Oviposition preference of three lepidopteran species is not affected by previous aphid infestation in wild cabbage

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Abstract

Several studies have shown that pre-infestation with aphids can improve plant quality for herbivorous caterpillars. This effect is often explained by the negative crosstalk between specific plant defence, signal-transduction pathways induced by aphids and caterpillars, respectively. However, in these studies caterpillars are introduced on the plants by the researcher, whereas in nature, the adult mother often chooses the food plants for her offspring. According to the preference–performance hypothesis adult females should choose oviposition sites that result in optimal performance and survival of their offspring. In this study, we investigated whether three lepidopteran species—Pieris brassicae (L.) (Pieridae), Plutella xylostella L. (Plutellidae) and Mamestra brassicae L. (Noctuidae)—prefer aphid-infested over clean plants. Adult females of the three species was given the choice between wild cabbage (Brassica oleracea L., Brassicaceae) plants infested with aphids, Brevicoryne brassicae (L.) (Hemiptera: Aphididae) for 3, 7, or 14 days vs. non-infested clean plants. Pieris brassicae females was also given the choice between plants dually infested with B. brassicae aphids and P. xylostella caterpillars when the order of infestation was varied. For oviposition, adult females of all three species did not discriminate between aphid-infested and clean plants, irrespective of the duration of aphid infestation. Also, P. brassicae females did not discriminate between sets of dually infested plants, irrespective of the order of infestation. Several mechanisms are discussed that could explain this lack of preference.

Introduction

Herbivory by insects can induce changes in morphological, physiological, chemical, and growth characteristics of plants (Ohgushi, 2005; Schoonhoven et al., 2005). Feeding by herbivores often reduces palatability of tissues and changes food plant quality for insect herbivores that are already feeding on the plant or for those that subsequently colonize these plants (Poelman et al., 2008; Harvey & Gols, 2011). Denno et al. (2000) provided evidence for plant-mediated competition between two planthopper species, where herbivory by one species reduced the nutritional quality of plants for the subsequently arriving species asymmetrically. In another study, Nakamura et al. (2003) reported that aphid and leaf beetle abundance increased on plants that were already infested with gall-forming midges. This effect was attributed to compensatory lateral shoot growth of the plants which have a higher nutritional value and are, therefore, preferred by aphids and leaf beetles (Nakamura et al., 2003). Thus, changes in plant traits induced by herbivores that arrive early in the season, may alter the relationship between the plant and subsequent herbivores, which in turn may have consequences for community structure and composition (Agrawal, 2000; Martinsen et al., 2000; Van Zandt & Agrawal, 2004; Viswanathan et al., 2007; Poelman et al., 2010; Ohgushi & Hamback, 2015).

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The direction of these plant-mediated indirect effects on subsequent herbivores has been shown to depend on the feeding guild of the inducing herbivore, which is predominantly investigated for chewing caterpillars vs. piercing-sucking aphids (Agrawal, 2000; Rodriguez-Saona et al., 2005; Poelman et al., 2008; Soler et al., 2011; Li et al., 2014). Generally, early-season herbivory negatively affects the performance of a second herbivore when both herbivore species belong to the same feeding guild, and facilitate secondary herbivores when they belong to different feeding guilds (Nakamura et al., 2003; Soler et al., 2012; Li et al., 2014). The underlying mechanism for this interguild herbivore facilitation is based on the fact that herbivores belonging to different feeding guilds activate distinct hormone-regulated signal-transduction pathways which may interact negatively when triggered simultaneously or sequentially (Pieterse et al., 2009; Mouttet et al., 2013). The key hormones involved in plant defence induction are jasmonic acid (JA) and salicylic acid (SA), which are differentially activated by chewing and piercing-sucking herbivores, respectively (Erb et al., 2012; Pieterse et al., 2012; Stam et al., 2014).

