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ORIGINAL ARTICLE

Special Issue: Insect-Plant Relationships

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

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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 behavioral 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 parasitized *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 behavioral 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.

KEYWORDS

carry-over effects, climate change, extreme weather events, fluctuating temperatures, heat shock, host preference, Hymenoptera, Ichneumonidae, larval endoparasitoid, Lepidoptera, parasitoid, Plutellidae

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 & 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., 2016). 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; Stoks et al., 2017; Harvey et al., 2020). 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 (Speights et al., 2017; Bai et al., 2019). 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 vs. mild periods both within and between days (Ma et al., 2018; Bai et al., 2019; 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 (Jervis et al., 2005; Harvey et al., 2020). Thermal sensitivity increases with increasing trophic level (Voigt et al., 2003). Therefore, parasitoids are expected to be acutely impacted by EXT (Hance et al., 2007; Furlong & Zalucki, 2017). Parasitoid foraging strategies have received a lot of attention, and a vast body of literature is available (Wajnberg et al., 2008; de Rijk et al., 2013; Aartsma et al., 2019). 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 behaviors exhibited by its host (Le Lann et al., 2014; Moiroux et al., 2016). Moiroux et al. (2015) found that the foraging behaviour of *Aphidius ervi* Haliday on *Macrosiphum euphorbiae* Thomas 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 and 20 °C). These results were interpreted as risk-prone behavior caused by a decrease in the expected reproductive success due to reduced expected survival (Wajnberg et al., 2008). *Leptopilina heterotoma* Thomson exhibited increased patch residence time and superparasitism (i.e., oviposition in an already parasitized 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 behavior (Steinberg et al., 1992). This matches with life-history theory, which predicts that an individual should adapt its behavior to its physiological status in order to maximize 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 (Stoks et al., 2017; Ma et al., 2021).

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 (Talekar & Shelton, 1993; Furlong et al., 2013). We hypothesize 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 2 and 4 days at 25 and 20 °C, respectively. *Diadegma semiclausum* is among the main parasitoids of *P. xylostella* and has been introduced in many countries as a biocontrol agent (Talekar & Shelton, 1993). It is a solitary endoparasitoid that consumes the host entirely before pupating (Harvey, 2015). This parasitoid species can parasitize 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; Brassicaceae) in separate rooms under constant conditions (22 ± 2 °C, 40–50% r.h., L16:D8 photoperiod). 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 20 *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* from the stock colony. The parasitized 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 (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 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

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

Hours	Reference (°C)	Heatwave (°C)
00:00–05:00	15	18
05:00–11:00	20	25
11:00–15:00	25	33
15:00–21:00	20	25
21:00–00:00	15	18

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 L16:D8 photoperiod corresponding to early summer conditions in The Netherlands. The parasitism rate of *D. semiclausum* was then compared at three temperatures (20, 25, 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 and 33 °C).

Experiment 1: direct effect of temperature

Thirty early second instar (L2) and third instar (L3) *P. xylostella* (15 per instar), taken from the stock colony, were placed on an excised young fully developed Brussels sprouts leaf in a plastic container (115 × 188 mm, volume 770 ml; Gédé Verpakkingen, Limmen, 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 turgid 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, 2- to 4-day-old female *D. semiclausum* were kept in a separate cage along with males (1:1 sex ratio) 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, 25, 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 3 h. Foraging time was set to 3 h 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×. The replicates were conducted over several days, allowing to perform all treatments in each of the climate cabinets randomly. The bioassays were performed between 09:00 and 16:00 hours.

Experiment 2: indirect effect of temperature

As in experiment 1, the parasitism rate of *D. semiclausum* was compared at three temperatures (20, 25, 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 3-day-old *D. semiclausum* females were kept with males (1:1 sex ratio) 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* were selected and placed on a young fully developed Brussels sprouts leaf inside a container (as in experiment 1). The *P. xylostella* larvae were placed back into the two climate cabinets at either 20 or 25 °C alongside the parasitoid cages ([Table 1](#)). Then the temperature was set at either 20, 25, or 33 °C. After 30 min of acclimation to the new conditions, a single naïve female *D. semiclausum* was released in each container for 3 h. 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 18–21 replicates accumulating over time, allowing to switch the thermal pre-treatment and temperature treatments between all climate cabinets. All bioassays were performed between 11:00 and 14:00 hours.

Variables measured

All host larvae were dissected under a Zeiss SR stereomicroscope (Oberkochen, Germany), and the presence/absence of parasitoid eggs was recorded. The occurrence of superparasitism (if >1 egg was found per host) was also recorded.

