

## Tolerance of an aphid species towards dietary sucrose-to-amino acid ratios and recovery from suboptimal nutrition

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### Abstract

Aphids feed on the phloem sap of their host plants. The chemical composition of this sap differs between plant species and is modulated by environmental factors. To understand why *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is able to infest various plant taxa, we investigated how aphids of this species respond to various sucrose-to-amino acid ratios of their diet. Moreover, we studied whether they are able to recover from periods with suboptimal nutrition. Preference and performance bioassays were performed using artificial diets with sucrose-to-amino acid ratios of 2.4:1 (optimal 'control' diet), 4.8:1 ('high sucrose'), 1.2:1 ('high amino acids'), or 1:0 ('no amino acids'). Also, the capacity to recover from periods on suboptimal diet (i.e., the 'no amino acids' diet) was assessed. In four-choice assays, both nymphs and adults of *M. persicae* were rarely found on the 'no amino acids' diet and they were similarly distributed on the other diets. As long as amino acids were available, the sucrose-to-amino acid ratio had only minor effects on aphid development and reproduction. On the suboptimal diet, nymphs survived, but with almost no weight gain over time. After transfer to the 'control' diet, they gained weight and reproduced with only low fitness deficits compared to aphids kept on the 'control' diet for the whole time. The capacity to survive under suboptimal nutrition and recover from it was dependent on the length of the period on the suboptimal diet. The ability to cope with different dietary sucrose-to-amino acid ratios and to withstand suboptimal nutrition for several days may contribute to the broad host plant spectrum of *M. persicae* and may explain why this species is a serious pest in agricultural systems.

### Introduction

Several aphid species are serious agricultural pests. They reproduce parthenogenetically as viviparous females at least during parts of their life cycle and exhibit short generation times, leading to rapid population growth. Moreover, they negatively affect plant performance via withdrawal of phloem sap, transmission of phytopathogenic viruses, and release of honeydew that may be colonized by fungi and reduces light transmission (Guerrieri & Digilio, 2008). Aphids specifically target and consume the phloem sap of their host plants using specialized piercing-sucking mouthparts (Guerrieri & Digilio, 2008). However, plant phloem sap is a challenging diet, mostly

due to its unfavorable composition of carbohydrates in relation to amino acids (Douglas, 2006).

Aphids generally rely on nitrogen-containing amino acids, which only occur in low concentrations in phloem sap. Moreover, the ratio of essential to non-essential amino acids of this sap is often unfavorable (Douglas, 2006). In contrast, sucrose is usually highly concentrated in the phloem sap. Aphids use this carbohydrate as a carbon source and for respiration (Rhodes et al., 1996), but it imposes osmotic challenges due to its high concentration (Douglas, 2006). Mechanisms of nutrient regulation by herbivorous insects, including aphids (Behmer, 2009), may explain how aphids balance the costs of excess uptake of sucrose to assimilate amino acids that are available in limiting concentrations. Indeed, aphids evolved several adaptations to exploit plant phloem sap as a food source. They harbor endosymbionts that assist in the biosynthesis

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of (essential) amino acids (Sandström & Moran, 1999; Gündüz & Douglas, 2009). Moreover, aphids possess osmoregulatory mechanisms to reduce the osmotic pressure of the ingested diet. They show gut sucrase activity, form larger oligosaccharides, and excrete excess carbohydrates via honeydew (Fisher et al., 1984; Karley et al., 2005; Douglas, 2006). Furthermore, aphids ingest plant xylem sap, which has a lower osmotic pressure than phloem sap, under certain conditions (Pompon et al., 2011). Thus, the sucrose-to-amino acid ratio of the phloem sap is a very important factor that may influence the aphid's physiology and performance.

The chemical composition of plant phloem sap is highly variable across space and time at multiple scales, imposing additional challenges on aphids (Douglas, 2006). It differs between plant taxa (Wilkinson & Douglas, 2003; Nadwodnik & Lohaus, 2008) and plant parts (Jakobs & Müller, 2018) and is affected by the developmental stage of the plant (Karley et al., 2002) as well as by environmental factors, for example, salinity of soil water (Pommerrenig et al., 2007). In response to aphid feeding, the phloem sap composition can be modified in terms of plant defenses and/or nutrient levels (Giordanengo et al., 2010; Elzinga et al., 2014; Cao et al., 2016; Jakobs et al., 2019), leading to varying chemical compositions with time of infestation or aphid density. These factors contribute to both spatial and temporal differences in aphid development and reproduction (van Emden et al., 1969; Karley et al., 2002; Jakobs et al., 2019). Indeed, using artificial diets, it was reported that the chemical composition of the diet affects aphid preference and/or performance (e.g., Dadd & Mittler, 1965; Hewer et al., 2010) and dietary needs are species-specific (Krieger, 1971). There is also strong evidence that the chemical composition of natural plant phloem sap influences aphids (e.g., Ponder et al., 2000; Karley et al., 2002).