JA–SA negative crosstalk, however, is not ubiquitous for all plant and interacting caterpillars and phloem-feeding species (Mouttet et al., 2013; Ali & Agrawal, 2014; Li et al., 2016a). This suggests that defence-signalling interactions in response to multiple attack by insects belonging to different feeding guilds are often more complicated than portrayed above (e.g., Mouttet et al., 2011; Kroes et al., 2015; Li et al., 2016a) and may involve the activity of other hormones as well as insect-related effectors (Robert-Seillaniantz et al., 2011; Erb et al., 2012). Moreover, a meta-analysis showed that feeding guild is not necessarily a good predictor determining competitive interactions between insect herbivores (Kaplan & Denno, 2007). In addition, the severity of the attack depending on herbivore density (Kroes et al., 2015; Poncio et al., 2016) and the order of attack by different herbivores (Voelckel & Baldwin, 2004; Erb et al., 2011; Soler et al., 2011; Mouttet et al., 2013) may have a substantial impact on how a plant responds to attack by more than one species.

A recent study has shown that attack early in the season by phloem-feeding aphids only affects the community associated with aphids (predators and parasitoids) and has no effect on chewing herbivores such as caterpillars or their natural enemies (Li et al., 2016b). Interestingly, a laboratory study (Li et al., 2014) using the same system as in a field study (Li et al., 2016b) suggested that aphid infestation preceding introduction of caterpillars can facilitate the performance of the caterpillars. This contrasting result motivated us to perform the present study. In laboratory studies, resistance-inducing insects are usually introduced onto the plant by the researcher, whereas in nature adult females usually select host plants. According to the preference–performance hypothesis (Jaenike, 1978; Thompson, 1988), adult insect herbivores should select host plants and conditions that result in optimal offspring development. A meta-analysis largely confirmed this hypothesis for several insect orders including the Lepidoptera, and also revealed that there are exceptions and that the support for this hypothesis is stronger for oligophagous than for mono- phagous and polyphagous insects (Gripenberg et al., 2010). Contrastingly, Berenbaum & Feeny (2008) reported a positive correlation for only 50% of the studies investigating this for Lepidoptera. Some of the inconsistencies in these patterns may be explained by difficulties in determining preference and performance (Thompson, 1988).

The aim of this study was to investigate oviposition preference of adult female phytophagous insects for host plants that were or were not already infested with aphids. We used the same system as in the previous study (Li et al., 2014), consisting of wild cabbage plants (Brassica oleracea L., Brassicaceae), the aphid Brevicoryne brassicae (L.) (Hemiptera: Aphididae), and adult females of three different Lepidoptera species, Mamestra brassicae L. (Noctuida), Plutella xylostella L. (Plutellidae) and Pieris brassicae (L.) (Pieridae). Lepidoptera choose their oviposition sites using chemical cues (volatile or contact), visual cues, or a combination of the two (Thompson & Pellmyr, 1991). Based on the results of our field study (Li et al., 2016b), we predict that for oviposition the adult Lepidoptera used in this study do not prefer aphid-infested over control plants, despite the fact that caterpillars may develop better on aphid-infested plants (Soler et al., 2011; Li et al., 2014). We also varied the duration of previous aphid exposure to reveal whether aphid density or the duration of aphid feeding had an effect on oviposition preference. Both B. brassicae aphids and P. xylostella caterpillars colonize cabbage plants early in the season in The Netherlands (Broekgaard et al., 2008; Kos et al., 2011; Li et al., 2016b). In an additional experiment, we tested whether dual infestation with aphids (B. brassicae) and caterpillars (P. xylostella) that were either introduced simultaneously or temporally separated, affected oviposition preference of P. brassicae females.

Wild cabbage populations grow naturally along the Atlantic coast line of England (UK) and they differ considerably in foliar glucosinolate profiles (Moyes et al., 2000; Harvey et al., 2011) and volatile emission in response to herbivory (Gols et al., 2011). Glucosinolates are secondary plant metabolites characteristic of brassicaceous plant species (Fahey et al., 2001) and have been shown to play an important role in mediating interactions with insects (Gols & Harvey, 2009; Hopkins et al., 2009; Winde & Wittstock, 2005).
All insects used in this study are naturally associated with the wild cabbage populations in England (Moyes et al., 2000; Newton et al., 2009b). Host acceptance for oviposition in all three herbivore species is based on olfaction and/or contact chemoreception (Justus & Mitchell, 1996; Rojas et al., 2000), which for the specialists is strongly influenced by the presence of glucosinolates (van Loon et al., 1992; Renwick et al., 2006). To determine whether oviposition preference was consistent despite the differences in foliar glucosinolate chemistry and volatile emissions, plants used in the bioassays originated from two wild cabbage populations that exhibited the most contrasting results on plant–insect interactions in previous studies (Gols et al., 2008; Harvey & Gols, 2011; Ode et al., 2016).

