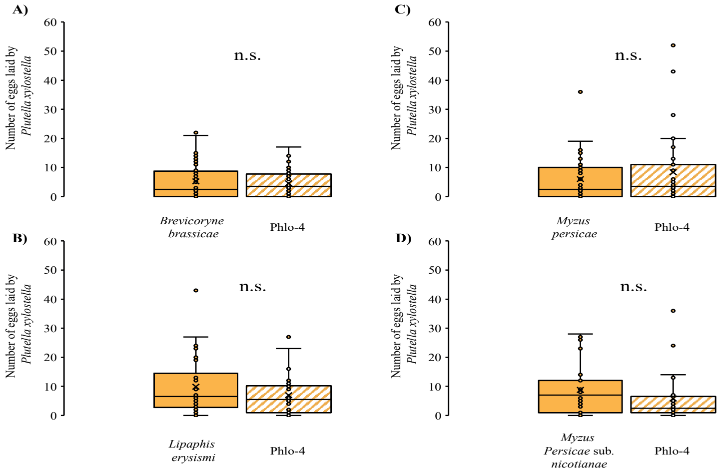
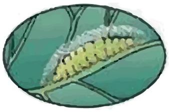


Figure S4: Number of eggs laid by *Plutella xylostella* moths on each *Brassica nigra* leaf of the two-choice assay between A. Plants treated with the phloem feeder *Brevicoryne brassicae* and plants treated with the highest species richness of phloem feeders (Phlo-4); B. Plants treated with the phloem feeder *Lipaphis erysismi* and plants treated with the highest species richness of phloem feeders (Phlo-4); C. Plants treated with the phloem feeder *Myzus persicae* and plants treated with the highest species richness of phloem feeders (Phlo-4); D. Plants treated with the phloem feeder *M. persicae* subsp. *nicotianae* and plants treated with the highest species richness of phloem feeders (Phlo-4). Boxplot height corresponds to the first and third quartiles (Q1 and Q3), and the middle line to the median. n.s.=non-significant differences (*P* > 0.05; Wilcoxon signed rank test).



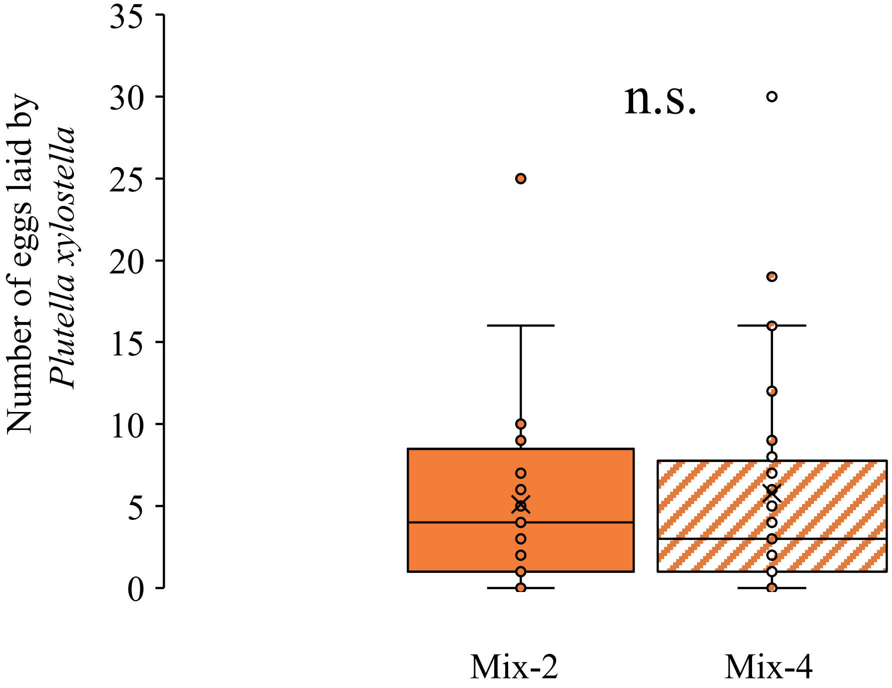


Figure S5: Number of eggs laid by *Plutella xylostella* moths on each *Brassica nigra* leaf of the two-choice assay between plants treated with the two or with four species of a mix of phloem feeders and chewers (Mix-2 or Mix-4). Boxplot height corresponds to the first and third quartiles (Q1 and Q3), and the middle line to the median. Asterisks above the boxplots show significant differences. n.s.=non-significant differences ($P > 0.05$; Wilcoxon signed rank test).

Table S1: Herbivore combinations included in the two-choice oviposition experiment 1. The experiment was divided in four blocks over time. Each comparison was repeated 12 times per block, making a total of 48 replicates per choice.

Leaf 1	Leaf 2
Con-0	Phlo-4
Con-0	Chew-4
Con-0	Mix-4
Phlo-4	Chew-4
Phlo-4	Mix-4
Chew-4	Mix-4

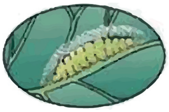
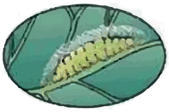


Table S2: Herbivore combinations included in the two-choice oviposition experiment 2. The experiment was divided in four blocks over time. The phloem feeder and chewer richness comparisons were repeated eight times per block, making a total of 32 replicates per choice. Each of the 48 Mix-2 Mix-4 comparisons was repeated only once. Phloem feeding aphids *Brevicoryne brassicae (Bb), Lipaphis erysimi (Le), Myzus persicae (Mp), Myzus persicae* subsp. *nicotianae (Mpn)*. Chewers: *Athalia rosae (Ar), Mamestra brassicae (Mb), Phaedon cochleariae (Pc), Pieris brassicae (Pb).*

Leaf 1	**Leaf 2**
Phlo-1	Phlo-4
Bb	*Bb+Mp+Mpn+Le*
Mp	
Mpn	
Le	
Chew-1	Chew-4
Ar	*Ar+Pb+Pb+Mb*
Pb	
Pc	
Mb	
Mix-2	Mix-4
BbMb	*BbMp MbAr*
BbAr	
MpMb	
MpAr	
BbPb	*BbMp PbPc*
BbPc	
MpPb	
MpPc	
BbMb	*BbMpn MbPc*
BbPc	
MpnMb	
MpnPc	



Leaf 1	**Leaf 2**
BbAr	*BbMpn ArPb*
BbPb	
MpnPb	
MpnAr	
BbMb	*BbLe MbPb*
BbPb	
LeMb	
LePb	
BbAr	*BbLe ArPc*
BbPc	
LeAr	
LePc	
MpMb	*MpMpn MbPb*
MpPb	
MpnMb	
MpnPb	
MpAr	*MpMpn ArPc*
MpPc	
MpnAr	
MpnPc	
MpMb	*MpLe MbPc*
MpPc	
LeMb	
LePc	

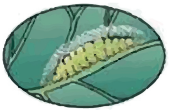
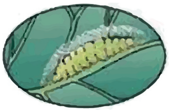


Table S3: Overview of replicates included for the oviposition experiments. “Total” indicates the total number of replicates performed. “No response” indicates the number of set-ups where the moths did not lay any egg. The “No choice” indicates the set-ups where the moths laid more than 50% of the eggs on the oviposition cages instead than on the two choice leaves. The “No response” and “No choice” columns were excluded from the data analysis. The “Included” column represents the number of replicates included in the analysis. Phloem feeding aphids *Bb= Brevicoryne brassicae, Le= Lipaphis erysismi, Mp= Myzus persicae, Mpn= Myzus persicae* subsp. *nicotianae, Ar= Athalia rosae, Mb=Mamestra brassicae, Pb= Pieris brassicae, Pc= Phaedon cochleariae*.

Comparisons	Total	“No response”	“No choice”	Included
Con-0 vs Phlo-4	48	9	0	39
Con-0 vs Chew-4	48	6	1	41
Con-0 vs Mix-4	48	10	0	38
Phlo-4 vs Chew-4	48	7	1	40
Phlo-4 vs Mix-4	48	7	2	39
Chew-4 vs Mix-4	48	14	1	33
Phlo-1 vs Phlo-4/Total	128	26	3	99
Bb vs Phlo-4	32	6	0	26
Le vs Phlo-4	32	6	0	26
Mp vs Phlo-4	32	6	3	23
Mpn vs Phlo-4	32	8	0	24
Chew-1- Chew-4/Total	128	36	5	87
Ar vs Chew-4	32	6	2	24
Mb vs Chew-4	32	10	2	20
Pb vs Chew-4	32	11	0	21
Pc vs Chew-4	32	9	1	22
Mix-2 vs Mix-4	48	20	0	28



Chapter 7.

General discussion

Introduction

Phenotypic plasticity is defined as the ability of one genotype to produce a range of phenotypes in response to environmental variables (Agrawal, 2001; Whitman and Agrawal, 2009). In essence, phenotypic plasticity is the result of biochemical and physiological processes affecting morphology, behaviour and life history. Broadly, phenotypic plasticity encompasses all environmental modulations of gene expression, that result in the expressed phenotype (Schlichting & Smith, 2002). Even maintaining a trait constant across an environmental gradient often requires altering other traits (Whitman and Agrawal, 2009). The phenotype expressed by an individual represents a combination of multiple traits, each with its own degree of plasticity in response to environmental fluctuations, and linked by developmental constraints, and energetic trade-offs dictating the allocation of limited resources to different functions (Flatt & Heyland, 2011; Iltis et al., 2019). The expressed phenotype drives the organism's realised fitness in a given environment, influencing its realised ecological niche within the community (Berg & Ellers, 2010; Dee et al., 2020).

