Plant-mediated species networks: the modulating role of herbivore density

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Abstract. 1. When herbivores of distinct feeding guilds, such as phloem feeders and leaf chewers, interact, the outcome of these interactions often shows facilitation. However, whether this facilitation turns into competition at stronger herbivory pressure remains unknown.

2. Using an integrative approach that links ecological processes (behavioural choices of insects) with physiological plant mechanisms (nutrient and phytohormone levels) for the wild crucifer Brassica nigra (L.) Koch., this study evaluates preferences of leaf chewers for plants previously infested with several densities of the specialist aphid Brevicoryne brassicae L. (Hemiptera, Aphididae). As leaf chewers, four species of caterpillars (Lepidoptera) were selected that differ in their degree of specialisation in crucifers.

3. These results show that, whereas at low and medium aphid densities caterpillars displayed a preference for aphid-infested plants or no preference, at high aphid infestation density, all four species of caterpillar preferred uninfested plants, with a significant difference for Pieris rapae and Mamestra brassicae.

4. In contrast to our expectation, the consistent preference for uninfested plants at a high aphid density could not be associated with a decrease in plant nutrition. However, while jasmonate concentrations [i.e. 12-oxo-phytodienoic acid and jasmonic acid (JA)] at medium aphid-density infestation decreased compared with low levels of infestation, at high infestation level, the jasmonates JA as well as JA conjugated with the amino acid isoleucine were present at higher levels compared with low-infestation treatments.

5. This work provides evidence that positive interactions observed in herbivore communities can be transient, leading to negative interactions mediated by changes in plant defences rather than in plant nutrition.

Key words. Cross-talk, facilitation, multiple attack, phytohormones, plant defences, plant–insect interactions.

Introduction

Understanding the forces that drive the assembly and functioning of terrestrial communities is a primary goal in ecology, and the discovery of plant chemicals mediating interactions between plants and insects has been a major contribution to this quest. A well-established concept in this field is that sophisticated direct and indirect plant defences are induced in response to herbivory, to help plants to cope with insect attack. In response to herbivory, plants produce phytotoxins that impair insect growth and development (direct defences, i.e. targeting the herbivores) (Karban & Baldwin, 1997; Schoonhoven et al., 2005). At the same time, plants emit volatile blends that are exploited as host-location cues by insect parasitoids that develop on/inside the body of the herbivores and ultimately kill them (indirect defences, i.e. targeting the attraction of the herbivore’s attackers) (Turlings et al., 1990; Vet & Dicke, 1992; Dicke & Baldwin, 2010). More recent insights into the mechanisms of biosynthesis and gene expression underlying plant defences have provided a basis to mechanistically understand patterns of ecological interactions...
further, opening up exciting new directions in this field (Kessler & Baldwin, 2002; Howe & Jander, 2008; Erb et al., 2009; Dicke & Baldwin, 2010; van Dam & Heil, 2011; Rasmann et al., 2012; Pineda et al., 2013; Soler et al., 2013; Casteel et al., 2015; Onkokesung et al., 2016). Besides plant defences, plant primary chemistry has also long been established to mediate interactions between plants and insects. Primary plant compounds such as nitrogen are of crucial importance for insect growth and development, and it is well established that the nutritional status of plants can also change in response to herbivory and thus influence the performance of the current and subsequent attackers (Mattson, 1980; Schwachtje & Baldwin, 2008). Consequently, plant defences and primary nutrients effectively link many species together into complex networks of organisms in terrestrial communities (Stam et al., 2014).