**Materials and methods**

**Plants**

Seeds were collected from two wild populations of B. oleracea located at sites known as Kimmeridge (KIM; 50°35′N, 2°03′W) and Winspit (WIN; 50°36′N, 2°07′W) in Dorset, UK. Seeds were sown 4 weeks prior to aphid infestation. One week after sowing, individual seedlings were transferred to 1.1-l pots filled with potting soil (Lentse Potgrond no. 4; Lent, The Netherlands). Plants were watered every other day until they were 3 weeks old, after which watering occurred daily. Plants were grown and experiments were conducted in a greenhouse at 22 ± 3 °C, 50–70% r.h., and L16:D8 photoperiod.

**Insects**

Plants used in the experiments were infested with the cabbage aphid (B. brassicae), a phloem-feeding herbivore specialized on brassicaceous plants, including wild B. oleracea (Newton et al., 2009a). The lepidopteran species used in the oviposition preference experiments were P. xylostella (diamondback moth) and P. brassicae (large cabbage white), of which the caterpillars primarily feed on brassicaceous plants, and the generalist herbivore M. brassicae (cabbage moth), of which the caterpillars have a much broader food range. Both P. brassicae and M. brassicae lay eggs in clutches, whereas P. xylostella lays single eggs.

All insects used in the experiments were reared in controlled large cabbage moth) and M. brassicae females were 5–13 days old, and M. brassicae females were 2–5 days old.

**Experiment 1: Single infestation with aphids**

We tested for the effect of aphid infestation per se, as well as the duration of aphid infestation on oviposition preference of lepidopteran species. Five adult aphids (B. brassicae) were introduced onto the test plants and incubated for 3, 7, or 14 days before they were used in the behaviour assay. Aphids were placed on the youngest, fully expanded leaf and were allowed to move and reproduce freely. To constrain aphids to plants, plants were covered with BugDorm nylon sleeve nets (48 × 60 cm; MegaView Science, Taichung, Taiwan), which were supported by four wooden sticks and tightly sealed around the rim of the pots. Control plants were not infested with aphids and were maintained under the same conditions as the aphid-infested plants.

Prior to being used in bioassays, the sleeve nets were removed and the aphids on the infested plants were counted. Single aphid-treated plants were then placed in a foldable cage (35 × 35 × 60 cm; Vermandel, Hulst, The Netherlands) together with a non-infested control plant of the same population (KIM or WIN) and age. For P. xylostella and P. brassicae, one mated female was released into the test cage. Experiments with P. brassicae were carried out during daytime starting at 10:00 hours until 13:00 hours on the same day. If there were no eggs deposited at 13:00 hours, the experiment was prolonged until 16:00 hours, or 10:00 hours the following morning. As P. xylostella females lay eggs only during the scotophase, experiments with this species were carried out overnight starting at 16:00 hours and ending at 09:30 hours the following morning. For M. brassicae, which is also active during the scotophase, an unmated male/female couple was released into the test cage for ca. 41.5 h (from 16:00 till 09:30 hours on the 2nd day) to allow mating and oviposition. Aphids remained on the plant throughout the experiments. Following the oviposition period, the eggs on both the aphid-infested and the control plant were counted. Adult females were only used once. The number of replicates per population*aphid infestation duration varied between 11 and 19 for P. xylostella, 12–17 for M. brassicae, and 19–25 for P. brassicae.