Environmental fluctuations, both biotic and abiotic, are common in natural ecosystems and often affect ecological interactions. Temperature has unavoidable thermodynamic effects on biochemical reactions, at the basis of physiological mechanisms. Thus, the ambient thermal conditions are key for individual fitness and population dynamics of ectothermic organisms (Angilletta, 2009; Huey & Berrigan, 2001). With ongoing climate change, the frequency and magnitude of extreme events, such as heatwaves, are predicted to increase (Fischer and Schär, 2010; Pachauri et al., 2014; Wang and Dillon, 2014). Insects are particularly susceptible to extreme events, having consequences, for example, on their survival and fitness (Colinet et al., 2015; Vasseur et al., 2014; Zhu et al., 2019). Phenotypic plasticity is considered one of the mechanisms allowing insects to cope with thermal fluctuations and extremes and potentially adapt to future climatic conditions (Noer et al., 2022; Wang & Althoff, 2019). Plastic responses to environmental fluctuations can be anticipatory, continuous, or delayed as well as reversible or permanent (Whitman and Agrawal, 2009). Usually morphological and developmental plasticity is considered irreversible as many morphological traits are fixed once the individual reaches adulthood (e.g., body size). Other traits represent reversible plastic adaptations and can track environmental change throughout the individual life cycle (Abram et al., 2017; Whitman and Agrawal, 2009).

This thesis project aimed at assessing the effects of ecologically relevant thermal regimes on phenotype expression in insects and its impact at different trophic levels. I used a well-studied host-parasitoid system comprising the insect herbivore *Plutella xylostella* L. (Lepidoptera: Plutellidae) and two of its larval endo-parasitoids, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* Haliday (Hymenoptera: Braconidae). Here, I discuss the findings of this research project and their ecological implications for species interactions in prospective future climatic conditions (Figure 1).

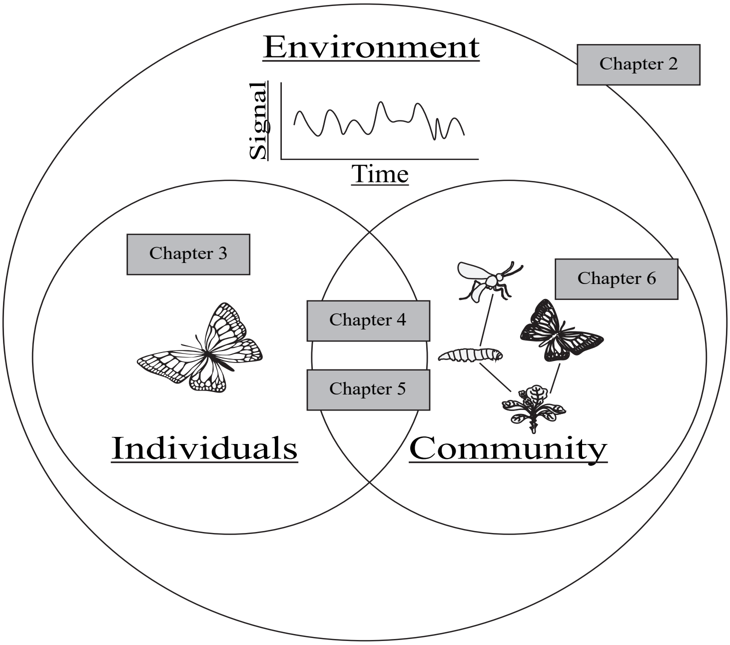


Figure 1: Schematic representation illustrating the research focus of the chapters in their ecological context.

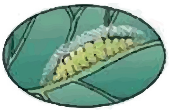
Phenotypic plasticity in response to thermal fluctuations and high-temperatures

Temperature drives the rate of biochemical reactions altering species metabolism and physiology (Brown et al., 2004). However, insects have evolved mechanisms for adaptive plasticity to cope with thermal variation and extremes (e.g., acclimation, tolerance) (Angilletta, 2009; González-Tokman et al., 2020). Many studies have emphasised the importance of these mechanisms in modulating insect responses to heat shocks (Bahar et al., 2013; C. Chen et al., 2019b; Terblanche et al., 2010; Xing et al., 2021). Thermal history, meaning the temperature experienced by an individual throughout its life, seems to play a crucial role in thermal tolerance (Cavieres et al., 2016; Kellermann et al., 2017; Pincebourde and Casas, 2015).

Progressive exposure to increasing temperatures enables acclimation processes allowing the insect to tolerate potentially harmful temperatures (González-Tokman et al., 2020; Manenti et al., 2018). Acclimation is mostly driven by biochemical mechanisms, notably by the production of Heat Shock Proteins (HSPs) that are chaperone molecules preventing protein denaturation in response to stress (González-Tokman et al., 2020; Sørensen et al., 2003; reviewed in Chapter 2). Furthermore, thermal fluctuations simulating short exposure to high temperatures followed by milder temperatures were found to mitigate the effects of thermal stress (Bai et al., 2019; Ma et al., 2018). A 4 h exposure to 33°C did not alter *D. semiclausum* parasitism success at any of the subsequently tested temperatures (20°C, 25°C, and 33°C; Chapter 3). However, foraging at 33°C negatively affected *D. semiclausum* parasitism success compared to foraging at 20°C and 25°C. When *P. xylostella* experienced heatwave conditions, it achieved higher lifetime fecundity than in the reference thermal conditions (Chapter 4). This supports the trophic-rank hypothesis on lower thermal tolerance of higher trophic levels (Furlong & Zalucki, 2017; Holt et al., 1999; Voigt et al., 2003). The relation between *D. semiclausum* parasitism performance and temperature follows the expected shape of the thermal performance curve (Chapter 3). Thermal performance curves display the relationship between the body temperature and a given performance (reviewed in Chapter 2). Interestingly, temperature did not influence *D. semiclausum* or *C. vestalis* parasitism performance in a subsequent foraging experiment (Chapter 5). The contrasting results between Chapters 3 and 5 may have resulted from the differences in conditions during the behavioural bioassays. In the experiments described in Chapter 5, the parasitoids could forage for their hosts on fully grown Brussels sprouts plants of 8-week-old, instead of an excised leaf as in Chapter 3, allowing both wasps and caterpillars to express a larger suite of behaviours. The experimental design did not allow the direct measurement of behavioural responses of either hosts or parasitoids, but only the end result of parasitism behaviour. However, several studies reported changes in behaviour in response to temperature (Augustin et al., 2020; le Lann et al., 2014a; Moiroux et al., 2016).

Behaviour is a highly plastic trait and can continuously adapt to match environmental conditions. Temperature affects many components of parasitic or predatory behaviour such as defensive behaviour, host or prey-encounter, attack rates and feeding rates (Gvoždík and Boukal, 2021; Lemoine et al., 2014; Moiroux et al., 2016). Changes in thermal conditions were also found to alter host-selection behaviour, and sex ratio of eggs laid by parasitoids (Moiroux et al., 2015, 2014). In contrast, in our studies, the proportion of *P. xylostella* second and third instar larvae parasitised by *D. semiclausum* at 20°C, 25°C, and 33°C was not affected by temperature (Chapter 3). Host-selection behaviour is influenced by the parasitoid's physiological status and its perception of environmental quality (Giraldeau & Boivin, 2008). Exposure to higher temperatures causes a decrease in survival, thus altering foraging strategies by the parasitoid to maximise its reproductive success (Giraldeau and Boivin, 2008; Moiroux et al., 2015). I argue that differences between the second and third larval instar of *P. xylostella* in the sense of parasitism success and larval development are minor for *D. semiclausum*. Indeed, koinobiont parasitoids regulate their host development to their advantage, thus mitigating differences in host quality due to host developmental stage (Brodeur & Boivin, 2004; Harvey et al., 2004).

Behaviour can strongly influence host-parasitoid interactions as well as insect thermal tolerance (Abram et al., 2017; Augustin et al., 2020). Insects are able to sense their thermal environment and adapt their behaviour (reviewed in González-Tokman et al., 2020). Behavioural thermoregulation is a well-known mechanism by which insects modulate their body temperature (May, 1979). Several species of lepidopteran larvae are known to behaviourally thermoregulate by grouping (i.e., gregariousness) and basking in the sun (Johns et al., 2012; Karban, 1998;



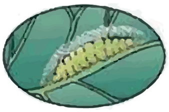
McClure et al., 2011). On the other hand, insects can move away from potentially harmful temperatures by selecting shaded or cooler areas (Caillon et al., 2014; Pincebourde & Woods, 2020). The benefits of behavioural thermoregulation in mitigating thermal variation are correlated to the heterogeneity of the thermal environment and the capacity of the insect to exploit it. The thermal heterogeneity can result from spatial or temporal variation creating a mosaic of temperatures prevailing on different parts of the plant (Cook & Dixon, 1964; Pincebourde & Woods, 2012). Although there is a consensus acknowledging the importance of microclimatic conditions on insect community dynamics, little is known about the influence of spatiotemporal variation in thermal conditions on insect movements or activity which represents a promising avenue for future research. However, some species such as *P. xylostella*, a small green caterpillar, rely on their cryptic phenotype to escape predators, which may influence its thermoregulatory behaviour (Angilletta, 2009; Choutt et al., 2011; Greeney et al., 2012).

We have seen that thermal conditions can alter the phenotypes of insect species by altering their physiology and behaviour, although the magnitude of effects is species- and trait-specific (Chapters 2, 3, 5). Species do not live in isolation but constantly interact with a multitude of other species creating complex community structures. One species' plastic response alters the environment of another species. Hence, thermal conditions are likely to alter species interactions creating feedback and cascading loops throughout the ecosystem. Thus, phenotypic plasticity plays an important role in community and population dynamics (Boukal et al., 2019; Tituskin et al., 2021).