During the 1980s and 1990s, a large body of studies investigated plant–insect interactions, based on systems consisting of a plant and a single insect species per trophic level (i.e. plant–herbivore–parasitoid trophic food chains). These studies have provided a comprehensive understanding of the processes that can be triggered in plants in response to insect attack, and how such processes can feed back on the attacking species. Yet, given the complexity that characterises insect communities, this relatively simplified scenario is uncommon in nature. More recent evidence shows that plant–insect interactions can dramatically change when only a second herbivore species comes to the scene, and the induction of plant defences can significantly change under multiple attack (Rodriguez-Saona & Thaler, 2005; Kaplan & Denno, 2007; Dicke et al., 2009; Soler et al., 2013; Onkokesung et al., 2016). For example, the induction of cardiacinols, defensive compounds in milkweed, was attenuated after attack by monarch caterpillars when aphids had previously colonised the plants, and this was associated with an improved caterpillar performance in aphid-infested plants compared with control plants (Ali & Agrawal, 2014). The activated immune response of plants to insect herbivores is under the control of a limited number of interconnected phytohormone-regulated signalling pathways, which provide plants with the ability to fine-tune defence responses geared towards the attacker (Pieterse et al., 2012). The two major phytohormones underlying plant immune responses are jasmonic acid (JA) and salicylic acid (SA) (Walling, 2000; de Vos et al., 2005; Pieterse et al., 2012). In general, chewing insects such as caterpillars trigger the JA pathway, while phloem-feeding insects such as aphids induce the SA pathway. These pathways often display negative cross-talk: an increase in the level of one of the phytohormones interferes with the induced defences under the control of the other phytohormone (Leon-Reyes et al., 2010; Pieterse et al., 2012; Thaler et al., 2012). Therefore, when JA- and SA-inducing attackers feed on the same plant, the defences triggered against the first attacker may reduce the defence capacities against the subsequent attacker with distinct feeding behaviour (Stout et al., 1998; Zhang et al., 2009; Rodriguez-Saona et al., 2010; Thaler et al., 2012; Soler et al., 2013). Yet the number of case studies about the role of cross-talk of signalling pathways in plant defences under multiple attack is limited and only a few are available for non-model systems (Zhang et al., 2009; Ali & Agrawal, 2014; Li et al., 2014). This clearly exemplifies the need to expand from single species interactions into more realistic frameworks that incorporate multiple players.

Studies on plant-mediated interactions between multiple attackers are typically conducted under single infestation densities and sequences of attack, which provide a rather static view of these interactions. While these studies have generated excellent insights into the ecology and mechanisms underlying more complex linkages between plants and the communities of organisms associated with them, density of infestation is largely forgotten in the contemporary studies that address more complex ‘plant-mediated species networks’. Our study explores this aspect and provides evidence of a potentially regulatory role of density of infestation in modulating interactions between plants and insects with contrasting feeding behaviours/guilds. Using the wild cruciferous plant *Brassica nigra* and an assemblage of commonly occurring insect herbivores as a model system, and an integrative approach that links ecological processes with physiological mechanisms underlying plant responses, we show how the feeding preferences of commonly occurring crucifer leaf feeders are modulated by the presence and density of phloem feeders. The crucifer specialists *Pieris rapae* (L.) and *Pieris brassicae* (L.) (Lepidoptera, Pieridae), the less selective crucifer specialist *Plutella xylostella* (L.) (Lepidoptera, Plutellidae), and the generalist *Mamestra brassicae* L. (Lepidoptera, Noctuidae) represent the leaf chews community, and the aphid *Brevicoryne brassicae* represents the phloem feeders. We hypothesised that the leaf chewers prefer to feed on plants previously infested by aphids where jasmonate-related defences are compromised (Soler et al., 2012a), but that this preference will change at higher aphid densities, when the concentration of essential primary nutrients is depleted. Additionally, we expect that the more specialised the herbivore, the more adapted it will be to changes in primary and secondary chemistry of its host plants (Cornell & Hawkins, 2003), and therefore the less it will be affected by previous herbivory.

### Materials and methods

#### Plants and insects

The experimental system of this study consists of the wild plant *Brassica nigra* (Brassicaceae) and an assemblage of insect herbivores that are commonly feeding on brassicas: *B. brassicae*, *P. brassicae*, *P. rapae*, *P. xylostella* and *M. brassicae*. The plants were grown in a greenhouse over a period of 4–5 weeks, at 21 ± 3°C, 70 ± 10% RH and LD 16:8 h. Natural daylight was supplemented by metal-halide lamps (225 μmol s⁻¹ m⁻² photo-synthetically active radiation). Insect cultures were maintained at the Laboratory of Entomology of Wageningen University, the Netherlands. All insect species were reared on *B. oleracea* L. cultivar *gemmifera* cv. Cyrus under the same conditions as described earlier.