**Experiment 2: Order of arrival of herbivores on dually infested plants**

In the second experiment, we tested the effect of dual infestation with both aphids (B. brassicae) and caterpillars (P. xylostella) on oviposition preference of P. brassicae. As the availability of insects was limited, oviposition preference was only tested for P. brassicae butterflies on WIN plants. Plants were infested with five adult (B. brassicae)
aphids, as in the previous experiments, and three L2 *P. xylostella* caterpillars. Three treatments were compared: (1) infestation with *B. brassicae* aphids for 7 days followed by an additional infestation with *P. xylostella* caterpillars for another 7 days, (2) infestation with *P. xylostella* caterpillars for 7 days followed by an additional infestation with *B. brassicae* aphids for another 7 days, and (3) simultaneous infestation with *B. brassicae* aphids and *P. xylostella* caterpillars for 7 days. Both species were introduced on the same leaf, i.e., the youngest fully expanded leaf at the time of the first insect introduction. In treatments 1 and 2, the herbivore that was introduced first remained on the plant when the second herbivore was added. In treatment 2, caterpillars and pupae were removed from the plant after 7 days and were replaced by three new L2 caterpillars to maintain feeding damage to occur. After 14 (treatments 1 and 2) or 7 (treatment 3) days following infestation, plants were placed in foldable cages (35 × 35 × 60 cm) and the oviposition preference of *P. brassicae* was determined as described for experiment 1. Treatments were tested pairwise (treatment 1 vs. 2, 2 vs. 3, and 3 vs. 1). Numbers of replicates varied between 12 and 16.

**Data analysis**

Egg laying varied considerably among and within species. Using a general linear model ANOVA, we first determined whether the number of eggs laid on infested plants or the overall number of eggs differed with aphid infestation duration and plant population. Neither aphid infestation duration nor plant population had an effect on overall egg deposition. We then gave equal weight to oviposition decisions of each individual by scoring the oviposition response of an individual as a preference for the aphid-infested plant, if a female laid more eggs on this plant (scored as 1), or as a preference for control plants (scored as 0). To determine whether the proportion of females that preferred the aphid-infested over the control plant varied with the duration of aphid infestation (3, 7, or 14 days), between the two plant populations (KIM vs. WIN), and among the three herbivore species (*P. xylostella*, *M. brassicae*, and *P. brassicae*), we used a generalized linear model (GLM) with a binomial distribution and a logit link function. Herbivore species, plant population, and aphid infestation period were entered as fixed factors in the statistical model. *Pieris brassicae* and *M. brassicae* lay eggs in clutches. To determine whether the number of egg clutches laid on control and aphid-treated plants varied with aphid infestation or plant population, the proportion of clutches laid on aphid-infested plants out of the total egg clutches was also compared using a GLM as described above. Here, we did not control for the number of clutches as these are less variable than total egg numbers. Estimates of the model term means also allow for testing whether there is preference for one of the two plants offered in a specific plant combination, i.e., *H*~0~: logit = 0 based on Z-tests.

In experiment 2, oviposition preference for three treatments was compared pairwise. Data of this experiment were analysed using the same statistical approach as in experiment 1, but here preference proportions for one treatment (e.g., simultaneous infestation) were compared when offered with each of the other two treatments (e.g., sequential infestation with aphids first followed by caterpillars, and infestation with caterpillars first and aphids second). Data were analysed using SAS v.9.3 (2002–2010, SAS Institute, Cary, NC, USA).

**Results**

**Effect of aphid infestation duration**

Aphids accumulated with increasing infestation duration (linear regression on log-transformed aphid numbers: \( F_{1,299} = 1.228, P < 0.001, R^2 = 0.81; \) Figure 1). As mentioned, the total number of eggs laid varied considerably (range, median, mean for *P. brassicae*: 3–189, 74, 78.6; for *P. xylostella*: 1–56, 6, 8.6; for *M. brassicae*: 4–975, 293, 307), but they did not differ depending on plant population or aphid infestation duration (Table 1).

Females of *M. brassicae* laid significantly more eggs on WIN plants infested with aphids for 7 days, and

![Figure 1](image_url)

**Figure 1** Mean (± SE) aphid counts at three time points (3, 7, and 14 days) following initial infestation with five adult *Brevicoryne brassicae* of wild cabbage plants originating from two populations (Kimmeridge and Winspit). Plants were used in oviposition preference assays with three herbivore species, *Plutella xylostella* (11–19 plants), *Mamestra brassicae* (12–17 plants), and *Pieris brassicae* (19–25 plants) and counts are presented for each of the three herbivores separately.
Table 1  Statistical analysis of eggs laid by Pieris brassicae, Mamestra brassicae, and Plutella xylostella on plants that were or were not already infested with Brevicoryne brassicae aphids for 3, 7, or 14 days. The response variables in the analyses were the number of eggs laid on aphid-infested plants (= treatment), the total number of eggs laid, and the number of egg clusters (P. brassicae and M. brassicae only) laid on aphid-infested plants out of the total number of egg clusters. The explanatory variables were plant population (Kimmeridge vs. Winspit), aphid infestation period (3, 7, or 14 days), and their interaction term.

