

Body size and the temporal sequence in the reproductive activity of two species of aphidophagous coccinellids exploiting the same resource

ALOIS HONĚK¹, ANTHONY F.G. DIXON² and ZDEŇKA MARTINKOVÁ¹

¹Research Institute of Crop Production, Drnovská 507, CZ 16106 Prague 6 – Ruzyně, Czech Republic; e-mail: honek@vurv.cz

²School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

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Abstract. The hypothesis that small species of aphidophagous coccinellids need lower aphid population densities for reproduction than large species (Dixon, 2007) was tested in the field. In 2006 we compared seasonal changes in the oviposition behaviour of two coccinellid species regularly found in cereal stands in central Europe, the large *Coccinella septempunctata* L. and the small *Propylea quatuordecimpunctata* (L.). Adults of both species were collected at 2–3 day intervals from stands of winter wheat and spring barley, females were allowed to deposit eggs for 24 h following collection and the percentage that laid eggs and the number of eggs were recorded. Both species colonized the cereal crop simultaneously in the middle of May. After colonization of the crop, while the aphid density was still low, few females of *C. septempunctata* oviposited and laid only a few eggs. Oviposition increased up to a maximum c. 1 month later and was closely associated with prey abundance. Of the females of *P. quatuordecimpunctata*, whose mass is about one quarter of that of the former species, the percentage ovipositing and number of eggs laid varied less in time and was less associated with prey abundance than in *C. septempunctata*. As predicted by theory, the small *P. quatuordecimpunctata* was more effective at exploiting the lower prey densities as it produced proportionally more of its eggs during the early stages of the aphid infestation than the larger *C. septempunctata*.

INTRODUCTION

As in most insects, reproductive activity in aphidophagous coccinellids varies throughout their lifetime. The changes in reproductive activity with age of several species have been studied under laboratory conditions. Female reproductive activity is generally triangular in shape and either peaks shortly after the completion of the teneral period and then decreases (Dixon & Agarwala, 2002) or peaks later and is more symmetrical in shape (cf. Kawachi 1985; Dixon, 2000; Pervez et al., 2004). Less is known about the course of oviposition in nature where it may be affected by photoperiod, temperature, voltinism and food availability. The latter is particularly important because aphid abundance varies in time and space, in an unpredictable way (Majerus & Kearns, 1989; Dixon, 1998). The factors affecting aphid abundance vary between years and annual variation in aphid reproductive success affects long term trends in the abundance of coccinellids (Carter et al., 1982).

Recently it was argued that body size in coccinellid species is important in determining when they start ovipositing in aphid colonies in the field. It was suggested that small species of coccinellid need fewer aphids per unit area for them to capture sufficient prey per unit time to mature and lay eggs than large species (Dixon, 2007). As a consequence it is expected that small species will be better at exploiting the low initial aphid population densities and will start laying eggs at lower aphid densities. To test this hypothesis the temporal variation in the oviposition behaviour of two species was compared, the small

Propylea quatuordecimpunctata (L.) (mean dry mass 3.7 mg) and large *C. septempunctata* L. (15.4 mg). Both species regularly occur in cereals infested with aphids (Honěk, 1979). Unfortunately, in 2006 it was not possible to obtain good estimates of the abundance of these two species because their relative rarity precluded sampling using sweep nets or direct counts. It was only possible to establish the initial appearance and disappearance of the species from the cereal stands and the variation in egg production during the season. Preliminary studies in 2004 and 2005 revealed that the median daily oviposition of *C. septempunctata* calculated for the whole female population (including non ovipositing individuals) peaked within a few days of the end of June and increased before and decreased after this period (Honěk et al., 2008).

The aim of this study was to test Dixon's (2007) hypothesis that body size determines the temporal sequence of reproductive activity among coccinellid species exploiting the same resource. In order, to compare the temporal variation in the reproduction of both species, the following were determined: (i) species presence/absence in the crop and variation in abundance over time, (ii) the proportion of females that oviposited and (iii) number of eggs laid per female per day. The proportion of the total number of eggs that are laid at different times during the oviposition period of each species were then calculated and the differences between species tested.

