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Chapter 3

Direct and plant-mediated effects of simulated rain downpours on insect herbivore performance

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Submitted

Abstract

Changes in the frequency, duration and intensity of rainfall events are among the abiotic effects predicted under anthropogenic global warming (AGW). Heavy downpours may profoundly affect the development and survival of small organisms such as insects. Here, we examined direct (physically on the insects) and indirect (plant-mediated) effects of simulated downpours (thundershower intensity) on the performance of a small (*Plutella xylostella*) and a large (*Pieris brassicae*) lepidopteran insect herbivore feeding on black mustard (*Brassica nigra*) plants. Host plants were exposed to four different rainfall regimes: (1) none, (2) prior to caterpillar infestation (indirect, plant-mediated effects), (3) during caterpillar infestation (mainly direct rainfall effects), and (4) both situations, applied as a single long (20 min) or as three short (5 min) daily rainfall events. Rainfall had a strong negative direct effect on the survival of the small *P. xylostella*, but not on that of the larger *P. brassicae*. Direct effects of downpours consistently increased development time of both herbivore species, whereas effects on body mass depended on herbivore species and rainfall frequency. Caterpillar disturbance by rain and microclimatic cooling by 5 °C, recorded using a thermo-detector, may explain extended immature development. Indirect, plant-mediated effects of rainfall on the herbivores were generally small, despite the fact that sugar concentrations were reduced and herbivore-induced increases in secondary metabolites (glucosinolates) were enhanced in plants exposed to rain. Changes in the intensity of precipitation events due to climate change may impact the survival and development of insect herbivores differentially. Broader effects of downpours on insects and other arthropods up the food chain could seriously impair and disrupt trophic interactions, ultimately destabilizing communities.

Keywords: rainfall; plant-insect interactions; development; survival; *Brassica nigra*; *Plutella xylostella*; *Pieris brassicae*

Introduction

Species interactions, such as those occurring between plants and insects, are not only affected by the biotic environment (e.g. presence of competitors and predators, quality and quantity of resources), but also by the abiotic environment (Schoonhoven et al., 2005). Temperature, wind, precipitation, and humidity have profound effects on plant-herbivore interactions at different temporal and spatial scales (Bezemer, Jones, & Knight, 1998; Chen et al., 2018; Harrington, Woiwod, & Sparks, 1999; Louis M Schoonhoven et al., 2005; Shure et al., 1998) and these in turn, may have consequences for population and community dynamics (Kingsolver, 1989; Ritchie, 2000). Moreover, small-scale heterogeneity in microclimatic patterns, as well as weather and large-scale climatic patterns have long been recognized as major factors influencing population dynamics of small ectothermic arthropods (Andrewartha & Birch, 1954; Bale et al., 2002; Uvarov, 1931). Studies of the impact of Anthropogenic Global Warming (AGW) on ecological processes have largely focused on the effects of elevated mean temperature and/or atmospheric concentrations of CO₂ (Christidis, Stott, & Brown, 2011; Norby & Zak, 2011; Zavala, Nabity, & DeLucia, 2013). However, global and regional patterns of precipitation are also expected to change with rising temperatures (IPCC, 2014). For example, in Europe, future summers are predicted to be longer and drier with shorter but more intense extreme rainfall events such as thunderstorms (Gadian et al., 2018). To underline this, June-August (summer) of 2018 was the hottest in Europe by a wide margin, coming in at 2,16 degrees C above the 1910-2000 mean, and was punctuated by

an extended drought (Severe Weather Europe, <http://www.severe-weather.eu/news/record-breaking-summer-in-europe-the-summer-of-2018-breaks-land-temperature-record/>). Intense heat generates convection that leads to severe thunderstorms and attendant downpours (Brooks, 2013). Short-duration extreme weather events are predicted to increase by approximately 14% with every one degree increase in temperature (Lenderink & van Meijgaard, 2008). Heavy rain can seriously affect the behavior and development of small organisms such as insects and other arthropods (Fink & Vökl, 1995, Kamata & Igarashi, 1994). Moreover, seasonal rainfall across years can cause a reduction in herbivore abundances and lead to shifts (i.e. simplification) in trophic structure, thus, modifying community-level interactions (Suttle, Thomsen, & Power, 2007; Zhu et al., 2014).

As AGW affects abiotic conditions in multiple ways, knowledge on how various abiotic factors influence individual species and interactions with other species is becoming increasingly important. Abiotic factors directly or indirectly affect insect herbivores feeding on their host plants. For instance, heavy rainfall can dislodge insects from the plants (Kobori & Amano, 2003) and can also affect microclimatic conditions (through cooling) (Dobkin et al., 1987; Kamata & Igarashi, 1994), which, like temperature in general, strongly affects the developmental rate of ectotherms. Indirect or plant-mediated effects of abiotic factors on insect herbivores may occur if these factors influence plant traits determining food plant quality for insect herbivores. For instance, variation in temperature and rainfall may change levels of primary and secondary metabolites in plant tissues that are consumed by the herbivores, affecting their growth and development (Jamieson et al., 2017). It has also been reported that the emission of volatile plant metabolites is affected by rainfall, relative humidity and temperature (Loreto & Schnitzler, 2010; Vallat, Gu, & Dorn, 2005). Changes in the emissions of

plant volatiles could potentially affect the ability of insect herbivores to find host plants when these changes concern specific cues important for foraging behavior. In addition it may affect the foraging behavior of higher trophic-level arthropods such as predators and parasitoids that is mediated by the volatiles emitted in response to herbivory (Dicke, 2016). Thus, downpours may strongly affect species interactions.

With the exception of temperature, abiotic factors, such as rainfall and wind, are rarely considered in laboratory and greenhouse experiments investigating insect-plant interactions. However, in the field, organisms have to deal with both the biotic and abiotic environment. By greatly reducing, eliminating or making abiotic factors static in lab experiments, some of the results may be open to conjecture. Therefore, it is important to determine the relative importance of abiotic and biotic factors when studying plant-herbivore (multitrophic) interactions. In a previous study (Chen et al., 2018), we showed that wind-exposure can extend larval development time of small and large lepidopteran herbivores, *Plutella xylostella* (L.) (Plutellidae) and *Pieris brassicae* (L.) (Pieridae), respectively. Interestingly, wind-exposure also reduced adult biomass of *P. xylostella*, whereas butterflies of *P. brassicae* were heavier on wind-exposed plants. Plant-mediated effects were relatively small (Chen et al., 2018).

Here, we examined the effects of simulated heavy short-term rainfall exposure on the survival and development of the same two herbivore species *P. xylostella* and *P. brassicae*, on one of their important natural food plants, *Brassica nigra* (L.) (Brassicales: Brassicaceae). The rainfall events simulated short-term heavy downpours like those that accompany strong thunderstorms. Experiments were performed in a greenhouse and applied at two different daily durations, one longer (20 min) downpour or three shorter (5 min) downpours, in two experiments separated in time. To separate direct and indirect, i.e. plant-mediated, effects of rainfall on insect development, exposure to rain was

separated into two phases: 1) rain exposure during the growth phase of the plants before insects were introduced and started feeding on the plants and 2) the phase during which the insects were feeding on the plants. Plants in each experiment were exposed to one of four rainfall regimes (1) no rain, (2) only rain during phase 1, (3) only rain during phase 2 and (4) rain during both phases.

