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Plant quantity affects development and reproduction of a gregarious butterfly more than plant quality

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Abstract

The larvae of insect herbivores feed on plants that may vary nutritionally (qualitatively and/or quantitatively) over the course of insect development. Plant quality may change in response to interactions with the biotic environment that in turn may affect development and biomass of the insects feeding on these plants. However, the larvae of many gregariously feeding herbivores feed on comparatively small plants with limited biomass and may also experience variation in the quantity of plant food available. *Pieris brassicae* L. (Lepidoptera: Pieridae) is a gregarious butterfly species laying clutches of 10–150 eggs that are often laid on small brassicaceous food plants, including the plant used in this study, *Brassica nigra* L. (Brassicaceae). A single *B. nigra* plant provides insufficient resources for the development of an entire brood of *P. brassicae*. In this study, we investigated the effect of both plant quality and quantity on the performance of *P. brassicae* when feeding on *B. nigra* plants. When we compared the effects of changes in plant quality induced by (1) aphid infestation, (2) exposure to pathogenic and non-pathogenic bacteria, and (3) inbreeding depression, which are all biotic stresses known to change plant quality, pupal mass and larval development time of *P. brassicae* were fairly similar. We then examined the effects of quantitative food constraints during immature development on pupal mass, which correlated strongly with adult size, longevity, and fecundity. Female pupal mass, longevity and fecundity were negatively correlated with the duration of starvation during larval development. No significant effect of male starvation was found on female reproduction and longevity. Thus, *P. brassicae* larvae were more affected by quantitative than by qualitative constraints in terms of pupal mass, which strongly correlated with female reproduction.

KEYWORDS
Brassica nigra, Brassicaceae, defence induction, feeding constraints, insect herbivores, insect-plant interactions, Lepidoptera, Pieridae, *Pieris brassicae*, plant nutritional quality, plant stress, reproduction

INTRODUCTION

In many holometabolous insects, such as butterflies, nutrients vital for growth, development, and reproduction are obtained during the larval stage, whereas adults primarily feed on resources like nectar for maintenance and energy. This clear distinction between dietary requirements in relation to life stage has pronounced consequences for the effects of nutritional quality and quantity of food consumed during both the larval and adult stages. Food plant quality for insect herbivores may change during plant and insect development in response to interactions with the biotic and abiotic environment (Karban & Baldwin, 1997; Schoonhoven et al., 2005; Barton & Koricheva, 2010). For instance, infestation with other plant antagonists, such as plant pathogens or other herbivores, can affect the quality of the host plant for insects feeding simultaneously or sequentially on the same host plant through changes in primary and secondary metabolite concentrations and morphological traits (Schoonhoven et al., 2005). Inbreeding depression results in loss of heterozygosity and expression of recessive deleterious genes causing reduced plant quality. Changes in these traits have also been shown to affect resistance to herbivores (Carr & Eubanks, 2002; Delphia et al., 2009; Prill et al., 2014). Approximately 80% of insect herbivore species is specialised in the sense that they feed only on species belonging to the same family of plants and are well adapted to defensive plant traits such as secondary metabolites that are phylogenetically conserved (Bernays & Graham, 1988; Schoonhoven et al., 2005; Loxdale et al., 2011). As a result of this, changes in resource quality may only have limited effects on the performance of specialist herbivores.

Whereas the effects of plant quality on herbivore performance are very well studied, much less attention has been paid to the amount of food necessary for larval development. Although most lepidopteran species are solitary, approximately 10% feed in groups (Stamp, 1980). Some butterfly species feeding on small ephemeral plants reduce their clutch size to account for limited food availability (Myers, 1980; Pilson & Rausher, 1988; Damman, 1991), or else the mother preferentially lays broods on plants growing in large clustered stands. However, gregarious lepidopterans sometimes oviposit on plants that grow in looser assemblages, without connecting canopies, or even in isolation (Fei et al., 2014, 2016). Under these conditions, the progeny may exhaust the plant before they have completed development. If they are unable to locate new food plants in the vicinity they may starve or are forced to pupate at a very small size (Andrewartha & Birch, 1954; le Masurier, 1994; Fei et al., 2016).

Insect performance is frequently quantified based on indirect or direct fitness correlates. Indirect correlates include egg-to-adult development time and pupal/adult body mass, whereas precocious mortality and reproductive success are direct correlates (Brown et al., 1993; Abrams et al., 1996). Prolonged development may enhance the risk of mortality before reproduction (Benrey & Denno, 1997) and reduced adult size/biomass is frequently correlated with reduced adult fecundity (Honěk, 1993) but see Leather (1988). Few studies have thus far investigated the effects of plant quality and quantity on realized fertility in lepidopteran species and even fewer have measured this for male conspecifics (Awmack & Leather, 2002; Boggs & Freeman, 2005). Variation in larval diet quality may influence the allocation of resources to somatic and reproductive tissues during metamorphosis (Awmack & Leather, 2002; Boggs, 2009). Males of many lepidopteran species transfer accessory gland products during mating (Wiklund et al., 1993; Vahed, 1998; Watanabe, 2016) and the nutritional quality of these substances, as well as sperm numbers, can be influenced by the larval diet (Cook & Wedell, 1996; Boggs & Freeman, 2005; Simmons, 2012). In the female, these substances can serve as nutrients for maintenance of somatic tissues and egg maturation, which in turn benefit fecundity (South & Lewis, 2011; Watanabe, 2016).

