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The influence of mixed aphid diets on larval performance of *Coccinella septempunctata* (Col., Coccinellidae)

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Abstract: Three questions regarding possible benefits of mixed diets for the specialist aphid predator, *Coccinella septempunctata* larvae were investigated. (1) Do aphids species from different host plants complement each other nutritionally? (2) Is a mixed diet of high-quality aphids beneficial? (3) How does the quality of mixed diets depend on the quality of constituent species? All mix-combinations of aphid species of high (*Metopolophium dirhodum*), intermediate (*Myzus persicae*), and poor food quality (*Aphis sambuci*), and the three single-species diets were compared. A mixed diet of two high-quality species (*Sitobion avenae* and *M. dirhodum*) was also compared with single-species diets. Larvae that were given pure *A. sambuci* and a mixed diet of *A. sambuci* + *M. persicae* died within 18 days and none of the larvae developed to fourth instar. *Metopolophium dirhodum* was generally of higher quality as food than *M. persicae*, whereas the mixed diet of *M. dirhodum* + *M. persicae* was intermediate. *Sitobion avenae* and *M. dirhodum* were found to have approximately the same food value. *Coccinella septempunctatam* larvae that were offered a mixed diet of these two high-quality aphids gained no extra advantage. Overall, no benefit from mixing of aphid species was found. The quality of mixed diets depended on the quality of the constituent species.

1 Introduction

Benefits of dietary mixing are known for many generalist insectivores (Reichert and Harp, 1987; Waldbauer and Friedman, 1991; Wallin et al., 1992; Bernays et al., 1994; Borg and Toft, 2000), but it is still doubtful whether this extends to specialist predators. In an earlier study (Hauge et al., 1998), larvae of the aphid specialist *Coccinella septempunctata* (L) (Col., Coccinellidae) raised on a mixed diet of the three cereal aphid species *Metopolophium dirhodum* (Walker), *Sitobion avenae* (F) and *Rhopalosiphum padi* (L) showed intermediate performance between others raised on single-species diets of high-quality *M. dirhodum* or *S. avenae*, and the lower-quality (though still essential) diet of pure *R. padi*. Thus, they suffered from the inability to exclude the relatively low quality *R. padi*. One reason for the lack of a mixing benefit may be a great similarity in the nutrient composition of the aphids because they were raised on the same host plant (wheat seedlings). The possibility exists that aphids raised on different hosts have a more diverse nutrient composition that might lead to improved performance of the ladybird larvae. In addition, the possibility that a mixed diet of the two high-quality aphids is advantageous was not tested in the earlier experiments. Lack of a mixing benefit could be due to the inclusion of a lower-quality aphid which might counteract a possible benefit of the two high-quality species. For example, Nielsen et al. (2000) found that *R. padi* induced increased activity of the detoxifying enzyme glutathione peroxidase, whereas *M. dirhodum* did not, indicating that *R. padi* contains a substance which is toxic to *C. septempunctata* larvae.

In this paper we address three questions. (1) Can a mixed diet composed of aphids raised on different host plants be more advantageous for a specialist aphid predator such as *C. septempunctata* larvae than single species diets? (experiment 1). (2) Can a mixed diet of two high-quality cereal aphid species raised on the same host plant be more advantageous for *C. septempunctata* larvae than single species diets? (experiment 2). (3) Is the effect of dietary mixing dependent on the food quality of the constituent species? This is answered by using various combinations of essential and toxic (cf. Hodek and Honěk, 1996) aphids in experiments 1 and 2. Fitness-related life history parameters were used to indicate diet quality as in Hauge et al. (1998).

2 Materials and methods

New generations of ladybirds were reared from adult *C. septempunctata* collected around Århus, Denmark, at the beginning of August 1998. The animals were kept as a pool population and fed *M. dirhodum ad libitum*. The *M. dirhodum* culture was reared on wheat seedlings of mixed cultivars.

Newly hatched (± 12 h) coccinellid larvae used in these experiments were randomly divided into the diet groups. Individual larvae were isolated in plastic tubes (height, 8.1 cm; diameter, 3.4 cm) with approximately 1 cm plaster with charcoal on the bottom to maintain high humidity. The tubes were covered with a rubber-foam stopper. The larvae

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were kept at 24 ± 0.5°C and a 16 h light:8 h dark photoperiod. Aphids were removed from their host plants daily and offered to the larvae *ad libitum*. The mixed diets consisted of equal numbers of the aphid species. All aphid species used in both experiments had similar size ranges. The larvae were checked daily for deaths, moults and pupation, and food remains were removed. The pupae were checked daily to obtain the exact day of emergence of the adult ladybirds. After ecdysis the ladybirds were weighed (fresh weight) and sexed according to Baungaard (1980).