#### Experimental setup

We studied feeding and oviposition preferences of the leaf chews (Lepidoptera), both the larval and adult stages, for uninfested plants and plants previously infested with aphids at
different densities (insect behaviour), and plant primary chemistry and defensive signalling to aphid attack (plant responses). The aim was to test the hypothesis that aphid density determines the feeding preferences of secondarily attacking leaf chewers, and that the interplay between phytohormones and nutrition modulates these preferences. Six days prior to the preference assays, plants were infested with 15 newly emerged first-instar nymphs (low-density treatment), three to four adults (medium density), six to eight adults (high density), or were kept uninfested to serve as controls. The aphids were carefully placed on the youngest fully developed leaf using a soft brush (number 0 for newly emerged nymphs and number 2 for adults), whose petiole was previously surrounded by a cotton-wool strip as a physical barrier to discourage aphid migration to other leaves. Aphids established and fed on the infested leaf during the course of the experiment. At the time at which the preference assays were performed, i.e. 6 days after infestation, the initial infestations effectively resulted in an average (± SD) of 14 ± 1 fourth-instar nymphs, 71 ± 20 mixed instars of aphids and 129 ± 23 mixed instars of aphids, referred to as ‘low’, ‘medium’ and ‘high’ density, respectively. The low density is the minimum density for which there is evidence that it can result in facilitation of the leaf chews P. brassicae (Soler et al., 2012a), while the medium and high densities correspond to numbers of aphids commonly observed in the field at different stages of infestation.

Insect behaviour

The feeding and oviposition preferences of the lepidopterans were studied individually, species by species, using two-choice behavioural bioassays as described in Soler et al. (2012b). Caterpillar preferences were assessed in so-called ‘behavioural rooms’, at 21 ± 2 °C and 60 ± 10% RH and without windows to avoid sunlight influence, and with artificial light provided from 1 m above the setup by four high-frequency fluorescent tubes (TL-D 58 W Philips, Eindhoven, the Netherlands) at an intensity of 60 ± 5 μmol photons m⁻² s⁻¹. First, to study caterpillar preferences, pairs of aphid-infested and uninfested plants were distributed pairwise on experimental tables. The first systemic leaf above the aphid-infested leaf and the corresponding physiologically similar leaf of the uninfested plants were connected by 4 cm² cardboard bridges that were placed horizontally. Larvae were individually released in the middle of the bridges and observed continuously until a choice was made. Choice and time between caterpillar release and climbing onto one of the two offered leaves were recorded. From preliminary observations and previous studies (Soler et al., 2012b) we know that the caterpillars hardly move away from the selected food source, so climbing onto a leaf – initial selection – can be used as an accurate indicator of feeding preference. Newly hatched larvae were selected for the behavioural assays, to avoid previous contact with plant tissue and thus potential food induction. Fifteen to 19 plant pairs and a minimum of 240 caterpillars were tested per species (with 10–25 caterpillars tested per plant pair). To avoid any possible influence among the choices of conspecifics, larvae were not allowed to feed on the bridged plants but were removed immediately after climbing onto the plant. To avoid any potential influence of chemical cues left on the bridges, new bridges were used for all larvae. All larvae were responsive and moved towards one of the two offered plant treatments within a period of half an hour.

Subsequently, we assessed oviposition preferences of the butterflies or moths for uninfested plants versus plants infested at the medium aphid density. Oviposition preferences of butterflies or moths were studied in a greenhouse under the same experimental conditions specified earlier for plant growth. In flight tents (75 × 75 × 115 cm), 2-day-old naïve female butterflies or moths were offered pairs of uninfested plants and plants infested with aphids. Plants were treated as described earlier. Twenty-four hours after releasing the butterflies or moths, plants were inspected for the presence of eggs/egg clutches, and the number of eggs laid on each plant treatment was recorded. Mamestra brassicae left most of the eggs on the walls of the experimental cages, and those eggs were also recorded. The oviposition preference of each lepidopteran species was studied separately in a two-choice setup, using two females per cage, each cage with one control and one aphid-infested plant (n = 6 cages) per lepidopteran species.

Plant responses

Because it requires destructive sampling, a separate experiment was designed to study local and systemic plant responses to aphids and aphid density of attack. Six days after aphid infestation, the infested leaves (so-called ‘local leaves’), the three leaves above (so-called ‘systemic leaves’) and comparable leaves of control uninfested plants were sampled. This time point of 6 days coincides with the time at which the behavioural experiments were carried out, and this therefore allowed us to elucidate the quality, defence and nutrition of the plant when caterpillars and butterflies/moths selected food/oviposition sources. Leaves were quickly placed into aluminum paper envelopes and immediately frozen in liquid nitrogen and stored at −80 °C for subsequent analysis. Eleven plants per treatment were prepared; plants were grown as described earlier and the densities and periods of aphid infestation were the same. The concentration of the phytohormones SA and JA, and the jasmonate derivatives 12-oxo-phytodienoic acid (OPDA) and JA conjugated with the amino acid isoleucine (JA-Ile) were measured. Phytohormone analysis was carried out following the method of Camañas et al. (2012). The concentration of the macronutrients nitrogen (N) and carbon (C), and the micronutrients phosphorus (P), potassium (K), magnesium (Mg) and sodium (Na) were also assessed from the same samples, following the method of Temminghoff and Houba (2004).