We hypothesize that 1) the direct effects of intensive rainfall such as physical disturbance and changes in microclimate negatively affect herbivore performance, 2) the direct effects of rainfall are stronger than the indirect plant-mediated effects and 3) the effects of rainfall are stronger in the micro-moth than in the macro-butterfly. As indicators of plant quality we measured foliar sugars and glucosinolates, which are representatives of primary and secondary metabolites, respectively, and are both considered important for insect performance (Awmack & Leather, 2002; R. J. Hopkins, N. M. van Dam, & J. J. van Loon, 2009; Scriber & Slansky, 1981). We also measured temperature close to the leaf surface before and after the simulated rainfall events. Our results are discussed in relation to the effects of predicted changes in extreme weather events on insect-plant interactions in Europe under AGW by the IPCC (2014)

Materials and Methods

Study system

Plants - Black mustard, *B. nigra*, is a widespread brassicaceous plant species native to Eurasia. This early-successional plant species originated from the Middle East and is common in a range of climatic regions, including hot regions of southern Europe (e.g. the Mediterranean region) and cooler regions of central/northern Europe (Prakash & Hinata, 1980). Like other members of the Brassicaceae family, it produces secondary compounds known as glucosinolates (GS) which are expressed in both shoot and root

tissues of the plant (Fahey, Zalcmann, & Talalay, 2001). GS can act as feeding deterrents or exhibit negative effects on the growth and development of non-adapted phytophages and their natural enemies, while they can be used as feeding and oviposition cues by specialist enemies (Gols & Harvey, 2009; Hopkins, van Dam, & van Loon, 2009). Concentrations of these compounds are also known to change in response to herbivory (Textor & Gershenzon, 2009). We measured GS, as well as sugar concentrations, in leaf tissues in plants exposed to the various rainfall and herbivore treatments described below to reveal how secondary and primary metabolites respond to rain and insect feeding.

Plants were grown from seeds and one week-old seedlings were transferred to 2-L pots (one plant per pot) filled with potting soil (Lentse potgrond no. 4: Lent, the Netherlands). Plants were grown in a greenhouse (22 ± 3 °C, 50%-70% r.h. and a 16-h photoperiod). Simulation of rainfall and age of plants are described in the *Experimental protocol* below.

Insect herbivores - The microlepidopteran diamondback moth *P. xylostella* is a European moth probably originating from the Mediterranean region or southern Africa that has spread worldwide (Furlong et al., 2013). It is one of the most important pests of cruciferous crops in the world (Talekar & Shelton, 1993). The large cabbage white *P. brassicae* is a macrolepidopteran species with a wide distribution in Eurasia and is notorious for the voracious way in which the caterpillars defoliate cabbages (Feltwell, 1982). Like *P. xylostella*, caterpillars of *P. brassicae* mainly feed on brassicaceous plant species (Feltwell, 1982). In contrast with the solitary feeding *P. xylostella*, caterpillars of *P. brassicae* feed gregariously and produce thick silk mats which facilitate tight attachment to the leaf. Caterpillars of both species were obtained from the laboratory of Entomology, Wageningen University. They were maintained on cabbage plants

(*Brassica oleracea*, var. *gemmifera*, cv. *Cyrus*) in climate rooms at 22 ± 2 °C, 50%-70% r.h. and a photoperiod of 16 h.

Experimental protocol

To study the effects of simulated downpours on plant-herbivore interactions, we conducted a factorial experiment in a greenhouse at Unifarm, Wageningen University. Rainfall was simulated using nozzles (full cone Hardi nozzles, type 1553, Homburg Holland, Stiens, The Netherlands) with an intensity of ~ 20.9 mm water per h. The nozzles were attached to a frame approximately 2 m above movable tables (80 × 80 cm, one nozzle per table). In total, there were twelve tables with simulated rainfall and twelve tables without simulated rainfall which were each placed in rows of six tables (Fig. 1). Rows of rain-exposed and non-exposed tables were alternated. The experiment was performed twice. In the first experiment, the frequency of rain was set at once a day for 20 min at 9 AM. In the second experiment, the frequency of rain was increased to three times a day (9 AM, 2 PM and 7 PM,) and the duration of each rain shower was reduced to 5 min. Each pot was covered with tin foil to avoid nutrients leaching from the soil. Plants that were not exposed to rain were also covered with tin foil and placed in saucers to allow watering which was done by hand once a day.

Rainfall-exposure treatments were divided into two phases: growth phase of the plants before introduction of the insects (phase 1) and insect exposure phase (phase 2). Plants were allocated to one of the following four rainfall treatments (Fig. 1): (1) control (no exposure to rainfall), (2) exposure to rainfall only during phase 1, (3) exposure to rainfall only during phase 2, and (4) exposure to rainfall during both phases. These four precipitation regimes allowed us to separate the indirect, plant-mediated effects of simulated rainfall (2,4 vs. 1,3) from predominantly direct effects of simulated rainfall

(3,4 vs. 1,2) on the herbivores. Tables were moved within the greenhouse according to their assigned rainfall treatment (see Fig. 1). In the single rain shower experiment, phase 1 was initiated 3 weeks after seedlings were transplanted and lasted 7 days whereas in the triple-shower experiment phase 1 was initiated 1 week after seedlings were transplanted and lasted 21 days. In both experiments, caterpillars were introduced when the plants were 4 weeks old. Phase 2 in which plants were exposed to herbivory lasted approximately 7 days for *P. xylostella* and 14-days for *P. brassicae* due to differences in larval development time (see below).

The experiment was set up according to a block design comprising 24 blocks (=tables) with six replicates per rainfall treatment, each containing nine plants of which two were not exposed to herbivory (control) and 7 were exposed to one of the two herbivore species. On each of three plants 10 early L2 larvae of *P. xylostella* were introduced (L1 larva of *P. xylostella* are difficult to handle as they are mining in the leaves), and on each of four plants 10 early L1 larvae of *P. brassicae* were introduced. The 216 plants in total were randomly allocated to blocks and treatments within blocks. The insect herbivores were allowed to move and feed freely on the plants. Plants were not touching preventing dispersal of insects to adjacent plants. When caterpillars of *P. brassicae* reached the third instar, their numbers were reduced to three per plant to ensure sufficient resources for caterpillar development. When larvae reached their final instar (fifth for *P. brassicae* and fourth for *P. xylostella*), plants with the same herbivore species within blocks were placed together in a netted cage (40 × 40 × 60 cm, Vermandel, Hulst, The Netherlands). At this point, exposure to rainfall ended. This was done at day 7 on plants with *P. xylostella* and at day 14 on plants with *P. brassicae* after phase 2 had started. Pupae of *P. xylostella* and *P. brassicae* were collected per cage and transferred to Petri dishes. Development time until eclosion and offspring sex were

recorded. Newly emerged adults were frozen immediately and weighed individually on a Mettler-Toledo (Columbus, OH, USA) microbalance. The number of caterpillars that survived to the pupal (*P. xylostella*) and the adult stage (*P. brassicae*) were counted. Development time from L1 (*P. brassicae*) or L2 (*P. xylostella*) to adult eclosion was recorded in days.