In experiment 1, larvae from the second generation after the field-collected animals were divided into seven diet groups with $n = 30$ in each. The diets consisted of the two essential aphid species *M. dirhodum* and *Myzus persicae* (Sulzer), one toxic (cf. Hodek, 1956; Blackman, 1965) species *Aphis sambuci* (L), and all combinations of these (table 1). *Metopolophium dirhodum* were reared on wheat seedlings and *M. persicae* on *Capsicum annuum* L.; both had been kept in the laboratory for several years. *Aphis sambuci* were collected in the field and reared in the laboratory on *Sambucus nigra* L. cuttings for several generations.

In experiment 2, larvae from the seventh generation after the field-caught individuals were used. Three groups were raised on *M. dirhodum*, *S. avenae* or a mixed diet of these ($n = 50$ in each). Both aphids had been reared on wheat seedlings of mixed cultivars.

3 Results

3.1 Experiment 1: mixed diets of aphid species from different host plants

Survival through all developmental stages (table 1A) showed a significant overall treatment effect ($\chi^2 = 105.59$, d.f. = 4, $P < 0.0001$). On the pure *A. sambuci* diet and the mixed diets including *A. sambuci*, none or very few survived. Highest survival was on pure *M. dirhodum*, followed by pure *M. persicae* and the mix of *M. dirhodum* + *M. persicae*.

In the following analyses the single species diet of *A. sambuci* and the mixed diet group of *A. sambuci* + *M. persicae* were excluded. In the remaining five treatment groups, the sex distribution of the adult ladybirds was roughly 1:1. No significant difference in the adult weight of males and females was found [two-way analysis of variance (anova), sex: $F = 0.73$, d.f. = 1, $P = 0.40$; diet*sex interaction: $F = 0.67$, d.f. = 3, $P = 0.57$). Accordingly, sex was neglected as a factor.

Significant overall treatment effects were found in the larval developmental time (Kruskal–Wallis test, $\chi^2 = 43.54$, d.f. = 4, $P < 0.0001$ and in the total developmental time (Kruskal–Wallis test, $\chi^2 = 44.20$, d.f. = 4, $P < 0.0001$. Larvae that were offered *M. dirhodum* or *M. dirhodum* + *M. persicae* had the shortest larval and total developmental times (table 1A). Larval time on pure *M. persicae* or on *M. dirhodum* + *M. persicae* + *A. sambuci* were significantly longer. Total developmental time on the mixed diet of all three aphid species was significantly longer than the four other diet groups. Larvae offered a single-species diet of *M. persicae* and larvae fed a *M. dirhodum* + *A. sambuci* mix was intermediate regarding total developmental time.

Table 1. *Survival to adulthood, larval times, total developmental times and adult weights (Mean ± SE), of* C. septempunctata *larvae on different aphid diets*

Aphid diets	*n*	No. survived [1]	Larval time (days) [2]	Total developmental time (days) [2]	Adult weight (mg) [3]	Males	*n*	Females	*n*	Difference between sex (P-values) [4]
A: Experiment 1										
Metopolophium dirhodum	30	26^{a}	11.27 ± 0.20^{a}	15.27 ± 0.22^{a}	25.54 ± 0.84^{a}	—	—	—	—	—
Myzus persicae	30	20^{ab}	12.95 ± 0.17^{b}	16.70 ± 0.15^{b}	18.88 ± 0.67^{b}	—	—	—	—	—
Aphis sambuci	30	0^{c}	—	—	—	—	—	—	—	—
M. dirhodum and *M. persicae*	30	18^{b}	11.22 ± 0.21^{a}	15.06 ± 0.12^{a}	25.44 ± 0.81^{a}	—	—	—	—	—
M. dirhodum and *A. sambuci*	30	2^{c}	$13.50 \pm 0.50^{a,b}$	17.00 ± 0.00^{b}	14.58 ± 1.78^{b}	—	—	—	—	—
M. persicae and *A. sambuci*	30	0^{c}	—	—	—	—	—	—	—	—
M. dirhodum, M. persicae and A. sambuci	30	5^{c}	17.80 ± 0.38^{b}	21.60 ± 0.51^{c}	16.14 ± 1.21^{b}	—	—	—	—	—
B: Experiment 2										
M. dirhodum	50	27^{a}	11.26 ± 0.17^{a}	15.33 ± 0.19^{a}	27.57 ± 0.95^{a}	24.76 ± 1.24^{a}	12	29.83 ± 1.11^{a}	15	0.0050
S. avenae	50	35^{a}	12.09 ± 0.26^{b}	16.11 ± 0.27^{b}	28.09 ± 0.73^{a}	24.78 ± 1.13^{a}	11	29.60 ± 0.76^{a}	24	0.0012
M. dirhodum and *S. avenae*	50	34^{a}	11.41 ± 0.20^{c}	15.18 ± 0.17^{a}	28.63 ± 0.90^{a}	26.32 ± 1.05^{a}	18	31.56 ± 1.19^{a}	15	0.0024