Polyphagous aphid species infest several host plant taxa and thus encounter diverse chemical profiles in their phloem diet, with which they need to cope. Especially nymphs can encounter phloem sap of low quality, if no better plant (part) is available when they are deposited or if the phloem sap quality changes rapidly. Due to their low mobility, nymphs can hardly escape such situations. To our knowledge, it has not yet been investigated whether nymphs of polyphagous aphid species can cope with diets of low nutritional quality and recover from such periods later on. Thus, the objectives of the current study were to investigate: (1) how the sucrose-to-amino acid ratio of an artificial diet affects the choice, survival, development, and fitness of a polyphagous aphid species, and (2) whether nymphs are able to survive on suboptimal diet and recover from this, depending on the length of the period on the

suboptimal diet. The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), was used as study species, because the basic nutritional requirements of this species are known and it can be reared on artificial diets allowing dietary manipulations (Dadd & Mittler, 1966). Moreover, as a serious pest on many crops worldwide, this species is of high economic importance (van Emden et al., 1969). We hypothesized that, in a choice assay, *M. persicae* aphids mainly settle on diets with amino acids and with low sucrose-to-amino acid ratios. Moreover, we expected that they can quite well develop on diets with varying sucrose-to-amino acid ratios. Furthermore, we hypothesized that nymphs can survive for several days on suboptimal diet and recover from such periods without fitness losses, but that this capacity depends on the length of the period on the suboptimal diet. These expectations were based on the fact that this species successfully infests a broad range of host plant species that largely differ in the chemical composition of the phloem sap. The present study improves our general understanding of the capacities of *M. persicae* to cope with varying diets and of factors explaining its immense pest potential.

## Materials and methods

### Aphid species

*Myzus persicae* were collected from infested *Brassica rapa* L. ssp. *pekinensis* (Brassicaceae) plants in a greenhouse. Subsequently, they were reared for several generations at room temperature and L16:D8 photoperiod on *Plantago lanceolata* L. (Plantaginaceae) plants of various ages grown in a 1:1 mixture of river sand and soil (Fruhstorfer Pikiererde; Hawita Group, Vechta, Germany). The genetic variability within this aphid population is not known. Plants were regularly exchanged to avoid the production of alate (winged) aphids. Under the conditions described, populations consisted of parthenogenetically reproducing viviparous females, as it is the case during spring and summer in temperate regions. For the experiments, apterous (unwinged) morphs that were randomly taken from the plants were used.

### Artificial diet

The experiments with *M. persicae* were done using an artificial diet, which is suitable to rear this aphid species for several generations. It contained sucrose (438 mmol l<sup>-1</sup>), 20 amino acids (total concentration: 181 mmol l<sup>-1</sup>), vitamins and vitamin-like compounds, KH<sub>2</sub>PO<sub>4</sub>, MgCl<sub>2</sub>, and trace elements (Fe, Zn, Mn, and Cu, provided as Na-EDTA chelates) in Millipore-H<sub>2</sub>O (Dadd & Mittler, 1966). Compounds were obtained from Merck (Darmstadt, Germany), Sigma-Aldrich (Steinheim, Germany), AppliChem

(Darmstadt, Germany), Adob (Poznań, Poland), Alfa Aesar (Karlsruhe, Germany), Serva Feinbiochemica (Heidelberg, Germany), Roth (Karlsruhe, Germany), and SAFC (St. Louis, MO, USA). This optimal 'control' diet had a molar sucrose-to-amino acid ratio of 2.4:1 (mol: mol). In addition, diets with ratios of 4.8:1 ('high sucrose'; double sucrose concentration compared to the 'control' diet), 1.2:1 ('high amino acids'; double amino acid concentration), and 1:0 (no amino acids) were prepared, with all other compounds kept constant. The relative composition of the amino acids was the same for all diets. This artificial diet had been optimized for *M. persicae* (Dadd & Mittler, 1966); thus, its composition differs from plant phloem sap. However, the concentrations of sucrose and amino acids as well as the sucrose-to-amino acid ratios of the first three diets are within the range of values found in the phloem sap of plants (Sandström & Pettersson, 1994; Wilkinson & Douglas, 2003; Douglas et al., 2006; Nadwodnik & Lohaus, 2008; Gündüz & Douglas, 2009; Lohaus & Schwerdtfeger, 2014). The 'no amino acids' diet seems to be unnatural, as it is unlikely that plant phloem sap is completely devoid of amino acids. However, very low availability of amino acids may occur, for example, when plants are highly nutrient-limited, free amino acids are rapidly unloaded from the phloem, or when other factors reduce the availability of free amino acids. The 'no amino acids' diet is thus suited to mimic a situation where the dietary supply is suboptimal, allowing conservative estimates of the potential of aphids to deal with such a situation. All diets were adjusted to pH = 7 with KOH and HCl and aliquots were stored at  $-80^{\circ}\text{C}$ .