Simulated rainfall was expected to affect the microclimate. Therefore, we measured the temperature of the leaf surface of plants in the middle of each block using a thermo-detector (Bosch PTD 1, Leinfelden-Echterdingen, Germany) during the second experiment. Temperatures were measured three times; before, immediately after, and one hour after the second rain shower at four consecutive days during phase 2.

Before the plants were placed in cages, leaf tissues were collected from all plants for chemical analyses (GS and sugars). Five leaf discs ($\text{\O} = 12 \text{ mm}$) were collected from the youngest fully expanded leaf of each plant. Leaf discs were taken from the tips of leaves (Agrawal & Fishbein, 2006). In each block, all leaf discs sampled from plants belonging to the same treatment were pooled, resulting in 3 samples (control, exposure to *P. xylostella*, and exposure to *P. brassicae*) per block and 6 samples per treatment. Samples were immediately submerged into liquid nitrogen and stored at $-20 \text{ }^{\circ}\text{C}$ until chemical analyses were conducted.

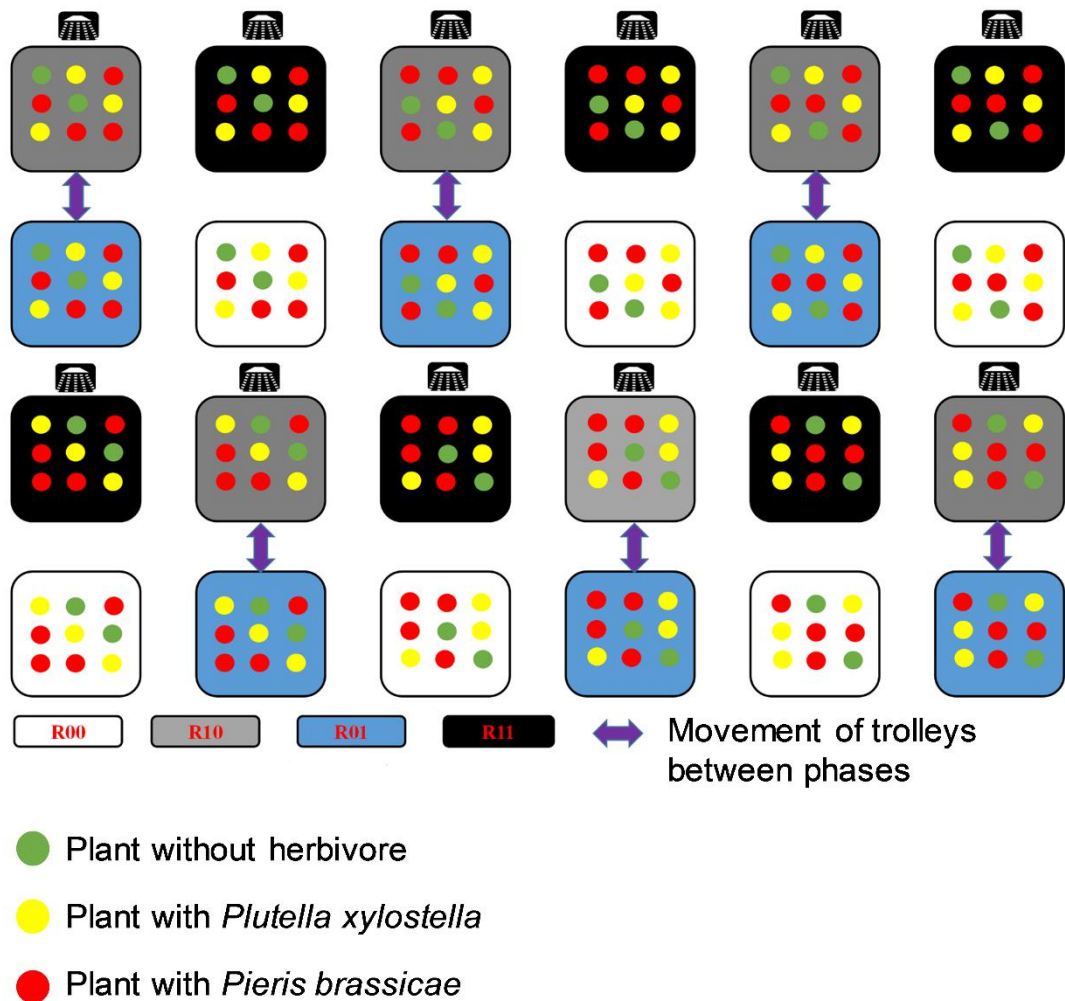


Fig. 1. Schematic diagram of the design of the simulated rainfall experiment. The experiment was divided into two phases: the growth phase of the plants (phase 1) and the phase in which the insects were feeding on the plant (phase 2). There were four rain treatments: (1) plants were not exposed to rainfall at all (R00), (2) plants were only exposed to rainfall during phase 1 (R10), (3) plants were only exposed to rainfall during phase 2 (R01), and (4) plants were exposed to rainfall throughout the experimental period (R11). Plants were placed on tables (=replicate) with nine plants of which three were infested with L2 larvae of *Plutella xylostella*, four were infested with L1 larvae of *Pieris brassicae* and two served as control (no herbivores).

Chemical analyses: glucosinolates and sugars

Samples were freeze-dried for three days (Labconco Freezone 12, Abcoude, The Netherlands) and ground to a fine powder using a Retch Ball Mill grinding machine (Retsch GmbH, Haan, Germany). A methanol extraction was used to extract both primary (sugars) and secondary metabolites (GS). Approximately 50 mg of dry ground leaf tissue was added to a 2 mL-Eppendorf tube. After adding 1 mL of 70% MeOH to each sample, the tubes were vortexed and boiled for 5 min, and then transferred to an ultrasonic bath for 15 min. Finally, tubes were centrifuged for 10 min at 10000 rpm and the supernatant was transferred to a clean and labeled 2-mL Eppendorf tube. The remaining pellet was extracted again with 1 mL 70% MeOH. The volume of the combined supernatants was adjusted to 2 mL with 70% MeOH. This extract was stored at -20°C until analysis. GS were analyzed using an HPLC as described by van Dam, Witjes, and Svatoš (2004). Sugar analyses were performed using an HPLC (Agilent 1260 Infinity Bioinert) equipped with an Antec Decade elite electrochemical detector (ECD) using a CarboPac PA1 (2 x 50 mm) guard column and a CarboPac PA1 (2 x 250 mm) main column. We used 100% 0.1 M NaOH (5.3 mL 50% NaOH/ 994.7 g MilliQ) as mobile phase. Ten µL of each extract (see above) was diluted with 990 µL MilliQ water. Five µL of the diluted extract was injected into the HPLC and was analyzed with a run time of 35 minutes. The column temperature was 20 °C and the flow rate was set to 0.25 mL/min. Sugar concentrations (µg/mg leaf dry mass) were calculated using calibration curves for each of the sugars in the concentration range of 2.5 – 10 µg/mL.