| Lepidopteran | Factor                 | Population | Infestation duration | Interaction |
|--------------|------------------------|------------|----------------------|-------------|
|              |                        | F or $\chi^2$ | d.f. | P   | F or $\chi^2$ | d.f. | P   | F or $\chi^2$ | d.f. | P   |
| P. brassicae | Eggs on treatment      | 0.35       | 1,120 | 0.55 | 0.12       | 2,120 | 0.88 | 0.21       | 2,120 | 0.81 |
|              | Eggs total             | 0.17       | 1,120 | 0.68 | 0.55       | 2,120 | 0.57 | 2.32       | 2,120 | 0.10 |
|              | Preference clusters    | 1.73       | 1     | 0.18 | 0.35       | 2     | 0.84 | 0.02       | 2     | 0.99 |
| M. brassicae | Eggs on treatment      | 0.03       | 1,81  | 0.86 | 0.15       | 2,81  | 0.86 | 2.06       | 2,81  | 0.13 |
|              | Eggs total             | 0.27       | 1,81  | 0.60 | 0.47       | 2,81  | 0.62 | 0.66       | 2,81  | 0.52 |
|              | Preference clusters    | 0.27       | 1     | 0.60 | 1.76       | 2     | 0.41 | 1.62       | 2     | 0.44 |
| P. xylostella | Eggs on treatment      | 0.19       | 1,89  | 0.66 | 0.15       | 2,89  | 0.86 | 0.23       | 2,89  | 0.79 |
|              | Eggs total             | 0.45       | 1,89  | 0.50 | 0.98       | 2,89  | 0.38 | 1.30       | 2,89  | 0.27 |

Egg numbers were analysed with a general linear model ANOVA (F) and the proportion of egg clusters on aphid-infested plants with a generalized linear model ($\chi^2$).

P. xylostella laid more eggs on WIN plants infested with aphids for 14 days (Figure 2A and B). Oviposition preference in relation to aphid infestation period and plant population varied the least for P. brassicae (Figure 2C). However, no consistent effects of aphid infestation or plant population were detected for any of the three herbivore species (GLM: $\chi^2 = 0.14$, d.f. = 2, P = 0.93). Irrespective of herbivore species, neither the duration of aphid infestation ($\chi^2 = 0.91$, d.f. = 2, P = 0.38) nor plant population ($\chi^2 = 0.36$, d.f. = 2, P = 0.54) had an effect on proportional oviposition preference and also none of the interaction terms was significant. Also, when for the two gregarious egg-laying species P. brassicae and M. brassicae the proportions of egg clusters were used as the response variable, aphid-infested and clean plants were equally preferred for oviposition (Table 1).

Figure 2  Oviposition preference of the three lepidopteran species (A) Mamestra brassicae, (B) Plutella xylostella, and (C) Pieris brassicae when adult females were given the choice between a wild cabbage plant infested with Brevicoryne brassicae aphids and a clean plant of the same population, Kimmeridge (KIM, grey bars) or Winspit (WIN, white bars). Plants were infested with aphids for 3, 7, or 14 days. Equal weight was given to oviposition decisions by scoring the oviposition response of each female as a preference for the aphid-infested plant if a female laid more eggs on this plant (scored as 1), or as a preference for control plants if more eggs were laid on the control plant (scored as 0). Preference is depicted as mean proportion (± SE) of choices for aphid-infested plants, which is the mean of the scores. Asterisks indicate pair-wise comparisons where preference proportions deviated significantly (Z-tests: P<0.05) from 0.5, representing the ‘no preference’ situation.
Effect of the order of arrival on dually infested plants

Irrespective of the alternative plant treatment in the choice assay, preference proportions of *P. brassicae* for simultaneously infested plants (GLM: $\chi^2 = 2.95$, d.f. = 1, $P = 0.086$), plants infested with caterpillars first, aphids second ($\chi^2 = 0.97$, d.f. = 1, $P = 0.32$), or plants infested with aphids first, caterpillars second ($\chi^2 = 0.44$, d.f. = 1, $P = 0.50$) were similar (Figure 3).