Larvae of the large cabbage white butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae) are specialized herbivores of plant species in the Brassicales (Feltwell, 1982). Adult *P. brassicae* females lay clusters of 10–150 eggs on the leaf undersides of young shoots and the larvae feed gregariously until they develop into the fourth instar, after which they initially disperse on the natal plant and eventually to adjacent plants (Feltwell, 1982; Smallegange et al., 2007). Most naturally occurring host plant species of *P. brassicae* are small, ephemeral annuals of which individual plants do not provide sufficient resources for the successful development of a complete brood (Fei et al., 2016).

The aim of this study is to compare the relative importance of larval food-plant quality and quantity on performance and reproduction of *P. brassicae* butterflies. We used black mustard, *Brassica nigra* L. (Brassicaceae), which is a natural host plant of *P. brassicae*. In a first series of experiments, we investigated the effect of qualitative variation in the host plant on larval performance (pupal mass and development time). We generated changes in plant quality by infestation with aphids, infiltration by pathogenic and non-pathogenic bacteria, and inbreeding depression, respectively. These conditions have all been shown to alter resistance to insects using the same seed batches of *B. nigra* and plant treatments as used in this study (Prill et al., 2014; Bonnet et al., 2017) and thus resource quality. We define resource quality, here plant quality, as any difference in the state or condition of a resource that affects growth and development of its consumers. To determine to what extent variation in pupal mass affects adult realised fecundity, we tested the effect of food deprivation during the final fifth instar on adult male and female reproductive success in a second experiment. We also investigated the effect of starvation during the final instar on female reproduction when they had mated with males that had been starved during the final instar. We hypothesize that (1) the effects of food plant quality on body mass of the dietary specialist *P. brassicae* are relatively small compared to

those caused by starvation (quantitative effects) during the final instar; (2) female body mass strongly correlates with reproductive output; and therefore (3) the effects on butterfly fitness are stronger in response to changes in plant quantity than to changes in plant quality.

MATERIALS AND METHODS

Insects and plants

Pieris brassicae originated from pupae collected from cabbage fields in the vicinity of Wageningen University (Wageningen, The Netherlands) and were reared on Brussels sprouts (*Brassica oleracea* var. *gemmifera* cv. Cyrus) in a climate-controlled room at 22 ± 2 °C, 50–70% r.h., and L16:D8 photoperiod. In all experiments, we used neonate caterpillars that were collected from the general rearing. *Brevicoryne brassicae* L. (Hemiptera: Aphididae) aphids that were used in the larval performance bio-assays also originated from cabbage fields near the university and were also reared on Brussels sprouts. Brussels sprouts plants for rearing of the insects and the starvation experiment were obtained from the general rearing maintained in a greenhouse (22 ± 4 °C, 50–70% r.h., and a photoperiod of at least 16 h).

Plant quality

Aphid treatment

Brassica nigra seeds originated from a natural population growing along the river Rhine in Wageningen. Plants were grown from seeds in 2-l pots filled with potting soil (Lentse Potgrond no. 4, Lent, The Netherlands) in a greenhouse set at 25 ± 2 °C, 50–70% r.h., and a photoperiod of at least 16 h. Plants were watered daily. Plants were infested with either 50 (18 plants) or 100 aphid nymphs (19 plants) when they were 3–4 weeks old. Aphids were placed on the youngest fully developed leaf and could move over the entire plant; we observed that aphid movement to other leaves was limited. Twenty plants that served as controls were not infested with aphids. Following incubation with aphids for 72 h, on each plant, five neonate *P. brassicae* caterpillars were introduced on the aphid-infested leaf or on a leaf of comparable development on control plants. Aphids were not removed. Caterpillars were allowed to move and eat freely within a plant until they reached the fifth and final instar and started to wander in search of a site to pupate. At this point caterpillars were collected and placed in small plastic boxes ($18 \times 13 \times 6$ cm) with some plant material of the plant on which they had fed previously. Caterpillars from the same plant were placed together. After pupation and the cuticle had hardened, the pupae were carefully removed from the boxes and weighed to the nearest mg and their development time to pupation was recorded in days.