MATERIAL AND METHODS

Species studied

Both species of coccinellid are consistently the most abundant species found in crops in the Czech Republic. *C. septempunctata* has been studied in great detail and there is a vast literature on its biology (Hodek & Honěk, 1996). Adults of this species overwinter at a variety of sites, but prefer forest margins or deforested hilltops. In the spring they migrate and colonize crops infested with aphids. Cereals are important because of the large acreage planted and the fact that they are usually heavily infested with aphids. Other crops that may be colonized by this coccinellid are alfalfa infested with *Acyrtosiphon pisum* Harris, or sugar beet or beans infested with *Aphis fabae* Scopoli. However, for various reasons the latter crops are of minor importance for *C. septempunctata* compared to cereals (Honěk, 1982a). Thus most of the adult *C. septempunctata* probably first colonize cereal crops after leaving their hibernation sites. Once they colonize cereal crops it is likely the females stay there until they die or the aphids become scarce.

The life cycle of *P. quatuordecimpunctata* is less well known. Observations on overwintering (Růžička & Vostřel, 1985; Hemptinne, 1989; Honěk, pers. obs.) and spring migration (Hodek, 1960; Honěk, 1982b) are contradictory and not easy to reconcile. *Propylea quatuordecimpunctata* migrates over a longer period to colonize crops, but is less specific in its crop preference than *C. septempunctata* (Honěk, 1985). This species is polyphagous (Kalushkov & Hodek, 2005) and has a relatively low fecundity (Bazzocchi et al., 2004). It is always present in cereal crops and may occasionally outnumber *C. septempunctata* (Honěk, 1979). Overall the abundance of both *C. septempunctata* and *P. quatuordecimpunctata* has decreased dramatically in recent years, presumably in response to changes in the agricultural landscape (Honěk & Martinková, 2005).

Study site

The study site was in fields at Prague – Ruzyně (50°05'N, 14°10'E, 340 m a.s.l. altitude). The area planted to cereals was subdivided into sections, each planted with different cereal or variety, and plots used for sampling were established within these sections. In 2006, the coccinellids were sampled in two sections planted with winter wheat and two with spring barley. The cereals were cultivated following recommended agricultural practices (Špaldon, 1982). No insecticides were applied during the study, and fungicides and liquid fertilizers were sprayed early in the season before aphids and coccinellids arrived and did not influence their population development. The cereals were naturally colonized by three common cereal aphid species, in decreasing order of abundance, *Metopolophium dirhodum* Walker, *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.). Aphid population densities were estimated at weekly intervals by a standard method (Honěk & Martinková, 2005). Six plots, 3

× 3 m, were established within one of the winter wheat sections where the coccinellids were collected. The plants were grown at the standard commercial density (3 plots) or thinned out so that the plants were growing 20–30 cms apart (3 plots). These represent the extremes of the range of plant densities recorded in cereal stands in the field (Honěk & Martinková, 1999). On each occasion, aphids on each plot were counted on 50–350 tillers, depending on aphid abundance, and the mean number per tiller calculated. For each date the average number of aphids (\pm SE) was calculated using mean numbers on particular plots. Numbers of all aphid species were pooled because coccinellid adults search the whole plant and make no distinction between aphid species. As growth of aphid populations was similar in all the cereal plots, at this site, estimates of aphid densities based on these plots give a good estimate of prey abundance at a particular time in each year.

Sampling female coccinellids

Coccinellid females were sampled at 2–3 day intervals, either by sweeping or collecting by hand from leaves or the ground, where they tend to bask in the sunshine during the morning and afternoon. Preliminary experiments revealed that the reproductive output of these coccinellids was not affected by the time of collection (morning vs. evening). The coccinellids collected in this way were sexed based on the morphology of the last sternite (*C. septempunctata*) or coloration of head and scutum (*P. quatuordecimpunctata*) (Rogers et al., 1971). As sex determination in the latter species is not completely reliable, the sex of the individuals that did not oviposit was verified by dissection.

Determination of reproductive activity and body mass

The females were immediately placed individually in 55 × 15 mm glass tubes, which were sealed with a plastic lid. Each tube contained a 40 × 13 mm piece of filter paper. The tubes were placed in a room kept at 25 ± 1°C and a natural photoperiod. Eggs were counted and removed at 1–3 h intervals to prevent cannibalism. Oviposition was recorded for a period of 24 h after capture by which time virtually all available eggs were deposited (A. Honěk, unpubl.). No food was provided and thus the females only laid eggs ready, or nearly ready, for oviposition at the time they were collected. The non-ovipositing females were dissected and the state of their ovarioles recorded. Females that did not lay eggs and were parasitized by *Dinocampus coccinellae* (Schrank) (Hymenoptera: Braconidae) were not included in the calculation of the percentage of ovipositing females. Some females were killed 24 h after collection and their dry body mass determined to a precision of 0.01 mg using an analytical balance.