#### Preference and performance bioassays: general methods

Preference (four-choice) as well as performance assays on the diets described above were performed modified after Stolpe & Müller (2016). Diets were offered between two Parafilm (Bemis Company, Neenah, WI, USA) layers that were stretched over the top of plastic rings. In preference assays, all diets (1 ml each) were simultaneously offered within the same ring (7.3 cm diameter) in four sachets of the same size; these sachets were prepared by pressing a cross into the Parafilm layers with a ruler. Performance assays, in which only one diet per ring was applied, were done in small (1.6 cm diameter; 80  $\mu\text{l}$  diet per sachet) and middle-sized (4.4 cm diameter; 800  $\mu\text{l}$  diet) cylinders for assays with single individuals and groups of aphids, respectively. The aphids were put directly on the bottom side of the sachet(s) and could feed on the diet from below. Green paper was placed on the top of the sachets to imitate leaves. The rings were put into Petri dishes. We call the whole setup, consisting of a ring

with Parafilm sachet(s) with diet(s), green paper, and the Petri dish, a 'cage' in the following. Within each assay, the orientation of sachets (preference assays) and positions of cages (performance assays) were randomized. Experiments were done under ambient conditions in the laboratory or in a climate cabinet (20  $^{\circ}\text{C}$ , 60% r.h., L16:D8 photoperiod; Percival Intel-lus Control System, CLF PlantClimatics, Emersacker, Germany). At least twice a week, cages with fresh diets were prepared and aphids were carefully transferred to the new sachets with a wettish brush. Dead individuals and exuviae were removed at least every other day and rings and Petri dishes were cleaned with tap water if necessary. To avoid density effects, nymphs produced by adults during the preference assays with groups of adults and by single individuals in the performance assays were removed at several time points (at 2, 4, 6, 8, 23, 25, and 27 h) and daily, respectively. In a few cases, individuals escaped or were killed by accident, that is, right-censoring occurred (see handling of right-censored data below). The assays with single individuals were terminated when all individuals had died.

#### Preference bioassays

Preference assays were done with groups of 10 nymphs (0–24 h old) or 10 adults ( $n = 12$  cages per experiment). Individuals were placed in the center between the four dietary sachets and aphids on the sachets were counted after 2, 8, and 29 h. At several time points (at 2, 4, 6, 8, 23, 25, and 27 h), dead individuals were replaced at the position where they were found by aphids kept on the same diet in additional cages and individuals that had escaped were replaced by individuals from randomly chosen additional cages at random positions.

#### Performance bioassays

For the performance assays, cages with 10 adult aphids ( $n = 15$ ) were prepared for each diet. Nymphs that were born within the first 24 h were removed and population dynamics in the 'population' cages were assessed by counting adults and nymphs every other day for 17 days ('population dynamics' experiment). One of the nymphs per cage that were removed within the first 24 h was used (same diet) for the assay series with single individuals in 'single' cages ( $n = 15$ ; 'individual performance' experiment). For these, survival, body weight, and number of nymphs were monitored daily. Body weight was assessed until first reproduction using a precision balance (ME36S; Sartorius, Göttingen, Germany); aphids were carefully taken from the diet using a wettish brush, weighed, and directly transferred back to the dietary sachet in the cage or in a new

cage with fresh diet (see above). Adult body weight was defined as the body weight at the day before the first reproduction. Generation time was defined as the time until first reproduction.

#### Measurements of aphid recovery from periods on suboptimal diet

To test whether *M. persicae* recovers from periods on suboptimal diet, single nymphs, which were produced by adults within 24 h on the respective diet, were either reared in small cages on the ‘control’ diet or on suboptimal diet (i.e., the ‘no amino acids’ diet) for four time periods (3, 6, 8, or 14 days) and then transferred to the ‘control’ diet ( $n = 20$  per diet combination treatment). The performance traits described above were monitored daily. As a proxy for the fitness of the individual aphids, the nymphs produced by these aphids were collected daily, placed into separate middle-sized cages (one cage for all offspring from one individual) with ‘control’ diet, and the size of the resulting offspring population was assessed daily for 49 days. To avoid space limitations, additional cages were used if population size exceeded 70 individuals, that is, populations were split and individuals put into two new cages.

#### Statistical analysis

Unless otherwise stated, all analyses were done with R v.3.4.2, 3.5.3, and 3.6.2 (R Core Team, 2017, 2019; packages *stats*, *survival*, *survminer*, *car*, *pgirmess*, and *multcompview*) using a significance threshold of  $\alpha = 0.05$ . The proportions of aphids (groups of nymphs or adults) on the sachets with diets in the four-choice preference assays were compared within each time point with Friedman rank-sum tests, followed by Wilcoxon signed-rank tests with Bonferroni–Holm correction of P-values. Only aphids that had settled on one of the sachets were included. For survival data collected in the performance assays, Kaplan–Meier curves were plotted including censored individuals and treatments were compared using overall log-rank tests followed by post hoc pairwise log-rank tests with Bonferroni–Holm correction. Adult body weight, generation time, number of nymphs, population size after 17 days, and offspring population size after 49 days were compared between treatments, including only treatment groups with  $n > 3$  data points. One-way ANOVA followed by Tukey’s honestly significant difference (HSD) tests were used for raw or  $\log_{10}$ -transformed data that showed normality of residuals and homoscedasticity (assessed by Shapiro–Wilk test, Levene test, and model plot diagnostics) ( $\log_{10}$ -transformed: data on generation time in the ‘individual performance’ experiment and population size after 17 days in the ‘population dynamics’ experiment of the performance assays as well as

data on adult body weight in the recovery experiment). Otherwise, Kruskal–Wallis tests followed by Kruskal–mc tests were applied. Adult body weight and generation time could only be assessed when individuals were not censored and did not die before the first reproduction. For the number of nymphs and offspring population size, data from censored individuals were not included.