Within columns and experiments, the same letters indicate no significant difference between treatments. [1]χ^2-test; numbers in this column are sample sizes for next three columns; [2]multiple comparisons after Kruskal–Wallis-tests, cf. Siegel and Castellan, 1988; [3]Fisher's LSD test; [4]*t*-tests.

There was a significant overall treatment effect in adult body weight (ANOVA, $F = 18.78$, d.f. $= 4$, $P < 0.0001$). Larvae offered pure *M. dirhodum* and a mix of *M. dirhodum* + *M. persicae* developed into significantly heavier adults than the larvae of the other diet groups (table 1A).

3.2 Experiment 2: Mixed diet of two high-quality aphid species

Larval survival showed no significant overall difference (table 1B) ($\chi^2 = 3.30$, d.f. $= 2$, $P = 0.19$). In all diet groups, the sex distribution was roughly 1:1. Larval time and total developmental time were not normally distributed; therefore a non-parametric two-way Scheirer–Ray–Hare extension of the Kruskal–Wallis test (SOKAL and ROHLF, 1995) was used. Differences in diet quality were expressed in the larval time (diet: $H = 18.42$, d.f. $= 2$, $P < 0.001$; sex: $H = 0.14$, d.f. $= 1$, $P > 0.70$; diet*sex interaction: $H = 0.32$, d.f. $= 2$, $P > 0.80$) and in total developmental time (diet: $H = 7.69$, d.f. $= 2$, $P < 0.05$; sex: $H = 0.12$, d.f. $= 1$, $P > 0.80$; diet*sex interaction: $H = 1.43$, d.f. $= 2$; $P > 0.30$). Because there were no significant effects of sex, the sexes were pooled in the analyses of developmental times.

Larvae that were fed pure *M. dirhodum* showed a faster larval and total developmental time than the pure *S. avenae* group. The mixed diet group was intermediate in larval time. Total developmental time in the mixed diet group was similar to that of the larvae that were fed *M. dirhodum*.

No significant overall difference in adult weight were found between groups (two-way ANOVA, diet: $F = 1.72$, d.f. $= 2$, $P = 0.18$; sex: $F = 32.06$, d.f. $= 1$, $P < 0.0001$; diet*sex interaction $F = 0.02$, d.f. $= 2$, $P = 0.98$). However, females of all diet groups were significantly heavier than the males (table 1B).

4 Discussion

Larvae offered a mixed diet of *M. persicae* + *M. dirhodum* or *S. avenae* + *M. dirhodum* did not obtain higher survival, faster development or increased adult weight than those on the best of the single-species diets. The value of the mixed diets were intermediate between the high quality diet of pure *M. dirhodum* and the slightly lower-quality diet of pure *M. persicae* and *S. avenae*, respectively. This lack of a mixing benefit may indicate a lack of nutritional complementarity in relation to the needs of the *C. septempunctata* larvae between *M. dirhodum* and *M. persicae*, even though they were raised on different host plants. Alternatively, ladybirds may have quite restricted dietary requirements, as expected from a specialist consumer. Furthermore, larvae offered the single-species diets of the two cereal aphid species *M. dirhodum* and *S. avenae* showed almost equal performance, and they gained no advantage from the mixed diet of these high-quality aphid species.

Thus, the lack of mixing benefit was shown when the mixed diet contained two essential high-quality aphid species, whether raised on the same or different host plants, and when the mixed diets consisted of an essential and a toxic aphid species. In all cases the quality of the mixed diet seemed to average that of the constituents, indicating lack of prey selectivity (cf. BLACKMAN, 1967; SENGONCA and LIU, 1994). Inclusion of a toxic prey in the diet therefore had a serious effect on larval fitness, even if a high-quality aphid was also available. Thus, if one species of the mixed diet is low quality or toxic, the value of the mix will be considerably lower than that of the best constituent. A similar results was obtained for a generalist predator, a wolf spider, by TOFT and WISE (1999).

Whether coccinellid larvae in nature are similarly constrained by the quality of prey species available is uncertain. In the field it is possible that the larvae will leave a patch with low quality or toxic prey and search for another patch with prey of higher food quality. This may be an alternative mechanism of prey selectivity adapted to the way aphids are usually distributed in the habitat, i.e. as single-species clumps of high density.

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