Short communication

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Suitability of Gramineous Plants as Food for the Phytophagous Ladybird *Cynegetis impunctata* L. (Coleoptera: Coccinellidae)

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Abstract: Six gramineous plant species, including cereal crops, were used as food for the ladybird beetle *Cynegetis impunctata* in a laboratory experiment. Five of them, including wheat and barley, were suitable for larval development, while *Phalaris arundinacea* L. was unsuitable as food. Larval development lasted 20–23 days. Females reared on leaves of wheat and *Agropyron repens* L. laid on average 153–182 eggs. Larvae and adults did not exhibit a preference to wild gramineous plants compared to cultivated cereal crops. Although wheat and barley represented suitable food for *C. impunctata*, this species is not a potential pest for these crops. This ladybird is wingless, has limited dispersion abilities and is vulnerable to agrotechnical cultivations that may destroy dormant adults in agricultural plots.

Keywords: grass, cereals, development time, body mass, fecundity, prey choice

Introduction

Several phytophagous ladybird beetles (Coccinellidae) of the genera *Epilachna* Dejean and *Henosepilachna* Li et Cook are major pests of cultivated plants and thereby are well-studied (Majerus 1994, Hodek, Honěk 1996). However, the vast majority of phytophagous coccinellids are not pests and never become pests. The first step to determine if a species can become a pest is to study its food spectrum (Kalushkov, Nedvěd 2010). A simple food chain analysis can show whether or not a species can be a potential pest. In Europe, there are several phytophagous ladybirds. *Subcoccinella quatuordecimpunctata* L. is a major pest on alfalfa in the south and southeast Europe and there is a lot of information about its life history (Ali 1979).

The aim of the present study is to assess the feeding preference of another European phytophagous ladybird, *Cynegetis impunctata* L., to several gramineous plants (Poaceae) and the suitability of these plants for the larval development and for the production of eggs, thus determining whether this species is a potential pest of cultivated cereal crops. These are the first data on the food ecology of this species.

Materials and methods

Laboratory cultures. Adults of *C. impunctata* were collected in weedy field margins near Sofia, Bulgaria, after overwintering, on warm sunny days in late March 2007. They were reared under laboratory conditions in glass jars of volume 750 ml, about 20 adults per jar. The glass jars were covered with nylon mesh. Adults were fed with leaves of wild *Poa pratensis* L. and wheat *Triticum vulgare* L. cultivated in pots under laboratory conditions. After 10-14

days, adults started to copulate. Insects were reared and all experiments were performed at 25 ± 2° C and 16L:8D photoperiod.

Fecundity: Mating pairs were separated in 18 cm Petri dishes. Twenty pairs were continuously fed with leaves of *Agropyron repens* and 20 pairs with *T. vulgare* collected from cereal fields around Sofia since the middle of April. Eggs laid were counted and removed from the jars daily, and some fresh plant leaves were added. The experiment was continued until all females died. When a male died, it was replaced with another male. Three females fed with *T. vulgare* and four females fed with *A. repens* died prior to laying eggs.

Larval development: Eggs obtained from these adults were separated in 9 cm Petri dishes (10-15 eggs per dish). The newly hatched larvae were reared in the same dishes and fed with leaves of one of the following tested plants: *P. pratensis*, *T. vulgare*, *A. repens*, *Phalaris arundinacea, Hordeum sativum* Jess and *Dactylis glomerata* L. collected in fields around Sofia. The larvae were supplied daily with fresh grass leaves. As third instar, they were transferred to 18 cm Petri dishes. The developmental time, survival of larvae and fresh weight of emerged adults were recorded.

Voltinism: Fifty newly emerged adults were reared under laboratory conditions, from the beginning of July (16L:8D), and fed with *P. pratensis*.

Food choice: To evaluate the preference for various plants, the choice experiment was performed: one leaf of each of two plant species was placed in 18 cm Petri dish lined with moist filter paper and fixed with entomological pins crosswise. The first treatment combined *A. repens* and *T. vulgare*, while the second one combined *P. pratensis* and *H. sativum*. In the middle of each of the four sections between leaves we placed either one larva of the fourth instar or one adult. The position of the insects was recorded every 6th, 12th, and 24th hours. Twenty replications with different individuals were performed (80 larvae and 80 adults were used).

Field observations. From the end of March till the end of October, twice a month, we observed visually the cereal fields and their weedy field margins near Sofia, Bulgaria, and České Budějovice, Czech Republic, and swiped the grassy vegetation with an entomological net (10 x 50 sweeps). These observations were carried out in 1997-1998 and 2006-2009.

The developmental time of larvae and adult fresh weight were analyzed by one-way ANOVA and post-hoc test. Fecundity was compared using t-test.

Results and Discussion

Laboratory experiments. Five host plant species, including wheat and barley, were suitable food for *C. impunctata*, with mortality less than 20 %. However, there was 100% mortality on *P. arundinacea*. The mean developmental time of larvae ranged between 20 and 23 days on the suitable host plants (Table 1). Larvae reared on the two cereals, *T. vulgare* and *H. sativum*, had significantly prolonged development (more than one day) in comparison to the three wild grass species (ANOVA: F_4=36, $p<10^{-4}$).