Statistical analyses

Data (insect performance, chemistry) from the two experiments in which different frequencies of rainfall were applied, were analyzed separately as they were performed

sequentially in time. We used rainfall during phase 1 (yes/no) and during phase 2 (yes/no) and their interaction terms as factors in the analyses to separate direct and indirect effects of rainfall. Variation among the caterpillars that were exposed to the same treatment within a block is a source of random variation and was treated as such in the statistical model. Effects of rainfall treatments on survival of *P. xylostella* larvae from L2 to pupation and for *P. brassicae* from L1 to the adult eclosion were analyzed using generalized linear models with a binomial distribution and logit link function, with rainfall treatments and their interactions as fixed factors. Development time from L2 (*P. xylostella*) or L1 (*P. brassicae*) to adulthood and adult body mass of the herbivores were analyzed using general linear mixed models with the same fixed factors as for the analysis of insect survival and blocks as a random factor. Data on sinigrin (the dominant GS in *B. nigra*, comprising >99% of the GS content) and total sugar concentrations in leaf tissues were also analyzed using general linear mixed models. In addition to the model terms included in the analyses of development time and adult body mass, herbivory treatment was included as well (none, *P. xylostella* and *P. brassicae*). Temperatures measured three times in each block during the second experiment were analyzed using a general linear mixed model with rainfall regime, time and their interactions as fixed factors. Blocks and date were entered as random factors. Multiple comparisons were conducted when any of the model terms were significant (Tukey-adjusted comparison). All analyses were performed in R version 3.4.0 (R Core Team 2017).

Results

Effects of rainfall on insect herbivore survival and development

Response of *Plutella xylostella* - When rain was applied as a single 20-min heavy shower once a day, exposure of plants to rain before herbivores were introduced onto the plants (phase 1) did not significantly affect survival of *P. xylostella* to pupation ($\chi^2_1 = 0.16$, $P = 0.70$; Fig. 2a). However, exposure to rain when *P. xylostella* was feeding on the plants (phase 2) significantly reduced survival to pupation by approximately 36% compared to survival on plants not exposed to rain ($\chi^2_1 = 58.5$, $P < 0.001$; Fig. 2a). Exposure to rain when the caterpillars were feeding on the plant also extended development time by approximately a half day ($F_{1,37} = 8.95$, $P = 0.005$; Fig. 3a). Both indirect (rain during phase 1) and direct (rain during phase 2) exposure to rain did not affect adult biomass (phase 1: $F_{1,37} = 0.02$, $P = 0.89$; phase 2: $F_{1,37} = 0.36$, $P = 0.55$; Fig. 3b).

Also when rain was simulated in three short 5-min showers each day, direct exposure of plants to rainfall (phase 2) significantly reduced larval survival to pupation ($\chi^2_1 = 179$, $P < 0.001$, Fig. 2c). This reduction in survival was even stronger (64% reduction compared to non-exposed plants) than in the first experiment with one daily rain event. However, this was dependent on plant-mediated effects on survival ($\chi^2_1 = 5.13$, $P = 0.02$), i.e. effects of rainfall during growth of the plants before the introduction of the herbivores (phase 1). Direct exposure to rain significantly extended development time (by >1 day) ($F_{1,31} = 31.7$, $P < 0.001$) and resulted in higher adult body mass of *P. xylostella* (by approximately 26 %) ($F_{1,31} = 13.8$, $P < 0.001$, primarily when they had fed on host plants that had not been exposed to rain during phase 1 (interaction between phase 1 and phase 2 is significant for development time ($F_{1,31} = 8.65$, $P = 0.006$) and adult body mass, ($F_{1,31} = 13.36$, $P < 0.001$)). Overall, development time to eclosion was longer and moths biomass was lower in the second than in the first experiment (Fig. 3).

Irrespective of the rain exposure treatments, females were approximately twice as heavy in both experiments (single rain showers: $F_{1,37} = 161$, $P < 0.001$; three rain showers: $F_{1,31} = 113$, $P < 0.001$; Fig. 3). Females also developed faster, but this was only significant in the experiment with three short daily showers (single rain showers: $F_{1,37} = 1.00$, $P = 0.32$, three rain showers $F_{1,31} = 9.52$, $P = 0.004$, Fig. 3).

Response of *Pieris brassicae* - In the single rain-shower experiment, direct exposure to rain slightly reduced survival of *P. brassicae* caterpillars to adulthood (by approximately 6 %) ($\chi^2_1 = 5.28$, $P = 0.02$, Fig. 2c), and this was affected by indirect exposure to rain (interaction between phase 1 and 2; $\chi^2_1 = 4.08$, $P = 0.04$; Fig. 2b). Development time of *P. brassicae* was not affected by rainfall exposure during phase 1 ($F_{1,20} = 2.16$, $P = 0.16$), but was extended by approximately one day when caterpillars were directly exposed to rain during phase 2 ($F_{1,20} = 34.9$, $P < 0.001$; Fig. 4a). Adults were lighter (by 3-18 %) when they had developed on plants exposed to rain, both indirectly (phase 1: $F_{1,20} = 8.98$, $P = 0.007$) and directly (phase 2; $F_{1,20} = 10.7$, $P = 0.003$; Fig. 4b).

Three short rain showers, neither indirect nor direct exposure to rain affected survival of *P. brassicae* caterpillars to adulthood (phase 1: $\chi^2 = 0.33$, $df = 1$, $P = 0.57$; phase 2: $\chi^2 = 0.91$, $df = 1$, $P = 0.34$, Fig. 2d). On average survival to adulthood was 89%. Development time was extended (by approximately a day and a half) when the caterpillars were directly exposed to rain during phase 2 ($F_{1,20} = 59.3$, $P < 0.001$), but also indirectly when plant were exposed to rain before the caterpillars were introduced (phase 1) ($F_{1,20} = 7.32$, $P = 0.01$, Fig. 4c). In contrast with the results for the single daily rain shower experiment, butterflies of *P. brassicae* were heavier when the caterpillars had developed on plants directly exposed to rain ($F_{1,20} = 7.38$, $P = 0.01$, Fig. 4d). Overall females developed faster (both experiments; $F_{1,20} = 42.6$, $P < 0.001$; $F_{1,20} = 15.2$, $P < 0.001$) and were heavier (only significant in single-rain shower experiment: $F_{1,20} = 22.4$,

$P < 0.001$) than males (Fig. 4). Immature development was slower in the second than in the first experiment.