Plasticity of species interactions in response to thermal fluctuation and high-temperatures

Phenotypic plasticity allows individuals to alter their interactions with individuals of other species (Berg & Ellers, 2010; Thierry et al., 2019). Plants, in response to insect herbivory, are capable to mobilise several defence mechanisms such as the production of secondary metabolites (Schoonhoven et al., 2005). Changes in plant chemical composition are well-known to affect the performance of members of the second (e.g., herbivore), third (e.g., parasitoids) and fourth trophic levels (e.g., hyperparasitoids). In warmer conditions, plants may alter their phytochemical composition inducing bottom-up effects on herbivores and parasitoids (Han et al., 2019; Philbin et al., 2021; Rosenblatt & Schmitz, 2016). High temperatures were found to increase the carbon-to-nitrogen ratio, and induce higher concentrations of secondary metabolites in leaves (Jamieson et al., 2015, 2017). The quantity and quality of the blend of herbivore-induced plant volatiles (HIPV), used by natural enemies to locate their prey or hosts, are also affected by temperature (Becker et al., 2015; McCormick, 2016). In a preliminary study the effect of a fluctuating temperature regime emulating heatwave conditions on the host-searching behaviour of *D. semiclausum* and *C. vestalis* females was tested in a choice assay performe d in a wind tunnel. The temperature treatments did not alter the orientation capabilities of either parasitoid species; both consistently preferred the host-infested over the clean plants (Bianchi & Gramsbergen, 2018).

A field experiment was conducted to assess the effects of varying degrees of community diversity (i.e., species identity and feeding guild) of *Brassica nigra* L. (Brassicaceae) on *P. xylostella* abundance and parasitism (Chapter 6). The proportion of *P. xylostella* larvae parasitised was



higher on plants harbouring the highest degree of community diversity (i.e., four species of herbivore feeding on the plant including both leaf chewers and phloem feeders). This result is likely to be driven by the higher detectability of HIPV due to the co-feeding of multiple herbivore species (Aartsma et al., 2019). Concomitantly, *P. xylostella* larval abundance was higher on the herbivore-infested plants, regardless of the community diversity treatment, than on plants with no herbivore community treatment. Several studies have shown an attraction of female moths and larvae toward certain volatile compounds emitted by herbivore-infested plants despite the higher risk of parasitism (Soler et al., 2012; Uefune et al., 2017). Herbivore induced plant volatiles and plant chemical composition mediate parasitism risks and the fitness of higher trophic levels (Jamieson et al., 2017). Although the experimental design of Chapter 5 did not measure the effects of temperature on volatile emissions and phytochemistry in interactions with the herbivore community on the abundance and parasitism of *P. xylostella*, other studies report changes in chemical profiles and volatile emissions in response to temperature (Dicke & Loreto, 2010; Kask et al., 2016; Truong et al., 2014). The phytochemical profile of a plant can influence the immune response of the hosts against their parasitoids (Poelman et al., 2014; Singer et al., 2014). The immune response of the host was also reported to be enhanced or weakened under warmer conditions depending on the species (Dievel et al., 2017; Iltis et al., 2019; Wojda, 2017). Encapsulation capacity under varying thermal conditions did not differ as no encapsulation responses were recorded at any of the tested thermal regimes (pers. obs.). Previous studies confirmed a reduction of immunity in parasitised *P. xylostella* larvae by *D. semiclausum* and *C. vestalis* at constant 25°C (Bae and Kim, 2004; Barandoc et al., 2010; Etebari et al., 2013; Huang et al., 2009b). This suggests that second-instar *P. xylostella* larvae were unable to mount a sufficiently strong immune response to prevent the development of the parasitoids, regardless of the thermal conditions. *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae) showed higher encapsulation under warmer constant rearing temperatures (Seehausen et al., 2018). I encourage further studies on the role of thermal variation and high-temperature extremes on plant-mediated bottom-up effects in tritrophic interactions, including host immune response while feeding on different plant species. A study on the development of *D. semiclausum* at different constant temperatures found that the parasitoid was unable to develop in its hosts at 30 when the latter was feeding on *Brassica napus* L. (Brassicaceae) (Dosdall et al., 2012). The responses of primary producers will play a key role in mediating the response of higher trophic levels to changing climatic conditions via, for example, altered microclimatic conditions and chemical phenotypes.

Thermal performance in a given thermal environment depends not only on species-specific thermal tolerance and affects trophic interactions but also non-trophic interactions such as competition. The extrinsic competition between parasitoid species was significantly altered by the interaction of temperature and species composition with *D. semiclausum* being a stronger competitor than *C. vestalis* at milder temperatures than at higher temperatures (Chapter 5). Previous studies have demonstrated that the thermal performance of one species was affected by competitive interactions (Davis et al., 1998b; Diamond et al., 2017). These results thus suggest that warmer and more frequent high-extreme heat events could re-arrange parasitoid species assemblages in various ways depending on the strength and variation of interactions (Amarasekare & Coutinho, 2014; Cebolla et al., 2018; Thierry et al., 2019). The ecological niches of these two parasitoids differ as *C. vestalis* is able to attack the larvae of several lepidopteran species while *D. semiclausum* specialises on *P. xylostella* larvae (Hiroyoshi et al., 2017; Sarfraz

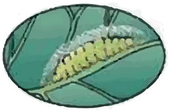
et al., 2005; Talekar & Shelton, 1993). As discussed earlier, thermal regimes will likely affect foraging behaviour, thereby altering the realised ecological niche of parasitoids. The strength and type of interactions depend in part on the degree of niche differentiation and the degree of environmental filtering between species. Environmental filtering represents the environmental pressure exercised on individuals to produce a specific phenotype (trait value), allowing for higher fitness, reducing the degree of niche differentiation between species (le Lann et al., 2014b; Outreman et al., 2018). High extreme thermal conditions impose strong constraints on the physiology of ectothermic organisms, reducing inter-individual trait variation in their expressed phenotypes, and increasing niche overlap between individuals (le Lann, Visser, et al., 2014; Romero-Mujalli et al., 2021). In contrast, evolution tends to reduce niche overlap and differences in thermal tolerance which may help maintain niche separation (Dajoz, 2006; Laughlin & Messier, 2015). For example, *D. semiclausum* and *C. vestalis* display different larval feeding strategies with the former consuming the entire host and the latter consuming host haemolymph and fat tissue (Yang et al., 1994; Yu et al., 2008). Furthermore, their reproductive strategies also differ as *D. semiclausum* produces large, although hydropic (yolk-poor) eggs (Huang et al., 2009a; Pourian et al., 2015), and *C. vestalis* produces smaller eggs, also yolk-poor (Yu et al., 2008). Despite both parasitoids producing yolk-poor eggs, the differences in egg size could translate into different ovigeny indices (i.e., fecundity at emergence/lifetime fecundity), and life-history strategies (le Lann et al. 2012, Chapter 4).

In the above sections, I discussed the thermal effects of fluctuating and high-extreme temperatures on individual insects and species interactions. I focused mainly on the immediate effects of temperature; however, thermal variation occurs on different time scales such as months, seasons and years. Moreover, with ongoing climate change, the frequency of high-extreme temperature events is predicted to increase (Fischer & Schär, 2010; Pachauri et al., 2014; Vasseur et al., 2014). Such events are highly unpredictable and may expose insects to stressful thermal conditions at specific stages. The thermal tolerance of individuals changes with ontogeny, driving different plastic responses and lagged ecological effects (Boukal et al., 2019; Bowler and Terblanche, 2008; Klockmann et al., 2017). Lagged effects influence future performance and phenomes displayed by individuals, and responses to future thermal conditions.

The effects of timing of extreme temperature events on species interactions

The timing and speed of plastic responses are critical in determining their adaptiveness (Romero-Mujalli et al., 2021). Indeed, in the context of climate change, extreme climatic events such as heatwaves can create sudden sporadic changes in thermal conditions, the effects of which on insect phenotypes and fitness will vary with the timing of occurrence of such extreme events relative to the insect life cycle (Zhang et al. 2015a,b; Fey et al. 2021). Moreover, the timing or time-course of warming can alter species interactions, thus driving complex responses of communities to asymmetric warming (Barton and Schmitz, 2018; Davis et al., 1998b; le Lann et al., 2021). It is, therefore, crucial to identify phenotypic plasticity at relevant temporal scales within different ecological contexts.

Although daily thermal variation can have short-term effects, they can mediate complex lagged effects on performance. Many components of fitness are dependent on the physiological status



of a given individual (Jervis et al., 2008). The larval phase is essential for resource accumulation allocated to adult reproduction and maintenance. Temperature can strongly influence the trade-offs between traits by altering the resource allocation to metabolism and to other traits such as egg maturation (Berger et al., 2008). Although the capacity of an individual to increase its upper-thermal limit seems restricted to a few degrees Celsius, many insect species are known to acclimate to the thermal conditions they experienced during their larval stages (Berrigan, 1997; Sentis et al., 2015). The metabolic activity of the parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera: Braconidae) reared at lower temperatures increased compared to individuals reared in warmer conditions (le Lann et al., 2011). These results are in line with the metabolic compensation hypothesis suggesting that insects are able to compensate for lower metabolic activity at low temperatures by increasing the density of mitochondria and enzymes (Pörtner, 2001). The metabolic compensation hypothesis is not always confirmed: results of another study comparing the metabolic activity of parasitoid populations originating from a warm and dry or a cool and wet environment did not support this hypothesis (Moiroux et al., 2012). The authors of the latter study argue that the evolution of metabolic rates was driven by resource availability. Indeed, higher metabolic rates in warm and dry environments resulted in a fitness advantage for parasitoids living in arid regions allowing them to cross longer distances between host patches. It most likely relied on the lipogenesis capacity displayed by adult parasitoid wasps. However, the capacity to synthesise *de novo* lipids during the adult stages is rather uncommon among parasitoid species (Visser & Ellers, 2008).