Bacteria treatment

Brassica nigra plants were grown similarly as described for the aphid-treated plants above. Three *Xanthomonas campestris* pathovars were selected based on their (in)ability to infect *B. nigra* plants. A virulent strain of *Xanthomonas campestris raphani* (referred to as Xcr-NL) causing disease symptoms was obtained from the Plant-Microbe Interactions group of Utrecht University (Utrecht, The Netherlands). A second *X. campestris raphani* (strain 5827, referred to as Xcr-Fr) was supplied by the French Collection of Plant Pathogenic Bacteria (CFBP, Angers, France). An avirulent strain, *X. campestris* pathovar *incanae* (strain 2527, referred to as Xci), only inducing a hypersensitive response at the site of infiltration, was also obtained from the CFBP in France. Plants were inoculated with a suspension of a single bacterial strain containing approximately 10^8 colony-forming units (cfu) ml^{-1} by infiltration using a needleless syringe (for details see Ponzio et al., 2016). Fresh inoculum was prepared by adding 250 µl of stock solution to 30 ml of Difco nutrient broth (Becton, Dickinson and Company, Sparks, MD, USA), and incubated in a shaker at 28 °C and 170 r.p.m. for 18–24 h. The broth was then transferred to a 50-ml tube and centrifuged at 3000 ***g*** for 10 min. The bacterial cells were resuspended in a 10 mM $MgSO_4$ buffer solution, and the OD_{600} was adjusted to 0.066 (approximately 10^8 cfu ml^{-1}). Ten 4- to 5-mm-diameter spots were infiltrated on the abaxial side of the largest fully developed leaf. Leaves of control plants were infiltrated with buffer only. All plants were incubated for 72 h in mesh cages ($35 \times 35 \times 60$ cm; Vermandel, Hulst, The Netherlands) which had been covered with transparent plastic for the first 24 h to ensure sufficiently high humidity for successful infection in a greenhouse at 25 ± 2 °C and 70% r.h. Disease symptom development was recorded on each plant 72 h after infiltration just before the caterpillars were introduced (for details see Ponzio et al., 2016). Plants infiltrated with the avirulent strain, *X. campestris* pathovar *incanae* only developed necrotic spots limited to tissue immediately around the infiltration site, whereas plants infiltrated with the virulent strains of *X. campestris raphani* all developed medium (up to a third of the leaf affected) to severe symptoms (over a third of the leaf affected). After 72 h of incubation, five neonate caterpillars were introduced on each plant. Caterpillar performance was determined as in the experiment with aphids. Experiments were performed in two blocks, seven or eight plants per treatment in each block. The blocks represented replicated experiments over time.

Plant inbreeding vs. outbreeding

Seeds from *B. nigra* originated from five populations in Dorset (UK). Inbred and outbred plant lines were obtained as described in Prill et al. (2014). Eleven maternal lines were used that were either inbred or outcrossed (22 lines in total). Plants were 6–7 weeks old when they were infested with

caterpillars. A previous study has shown that the developmental stage (vegetative, bolting, or flowering) of *B. nigra* plants has only marginal effects on larval performance of *P. brassicae* (Fei et al., 2017). Plants (n = 4–10 per plant line) of the 11 inbred and outbred families were equally divided over two greenhouses and were each infested with four neonate caterpillars and their performance was determined as described above.

Plant quantity

Approximately 400 second instar (L2) *P. brassicae* caterpillars obtained from the general rearing were transferred to mesh cages (35×35×60 cm) and were reared on Brussels sprouts plants until they developed into late L4 caterpillars. New plants were added when necessary. To obtain caterpillars that were in the same developmental stage, L4 caterpillars in apolysis were collected and transferred to a new cage with plants. The following day these caterpillars had moulted into the final instar which was set as day 0. On day 2, 100 caterpillars were collected from the cage and placed individually in Petri dishes (6 cm diameter) without food. Caterpillars were not deprived of food on day 1 because a previous study has shown that such a treatment causes all caterpillars to die (Fei et al., 2016). On the following days (day 3 and 4) 50 caterpillars were collected on each day and also placed individually in Petri dishes without food. Caterpillars in these Petri dishes were monitored until they pupated or died. Mortality or pupation was recorded in each dish and pupae were weighed. The remaining caterpillars usually developed into pupae on day 5 and are considered a control, non-starvation treatment.

Pupae of the same starvation cohort were placed together in mesh cages (35×35×60 cm) until they eclosed as adults. At this point, the females were collected and divided over two new cages: one group was allowed to mate with males that had experienced the same starvation regime and one group that was allowed to mate with non-starved control males. Mating was allowed for 1 day after which females were collected and placed individually in cages (35×35×60 cm) with a single Brussels sprouts leaf placed in a 10-ml vial filled with water and 10% sugar water provided in a blue cap with cotton wool, which is attractive to adult butterflies. Leaves with eggs were replaced once a day, and sugar water was refreshed every three days. Males of the respective starvation regime were added every 3 days, as *P. brassicae* females mate more than once, and left for 1 day. Oviposition was recorded until the females died. This starvation protocol was repeated 4× over a 4-month period. In total we recorded oviposition of 140 females of which 88 had mated with a non-starved male and 52 with a male from the same starvation group as the female. In the fourth replication of the experiment, we determined the length and width of one of the forewings of the females post-mortem as a measure of butterfly size (see statistical analyses).