Statistical analysis

The preference of caterpillars for plants with aphids and the effect of aphid density were analysed by modelling the binomial proportions with logistic regression, with the logit link function. Subsequently, within each density, and species by
species, binomial tests were used to determine whether preferences for plants with and without aphids differed significantly from a 50:50 distribution (two-tailed, \( \alpha = 0.05 \)). Oviposition preferences were analysed separately for each species with the Wilcoxon signed-rank test. The time spent by the caterpillars to select between food sources, and concentrations of primary plant compounds, phytohormones and derivatives were compared using a general linear model (ANOVA). Normality and homogeneity of variance were checked by inspection of the residuals after model fitting. Least significant differences were used as post hoc tests (\( P < 0.05 \)). All data were analysed with GENSTAT (version 14; VSN International, Hemel Hempstead, UK).

Results

Caterpillar feeding preferences

The feeding preferences of the caterpillars for *Brassica nigra* plants with and without aphids differed among aphid densities and the caterpillar species identity; caterpillars displayed a preference for plants infested with aphids or for control uninfested plants in a manner dependent on caterpillar species and aphid density (Fig. 1; interaction term aphid density \( \times \) species identity: \( F_{6,3} = 3.1, P = 0.005 \)). At the low aphid density (Fig. 1a), the preference for aphid-infested plants differed between caterpillar species (\( F_{1,8} = 2.4, P = 0.05 \)). While 69% of *P. brassicae* larvae significantly preferred aphid-infested plants over uninfested plants (\( P = 0.002 \)), *P. rapae, P. xylostella* and *M. brassicae* did not discriminate significantly between plants with and without aphids (50%, \( P = 0.50 \); 55%, \( P = 0.18 \); and 48%, \( P = 0.38 \), respectively). At the medium aphid density (Fig. 1b), all species significantly discriminated between plants with and without aphids, but the degree of preference for aphid-infested plants differed between species (\( F_{1,4} = 2.4, P = 0.05 \)). While 69% of *P. brassicae* larvae significantly preferred aphid-infested plants over uninfested plants (\( P = 0.002 \)), *P. rapae, P. xylostella* and *M. brassicae* did not discriminate significantly between plants with and without aphids (50%, \( P = 0.50 \); 55%, \( P = 0.18 \); and 48%, \( P = 0.38 \), respectively). At the medium aphid density (Fig. 1b), all species significantly discriminated between plants with and without aphids, but the degree of preference for aphid-infested plants differed between species (\( F_{1,4} = 2.4, P = 0.05 \)). A significant preference for aphid-infested plants over uninfested plants was recorded for *P. brassicae* (60%, \( P = 0.02 \)) and *P. rapae* (60%, \( P = 0.01 \)), while only 34% of *P. xylostella* larvae (\( P < 0.001 \)) and 38% of *M. brassicae* larvae (\( P = 0.02 \)) chose the infested plants. At high aphid density (Fig. 1c), there was an overall reduced preference for plants with...
aphids compared with uninfested plants, and the strength of this reduced preference did not differ significantly between species ($F_{1,7} = 0.9$, $P = 0.40$). But when we tested the preferences for plants with and without aphids for each species individually, the preference was statistically significant for $M$. brassicae and $P$. rapae, with 35% and 34% of larvae, respectively, choosing infested plants (both $P = 0.002$), but not for $P$. brassicae and $P$. xylostella (both 43%, $P = 0.09$).

Caterpillar food-selection behaviour

The time spent by the caterpillars choosing between plants with and without aphids and the general searching behaviour differed between species (Fig. 2). $P$. brassicae larvae spent significantly more time before making a choice (10.2 ± 0.3 min) than the other three species and it was the only species whose decision-making speed was influenced by the density of aphid infestation. Twice as much time was spent by the caterpillars discriminating between plants with and without aphids when plants were infested with a low aphid density compared with medium and high densities. This species showed what we described as ‘hesitation behaviour’ (Soler et al., 2012b); rather than moving directionally to one of the plants offered, as did the other three species, $P$. brassicae larvae crossed the bridge back and forth moving within close proximity of the leaves several times before climbing onto one of them.