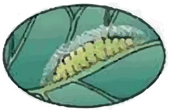
Most holometabolous species during their larval stage accumulate lipidic reserves that will be allocated in the adult stage to reproduction or maintenance (e.g., longevity) (Jervis et al., 2005). The energetic trade-off between life-history traits can mediate different plastic expressions and fitness outcomes under fluctuating thermal conditions, especially during ontogeny (Kingsolver et al., 2011). I tested the effects of shifts in thermal conditions between the larval and adult stages on several life-history traits of *P. xylostella*, *D. semiclausum*, and *C. vestalis* (Chapter 4). The results revealed complex interactions between the thermal conditions experienced throughout ontogeny, with species- and trait-specific responses. Overall, the host *P. xylostella* was not affected by heatwave conditions experienced during its larval stage, while exposure to this thermal condition during the adult phase enhanced its lifetime fecundity and lowered its body mass. All three species' egg maturation rates, measured as daily fecundity for *P. xylostella* and the number of mature eggs present in the ovaries at different ages for the parasitoid species, were affected by the interaction between the thermal conditions experienced during the larval and adult stages. For *P. xylostella*, this effect is predominant during the early adult life of the moth and declines with the moth's age. Although I did not measure physiological parameters, similar studies on thermal physiology suggest several potential mechanisms to explain these results. The life cycle modularity hypothesis holds that insects may isolate the physiological effects of environmental stress from one stage to the next. During ecdysis between larval instars, insects can physiologically mitigate the effects of stress via cellular restructuring (Y. Chen et al., 2019; Zhang et al., 2015a, 2015b). Moreover, *P. xylostella* displays a strong thermal tolerance and has colonised diverse climatic zones worldwide and is likely to have benefited from warmer conditions closer to its native conditions (Juric et al., 2017).

Egg maturation and oviposition are complex processes depending on physiological and behavioural responses affecting resource allocation to egg maturation and longevity (Denis et al.,

2011; Fischbein et al., 2013; O'Brien et al., 2004). The faster oviposition rate of female moths exposed to warmer conditions may have been driven by faster egg maturation and higher night-time activity (Chapter 4). The butterfly *Pararge aegeria* L. (Lepidoptera: Nymphalidae) displayed thermal constraints on egg maturation with faster egg maturation and oviposition rates in warmer night conditions (Berger et al., 2008). These results suggest that both egg maturation and oviposition behaviour are influenced by the timing of specific thermal conditions (e.g., night versus day temperatures). Night-time temperatures are predicted to increase at a faster rate than daytime temperatures, which will likely have unexpected consequences on the population dynamics of many species (G. Ma et al., 2021). The production of eggs requires lipidic resources often acquired during the larval stage even though lepidopteran species can use nutrients acquired during the adult stage for egg production (O'Brien et al., 2004). In the case of *P. xylostella,* I argue that due to the strong investment of the moth in early reproduction, most nutritional reserves for egg production originated from the larval phase. The experimental protocol included an *ad libitum* sugar source to the adult insects, which likely explains the overall high fecundity of *P. xylostella*. The effects of the thermal environment on behavioural trade-offs between foraging for food and searching oviposition sites represent an important avenue for future research and will provide valuable insights for biological control and species conservation.

The results of Chapter 4 showed species-specific thermal constraints on egg maturation in the two parasitoid species. Although both species showed an increase in egg load during early adult life, *D. semiclausum* produced fewer eggs than *C. vestalis* regardless of the thermal treatment. *Diadegma semiclausum* showed higher egg load when experiencing the same thermal conditions in both larval and adult stages, while egg loads were lower for wasps having experienced a shift in thermal conditions, this trend changed over time with wasps experiencing the reference treatment during the adult phase displaying higher egg load. *Cotesia vestalis* showed lower egg loads in individuals reared in the heatwave conditions compared to individuals reared in the reference thermal regime, with wasps experiencing the heatwave conditions in both stages showing the lowest egg loads over time. I argue that this difference arises from ecological strategies: *C. vestalis* larval development was shorter than for *D. semiclausum* and the former may have accumulated less energetic resources than the latter (Chapter 4). *Diadegma semiclausum* and *C. vestalis* display different degrees of syn-ovigeny and can mature or resorb eggs to adapt to their environments such as the abundance and quality of host patches and available time for foraging. *Diadegma semiclausum* is highly specialised and may display a stronger control over egg load depending on host-availability than *C. vestalis* (le Lann et al., 2012). Parasitoids are often considered egg or time-limited, meaning that they either produce not enough eggs compared to the availability of their hosts or that their lifespan is too short to lay all their eggs (Rosenheim, 1996; Sevenster et al., 1998). As a result, it is considered that the host availability, often patchily distributed in space and time, is a strong driver in the evolution of egg load throughout adulthood (Ellers et al., 2000; Harvey et al., 2001). The abundance and distribution of hosts in the environment may be affected by environmental variables, and their consequences on parasitoid reproductive strategies and species assemblage should be investigated further.

The sequence of temperatures experienced by an individual across its ontogeny may affect subsequent individual performance even over future generations (transgenerational plasticity). It results in complex effects as the thermal conditions lead to the expression of different phenomes by direct and lagged effects on the individual and by indirect effects on the biotic



environment such as host availability, or abundance of natural enemies. Indeed, community dynamics are composed of many direct and indirect interactions between plants, insect herbivores, predators and pollinators. The diversity of interactions between the species forming communities is strongly correlated with the productivity of communities (Miele et al., 2018).

The role of community context in species responses to extreme temperature events

Thermal variation influences the physiology and behaviour of individuals, having consequences for species interactions (Abram et al., 2017; Angilletta, 2009, Chapters 2, 3, 5). Predicting how climate change will affect ecosystem functioning and services is particularly challenging for ecologists. The diversity of species, interactions and functional roles that compose ecosystems are directly and indirectly susceptible to thermal variation (Vázquez et al., 2015). Species diversity is thought to buffer ecosystem functioning against environmental variation (Thébault & Loreau, 2005). However, evidence from a common garden study comparing the thermal response of four populations of *Melanoplus femurrubrum* De Geer (Orthoptera: Acrididae) and its predator *Pisaurina mira* Walckenaer (Araneae: Pisauridae) suggests that the species phenotypic plasticity displayed by each population does not overlap with the functional role, at the ecosystem level, of the native population (Rosenblatt et al., 2019). The responses of ecosystems to altered thermal regimes critically depend on the integrated effects of species-specific plastic responses at the community level. To assess the responses of ecological communities to thermal extremes, it is interesting to consider the species' realised ecological niches which can be viewed as the reflection of the expressed phenotypes. Ecological niches can be defined by a multidimensional space where each dimension represents a characteristic of the niche. Niches relate to the positioning of the species or individual in the ecosystem in temporal, spatial, and nutritional dimensions (Dajoz, 2006; Laughlin & Messier, 2015). Assessing the effects of thermal variation and extremes on the realised niche of species forming an ecosystem will provide key knowledge for species conservation and climate change mitigation measures.

Thermal variation and high-temperature extremes alter species' realised ecological niches through various mechanisms. Experimental warming of a community composed of several herbaceous plants, *M. femurrubrum* and *P. mira* over two years showed that higher night temperatures increased *P. mira* activity concomitantly reducing *M. femurrubrum* feeding rates resulting in a reduction in plant diversity. On the contrary, daytime warming had the opposite effects and *P. mira* retreated to the cooler lower canopy resulting in a spatial mismatch with *M. femurrubrum* that increased its feeding rates, increasing plant diversity by reducing the competitiveness of dominant plant species (Barton & Schmitz, 2018). Species interactions at the community level are often intricate and one species can influence indirectly the fitness of another via rippling effects mediated by several other species (Berg and Ellers, 2010; Gillespie et al., 2012; Tituskin et al., 2021). In a field study, the co-occurring herbivore species on *B. nigra* plants affected the distribution of *P. xylostella* larvae, its parasitism rate and the number of pollinators visiting each plant (Chapter 6). Interestingly, temporal blocks (i.e., two or four weeks after planting) had significant effects on the strength of these interactions as in the first block no effects of the co-occurring herbivore community on *P. xylostella* larval abundance were found whereas in the latter block such effects did occur. On the contrary, the parasitism rate of *P. xylostella* larvae was overall higher in the first block than in the second, although the effects of herbivore diversity on

parasitism rate remained unchanged (Chapter 6). Temperature records from the Veenkampen[3], Wageningen, The Netherlands, weather station indicate daily maximal and minimal temperatures fluctuating between 11-33℃ and 0.1-20.5℃, respectively, with an average daily temperature variation (i.e., maximal – minimal temperature) of 11.6℃. Although the effects of thermal variation were not analysed in this dataset, effects of temperature and herbivore species diversity on plant phytochemical profiles have been reported in other studies, suggesting that both factors are likely to interact to mediate community dynamics (Doghri et al., 2021; Kask et al., 2016; Mikkelsen et al., 2015; Truong et al., 2014). Ecosystems dynamics are marked by seasonality and the composition of the previous species assemblage influences the composition of the subsequent assemblage (Heinen et al., 2022; Stam et al., 2019). Thermal extremes and altered seasonal regimes such as milder winter periods and longer growing seasons have altered the phenology of many insects species and can lead to mismatches between interacting partners such as between hosts, parasitoids, pollinators and plant flowering time (Doi et al., 2017; Godfray et al., 1994; Senior et al., 2020). Altered thermal regimes at a seasonal scale are therefore likely to have strong legacy effects on community dynamics.