Eggs were counted and the leaves with the eggs were maintained in the vials with water in the greenhouse until the larvae hatched. The hatched larvae and the eggs that did not develop into larvae were also counted.

Statistical analysis

Larval performance

Variables (pupal mass and development time) were analysed for each of the three experiments separately. Pupal masses were analysed using linear mixed models. In all three experiments individual plants were infested with several caterpillars (4–5, depending on the experiment). To account for this, plant identity was included as a random factor in the statistical models. In the study examining the effects of aphid infestation, aphid exposure treatment (0, 50, or 100 aphids) was the main factor of interest. As some plants in all treatments, including control, were also found to be mildly infected by mildew and/or spider mites, presence/absence of these two infestations was included as two additional factors. The effect of bacterial infestation with *Xanthomonas* species was also analysed using a mixed model with bacterial treatment (four levels) and block (two levels) as main factors. The statistical model studying the effect of inbreeding contained inbreeding (yes/no), plant family (11 levels) and greenhouse compartment (two levels) as main factors. Assumptions of normality and equal variance were checked by visual inspection of the residuals. Development times were analysed using Cox proportional hazard regression (survival) analysis with the same explanatory variables as in the previous analysis. Here, we did not control for variation among plant individuals.

Starvation and lifetime reproduction

Larval survival and hatching (= offspring viability) in response to starvation regime were analysed using logistic regression, with a logit link function and a binomial distribution for errors. Pupal mass and lifetime reproduction were each analysed using a mixed general linear model with starvation regime of the female (three levels), starvation of the male (yes/no), and their interaction as explanatory variables, and replicate as random variable. The relationship between adult size and lifetime egg production was investigated in more detail in the last replicate. Here, we determined the surface area of butterfly forewings after the females had died. To determine whether forewing surface area, calculated as the product of the length and width of the forewing divided by 2, is a good predictor of butterfly mass, we starved additional larvae as described above and determined their pupal mass. At adult eclosion, these butterflies were frozen to death and their fresh mass was determined, as well as their forewing

length and width. We used linear regression to establish relationships between various size and reproduction attributes. Longevity was analysed using Cox regression with the same explanatory variables as in the mixed model. All analyses were performed in SAS v.9.3 (SAS Institute, Cary, NC, USA). In all models initially all interaction terms were included, but terms were progressively removed if they were not significant.

RESULTS

Plant quality

In the first experiment, we tested the effect of aphid infestation on plant quality in terms of larval development of *P. brassicae*. Neither aphid infestation, nor accidental mildew and/or mite infestation affected pupal mass or development time until pupation (Table 1). Mean (± SE) pupal masses among treatment groups were between 365 ± 9 mg for pupae that had developed on plants infested with 50 aphids and 385 ± 8 mg on control plants (Figure S1). Development times were fairly similar with mean values ranging between 13.0 ± 0.03 days on plants infested with 100 aphids and 13.1 ± 0.07 days on control plants and plants infested with 50 aphids (Figure S1).

Infection with *Xanthomonas* strains differing in virulence did not affect pupal mass (Table 1). Mean (± SE) pupal masses were between 394 ± 12 mg on plants infected with *X. campestris incanae* and 411 ± 12 on control plants (Figure S2). *Xanthomonas* infection did not affect development time to pupation (Table 1). However, development times differed between the two blocks by almost a day (Table 1, Figure S2).

In the third experiment, plant inbreeding had no effect on pupal mass (Table 1). The effect of inbreeding on larval development time interacted with plant family line (Table 1). Larval development tended to be faster on inbred than on outbred lines. For two families this pattern was reversed. Interestingly, the compartment in which the experiment had been conducted significantly affected both pupal mass and development time. Pupae were approximately 10% lighter and larval development was extended by almost 2 days compared to the results found for the insects in the other compartment (Figure S3).