Statistical analysis

The data were analyzed 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 analyzed the proportion of parasitized *P. xylostella* larvae using a generalized 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 function. Non-significant interaction terms ($P > 0.05$) were excluded from the model. Changes in the proportion of parasitized L2 vs. L3 *P. xylostella* instars were analyzed 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 parasitized L2 and L3 caterpillars. The model did not suffer from overdispersion. Non-significant interaction terms were excluded from the model.

The occurrence of self-superparasitism was analyzed using a Bernoulli GLM with a logit link function. Parasitoids that exhibited superparasitism behavior were analyzed vs. 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 analyzed. 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 super-parasitized hosts for which 3–4 eggs per larva were recorded and was excluded from analysis.

All analyses were performed in R v.4.0.2 (R Core Team, 2019). The figures were produced using the ggplot2 package (Wickham et al., 2019). The treatments main effects were extracted from the model output using the car package (Fox & Weisberg, 2019). When a significant effect of treatment was found, Tukey post-hoc tests were performed for pairwise comparison using the emmeans package (Lenth et al., 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 ($\chi^2 = 6.42$, d.f. = 2, $P = 0.040$) ([Figure 1](#)). The number of parasitized *P. xylostella* larvae across the three tested

temperatures resembles the expected shape of thermal performance curves (TPC) with more parasitized caterpillars at 25 °C than at 20 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 parasitized larvae between 20 and 33 °C is not significant.

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

Experiment 2: indirect effect of temperature

Temperature affected the overall proportion of *P. xylostella* caterpillars parasitized ($\chi^2 = 18.14$, d.f. = 2, $P < 0.001$), whereas the thermal pre-treatment had no effect on parasitism rates ($\chi^2 = 0.37$, d.f. = 1, $P = 0.55$). The proportion of

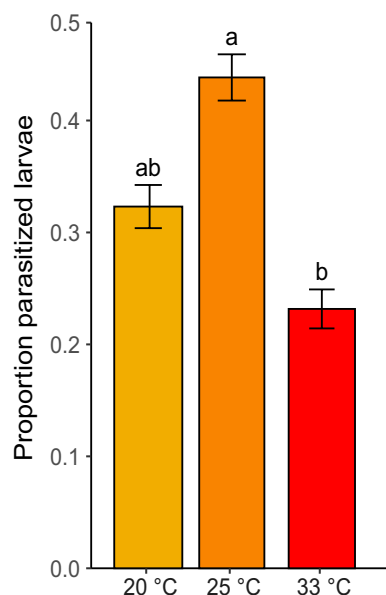
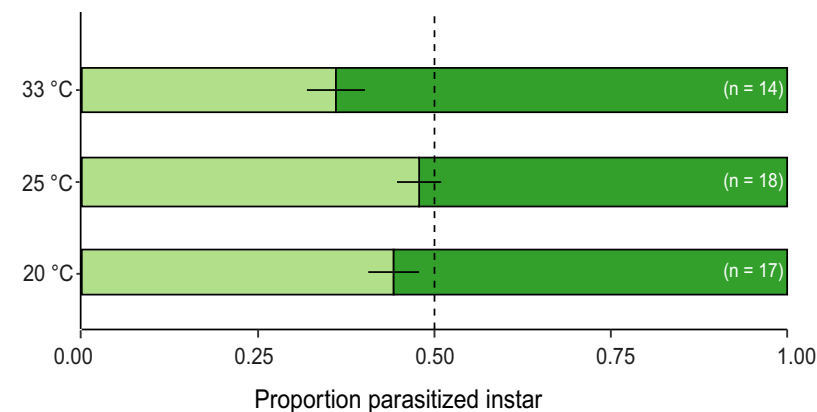


FIGURE 1 Mean (\pm SE) proportion of *Plutella xylostella* larvae parasitized by *Diadegma semiclausum* females at 20, 25, and 33 °C. Means capped with different letters are significantly different (Tukey-adjusted pairwise comparisons: $P < 0.05$).

FIGURE 2 Mean (\pm SE) proportions of parasitized L2 (light green) and L3 (dark green) *Plutella xylostella* instars at 20, 25, and 33 °C. 'n' indicates the number of tested *Diadegma semiclausum* female 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.



P. xylostella larvae parasitized at the three temperatures followed the same pattern as in the previous experiment, with a strong negative effect of high-temperature (i.e., 33 °C) exposure on *D. semiclausum* oviposition performance (Figure 3). No evidence of instar preference was detected for the thermal pre-treatments ($\chi^2 = 0.16$, d.f. = 1, $P = 0.69$) nor for temperature treatment ($\chi^2 = 2.00$, d.f. = 2, $P = 0.37$) (Figure 4).