Table 1. The effect of different cereal plants on larval development, adult weight and fecundity of *Cynegetis impunctata.* Means followed by the same letter are not significantly different from one another

Plant	Larvae n	Mean larval development days ± SD	Adult fresh weight*	Fecundity	
				Females n	Mean number of eggs ± SD
Poa pratensis	81	19.6 ± 1.8 a	♀ 7.34 ± 0.06 a ♂ 6.88 ± 0.06 a	-	-
Dactylus glomerata	82	20.6 ± 2.0 a	-	-	-
Phalaris arundinacea	69	100 % mortality	-	-	-
Hordeum sativum	77	22.7 ± 1.5 b	-	-	-
Agropyron repens	85	20.4 ± 2.1 a	♀ 7.28 ± 0.06 a ♂ 6.76 ± 0.04 a	16	182 ± 82 a
Triticum vulgare	75	21.8 ± 1.6 b	♀ 7.02 ± 0.05 b ♂ 6.72 ± 0.05 a	17	153 ± 91 a

* mean weight of 10 individuals

Both egg and pupa stages lasted 6 days each. The total development from egg to adult lasted 32–35 days. According to Honěk, Kocourek (1990), egg stage lasts 15-20% of the total preimaginal developmental time of ladybird beetles, larva stage lasts 55-65% and pupa stage lasts 20-25%. Our results show that both egg and pupa stages last about 19% of the total time, which is comparable to the published results, despite the fact that they were based mainly on aphidophagous coccinellid species. In another phytophagous ladybird, *S. vigintiquatuorpunctata*, egg and pupa stages last about 17 and 18%, respectively, of the total preimaginal developmental time (Ali 1979).

On two out of the three tested host plants, the weight of the freshly emerged males was significantly lower than the weight of the females (Table 1). The female weight was 7.28 mg when larvae were reared on wild grass *A. repens* and 7.34 mg on *P. pratensis*, while significantly less (7.02 mg) when the larvae were fed with wheat leaves (ANOVA: $F_2=28$, $p<10^{-6}$). Males did not differ significantly in weight among food treatments.

Particular food suitability is often displayed in parallel by more rapid development and higher achieved body weight, even in taxa quite different in their food type. Different aphid species had a positive effect on both larval development rate and adult fresh weight in several aphidophagous coccinellids (Kalushkov 1998, Ungerová *et al.* 2010).

Females of *C. impunctata* laid on average 182 eggs during their lifetime when fed with leaves of *A. repens* and 153 eggs when fed on wheat leaves (non significant difference t-test, t=1.15, p=0.28). The number is considerably lower than the average number of eggs laid by females of alfalfa ladybird *S. vigintiquatuorpunctata* (237 eggs at 23° C and 480 eggs at 28° C) (Ali 1979). Ali (1979) also tested four host plants as a food for *S. vigintiquatuorpunctata* and found differences in their suitability for both larval development and female fecundity.

The adults collected at the end of March lived till mid June in the laboratory conditions (mean longevity of egg laying females was 57 days). The newly emerged adults lived at most up to September at 25° C. Although they were reared during a long day (16L:8D) at 25° C, the adults did not mate and remained inactive. This indicates the presence of obligatory diapause and means that the population is strictly monovoltine.

The larvae and adults of *C. impunctata* did not show any significant preference for the wild grasses over the cultivated cereal crops. At the 6th hour of the experiment, the larvae and adults were more frequently found on the wild grasses, while at the 24th hour, most of them were found on the cereal leaves. We explain this shift as a simple function of the physical properties of the leaves, as the cereal leaves were thicker and still rich in water the next day.

Field observations. The first adults on wild gramineous plants were observed in the middle of March near Sofia and at the end of March near České Budějovice, after 5 to 7 sunny days with the temperatures being about 20° C. The adults started to mate in the middle of April near Sofia and from the end of April to the beginning of May near České Budějovice. The first eggs were found two weeks later. The larval development lasted from 25 to 35 days in Sofia and from 30 to 40 days in České Budějovice. Overwintered adults were observed till the end of May in Sofia and till the end of June in České Budějovice. They were distinguished by their dark maroon elytra, while newly emerged adults were paler, light brown colored.

No adults were observed on plants from the end of June near Sofia, and from the end of July near České Budějovice till October. If there were 5-7 warm sunny days in October following several cold days, adults could be observed again feeding on wild grass leaves. At the time when adults were not active, they were hidden amongst grass leaf litter, in the same places they had developed. No adults were collected near the end of the cereal fields even when there were up to 32 adults per 100 sweeps in surrounding weedy field margins.

During the last 10 years, the species has increased its population densities. In 1998, near Sofia, average 3 adults per 100 sweeps on the grass were collected, while in 2008 it was 11 adults. There were on average 48 individuals per 100 sweeps (adults and larvae) collected near České Budějovice in the second half of June 1997, while there were 85 individuals in 2009.

Conclusions

The leaves of wheat and barley are suitable but not preferable food for the phytophagous ladybird *Cynegetis impunctata*. There is a low risk that the species can become a pest similar to some *Epilachna*

or *Henosepilachna* species. *C. impunctata* is wingless and has a limited dispersion ability. Agrotechnical cultivations may easily destroy dormant adults on agricultural plots.

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