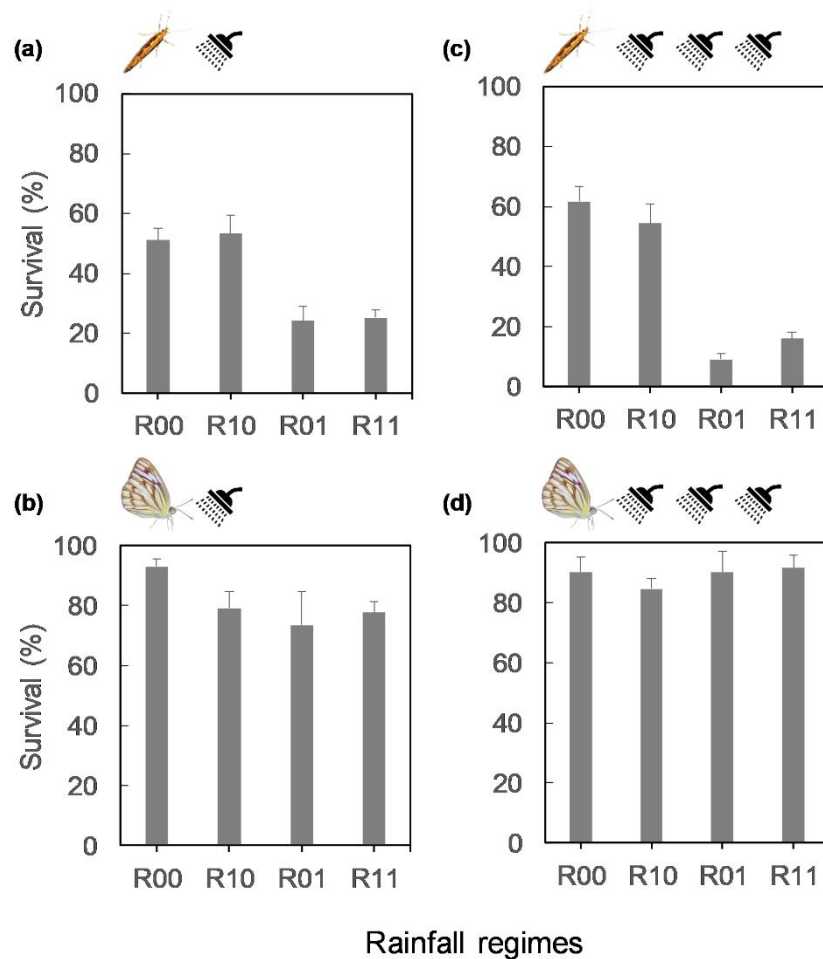


Fig. 2. Survival (means \pm SE) of *Plutella xylostella* from L2 to pupation (a, c) and of *Pieris brassicae* from L1 to adult eclosion (b, d) when developing on *Brassica nigra* plants exposed to different rainfall regimes: none (R00), only before caterpillars were introduced (R10), only after caterpillars were introduced (R01) or during both phases (R11). Insect herbivores were exposed to different frequencies of rainfall: once (20 min) per day (a, b), three times (5 min each) per day (c, d).

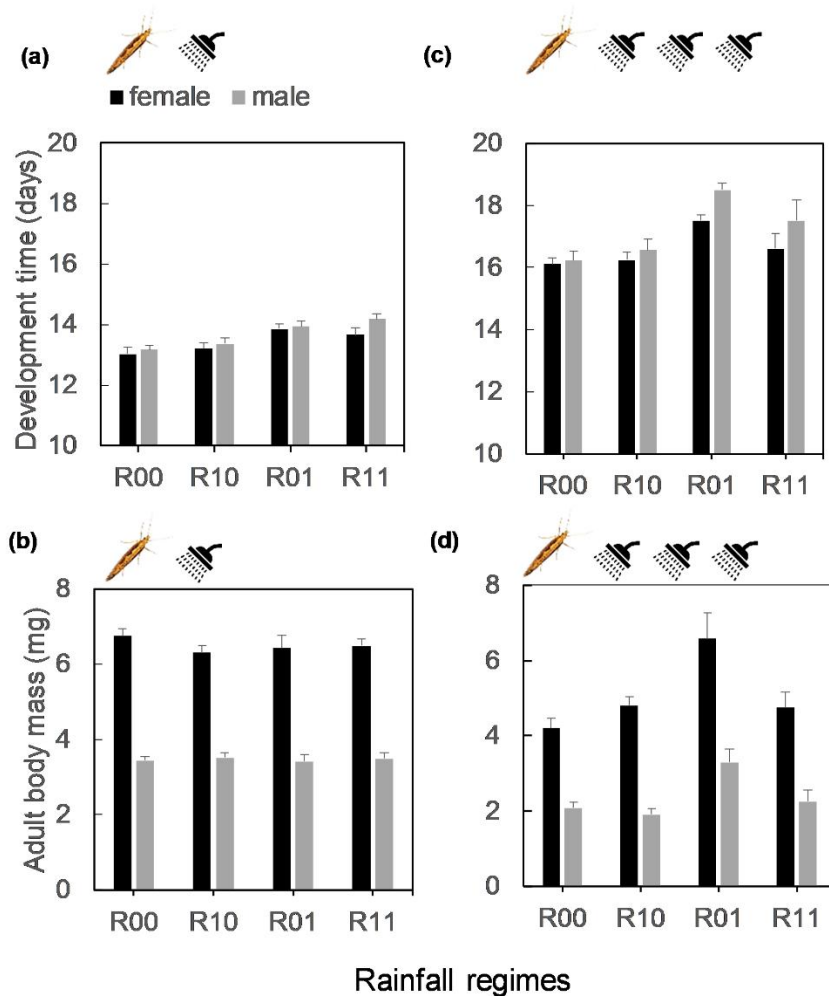


Fig. 3. Mean (\pm SE) development time from L2 to adult eclosion (a, c) and adult body mass (b, d) of *Plutella xylostella* on *Brassica nigra* plants exposed to different rainfall regimes: none (R00), only before caterpillars were introduced (R10), only after caterpillars were introduced (R01) or during both phases (R11). Note that axes of development time do not start at zero. Insect herbivores were exposed to different frequencies of rainfall: once (20 min) per day (a, b), or three times (5 min each) per day (c, d). Black bars: females; grey bars: males.