Analysis of results

Quantities determined or calculated are listed in Table 1. The results were pooled over periods of ten days with three such

TABLE 1. Parameters measured or calculated.

Parameter	Unit	Method of measurement or calculation
Daily oviposition	n	Number of eggs laid in the 24 h following collection in the field
Period specific oviposition	n	Daily oviposition averaged over periods of ten days starting from May 10 and categorized as May II, May III, Jun I, Jun II, Jun III, Jul I. (A)
Percentage of females ovipositing		Percentage of the females collected on a particular day that oviposited
Period specific percentage of females ovipositing		Percentage of females ovipositing averaged over each period of ten days, as was the average period specific oviposition (B)
Period specific fecundity	n	Average number of eggs laid per female per day in each period of ten days (C) C = A × B
Dry body mass	mg	Dry body mass after ovipositing in the laboratory for 24 h

TABLE 2. Numbers of females used to determine the reproductive activity of the ladybirds in particular ten day periods. Only non-parasitised individuals were used.

	May II	May III	Jun I	Jun II	Jun III	Jul I	Total
<i>C. septempunctata</i>	9	53	188	185	167	45	647
<i>P. quatuordecimpunctata</i>	3	12	66	92	10		183

periods in each month, e.g., Jun I, Jun II and Jun III. The numbers of females used for determining reproductive activity in each ten day period are given in Table 2.

A second order polynomial regression, $y = a_0 + a_1x + a_2x^2$ with the ten day periods as an explanatory variable and period-specific percentage of ovipositing females or period-specific oviposition as dependent variables was calculated to reveal the temporal trends in coccinellid oviposition. A correlation r^2 was calculated for each regression relationship.

For each species, the period-specific fecundities over the whole period of their presence in the cereal crop were summed and then the reproduction in each period expressed as a proportion of this total. The differences between the species in the trends in the cumulative proportion of the total reproduction were tested using ANCOVA, with cumulative proportion as an explanatory variable, species a dependent variable, and the period of ten days (Julian day is the 5th day of each period) a covariate.

RESULTS

Coccinella septempunctata

The factors determining the change in oviposition can be divided into two components, both of which varied over the period when eggs were laid.

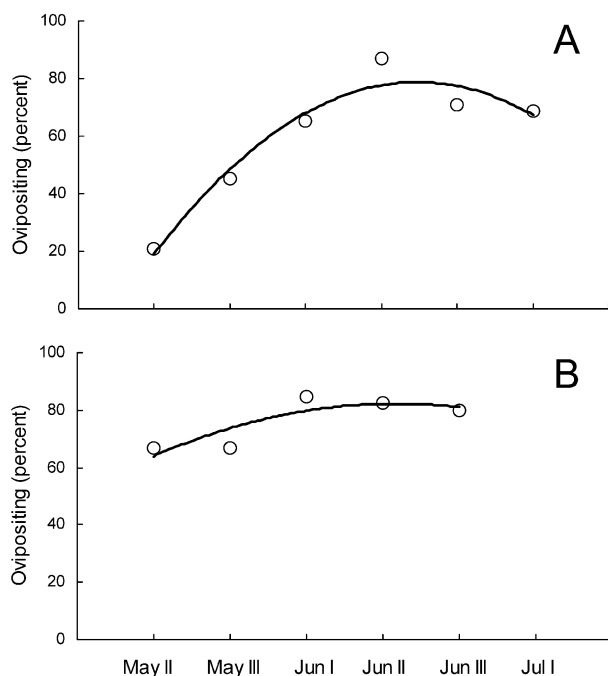


Fig. 1 Temporal changes in percentages of females that laid eggs in the samples of (A) *C. septempunctata* ($a_0 = 20.1$, $a_1 = 44.2$, $a_2 = -4.93$, $r^2 = 0.942$) and (B) *P. quatuordecimpunctata* ($a_0 = 50.6$, $a_1 = 15.2$, $a_2 = -1.83$, $r^2 = 0.732$) collected from the field on particular days. Results are for ten day periods from May to July.

(i) Percentage of ovipositing females (Fig. 1A)

Few females laid eggs at the start of reproduction shortly after this coccinellid arrived in the crop recently colonized by aphids. Nevertheless, ovipositing females were already present at the very beginning of crop colonization by aphids. The percentage of ovipositing females increased from an initial 20% in the second 10 d period in May to about 90% at the peak of reproductive activity in late June – early July. The ovarioles of the non-ovipositing females contained unripe eggs without chorions. At the end of the oviposition period (July 3–11), 42% of the females that did not lay eggs ($n = 36$) were parasitized by *D. coccinellae*.