Plant quantity

Starvation regime had a strong effect on larval survival to pupation ($\chi^2 = 862$, d.f. = 1, P<0.001). When larvae were deprived of food on the 2nd day after their final moult (day −4 in Figure 1), none of them survived, whereas the non-starved (day 0 in Figure 1) control larvae all pupated. Starvation regime had a significant effect on the mean pupal mass of the surviving larvae ($F_{3,132} = 243$, P<0.001). The starvation regimes generated pupae of which the weights ranged from 170 to 470 mg. The mean pupal mass of the butterflies increased with shorter starvation (Figure 2A). Close to 100% of the pupae eclosed as adult butterflies. Lifetime egg production was strongly affected by starvation duration of the females ($F_{3,130} = 31.3$, P<0.001), but not by that of their mates ($F_{3,129} = 0.42$, P = 0.52) or the interaction between female and male starvation regime ($F_{2,130} = 0.99$, P = 0.38). Lifetime egg production decreased from approximately 1100 in females that had not been starved prior to pupation to about 500 in females that had been deprived of food for 3 days prior to expected pupation (Figure 2B).

TABLE 1 Statistical analysis of the effect of infestation with *Brevicoryne brassicae* aphids (experiment 1), *Xanthomonas* bacterial infection (experiment 2), and plant inbreeding (experiment 3) on *Pieris brassicae* biomass and development time to pupation. Plants in experiment 1 were sometimes unintentionally co-infested with mildew or spider mites. These two factors were included as additional explanatory variables in experiment 1. Data on biomass were analysed using a mixed model ANOVA and data on development time using cox regression. Interaction terms were progressively removed from the model, if they were not significant

| Experiment	Factor	Biomass			Development time		
		F	d.f.	P	χ^2	d.f.	P
1	Aphid treatment	0.44	2,46.5	0.64	4.06	2	0.13
	Mildew	0.94	1,45.1	0.34	0.07	1	0.78
	Spider mites	0.76	1,47.2	0.38	1.85	1	0.17
2	*Xanthomonas* infection	0.59	3,43.2	0.62	4.22	3	0.23
	Block	0.14	1,15.6	0.71	10.8	1	0.001
3	Inbreeding (I)	0.07	1,99.7	0.78	0.08	1	0.76
	Family (F)	0.63	10,98.2	0.78	13.9	10	0.17
	Compartment (C)	36.7	1,99.7	<0.001	35.2	1	<0.001
	I*F	1.81	10,98.2	0.068	18.5	10	0.046
	I*C	0.28	1,99.7	0.59	0.57	1	0.44
	F*C	2.59	10,98.2	0.008	30.3	10	<0.001
	I*F*C	0.73	10,98.2	0.69			
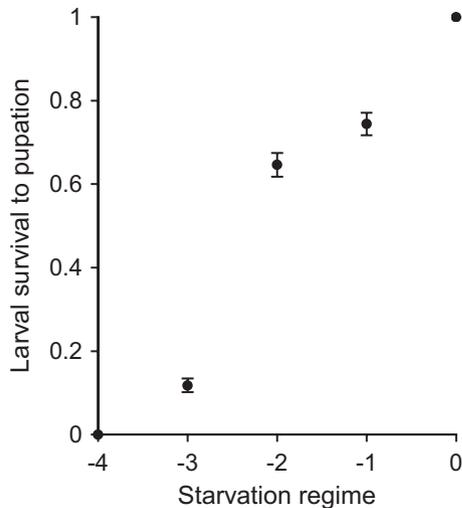
FIGURE 1 Effect of starvation regime during the final, fifth instar of *Pieris brassicae* on proportion survival to pupation. Cohorts of caterpillars were deprived of food on consecutive days following their final moult starting from day 1 following moulting (4 days before their expected pupation) to no starvation (controls). Means (± SEM) are based on model predictions. Number of pupae in each starvation treatment: day −4, n = 50; day −3, n = 390; day −2, n = 280; day −1, n = 262; day 0, n = 415.

The viability of eggs was high (on average 92.9 ± 1.3% of the larvae hatched), regardless of the starvation duration of the females ($\chi^2 = 1.48$, d.f. = 3, P = 0.68), of the males ($\chi^2 = 0.02$, d.f. = 1, P = 0.89), or their interaction ($\chi^2 = 3.04$, d.f. = 2, P = 0.22). Females lived longer with decreasing starvation duration ($F_{3,131} = 5.82$, P<0.001; Figure 2C). Starvation regime of the male mating partner did not affect longevity of the females ($F_{2,130} = 1.59$, P = 0.21).

Using linear regression, we established the relationship between adult fresh mass and pupal mass [$F_{1,31} = 155$, P<0.001; adult mass (mg) = −48 + 0.61 × pupal mass (mg), $r^2 = 0.83$], and pupal mass and forewing surface area [$F_{1,31} = 261$, P<0.001; forewing area (cm²) = 0.01 × pupal mass (mg) + 1.03, $r^2 = 0.89$]. In the plant quality experiments, we only measured pupal mass. The strong correlation between pupal mass and forewing surface area presented in Figure 3A shows that forewing surface area can serve as a proxy for adult size or mass. Based on these results we used forewing surface area as a proxy for adult biomass and established linear relationships between forewing surface area and lifetime oviposition and between forewing surface area and longevity of the female used in the last starvation replicate. Surface area correlated strongly with lifetime egg production ($F_{1,37} = 127$, P<0.001, $r^2 = 0.77$; Figure 3B), but not with longevity of the females ($F_{1,37} = 3.40$, P = 0.07, $r^2 = 0.06$; Figure 3C).