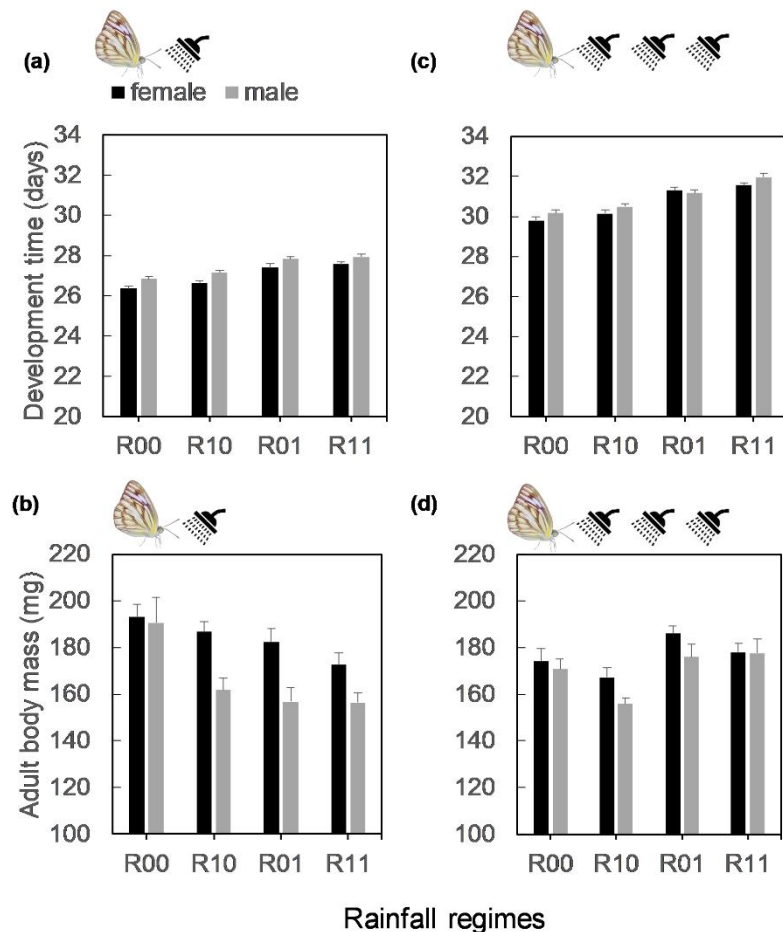


Fig. 4. Mean (\pm SE) development time from L1 to adult eclosion (a, c) and adult body mass (b, d) of *Pieris brassicae* on *Brassica nigra* plants exposed to different rainfall regimes: none (R00), only before caterpillars were introduced (R10), only after caterpillars were introduced (R01) or during both phases (R11). Note that axes do not start at zero. Insect herbivores were exposed to different frequencies of rainfall: once (20 min) per day (a, b) or three times (5 min each) per day (c, d). Black bars: females; grey bars: males.

Effects of rainfall on microclimate

Rainfall had a significant effect on the temperature of the leaf surface ($F_{3,20} = 10.5$, $p < 0.001$). In the treatments in which plants were exposed to rainfall during the insect feeding phase, when these measurements were performed (R01, R11, Fig. 5),

temperature immediately dropped by approximately 5°C and it took at least an hour to return to the original temperature (Fig. 5). No temperature drop occurred in the treatments in which plants were not exposed to rainfall, resulting in a significant interaction between rainfall regimes and timing: $F_{6, 253} = 36.2, p < 0.001$).

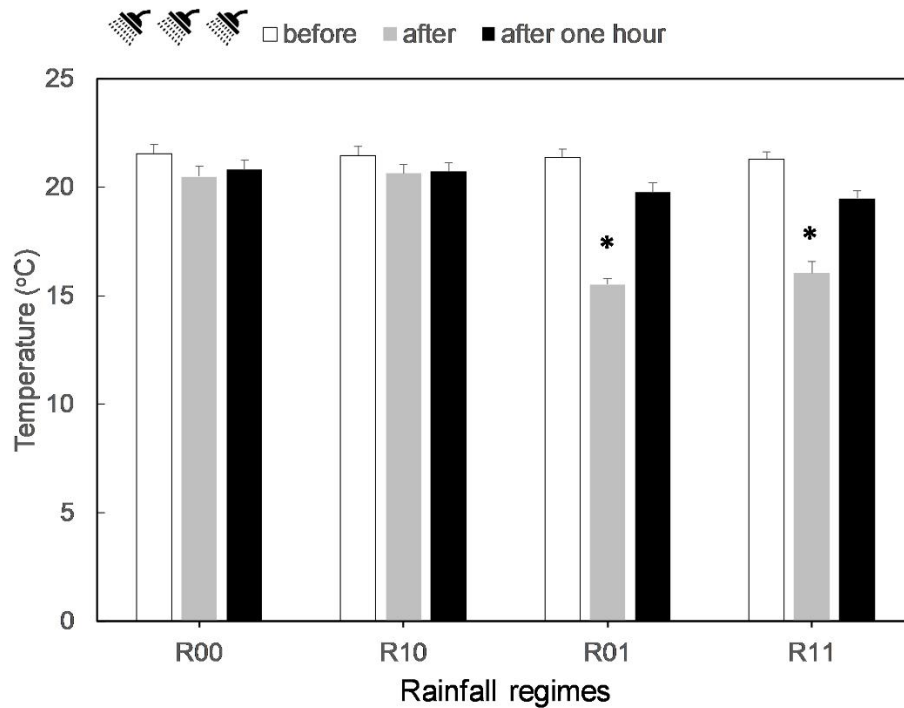


Fig. 5. Mean (\pm SE) temperature of leaf surface of the plants exposed to different rainfall regimes: none (R00), only before caterpillars were introduced (R10), only after caterpillars were introduced (R01) or during both phases (R11); and three different times: before, immediately after, and one hour after the second rain shower during phase 2. Note that only plants in treatments R01 and R11 were exposed to rainfall during the measurements, as these were the only treatments with rainfall during the insect feeding phase, when measurements were taken. The frequency of rainfall in R01 and R11 is three times (5 min each) per day. Asterisk above bars indicate that the treatment is significantly ($p < 0.05$) different from other treatments, based on Tukey-adjusted comparisons.

Effects of rainfall on chemical traits of Brassica nigra plants

Sinigrin - When rain was applied as a single long downpour daily, leaf concentrations of sinigrin, the dominant GS produced by *B. nigra*, were not affected by rainfall during phase 1 ($F_{1,20} = 3.02$, $P = 0.10$), but increased significantly during phase 2 when the caterpillars were feeding on the plants ($F_{1,20} = 18.5$, $P < 0.001$, Fig. 6a). Overall, sinigrin concentrations were higher in leaf tissues of plants exposed to herbivory (both species) than on plants without herbivores, and this effect was stronger when plants were also exposed to rain during the insect feeding phase (interaction between herbivory and presence or absence of rainfall during phase 2: $F_{2,40} = 6.82$, $P = 0.003$). The extent to which sinigrin was induced was similar for the two herbivore species (Herbivore effect, contrast *P. xylostella* vs. *P. brassicae*: $p > 0.05$). Compared to the first experiment, in the second experiment in which plants were exposed to three brief showers per day, sinigrin concentrations tended to be high irrespective of the rainfall regime and herbivore treatment (compare Fig. 6a and c). In the absence of rainfall during the insect feeding phase (R00, R10), sinigrin levels were not affected by *P. xylostella* and actually decreased in the presence of *P. brassicae* (Fig. 6c). By contrast, in the presence of rainfall during the insect feeding phase (R10, R11), sinigrin levels were slightly increased by caterpillar feeding, resulting in a significant interaction between herbivory and rainfall during the insect feeding phase ($F_{2,40} = 6.82$, $P = 0.003$, Fig. 6c).