Another layer of complexity is added by integrating micro-organisms and their effects on trophic interactions. Micro-organisms such as bacteria, viruses, fungi and microfauna such as nematodes play a key role in mediating many aspects of fitness, performance, species interaction and realised niche (Dicke et al., 2020; Frago et al., 2020). Many micro-organisms are involved in mutualistic associations that are either obligate or facultative with insect hosts and are referred to as symbionts (Dicke et al., 2020; Frago et al., 2020). In the case of obligate associations, the loss of symbionts due to high-extreme temperature events will lead to the death of the carrying individual. In the case of facultative associations, carrying symbionts may give access to new resources, protection against parasitoids and increase thermal tolerance (Corbin et al., 2017; Henry et al., 2013; J. A. Russell & Moran, 2006). Recent findings suggest that under warmer conditions, the protective advantage of carrying *Hamiltonella defensa* (Enterobacteriaceae) against the parasitoid *A. ervi* was lost in the aphid *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae) (Doremus et al., 2018; Higashi et al., 2020). Carrying symbionts often has a cost for individuals and the fitness advantage gained by harbouring symbionts will be mediated by the environmental conditions creating cryptic niches within communities and thus increasing the diversity of responses at the ecosystem level (Frago et al., 2020).

Overall thermal variation and extreme temperature events are likely to alter all levels of biological organisation directly at the trait and species level and indirectly via species interactions and realised ecological niches. The temporal dynamics of thermal conditions, from day to year, can have lasting consequences for populations and community dynamics; these consequences are mediated by the composition of species within the community itself.

Conclusions and future perspectives

Phenotypic plasticity is central to ecosystem functioning and cannot be neglected in predicting eco-evolutionary responses of organisms to new thermal conditions. This thesis highlights the importance of thermal variation and thermal history on species-specific fitness-related traits

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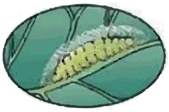
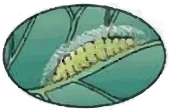
[3] Veenkampen.nl/data



and performance. In line with other studies, the findings suggest that on a short time scale (a few hours) the damage caused by high-extreme temperatures can be compensated or repaired during milder thermal periods (Bai et al., 2019; Ma et al., 2018). However, when the stress is prolonged for several days, different physiological mechanisms can occur, allowing acclimation to warmer conditions, e.g., accumulation of heat shock proteins and metabolic compensation (le Lann et al., 2011; Pazouki et al., 2016; Stanton-Geddes et al., 2016; Tian et al., 2021). Moreover, the biotic context in which species develop mediates the thermal performance of the focal species having rippling effects on community dynamics. The diversity of species interactions taking place in ecosystems makes predictions about community dynamics under climate change challenging. Indeed, the effects of the co-occurring herbivore-pollinator-parasitoid community of *B. nigra* suggest intricate multi-modal indirect effects on plant fitness. Including the mutualistic and antagonistic interactions with micro-organisms adds another level of variation and complexity to the response of organisms to changing thermal conditions.

The thermal environment influences ecological dynamics in countless ways, acting on phenotypic plasticity at all levels of biological organisation. I discuss promising avenues for future research on assessing species and community responses to thermal extremes such as heatwaves. Communities are often highly diverse, however, few species play a central role in the structuring and dynamics within these communities (Poelman & Kessler, 2016). Assessing the role of keystone species, phenotypic responses to changing environmental conditions as well as the cascading effects on community dynamics are likely to give valuable insights into the processes at play and their relative importance. As plants are at the basis of most ecosystems, they represent a common link between various species. Their chemical profiles play a major role in shaping insect interactions and community dynamics. However, little is known about the effects of the timing of extreme events on bottom-up processes on the (seasonal) dynamics of insect communities. This thesis focused on the effects of variable thermal conditions and their legacy effects on host-parasitoid interactions. However, behavioural plasticity, especially alteration of insect activity and spatial aggregation, within their relevant microclimatic scale will provide valuable data on the sensitivity of ecosystems to high-extreme temperature events. The microclimatic heterogeneity and the capacity of arthropods to move across the thermal landscape can mitigate or exacerbate the effects of changing thermal conditions (Pincebourde et al., 2007; Rebaudo et al., 2016; Tougeron et al., 2016). Moreover, more accurate measurements of microclimatic conditions will inform the modelling of temperature effects on physiology and plasticity of insects that are ultimately dependent on body temperatures (von Schmalensee et al., 2021). The fundamental knowledge generated could help create climate-resilient cropping systems, enhanced crop protection and improve conservation efforts.

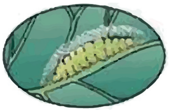
Thermal variation in natural ecosystems is characterised by periodic and stochastic fluctuations. The predictability of environmental variation is thought to favour adaptive phenotypic plasticity. In contrast, stochastic environments are thought to favour bet-hedging strategies. Bet-hedging produces various phenotypes in response to change itself and it is not necessarily adaptive (Bond et al., 2021; le Lann et al., 2021). Cyclical variation of environmental conditions seems to maintain higher intra-population trait variation than in stochastic environments, although populations originating from the latter displayed similar levels of plasticity (Bond et al., 2021; Park & Wootton, 2021). This further highlights the importance of periodicity of environmental variation for life-history trait variation. One of the consequences of anthropogenic



climate change is an increase in the frequency and magnitude of extreme weather events, thus an increase in stochasticity (Pachauri et al., 2014; Vasseur et al., 2014). Assessing the role of different types of plasticity such as bet-hedging, and trans-generational plasticity, as well as the influence of selective pressure imposed by an increase in environmental stochasticity on their evolution, will provide fundamental knowledge on the evolution of phenotypic plasticity and population resilience to climate change.

Acknowledgements

I thank my promotors prof. dr. Marcel Dicke, prof. dr. Joop J.A. van Loon and my co-promotor dr. Peter W. de Jong for their helpful suggestions on this chapter.



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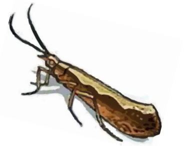

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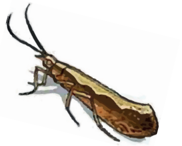

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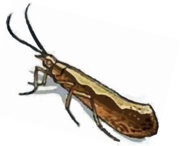

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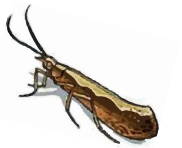

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Summary

Understanding the fundamental effects of climate change on ecosystems and the services they bring to human civilisations is essential to providing the necessary knowledge for conservation and climate-smart farming practices. Climate change alters many environmental parameters, such as temperature or precipitation. The alterations of these environmental parameters have strong consequences for all levels of ecological interactions, from species interactions to community dynamics. Temperature is a well-known and well-studied abiotic factor that influences ecosystem functioning. It has a major impact on ectothermic species such as plants or insects, whose body temperatures directly depend on ambient thermal conditions and influences many aspects of their fitness. Insects display a wide array of mechanisms allowing them to adapt to ambient thermal conditions. Phenotypic plasticity is the capacity of one genotype to produce different phenotypes in response to environmental conditions. It is a common mechanism by which individuals adapt to changing environments, and many studies have assessed the plasticity of insect phenotypic responses (e.g. metabolic rate, growth and body size) to changing thermal conditions. Their results give valuable insights into physiological, behavioural, and genetic aspects of phenotypic plasticity. However, in natural settings, temperature varies between years, seasons, or days. Moreover, climate change not only leads to an increase in global temperature averages but also leads to an increase in the occurrence of extreme weather events such as heatwaves. These extreme temperature events pose great challenges to ectothermic species, which are particularly vulnerable to these sudden variations in temperature.

Insects have vital roles in food webs, supporting a diversity of species as well as providing ecological services such as pollination and pest control. With increasing risks of worldwide biodiversity losses and especially the insect decline, it is of utmost importance to examine the role of phenotypic plasticity on insects' resilience to extreme temperature events. As ecosystems are networks of many species, it is essential to assess these plastic responses in a network context.

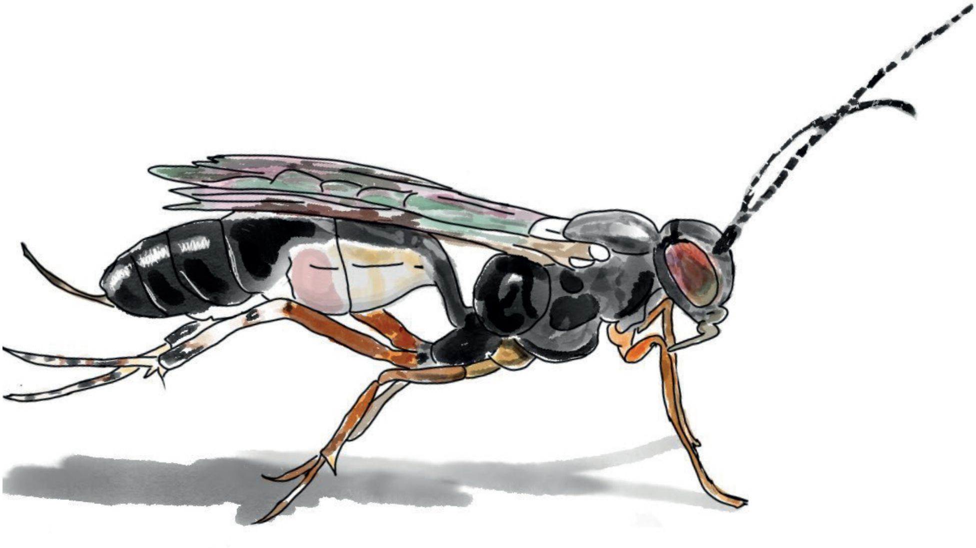
This thesis aimed to assess the phenotypic responses to two ecologically relevant climatic scenarios of temperature variation by a cosmopolitan herbivorous insect *Plutella xylostella* L. (Lepidoptera: Plutellidae), and two of its main associated parasitoids *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae) and *Cotesia vestalis* Haliday (Hymenoptera: Braconidae). *Plutella xylostella* is a specialist species feeding on brassicaceous plants; thus, my thesis included *Brassica oleracea* (Brassicaceae) as the first trophic level. One of the two climatic scenarios aimed at mimicking non-stressful thermal conditions, while the second simulated heatwave-like thermal conditions within the range of temperatures occurring in The Netherlands during June-July-August. I used temperature records from the Royal Netherlands Meteorological Institute (KNMI) from 1961 to 1990 to set the reference thermal fluctuations of the first thermal treatment. The minimum temperature of this treatment was 15℃ at night and followed a stepwise increase in temperature to peak at 25℃ at noon, cycling back with a stepwise decrease in temperature to 15℃. The heatwave-like thermal treatment displayed a rise in temperature of +3℃ and +8℃; thus, the minimum temperature was 18℃ at night, and the maximum temperature was 33℃ at noon. The thermal scenarios were simulated in climate-controlled cabinets with a 16L:8D photo: scotophase.