## Results

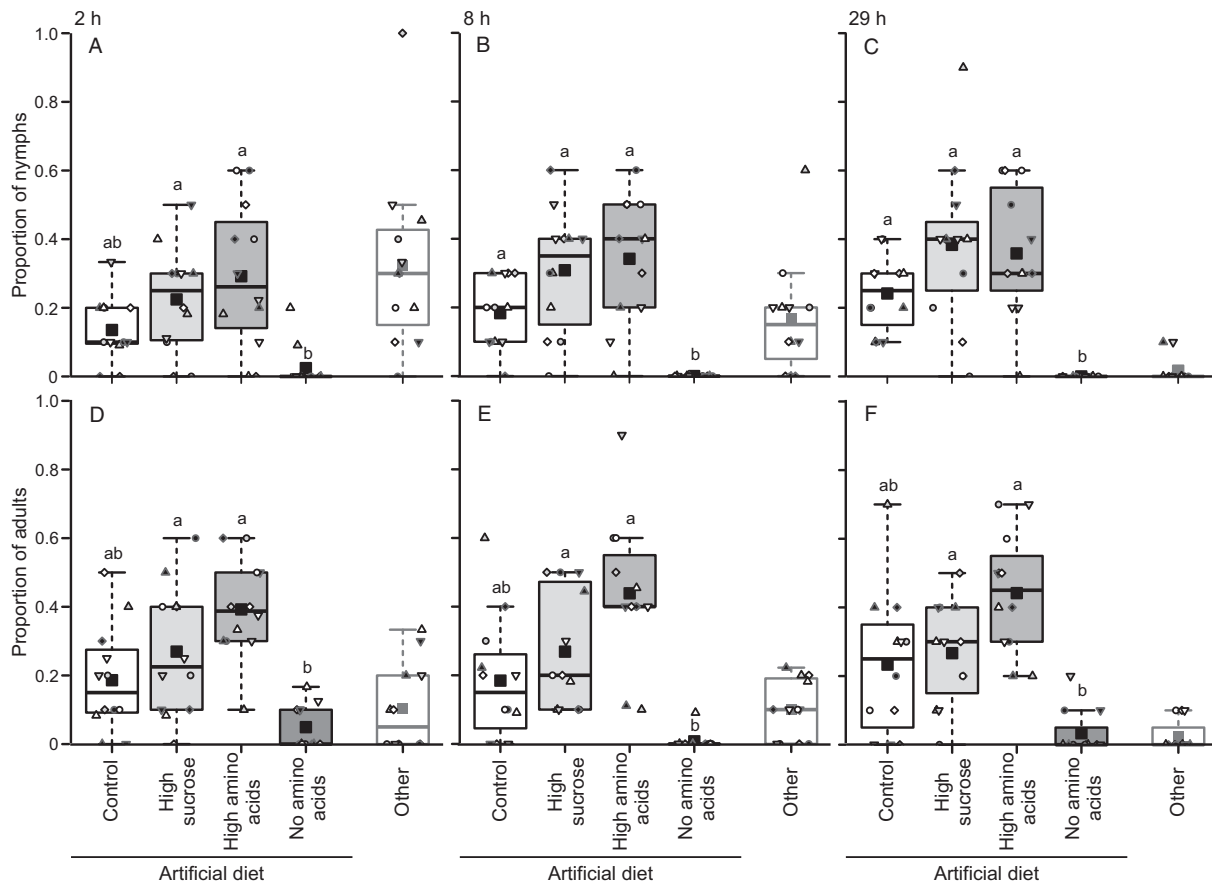
#### Aphid preferences for four diets

In the four-choice preference assays, both nymphs (Figure 1A–C) and adults (Figure 1D–F) were rarely found on the ‘no amino acids’ diet, and there were no differences in the proportions of aphids on the other three diets (Friedman rank-sum tests; nymphs: 2 h,  $\chi^2 = 17.2$ ; 8 h,  $\chi^2 = 21.1$ ; 29 h,  $\chi^2 = 22.7$ ; adults: 2 h,  $\chi^2 = 18.6$ ; 8 h,  $\chi^2 = 23.3$ ; 29 h,  $\chi^2 = 19.1$ , all d.f. = 3 and  $P < 0.001$ ; Figure 1). These patterns stayed similar over time (Figure 1). Most aphids had settled on one of the dietary sachets when their distribution was assessed, particularly at the final scoring.

#### Aphid performance on four diets

In the ‘individual performance’ experiment, aphid survival was lower on the ‘no amino acids’ diet compared to the other three diets (log-rank test:  $\chi^2 = 31.9$ , d.f. = 3,  $P < 0.001$ ; Figure 2A). None of the aphids on the ‘no amino acids’ diet survived longer than 32 days, whereas the last aphids on the other diets died ca. 50 days after onset of the experiment (Figure 2A). The weight of the nymphs on the ‘no amino acids’ diet only slightly increased and they did not deposit any offspring. Thus, the adult body weight (ANOVA:  $F_{2,33} = 2.10$ ,  $P = 0.14$ ; Figure 2C) and generation time ( $F_{2,33} = 1.83$ ,  $P = 0.18$ ; Figure 2E) could only be determined for aphids on the other three diets, between which these parameters did not differ. Adult weights ranged from 178 to 333  $\mu\text{g}$  (Figure 2C). The first nymphs were born after 9 days and most of the individuals deposited their first nymph within 15 days (Figure 2E). Single aphids deposited up to 39 nymphs (Figure 2G) and the number of offspring on the ‘no amino acids’ diet was lower than that on the other diets (Kruskal–Wallis test:  $\chi^2 = 26.6$ , d.f. = 3,  $P < 0.001$ ; Figure 2G).