$P$. xylostella and $M$. brassicae spent 1–2 min (1.1 ± 0.3 and 1.9 ± 0.3 min, respectively) selecting a plant, regardless of the number of aphids feeding on the infested plant. Almost immediately after being placed on the bridges, caterpillars of these two species moved directionally towards one of the two offered plant treatments. $P$. rapae spent twice as much time selecting a plant (4.2 ± 0.3 min) than did $P$. xylostella and $M$. brassicae caterpillars, also regardless of the density of aphids. Once placed on the bridges, these caterpillars first stayed still and only subsequently moved directionally towards one of the food sources offered.

Fig. 2. Caterpillar plant selection time. Time spent (average ± SE) by the larvae choosing between plants without aphids and plants with aphids infested at low (white), medium (grey) and high (black) densities. Letters above horizontal bars compare selection times between caterpillar species and capital letters below bars compare selection times within caterpillar species among aphid densities; different letters indicate significant differences ($P \leq 0.05$).

Fig. 3. Lepidopteran oviposition preference for Brassica nigra plants with and without aphids. Average proportion (± SE) of eggs/egg clutches laid on plants without aphids (white bars) and plants with aphids at medium infestation density (grey bars), ns, non-significant differences ($P > 0.05$, Wilcoxon test).

Adult oviposition preferences

In contrast to the juvenile stages, $P$. brassicae, $P$. rapae and $P$. xylostella adults did not discriminate between plants colonised by aphids and uninfested plants (Fig. 3; $Z = 6.5$, $P = 0.938$; $Z = 1$, $P = 0.125$; $Z = 9.5$, $P = 1$), selecting plants for oviposition irrespective of the presence of aphids. $M$. brassicae moths laid the majority of eggs on the walls of the cages (58%) rather than on the plants, and were therefore not further considered for oviposition preference analysis.

Plant responses

The concentration of OPDA differed between aphid infestation densities ($F_{3,70} = 4.3$, $P = 0.008$) and leaf type, i.e. local (infested leaves) or systemic (uninfested leaves) ($F_{3,70} = 26.1$, $P < 0.001$) (Fig. 4a). The concentration of OPDA was lowest in plants infested with aphids at medium density, in both systemic and local leaves, increasing again at high aphid infestation in systemic leaves. In the local leaves, OPDA levels were twice as high as in the systemic (uninfested) leaves (125 ± 7 and 63 ± 8 ng g⁻¹ plant dry weight, respectively, averaged across graphs). A similar pattern is observed for JA (Fig. 4b), but the interaction term aphid density × leaf type was significant in this case ($F_{3,70} = 5.2$, $P = 0.003$) due to the lack of aphid effects on the levels of JA in systemic leaves. In infested leaves, the concentration of JA was highest at the high aphid density. JA-Ile was detected exclusively in infested leaves, with levels approximately 10 times higher at the high aphid density than in the other treatments (Fig. 4c; $F_{3,8} = 16.4$, $P < 0.001$). The concentration of SA was similar in plants with and without aphids ($F_{3,8} = 0.23$, $P = 0.87$), for infested and uninfested leaves ($F_{3,8} = 0.05$, $P = 0.83$) (Fig. 4d). The percentages of the macronutrients (C, N) and micronutrients (P, K, and Mg) were not significantly influenced by aphid absence/presence and intensity of attack (Table 1).

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Fig. 4. Phytohormone concentration. Concentrations (mean ± SE; ng g⁻¹ plant dry weight) of 12-oxo-phytodienoic acid (OPDA) (a), jasmonic acid (JA) (b), JA conjugated with the amino acid isoleucine (JA-Ile) (c) and salicylic acid (SA) (d) measured at the moment the behavioural tests were carried out (6 days after the infestation with the aphids) on infested leaves (‘local leaves’; white circles) and uninfested leaves (‘systemic leaves’; grey circles). JA-Ile was not detected in systemic leaves. Significant differences between treatments (least significant difference test, \( P \leq 0.05 \)) within leaf type are indicated with different letters; ns, non-significant differences.