In chapter 2, I reviewed existing literature on the role of phenotypic plasticity in response to thermally fluctuating environments. I mainly focused on host and parasitoid responses to extreme temperature events. This review highlights the various types of plastic responses to changes in thermal conditions and their consequences on community dynamics. Thermal fluctuations can follow predictable or unpredictable patterns, with different implications for phenotypic plasticity and ecosystem dynamics. These various consequences arise from the constraints of past environmental conditions and the capability of a phenotype to continuously track environmental changes. In chapter 3, I designed an experiment to assess the effects of previous thermal conditions on the parasitism performance of *D. semiclausum*. Exposure to extreme temperatures can cause irreversible physiological damage leading to potential long-lasting effects and reducing subsequent performance. However, it may improve the individual resilience to subsequent exposure. Behaviour is a highly plastic trait, considered to enhance adaptability in a fluctuating environment. I aimed to assess the impact of previous exposure to extreme temperature on the oviposition success of *D. semiclausum*. Prior exposure to the heatwave-like thermal treatment did not significantly impact the egg-laying performance of *D. semiclausum,* suggesting it can recover from short thermal stress. Host-selection behaviour, measured as the percentage of parasitised second instar and third instar *P. xylostella*, was not affected by prior exposure to heatwave-like thermal conditions. However, as expected, the temperature that female *D. semiclausum* experienced during the foraging assays affected their parasitism performance. To further assess the influence of past thermal conditions on insect fitness, I exposed the larval and the adult stages of *P. xylostella*, *D. semiclausum*, and *C. vestalis* to either thermal treatment creating four thermal scenarios (Chapter 4). Exposure of larval and adult stages to different thermal environments had species- and trait-specific effects on life-history traits. *Plutella xylostella* showed a higher fecundity when experiencing heatwave-like thermal conditions during adulthood, while exposure to this treatment in the larval stage had no effect. On the other hand, both parasitoid species' egg maturation showed significant interactions between the thermal regimes experienced during the larval and adult stages. However, the thermal regimes applied during their immature stage did not affect the initial egg load (i.e., the number of mature eggs at emergence).

It is well known that temperature, influencing all levels of biological organisation, will also alter species interactions. However, the community species composition can mediate the thermal responses of a target species. When studying the extrinsic competition between *D. semiclausum* and *C. vestalis* under three temperatures, 22°C, 27°C or 33°C, the temperature experienced during the foraging assays, surprisingly, had no effect on the parasitism performance of either species foraging separately. When both species foraged simultaneously, parasitism by *D. semiclausum* was not affected at 22°C but decreased at 27°C and 33°C. The percentage parasitism achieved by *C. vestalis* was reduced in the presence of *D. semiclausum* at all temperatures. These results suggest that the competitiveness between *D. semiclausum* and *C. vestalis* is influenced by specific thermal effects due to species interaction. Together with other studies, these results highlight the importance of investigating the thermal responses at the community level.

In chapter 6, I present a field experiment aiming to investigate how the composition of an insect herbivore community differing in species richness and feeding guilds, affected *Brassica nigra* L. (Brassicaceae) fitness, its interactions with the pollinator community and the abundance of *P. xylostella* larvae as well as parasitism prevalence. The composition of the herbivore community affected the abundance of *P. xylostella*, its parasitism prevalence, and the pollinator community visiting the flowering plants. Plant fitness, measured as the number of seeds produced per plant, was strongly influenced by the composition of the herbivore community. A structural equation modelling of the interactive effects at the community level revealed a complex interplay of direct and indirect effects of the community composition on plant fitness. These indirect effects were mainly mediated via changes in the pollinator community visiting the plants. Although the experimental design of this experiment did not allow us to measure the effects of the thermal conditions on community-wide interactions, these results underline the importance of measuring the implications of altered thermal changes at the community level.

In chapter 7, I discuss the overall results of this thesis, interrelating and interpreting them in a broader ecological context. The temporal dynamics of thermal conditions, displaying predictable as well as stochastic patterns over time frames ranging from day to year, can have varying effects on the phenotypes expressed by individuals. This has repercussions on their realised ecological niches, altering populations and community dynamics. Moreover, species plasticity is affected by species composition within the community itself, a finding that needs to be taken into account in making predictions of population dynamics under future climatic scenarios.



Résumé

Comprendre les effets du changement climatique sur les écosystèmes et les services qu'ils apportent aux sociétés humaines est essentiel pour apporter les connaissances nécessaires à la conservation de la biodiversité et à l'amélioration des pratiques agricoles. La température est un facteur abiotique bien connu et étudié, influant sur le fonctionnement des écosystèmes. Elle a un impact majeur sur les espèces ectothermiques telles que les plantes ou les insectes, dont la température corporelle dépend directement des conditions thermiques ambiantes. Les insectes possèdent un large éventail de mécanismes leur permettant de s›adapter aux conditions thermiques de leur milieu. La plasticité phénotypique correspond à la capacité d'un génotype à produire différents phénotypes dans différentes conditions environnementales. Dans la nature, la température varie constamment selon les années, les saisons ou les jours. De plus, le changement climatique entraîne non seulement une augmentation des températures moyennes mondiales, mais également une augmentation de la fréquence des phénomènes météorologiques extrêmes tels que les canicules. Ces événements extrêmes posent de grands défis aux ectothermes, qui sont particulièrement vulnérables aux variations brusques de température. Les insectes jouent un rôle vital dans l'organisation des réseaux trophiques, nourrissant une diversité d'espèces et fournissant des services écologiques tels que la pollinisation ou la lutte contre les ravageurs des cultures. Avec le risque grandissant de perte de biodiversité à l'échelle mondiale et en particulier le déclin des insectes, il est important d'examiner le rôle de la plasticité phénotypique sur la résilience des insectes aux températures extrêmes. Comme les écosystèmes sont composés de nombreuses espèces interconnectées, il est essentiel d'évaluer cette réponse dans un contexte incluant les réseaux trophiques avec plusieurs espèces interconnectées.

Pour ce faire, cette thèse de doctorat évalue les changements phénotypiques en réponse à deux scénari climatiques sur un insecte herbivore cosmopolite, *Plutella xylostella* L. (Lépidoptère : Plutellidae), et deux de ses principaux parasitoïdes, *Diadegma semiclausum* Hellén (Hyménoptère : Ichneumonidae) et *Cotesia vestalis* Haliday (Hyménoptère : Braconidae). L'un des deux scénari climatiques visait à imiter des conditions thermiques non stressantes, tandis que le

second simulait des conditions thermiques de type canicule pouvant survenir aux Pays-Bas en juin, juillet et août. J'ai utilisé les relevés de températures de l'Institut Météorologique Royal des Pays-Bas (KNMI) de 1961 à 1990 pour définir les différents scénarios.

Dans le chapitre 2, j'ai réalisé une revue de littérature sur le rôle de la plasticité du phénotype engendré par des variations thermiques de différents types. Cette revue met en évidence les multiples réponses possibles ainsi que leurs conséquences sur la dynamique des communautés lorsque les conditions thermiques varient. Dans le chapitre 3, j'ai conçu une expérience pour évaluer les effets des conditions thermiques précédemment vécues par les individus *D. semiclausum* sur leurs capacités (présentes) à parasiter *P. xylostella*. Une exposition antérieure à un traitement thermique de type canicule n'a pas eu d'impact significatif sur les performances de ponte de *D. semiclausum*. Ceci suggère qu'un stress thermique court n'as pas ou peu d'effet ultérieur sur la capacité de cette espèce de parasitoïde à parasiter son hôte, et cela quel que soit le stade larvaire de ce dernier. Cependant, la température appliquée pendant les essaies influe sur les performances de *D. semiclausum*. Pour évaluer plus en détail l'influence des conditions thermiques passées, j'ai exposé les stades larvaires et adultes de *P. xylostella*, *D. semiclausum* et *C. vestalis* à l'un ou l'autre des traitements thermiques, créant ainsi quatre scénarios climatiques différents (chapitre 4). L'exposition des stades larvaires et adultes à ces environnements thermiques ont eu des effets spécifiques par espèces. *Plutella xylostella* a montré une fécondité plus élevée lorsqu'exposé à des conditions thermiques de type canicule durant l'âge adulte, alors que l'exposition à ce traitement pendant le stade larvaire n'a eu aucun effet. D'autre part, la maturation des œufs des deux espèces de parasitoïdes s'avère dépendante de la combinaison entre les régimes thermiques appliqués aux stades larvaires et adulte. Cependant, aucun des scénarios thermiques appliqués pendant le stade larvaire n'a eu d'effet sur la charge initiale en œufs (c'est-à-dire le nombre d'œufs matures lors de la sortie du cocon). Dans le chapitre 5, j'ai étudié la compétition extrinsèque entre *D. semiclausum* et *C. vestalis* sous trois températures, 22°C, 27°C et 33°C. La température appliquée pendant les essais n'a eu, étonnamment, aucun effet sur le parasitisme des deux espèces lorsqu'elles étaient séparées. Lorsque les deux espèces se trouvaient ensembles, le parasitisme par *D. semiclausum* n'était pas affecté à 22°C, mais diminuait à 27°C et 33°C. Le pourcentage de parasitisme atteint par *C. vestalis* a été réduit par la présence de *D. semiclausum* à toutes les températures testées. Ces résultats suggèrent que la compétitivité entre *D. semiclausum* et *C. vestalis* est influencée par des effets thermiques spécifiques dûs à l'interaction entre ces deux espèces. Dans le chapitre 6, je présente une expérience de terrain visant à étudier comment la composition d'une communauté d'insectes herbivores affecte *Brassica nigra* L. (Brassicaceae) (moutarde noire), ses interactions avec sa communauté d'insectes pollinisateurs, l'abondance de larves de *P. xylostella* ainsi que le pourcentage de parasitisme. La composition de la communauté d'herbivores a affecté l'abondance de *P. xylostella*, son pourcentage de parasitisme ainsi que la composition de la communauté d'insectes pollinisateurs. Une modélisation par équation structurelle au niveau de la communauté a révélé une interaction complexe avec des effets directs et indirects de la composition de la communauté d'insectes herbivores sur la quantité de graines de moutarde récoltée par plante. Dans le chapitre 7, je discute les résultats globaux de cette thèse, en les mettant en relation et en les interprétant dans un contexte écologique plus large. La dynamique temporelle des conditions thermiques, soit cyclique, soit stochastique, sur plusieurs échelles de temps a des effets variables sur les phénotypes exprimés. Cela a des répercussions sur les niches écologiques des individus, modifiant ainsi la dynamique des populations et des communautés d'insectes au sein des écosystèmes.