Discussion

When adult females of three lepidopteran species was given a choice between an aphid-infested vs. a clean wild cabbage plant, both plants were readily accepted for oviposition. Proportional preference was not affected when the aphid infestation period was increased from 3 to 7 to 14 days. In 2 out of the 18 treatment combinations, a significant preference was found for aphid-infested over clean plants – *M. brassicae* preferred WIN plants that were infested with aphids for 7 days, and *P. xylostella* preferred WIN plants that were infested with aphids for 14 days. However, the effect of aphid presence on oviposition preference in relation to plant population was not statistically significant. When *P. brassicae* females was given the choice between WIN plants that were simultaneously or sequentially infested with *B. brassicae* aphids and *P. xylostella* caterpillars they also did not distinguish between plant treatments.

Previous studies have shown that food plant quality for caterpillars of *P. brassicae* and *P. xylostella* and other Lepidoptera, but not *M. brassicae*, is enhanced when plants were infested with aphids before the caterpillars were introduced (Rodriguez-Saona et al., 2005; Soler et al., 2011; Ali & Agrawal, 2014; Li et al., 2014). Recently, it has also been reported that the density of aphids on plants co-infested with caterpillars can result in a dramatic shift in the behaviour and performance of insects interacting with these plants (Kroes et al., 2015, 2016; Ponzio et al., 2016; Li et al., 2017). Mechanistically, these shifts have been proven to be difficult to explain but point at the involvement of both SA and JA, and differential activation of transcription factors (Kroes et al., 2017).

Oviposition preference of adult mothers and offspring performance are not necessarily always positively correlated (Mayhew, 2001; Scheirs et al., 2005; Gripenberg et al., 2010). For Lepidoptera this has been found for roughly 50% of the studied species (Berenbaum & Feeny, 2008). Data presented here and those reported by Soler et al. (2012) and Li et al. (2014) suggested that adult preference and larval performance are not positively correlated in *P. xylostella* and *P. brassicae*. Sensory inputs guiding oviposition and feeding decisions, and the environments in which they are perceived, are quite different for the adult and larval stage and insect herbivores may also be neurologically constrained in their ability to differentiate qualitative aspects of host plants (Bernays, 2001). Location and acceptance of a plant for oviposition primarily relies on sensory cues, whereas larval performance is influenced by the actual intake of plant tissues resulting in exposure to a broad range of chemicals of which some may strongly impact on larval growth (Berenbaum & Feeny, 2008). Thus, natural selection acts on different traits in adult females optimizing oviposition, and in offspring optimizing growth and development. Females may also be

![Figure 3](image-url)
physically hampered to lay eggs on plants that are of high quality for her offspring. Moreover, oviposition decisions by adult mothers may optimize their own fitness and not necessarily that of their offspring (Mayhew, 2001). Physiological constraints, such as age and egg load, may further influence selectivity in egg-laying adult females (Sisterson, 2012). Other ecological factors, such as the availability of suitable host plants at the time of oviposition (Singer & Stireman, 2001) and the presence of or the risk of predation/parasitism by the herbivore’s natural enemies (Ohsaki & Sato, 1994; Singer et al., 2004) can also strongly influence female oviposition decisions.

Life-history traits, such as gregarious egg laying and dietary breadth, may also explain the strength of the link between adult preference and offspring performance (Gripenberg et al., 2010). For instance, a meta-analysis revealed that this correlation is stronger in oligophagous than in monophagous and polyphagous insects (Gripenberg et al., 2010). Aggregated egg laying is predicted to increase host plant selectivity as one bad decision by the mother determines the fate of a large number of her offspring. Both *P. brassicae* and *M. brassicae* lay eggs in clutches, but larvae of *P. brassicae* remain and feed together during the first three larval stages, whereas larvae of *M. brassicae* tend to disperse upon egg hatching. Moreover, although *P. brassicae* caterpillars are restricted to feed on glucosinolate-containing (mainly brassicaceous) plant species, *M. brassicae* caterpillars can feed on a range of species in various families. Mobility of the caterpillars combined with a broad dietary breadth may not strongly select for oviposition preference in *M. brassicae*, though at the plant species level a hierarchy in oviposition preference has been reported for *M. brassicae* (Rojas et al., 2000).