Table 1. Plant nutritional status.

| Aphid infestation | Control | Low   | Medium | High  | \( F_{31} \) | \( P \) |
|-------------------|---------|-------|--------|-------|-------------|-------|
| Macronutrients    |         |       |        |       |             |       |
| N                 | 4.7 ± 0.2 | 4.7 ± 0.2 | 4.4 ± 0.2 | 4.2 ± 0.2 | 1.16       | 0.32  |
| C                 | 40.5 ± 0.3 | 40.7 ± 0.3 | 40.5 ± 0.3 | 40.6 ± 0.3 | 0.09       | 0.96  |
| Micronutrients    |         |       |        |       |             |       |
| P                 | 0.6 ± 0.04 | 0.6 ± 0.05 | 0.6 ± 0.04 | 0.5 ± 0.04 | 0.69       | 0.56  |
| K                 | 2.8 ± 0.2 | 2.4 ± 0.2 | 2.6 ± 0.2 | 2.8 ± 0.2 | 0.67       | 0.57  |
| Mg                | 0.5 ± 0.02 | 0.5 ± 0.02 | 0.5 ± 0.02 | 0.5 ± 0.02 | 0.91       | 0.44  |

Concentration (mean percentage ± SE) of nitrogen (N), carbon (C), phosphorous (P), potassium (K), magnesium (Mg) and sodium (Na) in *Brassica nigra* plants (samples from uninfested leaves). Plants were either infested with aphids at low, medium and high densities, or uninfested (controls).

Discussion

Our study provides empirical evidence for the importance of herbivore density underlying plant-mediated interactions. The intensity of damage (density of attack) that initial attackers exert on a plant can have a major effect on subsequent attackers with contrasting feeding behaviour (neutral, positive, negative). These experiments also reveal that preference can be largely modulated by the density of the attackers. Whereas at low and medium aphid densities caterpillars showed behavioural responses ranging from attraction to neutrality with regard to aphid-infested plants, at high aphid density, all four caterpillar species preferred control plants over aphid-infested ones, with a significant difference for *P. rapae* and *M. brassicae*. Several studies have shown that facilitation is the most common outcome between phloem feeders and leaf chewers when the phloem feeders colonise the plant first, but single infestation densities are usually tested (Stout et al., 1998; Rodriguez-Saona et al., 2010; Soler et al., 2012a, 2012b; Ali & Agrawal, 2014; Li et al., 2014). Overall, these results support our hypothesis that preference for aphid-infested plants is characteristic at low/moderate aphid densities, with a switch to a preference for uninfested control plants at higher aphid densities.

The consistent preference for uninfested control plants that caterpillars exhibited at high aphid densities was not associated with a decrease in plant nutrition, either macro- or micronutrients (C, N, P, K, Mg). However, other studies have shown that aphid infestation can indeed decrease the leaf levels of nutrients...
such as N and K, affecting the performance of heterospecifics sharing the host plant (Johnson et al., 2009). Instead, the shift in attraction between aphids and caterpillars can be associated with an increased defensive response in the plant. Our results show that, while aphids at medium density seem to attenuate the defensive response against caterpillars as compared with aphids at low density, i.e. the jasmonate levels, at high infestation these levels are higher than in treatments with lower infestation levels. Although here we used the levels of several jasmonates as indicators of plant defence, the cues that are detected by the caterpillars are probably other compounds whose synthesis is regulated by the JA-signalling pathway (Pieterse et al., 2012; Stam et al., 2014). From the cross-talk perspective, it is interesting that no change in SA levels was observed. Previous results have also shown no changes in SA concentration in response to aphid attack, although SA-related genes are induced locally at the site of aphid feeding (de Vos et al., 2005). In our study, we analysed SA levels for whole leaves, and the local SA induction may have been obscured because of the dilution in the whole-leaf analysis (de Vos et al., 2005). Other studies showed a reduction of JA, possibly due to cross-talk mechanisms, despite evidence for an increase of SA at the same time point (Zhang et al., 2009; Soler et al., 2012a; Ali & Agrawal, 2014), so cross-talk effects between SA and JA signalling pathways may be more difficult to detect at the metabolic level than at the gene transcription level, especially when evaluating single time points. Along the same lines as our findings, a recent study found that, for A. italicana, the effect of aphids on the performance of P. xylostella depends on the density of the aphids infesting the plants, and that this effect was mediated by both SA- and JA-signalling pathways, as confirmed with knock-out mutants (Kroes et al., 2015). Cross-talk of signalling pathways has been proposed to be a powerful regulatory system to mount the most suitable defence response in plants (Pieterse et al., 2012). However, in the case of plant responses to multiple attackers, an enhanced preference or performance of leaf chewers may be maladaptive for the plant if caterpillars impose stronger fitness losses than phloem feeders (Heil et al., 2009; Ali & Agrawal, 2012).

