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Patch residence by aphidophagous ladybird beetles: Do specialists stay longer?

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ABSTRACT

Aphidophagous ladybird beetles are perceived as highly mobile organisms, with adults frequently moving between patches containing ephemeral aphid prey. Nonetheless more specialized ladybirds are likely to be more sedentary due to their greater tolerance of lower aphid densities. We tested the hypothesis that a conifer specialist, *Mulsantina picta*, would remain in patches (individual pine trees) longer than the generalist *Harmonia axyridis*. One hundred marked *M. picta* and *H. axyridis* were released onto single isolated Austrian pines infested with the aphid *Eulachnus rileyi* once in 2006 and once in 2007. On both occasions marked *H. axyridis* left the pine trees almost immediately, but marked *M. picta* continued to be detected for periods in excess of 10 days. Unmarked *H. axyridis* continued to be observed on the trees after the marked ladybirds had dispersed and in 2006 *H. axyridis* oviposition also continued after dispersal of marked individuals. In 2007, *M. picta* oviposition was observed to continue after *H. axyridis* oviposition had ceased. The factors that might be responsible for the differential dispersal of the two species from the pines either do not appear to satisfactorily explain the difference between them or are directly related to their degree of specialization. We therefore conclude that *M. picta*'s longer patch residence time is a consequence of it being more specialized. In the habitats in which they occur, longer patch residence times of specialist ladybirds and a tolerance of lower densities of their preferred prey are likely to make specialists better aphid biocontrol agents than generalists.

1. Introduction

Aphidophagous ladybirds are generally perceived as highly mobile organisms, with adults frequently moving between patches and habitats containing ephemeral aphid prey (Hodek and Honěk, 1996; Dixon, 2000). Nonetheless this view may be biased: the majority of well researched species are habitat- and dietary generalists (Sloggett, 2005), and more specialized ladybirds are likely to be more sedentary. Habitat- or prey species-specific adaptations that increase foraging and capture efficiency (e.g. Berthiaume et al., 2007; Sloggett, 2008a) make specialists more efficient predators of aphids in the habitats in which they live than generalists are. Specialists appear to be able to exploit lower aphid densities than generalists in comparable habitats, and this attribute argues that they are able to utilize the same patches of ephemeral aphids to reproduce for longer than generalists, which are restricted to periods of higher aphid density (Sloggett and Majerus, 2000; Sloggett, 2008b).

In general, adult ladybirds are expected to arrive and reproduce in aphid patches early on in their development. Reproducing too late will mean that there are insufficient aphids remaining to support development of the resulting ladybird larvae as aphid numbers decline (Hemptinne et al., 1992; Dixon, 1997). Due to their higher feeding efficiency, specialist adults are expected to arrive in patches and to begin to reproduce earlier than generalists, which will only be able to obtain enough food to support reproduction as aphids reach higher densities. Specialists also should be able to reproduce later. In this case, greater efficiency of larval foraging in specialists is as important as the high efficiency of adults: high efficiency of specialist larvae will permit them to continue to locate and catch enough prey to support development as aphids decrease to low numbers.

There have been many phenological studies of generalist ladybirds that show for how long adults reside and reproduce in different habitats (e.g. Iperti, 1965; Honěk, 1989), and there are also many studies of temporal changes in ladybird species in particular habitats, notably crops (e.g. Banks, 1955; Agarwala and Bardhanroy, 1999). However, these provide only a broad habitat-wide perspective on the lengths of time that ladybirds persist and reproduce for. They are uncommon for specialists and, to our knowledge, only one study has compared a specialist and a generalist in the same habitat. In this study in balsam fir plantations, it was the generalist, *Harmonia axyridis* that reproduced for marginally longer than the conifer specialist *Anatis mali*; however, *A. mali* was better synchronized with the aphid prey, *Mindarus abietinus* (Berthiaume et al., 2007). Field studies within individual patches are rarer (but see

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Dixon, 1997, Fig. 12.3, p. 209). This is perhaps understandable as in many habitats, a crop field for example, it is often unclear exactly what spatial scale constitutes a patch, and different ladybirds aggregate within the habitat at different spatial scales (e.g. Schellhorn and Andow, 2005). Unsurprisingly, given their overall underrepresentation in the literature, there do not appear to be any studies of the patch dynamics of specialist species. The assertion that specialists persist and reproduce in one place for longer than generalists has thus lacked any clear corroboration from the field.

A better understanding of more specialized ladybird species is certainly desirable. In contrast to phytophagous insects, the factors responsible for dietary or habitat specialization in insect predators have been less well understood (Bristow, 1988). Ladybirds have much to contribute to our overall understanding of predator specialization because their dietary range is easily characterized and much of their biology is, in general(ist) terms, well understood (Sloggett and Majerus, 2000; Sloggett, 2008b). Additionally, the potential of specialist ladybirds for control of pest aphids has to a great extent remained unexplored. Generalist species such as *H. axyridis*, *Coccinella septempunctata* and *Coleomegilla maculata* predominate in aphid biocontrol (e.g. Koch, 2003; Seagraves and Yeargan, 2006; Hodek and Michaud, 2008) and based largely on the numerous studies of such species it has been argued that ladybirds have often proven less effective than is desirable (Dixon, 2000, but see Hodek and Michaud, 2008). Additionally, introduced generalists undoubtedly have exhibited a variety of undesirable non-target effects beyond their target agroecosystems (e.g. Harmon et al., 2007; Koch and Galvan, 2008). Among coccidophagous ladybirds, specialists appear to provide better biocontrol than generalists (Dixon, 2000) and there are reasons for thinking that the same is likely to be true for aphidophagous ladybirds (Sloggett, 2005). Clearly longer patch residence times, at lower aphid densities, would be such a reason.