DISCUSSION

In this study, we asked 'to what extent does variation in pupal mass caused by variation in plant quality and
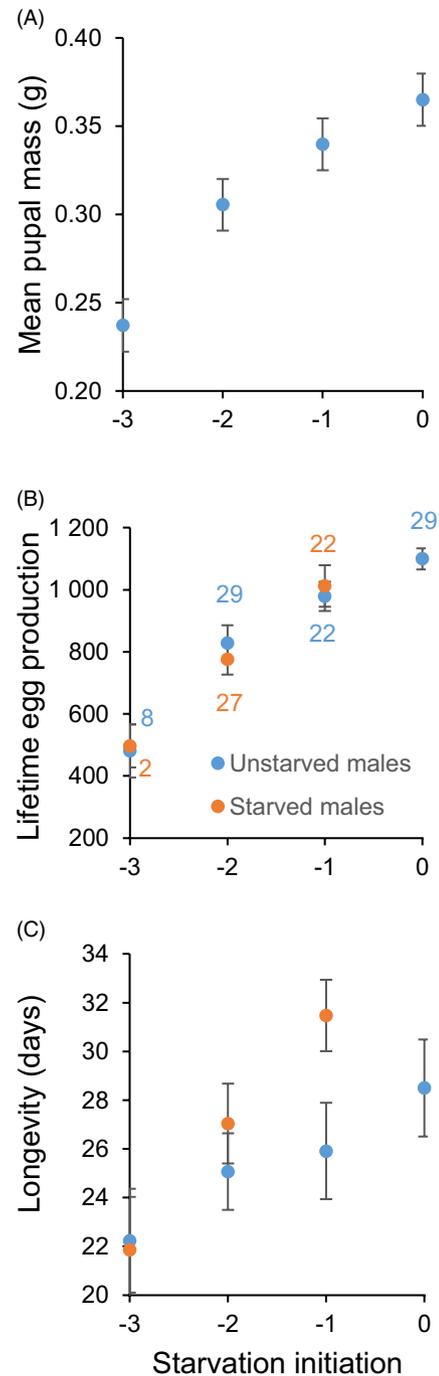

FIGURE 2 Effect of starvation regime during the final, fifth instar of *Pieris brassicae* on (A) pupal mass (g), (B) lifetime reproduction of the females (no. eggs), and (C) adult female longevity (days). Cohorts of caterpillars were deprived of food on consecutive days following their final moult starting from day 1 following moulting (4 days before their expected pupation) to no starvation (controls). There were no data for day −4 because none of the larvae survived to pupation (see Figure 1). Females were mated with a male that had been exposed as larva to the same starvation regime (starved males) or a non-starved control male (unstarved males). Means (± SEM) are based on model predictions. Total numbers of individuals per treatment are given in panel B. Note: Y-axes do not start at zero.

quantity, respectively, have ecological consequences in terms of lifetime egg production', assuming that a female is able to find suitable host plants throughout her life. We
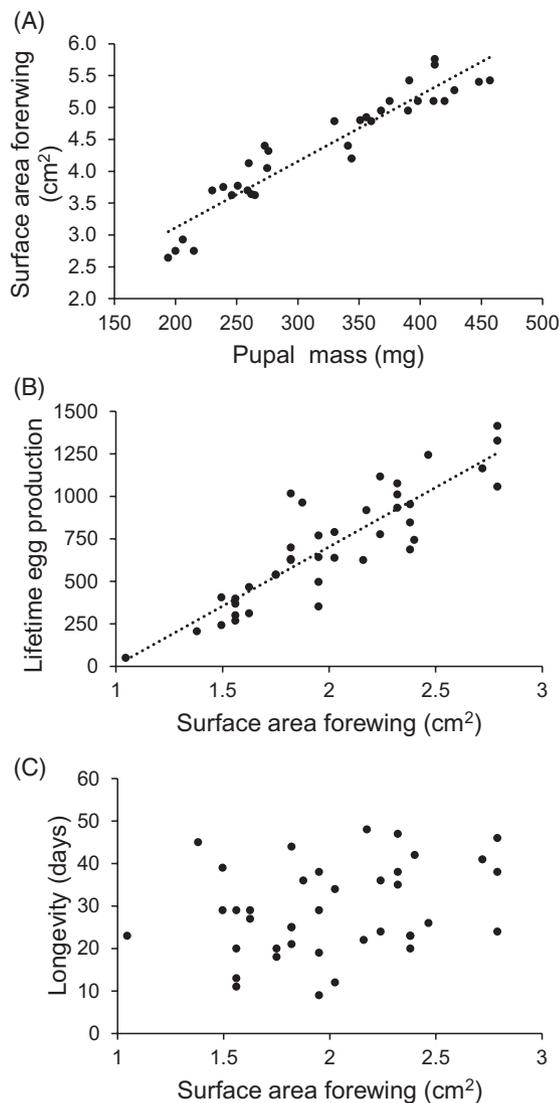
FIGURE 3 Relationships between biomass of *Pieris brassicae* butterfly females, their size (approximated by the surface area of the forewing), lifetime egg production, and longevity. (A) Pupal mass and size [linear relationship: forewing area (cm^2) = 0.01 × pupal mass (mg) + 1.03, r^2 = 0.89], (B) size and lifetime egg production [lifetime egg production = 697.8 × forewing area (cm^2) − 692.2, r^2 = 0.78], and (C) size and adult longevity. To obtain butterflies of variable sizes, they were deprived of food as caterpillar on different days following their final larval moult until pupation.