Sugars - When rainfall occurred once a day, total sugar concentration in leaf tissues was affected by rainfall regime and herbivory (Fig 6c). Sugar concentrations were lower in plants exposed to rain during phase 1 than plants not exposed to rain during this phase ($F_{1,20} = 18.3$, $P < 0.001$, whereas rainfall during phase 2 did not significantly reduce sugar concentrations further ($F_{1,20} = 2.82$, $P = 0.11$). Feeding by the herbivores reduced foliar sugar and this effect was stronger for *P. brassicae* than for *P. xylostella*,

irrespective of rainfall regime (Tukey HSD, $P < 0.05$). In the second experiment with three showers per day, the effects of rainfall regime and herbivory were similar but less significant than in the first experiment (Fig. 6d).

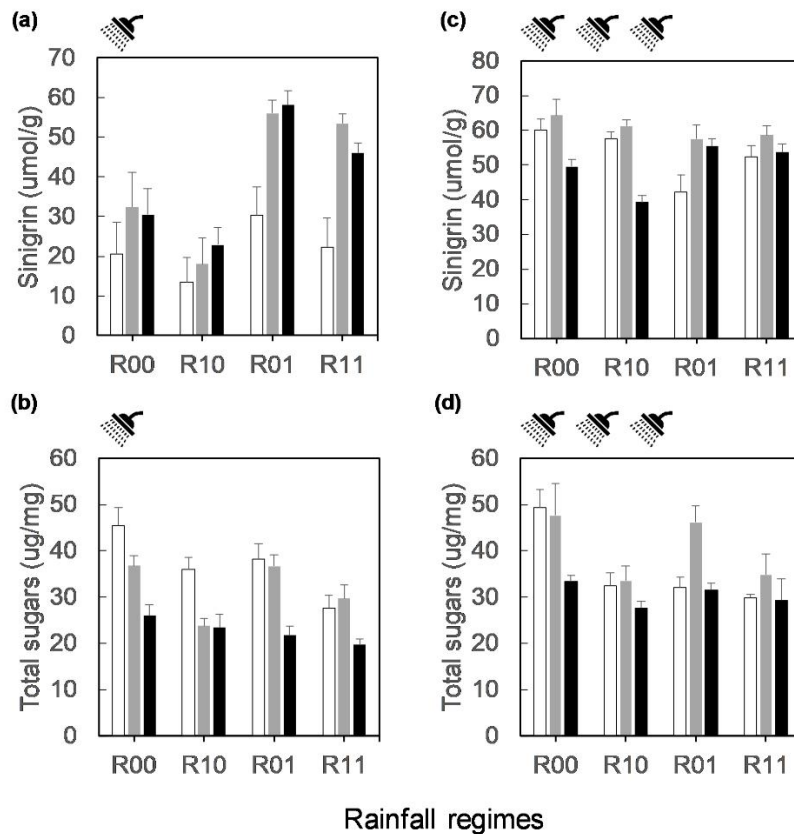


Fig. 6. Mean (\pm SE) concentrations of sinigrin (a, c) and total sugars (b, d) of *Brassica nigra* plants exposed to different herbivory treatments: none (white bars), *Plutella xylostella* (grey bars), *Pieris brassicae* (black bars); and rainfall regimes: none (R00), only before caterpillars were introduced (R10), only after caterpillars were introduced (R01) or during both phases (R11). Insect herbivores were exposed to different frequencies of rainfall: once (20 min) per day (a, b) or three times (5 min each) per day (c, d).

Discussion

Simulated rainfall, which mimicked downpours occurring during thunderstorms, had a profound negative effect on the survival of *P. xylostella*, and this effect was stronger when the frequency of rain events increased from one to three daily. By contrast, whereas the survival of *P. brassicae* was marginally affected by a single extended downpour daily, it was not affected when the frequency of downpours was increased to three per day but the duration of each downpour reduced. Direct exposure of insects on plants to rainfall also increased development time of the two herbivores, whereas the effects of rainfall on adult body mass were more idiosyncratic. Rainfall also affected leaf chemistry. During the earlier stages of plant ontogeny, before herbivores were introduced, rainfall significantly reduced the concentration of foliar sugars, whereas sinigrin concentrations increased more in response to herbivory when plants were also exposed to rain. However, in spite of these significant changes in leaf chemistry, indirect, plant-mediated effects of rainfall on the performance, i.e. development time and adult biomass, of the two specialist insects were generally small.

Species-specific differences in survival of *P. xylostella* and *P. brassicae* when exposed to rainfall may be due to the variation in the size and feeding behavior of the two herbivores. Larvae of *P. xylostella* are solitary and small, and, consequently, more likely to be susceptible to physical disturbance. Feeding in *P. xylostella* is characterized by bouts of tissue intake interspersed with periods of resting away from the feeding site. When disturbed, such as by the presence of natural enemies or wind, they often drop from the food plant along a silk thread, which is later used by larvae to climb back onto the plant. However, heavy rainfall may prevent this from occurring (Kobori & Amano, 2003), because the silk thread may be severed or the larvae drown in water pools and/or mud that accumulate beneath the plant. By contrast, larvae of *P. brassicae* feed

gregariously during the first three instars and are much larger than *P. xylostella* larvae of the same instar. Moreover, *P. brassicae* larvae tend to move much less between feeding bouts and produce silk beds with which they are tightly attached to the leaf. Therefore, *P. brassicae* larvae are in general less affected by physical disturbance. In a previous study we reported that caterpillars of *P. xylostella* are also more strongly disturbed by wind than the caterpillars of *P. brassicae* (Chen et al., 2018). The higher mortality of *P. xylostella* in response to rainfall, especially when occurring at higher frequencies (80% reduction), is even stronger than mortality caused by wind exposure (10%) (this study, Chen et al. 2018).

The development time of both herbivores was extended by both direct and indirect (plant-mediated) effects of exposure to rainfall whereas the effects on biomass were not very consistent as they differed depending on the frequency of rainfall as well as between the two herbivore species. The direct effect of rainfall on development time was possibly caused by the fact that during downpours caterpillars do not feed. Instead, given the risks of being physically displaced, they ‘hunker down’ on the plants as best as possible and remain inactive until the shower passes. Even after the rain shower ended, feeding was not immediately resumed. We also found that the temperature near the leaf surface dropped dramatically in response to the heavy showers, and it took more than an hour before the temperature returned to its original level. Metabolism and growth rate in ectotherms such as insects are strongly determined by ambient temperature. In addition to disturbance, microclimatic cooling caused by rain may explain the extended immature development times of the two herbivores. Previous studies have shown that in addition to the biotic environment, the microclimatic environment plays an important role in determining the population dynamics of insect herbivores (Dobkin et al., 1987; Walsh, 2017). Moreover, Walsh (2017) found that after a serious drought event, factors

influencing the microclimate better explained the population decline of an endangered butterfly species, the Karner blue butterfly (*Lycaeides melissa samuelis*) than the biotic factors. These results demonstrate the relative importance of abiotic factors on the survival of small ectothermic organisms.