Females of which the larvae feed on herbaceous plants, which is the case for the three herbivore species used in this study, may not be able to assess plant quality for their offspring as this may change rapidly over the growing season (Craig & Itami, 2008). In the study by Li et al. (2014), the performance of caterpillars was compared on plants that were treated with a single aphid density and aphids were introduced on the plant before the caterpillars. Kroes et al. (2016) showed that *P. xylostella* performed better on wild cabbage plants without aphids than on plants on which aphids and caterpillars had been introduced simultaneously. When *P. xylostella* performance was compared on *Arabidopsis thaliana* (L.) Heynh. plants in the absence of aphids and when aphids and caterpillars were introduced at the same time, a positive effect of *B. brassicae* on caterpillar performance was found at low aphid densities, and a negative effect at high aphid densities (Kroes et al., 2015). At higher densities, aphids may deplete resources affecting plant quality also for chewing herbivores. These results suggest that whether aphids may promote caterpillar performance is very subtle and depends on the time of arrival of both herbivores and its effect may wane when aphid densities increase. This prevents strong selection on adult females to find aphid-infested plants. In addition, in the dietary specialists *P. brassicae* and *P. xylostella*, the differences in food plant quality may often not be large enough to result in oviposition preference in the adult females (Gripenberg et al., 2010). This applies to the situation in which *P. brassicae* is given the choice between a clean plant and an aphid-infested plant as well as when plants are dually infested with both *B. brassicae* aphids and *P. xylostella* caterpillars but where the order of arrival is varied. However, specialist pierids, including *P. brassicae*, have been shown to distinguish subtle differences in plant quality when deciding where to lay eggs (Myers, 1985; Chen et al., 2004; Bruinsma et al., 2007). *Pieris brassicae* avoids to lay eggs on plant on which conspecific eggs are present and this is mediated by both visual cues and physiological changes in response to egg deposition (Rothschild & Schoonhoven, 1977; Blaakmeer et al., 1994; Fatouros et al., 2012). As caterpillars of *P. brassicae* feed gregariously and are voracious feeders, the impact on survival is large when eggs are laid on plants already infested with conspecific eggs. Hence, selection pressure in *P. brassicae* on discrimination ability of plants with conspecifics is strong.

One issue that is often raised when using laboratory-reared insect colonies in behavioural studies is that the rearing conditions may have reduced selectivity with regard to traits such as oviposition. In a recent study (Fei et al., 2016), however, it was shown that ‘choosiness’ is maintained in the same laboratory culture of *P. brassicae* as used in this study. Moreover, field-collected individuals are added to the cultures of *P. brassicae* and *P. xylostella* every 1 or 2 years. In addition, Li et al. (2016b) demonstrated that the effect of early aphid infestation under field conditions has no effect on colonization by Lepidoptera, which further corroborates the results of this study.

No difference was observed in oviposition preference of the three Lepidoptera species when the two *B. oleracea* populations were compared. Though in two cases aphid-infested WIN plants were preferred over clean plants, no systematic preference for aphid-infested plants was found for this population. These two populations differ significantly in glucosinolate chemistry and have been shown to differentially affect the development of various caterpillar species and their parasitoids (Gols et al., 2008; Harvey et al., 2011; Ode et al., 2016). In addition, these plant populations differ in inducibility of secondary chemistry in response to caterpillar feeding (Gols et al., 2011; Harvey & Gols, 2011), whereas the effect of aphid infestation on
plant quality for *P. xylostella* caterpillars was similar on three wild cabbage populations of which two are used in this study (Li et al., 2014). Glucosinolates, especially indole glucosinolates, stimulate oviposition of both *P. xylostella* (Sun et al., 2009) and *P. brassicae* (van Loon et al., 1992). Feeding by *B. brassicae* has been reported to only induce aliphatic glucosinolates in *A. thaliana* (Mewis et al., 2006). If this is also the case in wild *B. oleracea*, this could explain why *B. brassicae* infestation does not affect oviposition preference. Moreover, in a garden study with wild cabbage, the associated lepidopteran community was not affected by early-season infestation with *B. brassicae* aphids (Li et al., 2016b), which suggests that the potentially positive effects of early aphid infestation on insect performance are not strong enough to select for oviposition preference in lepidopteran herbivores.

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