found that variation in plant quality in response to the various biotic stresses had little effect on immature development of *P. brassicae* and pupal size. In contrast, starvation of the caterpillars during the final instar had a strong effect on survival to pupation, adult mass, and lifetime egg production of the female butterflies. Male investment in offspring production was not affected by starvation regime.

Immature development of insects is considered an important life-history trait affecting fitness of an individual (Scriber & Slansky, 1981). For instance, the risk of predation or parasitism is expected to be lower in faster developing individuals ('slow-growth–high-mortality'

hypothesis; Clancy & Price, 1987; Benrey & Denno, 1997; Williams, 1999). Plant quality is an important factor determining how fast herbivorous insect larvae can develop (Scriber & Slansky, 1981). More importantly, in holometabolous insects, such as species in the Lepidoptera, resources are predominantly acquired during the larval stage and as a consequence determine the size/mass of the adult stage. There were no (aphid-exposure and plant inbreeding) or only marginal (*Xanthomonas* infection) effects of plant quality on development time. Previous studies have shown that aphid infestation can improve plant quality for some chewing herbivore species (Rodriguez-Saona et al., 2005; Soler et al., 2011; Li et al., 2014), whereas infection with plant pathogens has more variable effects on plant quality either promoting or reducing growth of chewing herbivores (Stout et al., 1999). Necrotrophic pathogens, such as *X. campestris*, often inhibit the performance of chewing herbivores, whereas biotrophic pathogens facilitate this (Lazebnik et al., 2014; Bonnet et al., 2017). Plant inbreeding depression may reduce insect resistance through changes in plant quality (Carr & Eubanks, 2014; Prill et al., 2014). Interestingly, in the plant inbreeding experiment, in which replicates were allocated to two greenhouses, caterpillar cohorts developed slower (10%) and attained lower pupal masses (7%) in one of the greenhouses. Also, caterpillar development times differed in the two blocks that were conducted over time that tested the effects of bacterial infection. This result suggests that environmental conditions differed in the two greenhouses and blocks, respectively, though photoperiod, temperature, and relative air humidity were set at the same values. Moreover, inspection of data-log files revealed no anomalies in the actual values of, e.g., temperature. Subtle differences in the conditions (e.g., light intensity/quality) between the two greenhouses/ blocks clearly affected caterpillar growth and development. The effects of uncontrolled external or internal (e.g., insect strain) factors are often ignored in laboratory studies, but can clearly influence life-history traits (Kulminski et al., 2009; Gols et al., 2019).

For insects that are food-plant specialists, such as the caterpillars of *P. brassicae* in this study, moderate changes in plant quality may have relatively small impacts on indirect fitness correlates such as development time and biomass. Natural concentrations of host-plant-specific defensive compounds (i.e., glucosinolates in the case of brassicaceous plants) appear to have little or only marginal impact on growth and development of the specialist *P. brassicae* (Smallegange et al., 2007; Gols et al., 2009; Fei et al., 2017; Chen et al., 2019). *Pieris brassicae* has evolved an efficient detoxification mechanism to circumvent the toxic effects of glucosinolate breakdown metabolites that are produced in response to feeding damage (Wittstock et al., 2004). Host quality for insect growth does not only depend on concentrations of secondary metabolites, but also on concentrations of primary metabolites such as amino acids and minerals such as phosphorous and sulphur that are often limiting in plants. Fei et al. (2017) found that both