In the ‘population dynamics’ experiment, which started with 10 adults per cage, no population went extinct within 17 days. The nymphs that were deposited on the diets with amino acids became adults and deposited offspring, leading to exponential population growth in most cages and final population sizes of up to 218 individuals after 17 days (Figure 3A). In contrast, although nymphs were deposited on the ‘no amino acids’ diet, these did not reproduce and



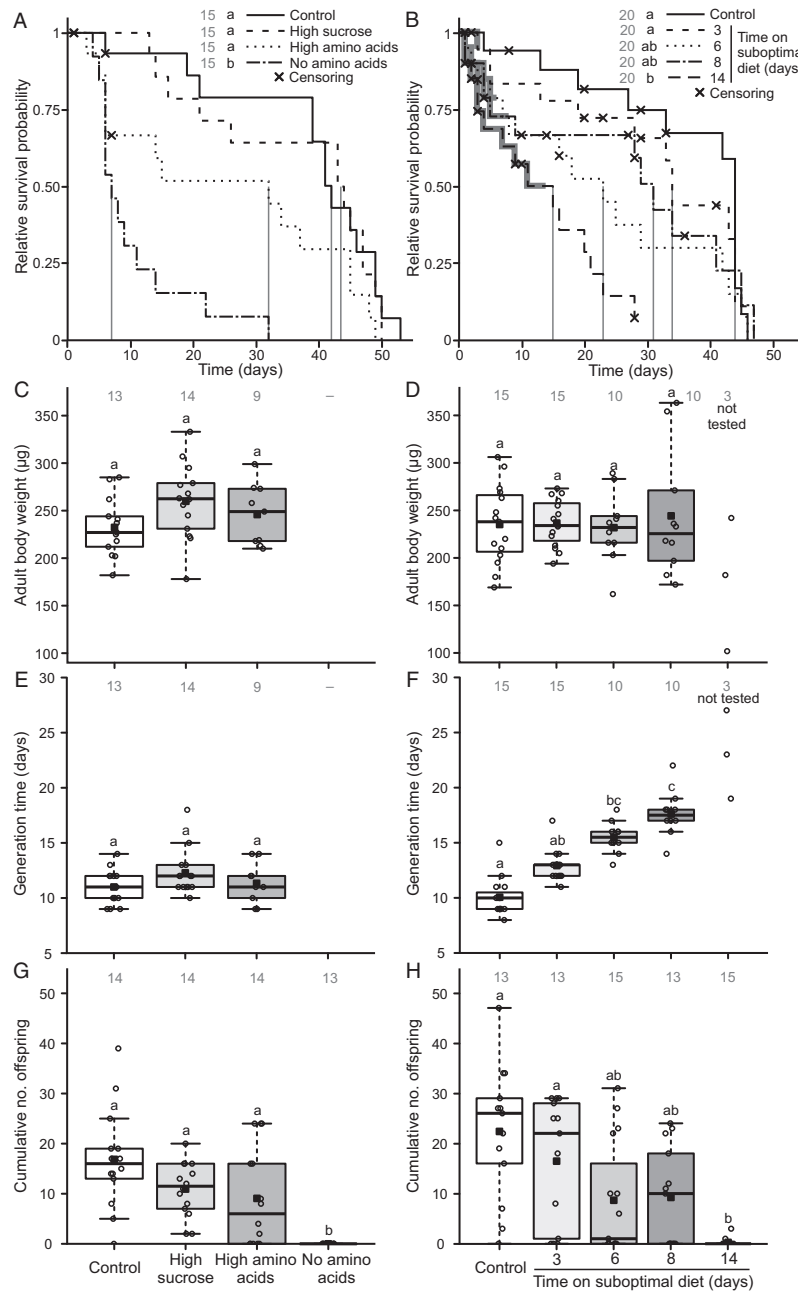
**Figure 1** Distribution of *Myzus persicae* (A–C) nymphs and (D–F) adults on artificial diet sachets in four-choice preference assays after (A, D) 2 h, (B, E) 8 h, and (C, F) 29 h. Each cage contained 10 aphids ( $n = 12$ ). Data are shown as box-whisker plots, with medians as horizontal lines, means as black squares, interquartile ranges (IQR) as boxes, and whiskers extending to the most extreme data points within  $1.5 \times$  IQR. Raw data points are shown as smaller symbols, with symbols of the same type representing data points belonging to the same four-choice cage. The group ‘other’ represents aphids that had not settled on any of the dietary sachets. Different letters capping different treatments indicate significant differences among medians (pairwise Wilcoxon signed-rank tests with Bonferroni–Holm correction:  $P < 0.05$ ).

individuals died earlier, leading to lower population sizes on the ‘no amino acids’ diet compared to the other diets after 17 days ( $F_{3,56} = 33.39$ ,  $P < 0.001$ ; Figure 3A).