Acknowledgements

A PhD project is always a challenging undertaking in which many more people are taking part than the PhD candidate alone. It is now time for me to formally thank all these wonderful people that have accompanied me, in one way or another, through my doctoral journey. It is never easy to find the right words to express all the gratitude I have for my family, friends, and colleagues; for their support, unconditional friendship, and love. You will find in this text my humble attempt at this exercise.

My PhD journey at the Laboratory of Entomology officially started in September 2017. However, it was already during my MSc thesis at the Netherlands Institute of Ecology that I first had the chance to meet the people that directed me towards a PhD in entomology. **Ana Pineda**, from the moment I met you from before my MSc minor to the productivity course at the end of my PhD, you have supported me. Thank you for your kindness and mindful help; it meant a great deal to me. **Martine Kos**, thank you for giving me the chance to enter the extraordinary world of insect sciences and for your enthusiastic supervision. **Jeffrey Harvey**, I always enjoyed our late evening science talk in the phytotron. Thank you for fanning my curiosity in ecology and entomology into a professional career. **Erik Poelman**, I had the chance first to meet you during my MSc thesis, and even though we did not get to collaborate closely, you always supported me in doing a PhD, and I am grateful. **Antonino Cusumano**, I first met you for a crash course on Y-tubing technicalities, and later we became colleagues and friends. And I can only thank you for all the good moments and memories during my PhD journey. **Maite Fernandez de Bobadilla**, it is impossible to express my feeling of gratefulness for all the energy you put into our friendship that gives me the confidence to do more. You did so much before, during and certainly after my PhD. Still, one example among many is your implication for my candidature for a PhD position at the Laboratory of Entomology. You not only sent me the PhD offer, but

you also pre-selected relevant scientific articles on the topic and invited me to one of the famous Ento parties (Thank you, **Patrick,** for the pizzas) to meet my "future" colleagues.

Despite all this, I applied for the position a few hours after its deadline. Luckily, I got a second chance and was invited for an interview. It was then that I first met the three musketeers that would become my promotors and co-promotor. It is truly an honour to have worked with you during these last five years, and I hope to have the luxury of meeting you again in my future career. **Peter de Jong**, it is a pleasure to have worked with you. You always brought a lot of happiness to every one of our meetings, whether formal or informal. **Joop van Loon,** your wisdom and talent for storytelling are unmatched, and I wish I could be a sponge and absorb every piece of knowledge you always carefully share at the opportune time. Thank you for all the inspiring science talks and for enriching my lexicon. **Marcel Dicke,** I am obliged for your patience and kindness during this PhD endeavour. Your expertise and academic sharpness make you an excellent mentor from whom I learned much about being a scholar.

A PhD candidate is nothing without his laboratory; of course, the Laboratory of Entomology (Ento for short) is one of the best examples you can find. Indeed, from the start, I have been immediately added to the happy Ento family with whom I shared so many beautiful times and will keep unforgettable memories. It is impossible to name them all, between the wonderful activities and parties organised by the party committee to the small pleasure of going for walks and coffees around campus, but rest assured that it meant a great deal to me, each and every time. In the beginning, I was a "junior" PhD candidate. It was first, **Karol, Gonzalo, Daan, Quint, Maite, Kay, Lucille, Jeroen A, Tim, Milena, Stijn, Yidong, Julia, Sandeep, Peter, Janneke, Tessa, Hans, Patrick, Jeroen S, Rieta, Yavanna, Shaphan, and the rearing team: Pieter, André, Kimmy**. They taught me what it meant to be part of the Laboratory of Entomology at Wageningen University. They were always at the lab or the greenhouse, taking care of the plants and insects. Being part of Ento does not stop at Campus; I got the chance to participate in Latin parties, Viking runs, and many more activities that enriched my life in such a way it is hard to describe. Of course, with time, I became a "senior" PhD candidate, and I had the pleasure to welcome many more PhD candidates and colleagues into the Ento family: **Els, Max, Filippo, Bram, Davy, Marieke, Maximilien, Parth, Alexander, Karen, Aidan, Julian, Hanneke, Julien, Alessandro, Kelly, Yvonne, Anna; Mitchel, Marcella, Luuk, Katherine, Alessia, Simone, Angelos, Miguel, Camilo.** With them, I got many more fantastic memories. This family is an ever-growing family that has always brought many new outstanding members over my five years at Ento. I have not made an exhaustive list of all the people. However, it is all of them that I thank for the help, the support, the laughter, the dinner, and all these little things in life that make a big difference.

I have unique stories about everyone in Entomology, but I will only tell a few memorable highlights (because it is customary and every good story needs a few). Thanks to the determination of **Katherine, Alessia and Joop**, I got the luxury of being part of the Ento Christmas Choir so that we could sing Christmas songs in French, Dutch, Spanish and, of course, English. I will keep excellent memories from our rehearsals and performance day at the Christmas dinner in front of the group with Joop as conductor. **Peter and Quint,** I had plenty of inestimable adventures with you whether it was for the Viking run, wandering around campus looking to identify all sorts of insects or simply talking about random science. **Filippo, Max, and Davy**, I could not have found better desk partners than you; thanks for the snacks, coffees, campus lunches and dinners and special foreign treats such as beers and liquors brought back from holiday trips. **Els**, thank you so much for all your small attention to me; I always enjoyed our little evening conversation in the ecology lab at the end of the work day. After Els, it was **Mitchell** to come in to take care of your hyperparasitoid rearing. You always brought positive energy to create our own little 6 PM Ecolab party time, which were priceless moments. Special acknowledgements to **Rieta** because we would go for a more than welcome glass of wine and chit-chat at Doppio every now and then. Last but not least, **Hans** and **Marieke,** thank you for having at least tried to get me running with you on Friday mornings. I know I joined only a few times (which were nicely spread over these five years), but I am thankful for your perseverance, patience and kindness.

Special dedication to **Alessia, and Katherine**, you quickly became very close friends, and you supported me to such an extent that I cannot find words strong enough to say how grateful and indebted I am. You are amazing persons, and I can only say thank you for absolutely everything.

Of course, I cannot forget my two MSc students **Francine** and **Cristina**. You brought a lot of dedication, creativity and enthusiasm to your projects, and it was a real pleasure to work with you.

Although the Ento family represented a considerable part of my PhD life, it did not have it all. And it is now time to break my PhD timeline and acknowledge the people supporting me from the sideline. **Belén, Gauthier, Francesco, and Paula**, you have been amazing friends, and together with Maite, we have created a strong friend community. We met during the MSc at Wageningen in 2015, and since then, we have supported each other even over distances. I have so many things I could thank you for that I will not even try to write them down. And you will, unfortunately, have to content yourself with this small thank you.

It was with Maite, Belén and Gauthier that I finally dared to start salsa classes. There are no better ways than dancing to feel better. There are more names than I can list, and I will thank everyone at **Salsa Wageningen and LAfrique** for the fantastic times, happy mood and self-betterment I got from you. You changed my life for the better.

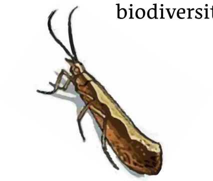
My dear Pomonians, **Diana, Vera, Ivette, Sofí, Varsha, Iris, Annanya, Dominique, Jin, Alison, Denisa and Emma**, how could I thank you properly for all the good things you gave me. Once again, I lack the words and creativity to express all my gratitude. You have welcomed me as an extra housemate and brightened my daily life with fantastic dishes, games and excursions. I will keep unforgettable happy memories of all of you.

Parmi tous mes amis qui m’ont accompagné durant mon doctorat, je me dois de réserver une mention plus que spéciale à **Laura, Ludivine, Adrien, Bastien, Hugo, Matthieu, Rémi, et Raphaël**. Votre amitié n’a jamais failli et je ne peux que vous remercier pour votre ténacité et persévérance. Pendant toutes ces années, j’ai souvent été absent, j’ai même complètement disparu et pourtant vous ne m’avez jamais oublié. Il est souvent plus difficile de seulement observer et être là que d’agir, vous l’avez fait à merveille, jusqu’à la fin et je vous en suis plus que reconnaissant. Vous êtes des amis en or, et irremplaçables.