(ii) Number of eggs laid per day (Fig. 2A)

This variable followed a trend similar to that of the percentage of ovipositing females. Shortly after colonizing aphid-infested crops, the number of eggs deposited by females was low, gradually increased up to a maximum which coincided with the period when the peak percentage of females oviposited. The peak of oviposition occurred at the end of the second 10 d period of June. The change in coccinellid oviposition paralleled the change in aphid abundance, which peaked on June 20 (Fig. 3).

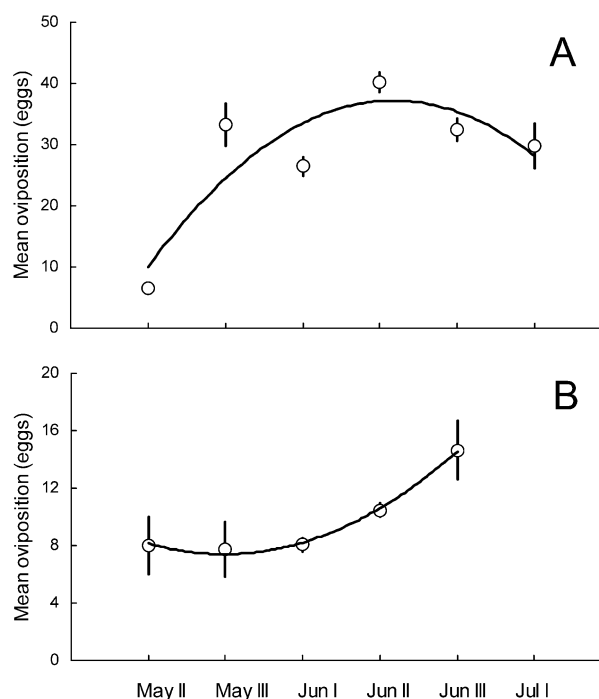


Fig. 2 Temporal changes in the mean number of eggs laid in 24 h ($O_x \pm SE$) by (A) *C. septempunctata* ($a_0 = -9.82$, $a_1 = 22.5$, $a_2 = -2.70$, $r^2 = 0.759$) and (B) *P. quatuordecimpunctata* ($a_0 = 10.5$, $a_1 = -3.09$, $a_2 = 0.78$, $r^2 = 0.994$). Results are for ten day periods from May to July.

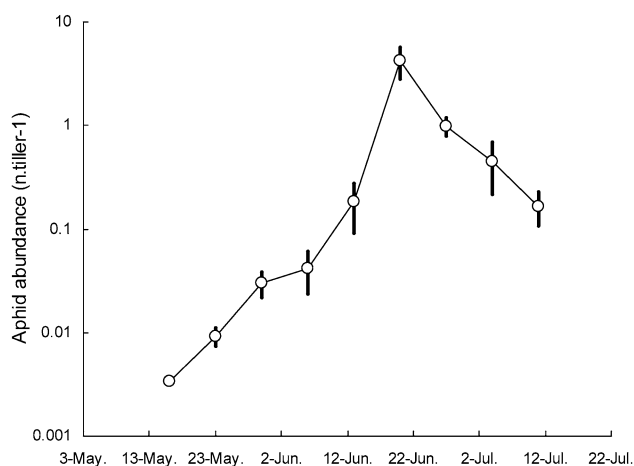


Fig. 3 Temporal changes in the average (\pm SE) cereal aphid abundance on 6 experimental plots at Prague Ruzyně, from early May to late July 2006.

Propylea quatuordecimpunctata

For *P. quatuordecimpunctata* the trends in the percentage of ovipositing females and daily oviposition differed from that recorded for *C. septempunctata*. Shortly after colonizing the aphid-infested crop (i) the percentage of ovipositing females was high and increased less during the season (Fig. 1B) and (ii) the daily oviposition increased up to the 3rd period of June, i.e. over the whole period they were present in the crop. The average daily oviposition in the final period was about twice that in the initial period, whereas in *C. septempunctata* it was 10 times greater (Fig. 2A, B).

Differences in the reproductive activity of the two species

Both *C. septempunctata* (first female captured on May 13) and *P. quatuordecimpunctata* (May 15) appeared in the cereal stands nearly simultaneously. Overwintered adults of *C. septempunctata* remained in the crop (July 10) later than those of *P. quatuordecimpunctata* (June 21). Comparison of the cumulative proportions of total reproduction achieved by both species over the period from middle of May to early July (Fig. 4) revealed a significant difference (ANCOVA: $F_{1,8} = 16.09$, $p < 0.005$). Overall *P. quatuordecimpunctata* laid most of its eggs earlier than *C. septempunctata*, because of its higher specific fecundity at the onset of oviposition.