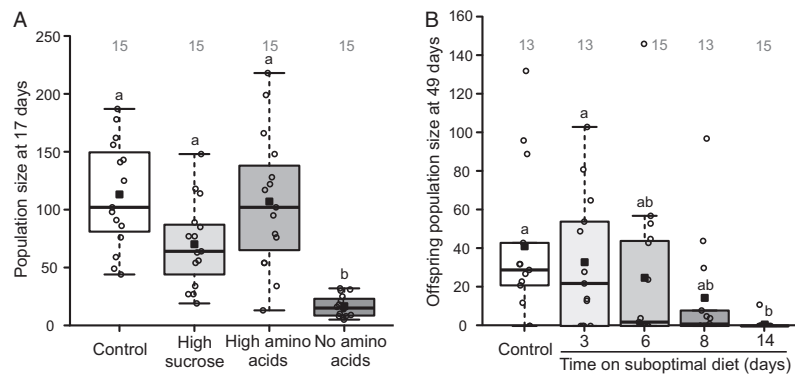
#### Aphid recovery from periods on suboptimal diet

In the recovery experiment, aphid survival was lower when they were kept for 14 days on suboptimal (‘no amino acids’) diet before transfer to the ‘control’ diet, compared to aphids that were not or only for 3 days on suboptimal diet (log-rank test:  $\chi^2 = 20.4$ , d.f. = 4,  $P < 0.001$ ; Figure 2B). During the time on the ‘no amino acids’ diet, nymphs gained much less weight compared to those on the ‘control’ diet, but after their transfer to the ‘control’ diet, they developed similarly (data not shown). Indeed,

aphids on the ‘control’ diet and those that were on the suboptimal diet for up to 8 days had comparable adult body weights (ANOVA:  $F_{3,46} = 0.08$ ,  $P = 0.97$ ; Figure 2D). The generation times strongly increased with the duration the aphids were kept on the suboptimal diet (Kruskal–Wallis test:  $\chi^2 = 38.2$ , d.f. = 3,  $P < 0.001$ ; Figure 2F). The number of offspring per individual and the overall sizes of the resulting offspring populations at 49 days ( $\chi^2 = 25.1$  and 19.6, both d.f. = 4,  $P < 0.001$ ) were lower when aphids experienced suboptimal diet for 14 days compared to aphids on ‘control’ diet and those that were reared on suboptimal diet for 3 days, whereas values for aphids that were kept on the suboptimal diet for 6 or 8 days were in between (Figures 2H and 3B).



**Figure 2** Performance of *Myzus persicae* aphids on (A, C, E, G) four artificial diets ( $n = 15$ ) and (B, D, F, H) optimal ‘control’ diet after spending time on suboptimal (‘no amino acids’) diet (i.e., recovery) ( $n = 20$ ). (A, B) Kaplan–Meier survival plots with crosses indicating right-censoring (i.e., individuals escaped or were killed by accident and thus survival data were only included for these individuals until the censoring event), vertical gray lines depicting time points at 50% survival, and (for B only) thick gray lines highlighting periods on suboptimal diet. (C, D) Adult body weights (i.e., fresh weights at the day before the first reproduction). (E, F) Generation times (i.e., time until first reproduction). (G, H) Cumulative number of offspring per aphid individual in experiments with single individuals. (C–H) Data are given as box-whisker plots, with medians as horizontal lines, means as black squares, interquartile ranges (IQR) as boxes, and whiskers extending to the most extreme data points within  $1.5 \times$  IQR. Raw data points are shown as smaller symbols. Gray numbers at the top of each plot represent sample sizes. Different letters capping different treatments indicate significant differences among me(di)ans [(A, B) Pairwise log-rank tests with Bonferroni–Holm correction, (C–E) Tukey’s HSD tests, (F–H) Kruskal–mc tests:  $P < 0.05$ ]. Sample sizes were reduced due to death before reproduction and/or due to censoring events.



**Figure 3** Population sizes of *Myzus persicae* aphids (A) on day 17 on four artificial diets ( $n = 15$ ; ‘population dynamics’ experiment, started with 10 adults) and (B) offspring population sizes on day 49 on optimal ‘control’ diet after spending time on suboptimal (‘no amino acids’) diet (i.e., recovery) ( $n < 20$ , as censored individuals were not included). Data are given as box-whisker plots, with medians as horizontal lines, means as black squares, interquartile ranges (IQR) as boxes, and whiskers extending to the most extreme data points within  $1.5 \times$  IQR. Raw data points are shown as smaller symbols. Gray numbers at the top of each plot represent sample sizes. Different letters capping different treatments indicate significant differences among means [(A) Tukey’s HSD test, (B) Kruskal-mc test:  $P < 0.05$ ].

## Discussion

In this study, we show that *M. persicae* aphids mainly settle on diets containing amino acids and are able to tolerate a broad range of dietary sucrose-to-amino acid ratios. Moreover, nymphs recovered from periods of up to 8 days on suboptimal (‘no amino acids’) diet with only minor fitness losses.