primary and secondary chemistry in different developmental stages of *B. nigra* and *Sinapis arvensis* L. food plants correlated poorly with *P. brassicae* performance, which was only marginally affected by plant developmental stage. A similar lack of correlation between phytochemical concentrations and *P. brassicae* performance was found in *B. nigra* plants exposed to wind and rain (Chen et al., 2018, 2019). This further supports our results that small to moderate changes in plant nutritional quality in response to the biotic and abiotic environment and those related to plant ontogeny and genetic make-up have little impact on larval growth and development of the dietary specialist *P. brassicae* and, as a result, on adult biomass. Alternatively, the changes were below the threshold to noticeably affect life-history traits of the herbivore. In contrast, pupal mass of the generalist gypsy moth, *Lymantria dispar* (L.), strongly varied with the host plant on which they had been reared (fivefold difference in pupal mass), and pupal mass strongly correlated with the number of eggs produced (Hough & Pimentel, 1978). Host plant quality for insect herbivores is more variable among plant species belonging to different families at least in term of plant secondary metabolites (Schoonhoven et al., 2005). Consequently, polyphagous insect herbivores feeding on plant species in different families are expected to experience more variation in plant quality affecting egg production compared with host specialists such as *P. brassicae* (Hough & Pimentel, 1978; Razmjou et al., 2014; Borzoui et al., 2018).

Fecundity in insects and ectotherms in general has been shown to correlate positively with body size (Honěk, 1993; Bauerfeind & Fischer, 2008; Kingsolver & Huey, 2008). We found that female butterflies emerging from pupae weighing 350 mg or more produced on average between 1000 and 1200 eggs in their life when continuously provided with a suitable substrate for egg laying and ad libitum availability of sugar and water. Differences in mean pupal mass in the plant quality experiments were relatively small; mean pupal mass varied between 360 and 400 mg, which is well within the range of *P. brassicae* pupal masses previously reported when the caterpillars had developed on *B. nigra* or on other brassicaceous plant species (Gols et al., 2008; Pashalidou et al., 2015; Fei et al., 2017). We did not measure fecundity of the butterflies in the experiments in which we determined the effects of differences in plant quality on pupal biomass. It is conceivable that butterflies eclosed from pupae with similar masses grown on plants exposed to biotic stress show differences in fecundity caused by qualitative nutritional differences affecting other life-history traits such as mating success and egg size (e.g., Moreau et al., 2006). Alternatively, the changes in nutritional quality caused by the various plant treatments in this study were too small to significantly affect growth and development of this specialist herbivore species.

Pieris brassicae caterpillars feed in aggregation and feed on relatively small herbaceous plants. Running out of food during this critical life stage is a potential risk for individuals of this species. Immature insect stages need to acquire sufficient nutrients and biomass before they can pupate and develop into an adult. This study and a previous one by Fei et al. (2016) show that final instars do not pupate if they are deprived of food during the 1st day after their final moult. Moreover, delayed starvation reduced the risk of premature death, and pupal biomass of surviving individuals was higher the later the larvae had been deprived of food. Most importantly, pupal mass and adult biomass strongly correlated with lifetime egg production. Compared to non-starved females, lifetime egg production was reduced by 50% in females that were starved 3 days before expected pupation. Similar patterns were found for starved *Speyeria mormonia* (Boisduval) caterpillars (Boggs & Freeman, 2005). Interestingly, larval hatching rate, which was >90%, was not affected by larval starvation regime. This result suggests that egg quality is retained at the cost of laying fewer eggs. Moreover, larval starvation did not reduce longevity of the females. This result contrasts with that found for two other lepidopteran species, *Plutella xylostella* (L.) and *S. mormonia*, of which larger individuals lived longer than smaller ones (Shirai, 1995; Boggs & Freeman, 2005). In our study, high reproduction by larger females may have reduced longevity (reproduction–longevity trade-off; Stearns, 1989; Roff, 2002).

The results of this research show that running out of food in the final instar has strong negative implications for lifetime fecundity of the dietary specialist *P. brassicae*. By far, the consequences of larval starvation on adult size exceed those resulting from variation in plant quality. Studies that investigate the effects of plant quality or resistance on performance often determine larval development time, growth, or biomass as proxies for insect fitness (Awmack & Leather, 2002). For obvious reasons, relatively few studies have investigated the relationship between biomass acquired during larval development and fitness of the adults, i.e., offspring production. If the effect sizes of plant quality on larval performance are relatively small, as is often found for well-adapted dietary specialists, their ecological significance on adult fitness may be overestimated, and requires that fitness consequences in terms of offspring production are quantified empirically rather than assumed. For herbivorous insects of which the immature stages feed gregariously on ephemeral plants, females are challenged not only to find suitable host plants for their offspring, but also to find plant patches that sustain growth of a single egg clutch.

AUTHOR CONTRIBUTIONS

Rieta Gols: Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (lead); supervision (lead); writing – original draft (lead); writing – review and editing (lead). Luuk Croijmans: Investigation (lead); writing – review and editing (supporting). Marcel Dicke: Resources (lead); writing – review and editing (supporting). Joop J.A. van Loon: Investigation (supporting); writing – review and editing (supporting). Jeff A. Harvey: Conceptualization (supporting); methodology (supporting); writing – review and editing (supporting).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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