The observed preferences were also dependent on the herbivore species, and to a certain extent on the dietary breadth of the attackers, considering that the four leaf chewer species represent a continuum rather than the classical generalist–specialist division (Ali & Agrawal, 2012). The importance of specificity in both the plant-induced responses and the effects of induction on the attacking herbivores has been highlighted in previous studies (Agrawal, 2000). In our study, whereas the interaction between aphids and the more specialised caterpillars (i.e. the two Pieris species) ranged from preference for aphid-infested plants (at low or medium aphid infestation) to preference for uninfested control plants (at high aphid infestation levels), caterpillars with a wider diet breadth exhibited fewer choices for aphid-infested plants. It has been proposed that at low levels of plant defence, specialist insects tolerate toxins better than generalist ones, but at higher levels of defence both types of herbivore are negatively affected by plant toxicity (Ali & Agrawal, 2012). Our results are in line with this hypothesis as, at medium aphid infestation, the jasmonate response was less than at high infestation levels.

Associated with this, at medium aphid density the more specialised caterpillars were attracted to infested plants (whereas the more generalist ones were attracted to uninfested control plants), but at high aphid density all species showed a preference for control plants over aphid-infested plants. In the field, the aphid B. brassicae is a common herbivore on crucifers, usually colonising plants before leaf chewers, but with a phenological overlap (Poelman et al., 2008). This may have led to an adaptation in the highly specialist Pieris species to better cope with, and even benefit from, the presence of a typically co-occurring aphid herbivore. Recently, the identity of plant species and plant populations (of one plant species) has been shown to be crucial in determining the strength of the facilitative interaction between aphids and caterpillars (Ali & Agrawal, 2014; Li et al., 2014), yet these aspects remain relatively ignored in the study of plant-mediated interactions in terrestrial communities.

While the juveniles of all four species of leaf chewers significantly discriminated between uninfested plants and plants infested by aphids at medium density, the adults selected plants for oviposition independently of the presence or absence of aphids. Aphid-infested plants can become a better food source for leaf chewers as a consequence of interference with JA signalling (Soler et al., 2012a, 2012b). Optimal oviposition theory predicts that females evolved to select those plants on which their offspring perform best, thus maximising their fitness (Jaenike, 1978). This ‘mother knows best’ principle is widely accepted in plant–insect ecology (Thompson, 1988; Gribenberg et al., 2010). By contrast, caterpillars are often considered to be immobile and unselective. However, more recent findings indicate that caterpillars can also engage in host-plant selection (Shikano et al., 2010) and can discriminate between conspecific plants that are uninfested and those that are infested by other herbivores (Soler et al., 2012b). We show that this is not an exception and extend these data to the community of lepidopterans that commonly occur in this system. Studies that specifically address the relative role of adults and juveniles in plant selection, considering the potential differential sensitivity of adults and juveniles to perceive differences in quality within conspecific host plants and the presence of competitive/facilitative counterparts, are urgently needed.

Our study shows the importance of a dynamic approach to understand how plant-mediated species networks assemble and function. In terrestrial systems, plants are exposed to multiple attackers that can influence plant physiology and chemistry (Stout et al., 2006; Dicke et al., 2009; Pieterse et al., 2012) and thus interact with diverse community players. These interactions are not fixed but rather dynamic, and we have provided preliminary evidence that they can considerably change over time when, for example, herbivore density increases. The majority of studies on host-plant selection by herbivorous insects have typically focused on preferences between different host-plant species that are undamaged. But insect attackers can trigger rapid changes in plant physiology and chemistry, even within conspecific plants, which can influence the suitability of the plants as a food source for other community members (Zhang et al., 2009). This work provides evidence of the importance of further extending from researching presence/absence of organisms, and include the dynamic aspect of herbivore densities (see
also Zhang et al., 2009; Kroes et al., 2015). We argue that manipulating herbivore density is a relatively simple alternative to gain insight into the dynamic and complex processes of plant defences against multiple attackers.

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