Our hypothesis that *M. persicae* rather settle on amino acid-containing diets than on a diet lacking amino acids, and that they settle on diets with lower sucrose-to-amino acid ratios, but develop successfully on diets with diverse sucrose-to-amino acid ratios was supported partially. Indeed, aphids were rarely found on the ‘no amino acids’ diet, but they were similarly distributed on the other diets. Likewise, in other studies, more *M. persicae* were found on diets with sucrose and amino acids than on diets with only sucrose (Mittler & Dadd, 1964; Mittler, 1967b). Besides differences in viscosity and osmotic pressure between the diets that may affect choice behavior (Hewer et al., 2010), aphids probably sense sucrose and amino acids. Indeed, several genes presumably encoding receptors involved in gustatory chemosensation, including sugar receptors, were found in different aphid species (Smadja et al., 2009; Kang et al., 2018; Robertson et al., 2019). Sucrose is crucial to induce and sustain feeding of various aphid species (Douglas et al., 2006; Hewer et al., 2010), whereas certain amino acids such as methionine, cysteine, and histidine are phagostimulatory for *M. persicae* (Mittler, 1970). The uptake of diets differing in concentrations of sucrose or amino acids by *M. persicae* follows optimum

curves and most amino acids enhance the acceptability of sucrose (Mittler, 1967a,b). Surprisingly, the distribution of aphids in our assays was similar on all three diets containing amino acids with various sucrose-to-amino acid ratios. This indicates that the amino acid concentration was sufficient for aphid physiological processes, probably due to endosymbionts assisting in the production of (essential) amino acids (Sandström & Moran, 1999; Gündüz & Douglas, 2009).

The performance of *M. persicae* on the ‘no amino acids’ diet was poor. Nymphs only slightly gained weight without reaching the adult stage and adults in the ‘population dynamics’ experiment only deposited few nymphs, similar as reported previously (Dadd & Mittler, 1965). In our study, nymphs probably fed somewhat on the ‘no amino acids’ diet, as they had mostly settled, produced some honeydew, and would probably have died much earlier without any food or water. However, feeding rates may have been reduced due to the lack of phagostimulatory amino acids (Mittler, 1970). Some of the amino acids that are considered to be essential for aphids (Douglas, 2006) are delivered by their endosymbionts (Sandström & Moran, 1999; Gündüz & Douglas, 2009). Only methionine, histidine, and isoleucine seem to be essential for symbiont-containing *M. persicae* (Dadd & Krieger, 1968). However, the symbionts also need metabolic precursors for the production of (essential) amino acids (Sandström & Moran, 1999; Douglas, 2006; Gündüz & Douglas, 2009; Price et al., 2014). Thus, symbionts associated with the aphids in our experiment probably could not meet the amino acid demands of their aphid hosts on the ‘no amino acids’ diet. There are several mechanisms that may have

allowed aphid survival, but constrained aphid growth on the diet lacking amino acids. On this diet, aphid metabolic activity was probably low, as many processes rely on nitrogen-containing compounds. Besides sucrose (Rhodes et al., 1996), aphids can also use amino acids (e.g., Wilkinson et al., 2001) as respiratory substrates. Due to the lack of amino acids in the ‘no amino acids’ diet, aphids probably rather used sucrose for respiration. Furthermore, endogenous proteins were likely degraded to amino acids. It is also possible that a resorption and/or arrest of embryos occurred, as reported for starved adult *Megoura viciae* Buckton (Ward & Dixon, 1982).

In contrast, on the three diets containing amino acids, aphids developed similarly. Aphid fecundity was possibly largely determined by the plants from which the mother aphids were taken due to telescoping generations. However, the lack of effects of the diet treatments on aphid survival and development was unexpected, because these parameters should be less dependent on the conditions the mother individuals experienced and be more affected by current nutritional factors. Although the composition of the diets had been optimized for *M. persicae* rearing (Dadd & Mittler, 1966), the sucrose and amino acid concentrations of these diets as well as the sucrose-to-amino acid ratios are within the ranges naturally observed in phloem sap of diverse plant species (Sandström & Pettersson, 1994; Wilkinson & Douglas, 2003; Douglas et al., 2006; Nadwodnik & Lohaus, 2008; Gündüz & Douglas, 2009; Lohaus & Schwerdtfeger, 2014). Thus, polyphagous *M. persicae* probably encounter similar diets under natural conditions. For *Acyrtosiphon pisum* Harris, a feeding specialist on Fabaceae, sustained feeding required a minimum sucrose concentration, whereas high sucrose levels led to failure of osmoregulation (Douglas et al., 2006). This species showed highest fecundities at 500–750 mmol l<sup>-1</sup> sucrose. The sucrose concentrations in our study (438 or 876 mmol l<sup>-1</sup>) were similar and clearly in a range allowing successful development and reproduction of *M. persicae*. Besides sucrose, the concentrations of amino acids are relevant for aphid performance. Aphids feed more and develop and/or reproduce better when more nitrogen is available in soluble form, for example, amino acids in phloem sap (van Emden et al., 1969; Ponder et al., 2000; Nowak & Komor, 2010), sometimes even when defensive compounds are present at higher concentrations as well (Cao et al., 2018). In addition, sucrose and amino acids can interactively affect aphids. Sucrose concentrations in plant phloem sap usually exceed aphid requirements, whereas amino acids are limiting (Douglas, 2006). Thus, aphids may ingest phloem sap until their amino acid pools are replenished and have to cope with the excess sucrose. Osmoregulation can be realized physiologically, for