Pour finir ces remerciements, il ne manque plus que ma très chère famille, mes parents; **Laurence et Jean-Pierre**, mon frère **Hugo**, ma tante **Brigitte**, et ma grand-mère **Huguette**. Vous non plus n'avez pas eu la tâche facile et pourtant vous avez été formidables pendant toutes ces années. Merci pour votre soutien infaillible, vos petites attentions et les colis qui m'ont beaucoup aidé pendant toutes ces années et surtout pendant les longs mois d'hiver Néerlandais et ce, depuis bien plus longtemps que le doctorat lui-même.

About the author

Thibault Pierre Marius Costaz was born on the 14th of September 1992 in Ardèche, France. He grew up in a countryside village, providing him with all sorts of adventures. Insects were already of great interest, from breeding race snails to finding stick insects in the bushes, although his mum never allowed him to rear ants inside the house. As a child, his first goal was to become a vegetable grower, but he first needed to learn how to count. Therefore he had to continue his primary education courses. Growing up, he developed a particular interest in science and enjoyed watching science documentaries. Later, he went to high school; LEGTA Le Valentin, Bourg-lès-Valence, France, that specialises in agriculture, where he obtained his Baccalaureat in science with a specialisation in biology and ecology. Following the advice of his parents and teachers, he applied to ISARA-Lyon, France, for an MSc in agriculture and food sciences and got accepted. His study advisor convinced him to apply for the double degree program in collaboration with Wageningen University, The Netherlands. Indeed Wageningen University was more oriented toward fundamental research. Before being selected for the double-degree programme, he had the chance to do a three-month internship at the University of Adelaide, Australia, in the soil biology laboratory under the supervision of prof. dr. Petra Marschner. He then completed an MSc in environmental sciences with a major in environmental system analysis at Wageningen University. His major MSc thesis was supervised by prof. dr. Wim de Vries focusing on the modelisation of tree carbon sequestration using foliar nutrient content based on a meta-analysis. Filled with enthusiasm from friends majoring in entomology and missing the agriculture domain, he decided to do a minor MSc thesis at the Netherlands Institute for Ecology (NIOO). There he studied the foraging behaviour and thermal tolerance of two aphid hyperparasitoid species (*Dendrocerus spp.* and *Asaphes spp.*) under the supervision of dr. Martine Kos. Thoroughly convinced about becoming an expert in biocontrol, he looked for a PhD in this field. In the meantime, he got offered a summer position as a field assistant within the soil living legacy project team, led by prof. dr. Martijn Benzemer at the Terrestrial Ecology department of NIOO. Soon after, he applied for a PhD project entitled “Safeguarding biological control in the face of climate change” under the supervision of prof. dr. Marcel Dicke, prof. dr. Joop van Loon and dr. Peter de Jong at the Laboratory of Entomology, Wageningen University. Starting on this project in September 2017, Tibo studied the impacts of heatwave-like events on the phenotypic plasticity of a worldwide pest and two of its main parasitoid natural enemies. To this end, he performed a combination of laboratory and field experiments. After his PhD, he wishes to pursue a scientific career in entomology with a particular focus on functional biodiversity, agroecology, and integrated pest management.



Publications

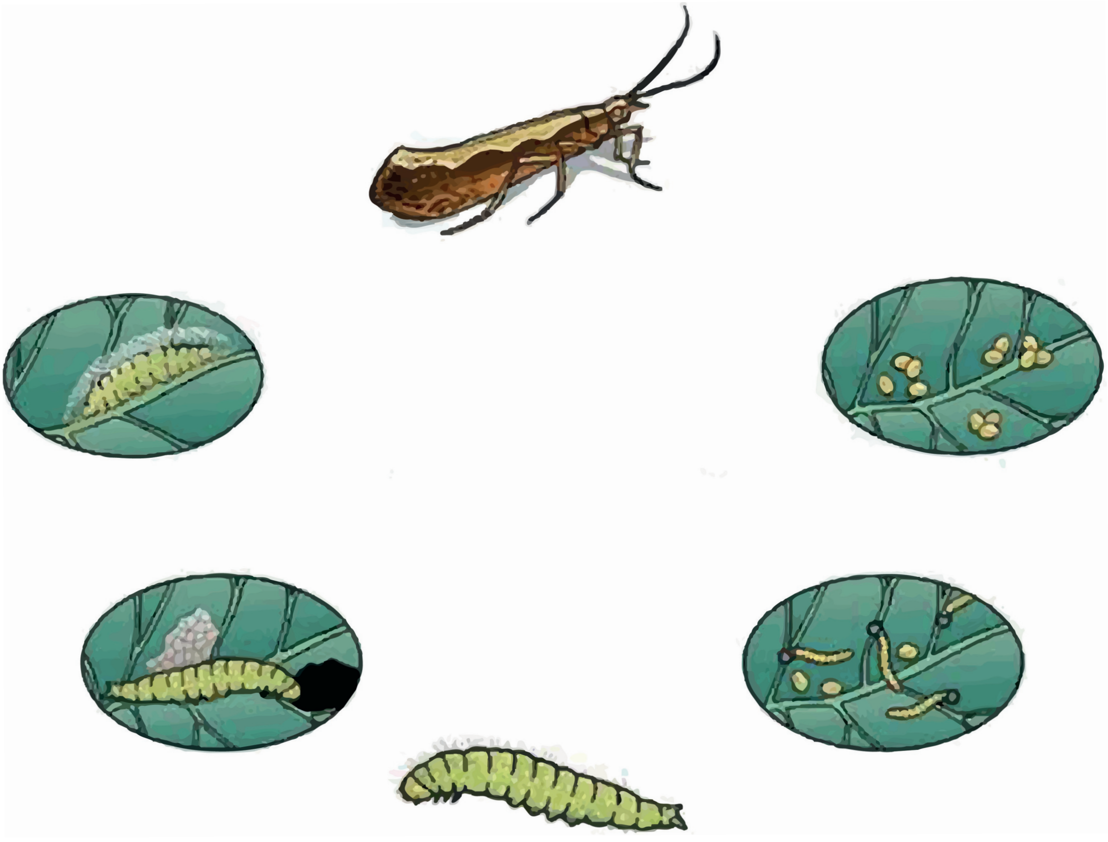
de Boer, J. G., Salis, L., Tollenaar, W., van Heumen, L. J. M., **Costaz, T. P. M.**, Harvey, J. A., Kos, M., & Vet, L. E. M. (2019). Effects of temperature and food source on reproduction and longevity of aphid hyperparasitoids of the genera *Dendrocerus* and *Asaphes*. BioControl, 64(3), 277–290. https://doi.org/10.1007/s10526-019-09934-4

Costaz, T. P. M., Gols, R., de Jong, P. W., van Loon, J. J. A., & Dicke, M. (2022). Effects of extreme temperature events on the parasitism performance of *Diadegma semiclausum*, an endoparasitoid of *Plutella xylostella*. Entomologia Experimentalis et Applicata. https://doi.org/10.1111/eea.13197 (Chapter 3 of this thesis)

Submitted

Costaz, T. P.M., de Jong, P. W., Dicke, M., van Loon, J J. A., It is about time: Effects of thermal fluctuations resulting from climate change on host-parasitoid interactions. (Chapter 2 of this thesis)

Costaz, T. P.M., de Jong, P. W., Harvey, J.A., van Loon, J J. A., Dicke, M., Gols, R. Temperature and density steer competitive outcomes between two sympatric endoparasitoids. (Chapter 5 of this thesis)



PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

It is about time: effects of thermal fluctuations resulting from climate change on host-parasitoid interactions

Safeguarding biological control in the face of climate change

Post-graduate courses (5.3 ECTS)

Multivariate analysis; PE&RC (2017)

Consumer-resource interaction: in times of global environmental change; PE&RC (2018)

Design of experiments; WIAS/PE&RC (2018)

Advances in intercropping: principles and implementation; PE&RC (2021)

Competence strengthening / skills courses (3.1 ECTS)

Scientific writing; WGS (2020)

Adobe InDesign; WGS (2021)

Mindful productivity for scientist; WGS (2021)

Reviewing a scientific manuscript; WGS (2021)

Scientific integrity/ethics in science activities (0.6 ECTS)

Research integrity; WGS (2021)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

PE&RC First year weekend (2018)

PE&RC Days (2018, 2019, 2021)
PE&RC Last year weekend (2021)

Discussion groups / local seminars or scientific meetings (8.2 ECTS)

Insect-plant-interaction workshop (2017)
29th Annual meeting of the Netherlands entomological society (2017)
Insect-plant-interactions (2017-2020)
30th Annual meeting of the Netherlands entomological society (2018)
Netherland annual ecology meeting (2018)
Netherland annual ecology meeting (2021)
Wageningen evolution and ecology seminar (2021)

International symposia, workshops and conferences (5 ECTS)

Symposium on Insect-Plant Interaction; online (2021)
Congreso Nacional de Entomología Applicada; Málaga, Spain (2022)

Committee work (2 ECTS)

PE&RC PhD council member (2018-2019)

Lecturing/supervision of practicals/tutorials (3.6 ECTS)

Insect ecology (2018-2019)
Insect-plant interaction (2018-2020)
Ecophysiology (2018-2020)

BSc/MSc thesis supervision (3 ECTS)

Behavioural responses of *Plutella xylostella* and its parasitoid *Diadegma semiclausum* in response to heat waves
Reproductive plasticity of a global pest *Plutella xylostella* and two of its parasitoids in response to extreme temperature events

The research presented in this thesis was performed at the Laboratory of Entomology of Wageningen University & Research (WUR).

Cover and illustrations designed by Sarah Haimes
Thesis layout designed by Felipe Hernando Tadeo Padilla Bruges
Printed by Proefschriftmaken, Wageningen, the Netherlands