The occurrence of self-superparasitism behavior was not affected, neither by the thermal pre-treatment ($\chi^2 = 0.47$, d.f. = 1, $P = 0.50$) nor by the temperature during parasitism ($\chi^2 = 2.80$, d.f. = 2, $P = 0.25$) (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

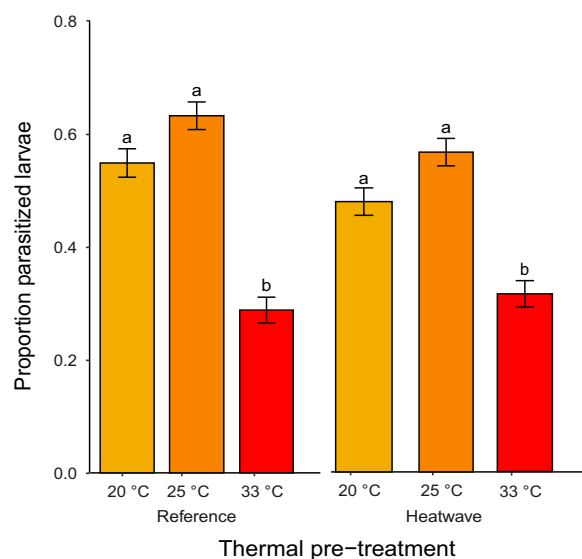


FIGURE 3 Mean (\pm SE) proportion of *Plutella xylostella* larvae parasitized by *Diadegma semiclausum* females at 20, 25, and 33 °C, for both the reference (left side) and heatwave (right side) thermal pre-treatments. Means capped with different letters are significantly different (Tukey-adjusted pairwise comparisons: $P < 0.05$).

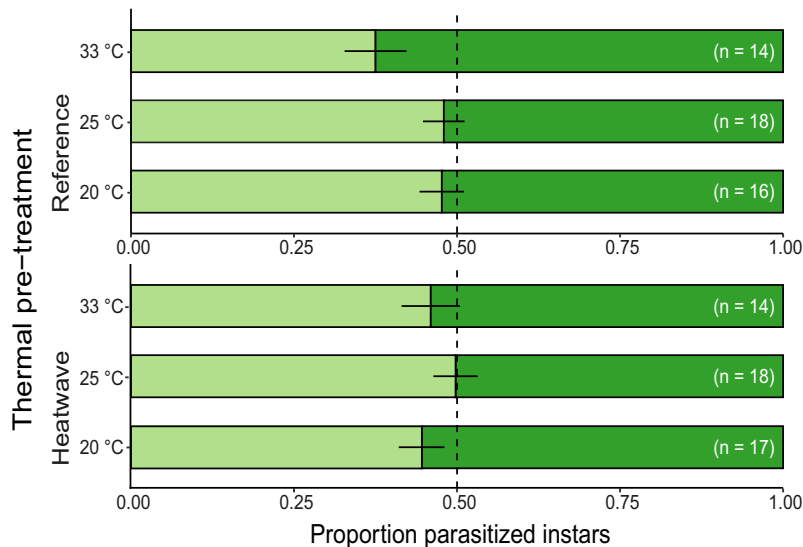


FIGURE 4 Mean (+ SE) proportions of parasitized L2 (light green) and L3 (dark green) *Plutella xylostella* instars at 20, 25, and 33 °C, for both the reference (top) and heatwave (bottom) thermal pre-treatments. 'n' indicates the number of tested *Diadegma semiclausum* female parasitoids, that each were offered 10 L2 and 10 L3 larvae.

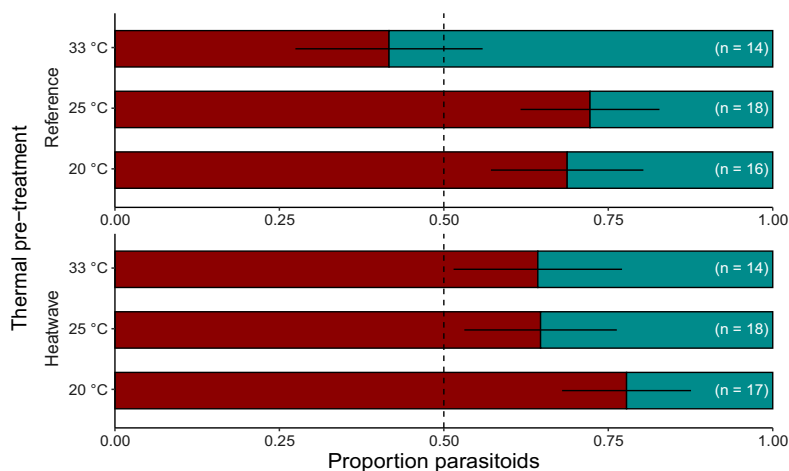


FIGURE 5 Mean (+ SE) proportion of *Diadegma semiclausum* parasitoids displaying superparasitism behavior (blue) or not (red) at 20, 25, and 33 °C, for both the reference (top) and heatwave (bottom) thermal pre-treatments. 'n' indicates the number of tested parasitoids. Females that did not lay eggs were not included in the analysis.