example, by conversion of sucrose to larger oligosaccharides and excretion (Fisher et al., 1984; Karley et al., 2005; Douglas, 2006), or behaviorally, for example, by feeding on xylem sap (Pompon et al., 2011). The similar development and reproduction of *M. persicae* on diets with sucrose-to-amino acid ratios ranging from 1.2 to 4.8 in the present study indicates that there was no evident trade-off between osmoregulation and performance. *Myzus persicae* can adjust their feeding rates (Mittler, 1967a) and/or the amount and/or chemical composition of honeydew (Fisher et al., 1984). In the present experiments, *M. persicae* may have used one or both of these options, as no xylem sap was available. In contrast to our study, the performance of the feeding specialist *A. pisum* was affected by the sucrose-to-amino acid ratio of the diet (Febvay et al., 1988; Simpson et al., 1995). In general, whether and how aphid performance is influenced by food quality probably depends on the species. Possibly, polyphagous species are more tolerant towards their diet than feeding specialists. Indeed, the performance of the Brassicaceae specialist *Brevicoryne brassicae* L., but not that of *M. persicae*, was affected by the amino acid concentration of plant phloem sap (Cole, 1997). However, intra-individual differences in phloem sap chemistry and specific aphid preferences have to be considered as well. Depending on the host plant species, *M. persicae* is mainly found on young or senescing leaves and flowers, probably because of high availabilities of nitrogen-containing solutes (van Emden et al., 1969). As the phloem sap composition may also differ between species and depend on environmental conditions at these preferred feeding sites, our finding that *M. persicae* is quite tolerant regarding the chemical composition of its diet may partly explain its pest status.

As expected, *M. persicae* nymphs were able to survive on and recover from suboptimal diets, with the effect depending on the duration of suboptimal nutrition. There are variable findings regarding the capability of aphid species to compensate for various poor diets experienced during nymphal stages (van Emden, 1977; Cailaud et al., 1994). Our study revealed that, although generation times increased along with the time on suboptimal diet, aphids recovered with nearly no (3 days on suboptimal diet) and only minor (6–8 days on suboptimal diet) negative effects on their fitness in terms of the number of offspring. Nymphs did not or only slightly gain weight on the ‘no amino acids’ diet, indicating that mainly respiratory catabolism took place (see above). However, after transfer to the optimal ‘control’ diet, aphids gained weight rapidly, reaching similar weights and offspring numbers as the aphids permanently reared on the ‘control’ diet. This indicates that metabolic and reproductive machineries were not irreversibly damaged.



Probably, the metabolic pools were replenished and the nitrogen-related metabolism was activated as soon as amino acids were available. That aphids were able to reproduce quite well after their transfer to the 'control' diet, producing large offspring populations, suggests that during the period of suboptimal nutrition, there was no or not much resorption of the embryos that were established in the nymphs before birth (telescoping generations). In future studies, it may be investigated how the aphids can recover so quickly after facing suitable diet again and be able to reproduce similarly as aphids on optimal 'control' diet.

The transferability of our findings based on experiments with artificial diets to natural field conditions is limited by the facts that the chemical composition of these diets does not resemble plant phloem sap, that aphid performance is generally lower on these diets than on plants, that aphid handling may have influenced the results, and that the aphids could not choose feeding sites in the performance and recovery experiments. Under natural conditions, *M. persicae* aphids can probably cope even better with varying dietary conditions and withstand longer periods on suboptimal diets than in our study, as they may search for phloem sap of higher quality, consume xylem sap intermittently, and rarely or never encounter phloem sap of such a low quality as the artificial diets in general and the diet without amino acids in particular. Especially alate aphids may be adapted to withstand periods of starvation or poor diets, which they experience during migration to new host plants. Indeed, for adult *Sitobion avenae* Fabricius, a higher survival of alate compared to apterous adults under starvation was found (Xu et al., 2012). Morphological and chemical plant defenses against aphids have to be considered as well (de Vos & Jander, 2009; Nalam et al., 2018; Dancewicz et al., 2020). In general, for herbivores, there is a trade-off between nutrient intake and ingestion of harmful compounds (Behmer, 2009). Thus, the outcome of plant-aphid interactions depends on whether the aphids can cope with or counteract these defenses (Elzinga et al., 2014; Züst & Agrawal, 2016). The potential of *M. persicae* to infest diverse plant taxa may be due to proteomic changes when switching host plants (Francis et al., 2006) and the expression of several detoxification enzymes (Ramsey et al., 2010). Infestation by aphids, including *M. persicae*, can also result in a modification of the nutritional composition of phloem sap (Giordanengo et al., 2010; Cao et al., 2016; Jakobs et al., 2019), which may indicate niche construction. Moreover, sterols occurring in natural phloem sap may affect aphid performance, as shown for *A. pisum* (Bouvaine et al., 2012). To better understand the potential to cope with and recover from suboptimal diets, the genetic and enzymatic repertoire of individual aphids

and its variability within and between populations should be taken into account in future studies.

In conclusion, this study provides evidence for a high tolerance of *M. persicae* towards varying dietary sucrose-to-amino acid ratios, accompanied by the capacity to withstand poor nutrition for several days and to recover from it. These factors, in addition to insecticide resistance (Bass et al., 2014), probably contribute to the broad host plant range and high pest potential of *M. persicae*. This study gives important insights into the nutritional flexibility of this aphid species, enabling to exploit diverse host plants and to withstand adverse conditions.

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