In both species, extended larval development time coincided with adults being heavier, but only in the experiment in which the caterpillars were exposed to three short rain events. This effect was more prominent for *P. brassicae* than for *P. xylostella*. In contrast, although development time of *P. brassicae* was also extended when exposed to a single rain event per day, adults in this treatment group were lighter. In a previous study (Chen et al. 2018), *P. brassicae* exposed to wind developed slower but attained more biomass. Predation of caterpillars by birds (great tits) was considerably reduced when reared on plants exposed to wind. We speculated that this phenotypic shift to a prolonged developmental program might be an adaptive response to reduced predation risk that is perceived by the feeding caterpillars that benefit significantly from larger size in terms of fecundity (Chen et al. 2018). Whether a similar scenario could hold for increased exposure to rain remains to be investigated. Heavy downpours are not only likely to have direct effects on insect herbivores, but on their natural enemies as well. Studies with birds (Radford, McCleery, Woodburn, & Morecroft, 2001; Robbins, 1981) and parasitoids (Fink & Volkl, 1995) showed a significant decrease in foraging activity of these natural enemies during rainy periods. If rainfall is also perceived by herbivores as reducing predation risk, as it does with wind, then this might explain trade-offs between traits such as development time and body size. However, the result of this study suggests that abiotic factors such as rainfall need to be prolonged or more frequently applied in order to induce phenotypic shifts, as this phenomenon was only observed in the experiment in which rainfall frequency was increased to three times per day.

Indirect effects of rainfall may affect food plant quality through both chemical and morphological changes. We measured concentrations of the GS sinigrin, a secondary plant chemical that is produced by all brassicaceous species (Fahey et al., 2001) and that has been shown to be important in providing resistance against a range of attackers including generalist herbivores (Gols et al., 2008; Jeschke et al., 2017). Concentrations of primary and secondary metabolites are dynamic and change in response to plant development, biotic conditions (e.g. herbivory), and abiotic conditions (Akula & Ravishankar, 2011; Barton & Koricheva, 2010; DeLucia, Nability, Zavala, & Berenbaum, 2012; Karban & Baldwin, 1997). For example, rainfall/drought-mediated changes in plant chemistry can influence insect performance by either an increase or decrease in plant defensive compounds (e.g. terpenoids, phenolics and GS) (Gutbrodt, Mody, & Dorn, 2011; Mattson & Haack, 1987; Shure et al., 1998). In plants exposed to both rainfall (single daily events) and herbivory, the induction of foliar levels of sinigrin was strongest (2 fold increase), whereas the induction of sinigrin by either herbivory or rainfall alone was not statistically significant. This result suggests that, in *B. nigra*, rainfall dramatically increased the sensitivity of GS metabolism to herbivory. However, specialist herbivores such as *P. brassicae* and *P. xylostella*, which both have efficient mechanisms to prevent exposure to the toxic GS breakdown products (Winde & Wittstock, 2011), are generally less affected by changes in GS, and this could be the reason that, despite these considerable changes in sinigrin concentrations in response to rainfall, indirect (plant-mediated) effects of rainfall on these insects were small. We therefore speculate that generalist insect herbivores, that are more strongly affected by changes in GS (Gols et al., 2008; Jeschke et al. 2017) may be more sensitive to such indirect, plant-mediated effects of rainfall. In the second experiment in which the frequency (but not duration) of downpours was increased, sinigrin concentrations were

high in all plants including those that were not exposed to rain and or herbivory. Sinigrin concentrations reached the same levels as those found in plants with the highest concentrations ($>50 \mu\text{mol/g DW}$) in the first experiment. These concentrations are high in comparison with results previously reported for *B. nigra* (Gols, van Dam, Raaijmakers, Dicke, & Harvey, 2009; van Dam, Witjes, & Svatos, 2004). It is not clear why the concentrations were higher in the second than in the first experiment, but it seemed that the conditions were less favourable for both the plant and the herbivores (on control plants, caterpillars developed faster and were heavier in the first than in the second experiment). The second experiment was conducted in a greenhouse in winter, whereas the first experiment was conducted in a greenhouse in autumn. Even in climate-controlled greenhouses, conditions, such as the quality and intensity of the artificial light for plant and insect growth may vary seasonally (Gols et al., 2007).

Sugar concentrations declined significantly in response to rain, but only during early development of plants before the insects were feeding on them. Sugar concentrations were also lower in leaf tissues that had been exposed to herbivory, especially feeding by *P. brassicae* reduced foliar sugar concentrations. Previous studies have shown that plant nutrients can be leached by rainfall (Schreiber, 1999; Tukey, 1970). Moreover, damaged leaves of plants are much more susceptible to leaching than undamaged leaves (Risley & Crossley, 1988; Tukey & Morgan, 1963). Feeding damage by *P. brassicae* caterpillars is more severe than feeding by *P. xylostella* caterpillars and this could explain the lower sugar concentration in leaf tissues damaged by the former herbivore species. Sugars such as sucrose and glucose are important feeding stimulants (Schoonhoven, van Loon, & Dicke, 2005). To what extent the reduced sugar concentrations and leaching of nutrients in general explain extended development and

changes in other fitness-correlates of insect herbivores feeding on rain-exposed plants merits further investigation.

Conclusions

Our results reveal that rain-exposure, via simulated downpours characteristic of thunderstorms and its frequency has profoundly negative effects on the survival of a micro-lepidopteran herbivore and affects secondary fitness correlates, mainly by extending larval development, of two insect herbivore species. The direct effects (disturbance, microclimatic cooling) of rainfall were more pronounced than the indirect, plant-mediated (phytochemical) effects. More extreme weather events, including short, intense downpours, as predicted by the IPCC (2014) under AGW, are likely to affect higher trophic levels as well. Asymmetric effects of rainfall on population dynamics of species within and across trophic levels, in turn, have consequences for the structure of the whole community. Climate change has far reaching effects on ecological processes ranging from effects on individual species to entire ecosystems (Harrington et al., 1999; Knapp et al., 2008; McLaughlin, Hellmann, Boggs, & Ehrlich, 2002; Walther et al., 2002). Our study emphasizes the importance of rainfall in understanding the effects of climate change on ecological responses in trophic chains and broader ecological communities. Future experiments should investigate combined effects of climate-related factors on plant-insect as well as multitrophic interactions.

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