DISCUSSION

Although the relative rarity of the two species in 2006 precluded a rigorous analysis of temporal differences in the time of their arrival in cereal crops, the results revealed clear temporal differences in their oviposition behaviour. Both species of coccinellid appeared to colonize the cereal stands at the same time as the aphids.

The percentage of *C. septempunctata* females that laid eggs and the number of eggs deposited varied during the season, in parallel with aphid numbers. On arrival in the crop aphid populations were sparse and only a small percentage of the *C. septempunctata* females laid a few eggs.

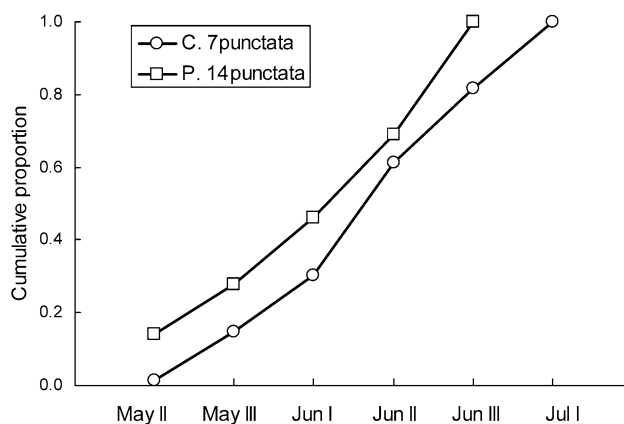


Fig. 4 Trends in the cumulative proportion of the total reproduction achieved in each ten day period during the course of the season by *C. septempunctata* and *P. quatuordecimpunctata* in 2006.

It is possible that those ladybirds that reproduced at this stage may have fed on aphids elsewhere prior to arriving in the crops (Banks, 1955, 1956). However, their very poor initial reproductive performance indicated they were markedly food-limited. The percentage of females laying eggs and the number of eggs laid increased and peaked at about the same time as aphid abundance. At the peak in aphid abundance, the few *C. septempunctata* females that did not lay eggs may have done so shortly prior to their collection, or may have been diseased or parasitized. Parasitized females were identified by dissection and excluded from the analysis. In addition, as no adults died during the period they were confined in tubes in the laboratory disease is unlikely to have affected oviposition. Therefore, their having exhausted their egg supply is the most likely cause of their not ovipositing in the laboratory. Prey availability was clearly the factor determining the number of eggs laid, which increased in parallel with the increase in aphid abundance.

Although generally similar, *P. quatuordecimpunctata* reproductive activity differed from that of *C. septempunctata* in several respects. After arrival in cereal stands the percentage of females that oviposited was greater than in *C. septempunctata* and did not increase so markedly with increase in aphid density. In addition, the initial number of eggs laid was relatively large compared to the maximum, unlike that recorded for *C. septempunctata*. Between arrival and peak performance, the average clutch of *P. quatuordecimpunctata* eggs doubled in size while that of *C. septempunctata* increased about 10 times. *P. quatuordecimpunctata* is thus capable of producing proportionally more of its eggs at low aphid densities than *C. septempunctata*. The sum of the period-specific fecundities revealed that *P. quatuordecimpunctata* laid a greater proportion of its eggs earlier than *C. septempunctata*. This difference results from the temporal variation in specific oviposition activity, with proportionally more eggs laid by *P. quatuordecimpunctata* in the initial stages of the aphid infestation than by *C. septempunctata*. That is, the results presented here accord with that predicted by theory. The small species, *P. quatuordecimpunctata* laid

proportionally more of its eggs when aphid population densities were low than the larger species, *C. septempunctata*.

Finally, one may ask about the frequency with which this phenomenon may occur in nature. Body size is a very important determinant of life history traits (Brown & West, 2000), but other factors contribute, including taxonomic affiliations, thermal requirements for development (Dixon et al., 2005), voltinism and habitat. These factors may cause local and temporal variation in species reproduction and presence, which require further investigation. As suggested by Sloggett (2005) there is a need to study a wider range of coccinellid species in order to determine whether the predictions about the importance of body size, which are based mainly on studies of few species ladybird of agricultural importance, apply generally to all ladybirds.

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