success. As hypothesized, *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. These discrepancies are likely due to the study design as, in the latter study, female parasitoids could forage for 24 h, 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 parasitized *P. xylostella* larvae at 20 °C was not significantly different from the proportion parasitized at 25 °C in both experiments of this study. Similarly, temperature did not significantly affect the proportion of *Sitobion avenae* Fabricius aphids parasitized by *Aphidius rhopalosiph* De Stefani-Perez exposed to temperatures between 15 and 25 °C (Le Lann et al., 2014). Temperature has a direct kinetic effect on insect metabolism, thus regulating insect performance. Insects are also able to integrate thermal information and adjust their behavioral response

accordingly (Abram et al., 2016). 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 behavior. 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., 4 h at 38 °C) occurred 3 days before the foraging assays (Valls et al., 2020). *Aphidius avenae* Haliday parasitism success after an exposure of 1 h to 36 °C followed by 24 h recovery at 20 °C led to a significant reduction in the number of parasitized 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, whereas 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 was only significantly reduced by previous exposure to extreme high temperature (>30 °C) of more than 24 h (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. rhopalosiphii* 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 et al., 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 showed that females attacked L2, L3, and L4 *P. xylostella* without any preference despite a lower survival for parasitized fourth instars (Nofemela & Kfir, 2007). Additionally, Gols & Harvey (2009) studied the effect of host size at parasitism on *D. semiclausum* development and found lower survival for parasitized L4 compared to L2P. *xylostella*. 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 behavior theory, to maximize their fitness under stressful conditions, female parasitoids are expected to attack more frequently lower quality hosts (Wajnberg et al., 2008; Moiroux et al., 2015). It is important to note that temperature can also alter host defensive behavior. Although host defensive behavior was

not quantified in this study, it was reported that higher temperature led to more intense defensive behavior from *S. avenae* (Le Lann et al., 2014). However, the host's increased defensive display was counterbalanced by the parasitoid's higher attack rate (Le Lann et al., 2014). The differences in results between this study and that by Moiroux et al. (2015) 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*. 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 koinobiont parasitoid, thus allowing the host to continue its development after parasitization. To some extent, they can exert control over host physiology to maximize their fitness (Gols & Harvey, 2009). 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 analyzed in the second experiment. The proportion of parasitoids expressing this behavior 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 behavior. 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 behavior 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 & 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 (van Alphen & Visser, 1990; Poelman et al., 2014; 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 (T.P.M. Costaz, pers. obs.). 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* superparasitized more often after a drop in barometric pressure (associated with reduced life expectancy) (Roitberg et al., 1993). This type of behavior can be interpreted as risk-prone behavior (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, maximize its fitness outcome by engaging in self-superparasitism. However, this was not confirmed by the data as the percentage of female parasitoids displaying

self-superparasitism in the heatwave pre-treatment was similar to the one foraging at 20 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 parasitized from unparasitized larvae. Temperature may alter *D. semiclausum*'s capacity to detect parasitized 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 behavior may only occur at higher stress temperatures. Abram et al. (2015) found that previous thermal stress prevented memory loss in the parasitoid *Trissolcus basalisi* (Wollaston). 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) (Voigt et al., 2003; Furlong & Zalucki, 2017; Stoks et al., 2017). 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 (Truong et al., 2014; Jamieson et al., 2017). Changes in the plant primary and secondary metabolites can, in turn, alter host physiology, affecting parasitoid larvae (Gols et al., 2008). Dossdall 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 the ability of *D. semiclausum* to control *P. xylostella* populations, thus increasing the risk of pest outbreaks (Hance et al., 2007; de Sassi & Tylianakis, 2012; Ma et al., 2015; Schreven et al., 2017).

AUTHOR CONTRIBUTIONS

Thibault Costaz: Conceptualization (equal); formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead). Rieta Gols: Conceptualization (equal); supervision (supporting); validation (equal); writing – review and editing (equal). Peter W. de Jong:

Conceptualization (equal); supervision (equal); validation (equal); writing – review and editing (equal). Joop J.A. van Loon: Conceptualization (equal); supervision (equal); validation (equal); writing – review and editing (equal). Marcel Dicke: Conceptualization (equal); supervision (equal); validation (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

All data are placed in the WUR repository so that they are freely accessible.

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