In this study, the patch residency of ladybirds in relation to specialization is assessed for two species, *H. axyridis* (Pallas) and *Mulsantina picta* (Randall), on aphid-infested pine trees. The dietary and habitat generalist *H. axyridis* breeds in a diverse array of arboreal and herbaceous habitats, including pines (e.g. Sasaji, 1980; Majerus et al., 2006), while *M. picta* is a conifer specialist (e.g. Gagné and Martin, 1968; Chapin, 1985). Their relative specialization is reflected in the aphid densities with which they are associated: while *H. axyridis* tends to reproduce just before or at peak aphid densities (e.g. Hironori and Katsuhiro, 1997; Osawa, 2000), *M. picta* is a dominant species in older pine trees, which have low aphid densities (Gagné and Martin, 1968). Given that their aphid density relationships are already known, a comparison of their residence time in patches is particularly desirable. In the work described here, marked individuals of both species are inundatively released on individual trees. The numbers of marked individuals observed during subsequent examinations of the trees are used to compare the patch residence times of the two species and to test the hypothesis that *M. picta* remains in patches for longer.

2. Materials and methods

2.1. The system

The system used comprised Austrian pines, *Pinus nigra* Arnold, the aphid *Eulachnus rileyi* (Williams) and the two ladybird predators in Lexington, KY, USA. The pine is a European species first introduced to North America in the 18th century and frequently used in urban and landscape settings (Burns and Honkala, 1990). Ornamental plantings are common in Lexington. The aphid *E. rileyi* predominates on *P. nigra* in Lexington; it is also a Palaearctic species that has been introduced to North America (Blackman and Eastop, 1994). This aphid has the potential to be a pest on ornamental pines, with heavy infestations causing yellowing of the needles and premature needle drop (Felt and Bromley, 1936). Although *E. rileyi* is not a North American native, it shares a variety of important features with North American pine aphids, notably members of the genus *Essigella*, including its spindle-like shape, small size and high mobility when disturbed (Blackman and Eastop, 1994). It is therefore reasonable to assume that interactions of American pine specialist ladybirds are similar with this species to those with some North American pine aphids. In Lexington, *E. rileyi* increase to peak densities in May, after which populations decline. During the course of the studies described here virtually no other aphids were observed, although small isolated colonies of an unidentified species of *Cinara* were found in very low numbers.

The two ladybirds, *H. axyridis* and *M. picta*, are the only species that regularly breed on the pine trees, although other species may occasionally visit them. The generalist *H. axyridis* is an introduced Asian species that has spread through much of North America (Koch et al., 2006). It established in Kentucky in about 1992 (Cottrell and Yeargan, 1998) and is now found breeding in a diverse array of habitats, including pines. The native specialist *M. picta* is strikingly variable in size and coloration over its range. Specimens from the south eastern United States, including Kentucky, are larger, with an orange ground color and (if present at all) heavy black markings, unlike the smaller, browner, less heavily marked specimens from more northerly states such as Michigan (Chapin, 1985; Sloggett and Zeilstra, personal observation). There is thus a possibility that *M. picta* is comprised of separate subspecies, although Chapin (1985) was not of this opinion; furthermore, the association with pines appears to be uniform throughout *M. picta*'s range.

2.2. Origin, breeding and storage of ladybirds for release

Mulsantina picta is not easily found in large numbers in Lexington and the collection of sufficient ladybirds for marking and release would be unfeasible. For this reason ladybirds were reared in the laboratory for release. Collections of *H. axyridis* and *M. picta* were made in Lexington late in 2005 and 2006 to rear stocks for release the following year. After collection, adult ladybirds were anaesthetized using CO_2 and sexed using abdominal characters: in both species, the terminal segment of the male exhibits a clear notch that is not present in the females, as occurs in many Coccinellini (e.g. Randall et al., 1992). Breeding commenced in January. At this time, adults were maintained as mated females or pairs in 100 mm diameter Petri dishes at a temperature of 20–22 °C and photoperiod of 16 h light to 8 h dark. They were fed daily on pea aphids, *Acyrthosiphon pisum* (Harris) and provided with a small piece of apple (0.5 cm^3) as a fluid source. The aphid is not a natural prey for *M. picta*, but the ladybird will readily breed on it, and *M. picta* larvae provided with *A. pisum* exhibit high growth rates and low mortality (Sloggett, personal observation). Petri dishes containing eggs were replaced each day and retained, and eggs were reared to adults as family groups. Rearing was carried out under similar conditions of food, light and temperature to the adults in the first year, but in the second year larvae were reared in greenhouse at higher temperatures and under natural winter conditions of shorter daylength. Newly enclosed young adult ladybirds were sexed as previously described, and fed on the same diet for a minimum of four days. They were then placed in a refrigerator in single-sex family groups until required; at this time they were provided with apple weekly as a fluid source. Neither species exhibits an obligatory overwintering diapause (Sloggett, personal observation), and refrigeration was used merely as a non-labor intensive, convenient means of storage.

2.3. Preparation of ladybirds for release

In 2006, ladybirds were removed from the refrigerator nine days prior to release, in late April. Experience gained in the first year suggested that *M. picta* took longer to begin to reproduce than *H. axyridis* after refrigeration and in 2007 *M. picta* were removed in early May (one month prior to release) and *H. axyridis* in mid May (two weeks prior to release). They were provided with aphids and apple under conditions similar to those previously described; however, the light cycle used after removal from the fridge was chosen to match that outdoors at the same time (and consequently lengthened every few days) to ensure that behavior on release was not affected by a sudden change of photoperiod.

Beetles were marked using paint taken from TexPen® markers thinned using a non-toxic turpentine substitute (Bates et al., 2005). In trials of a number of different inks, pens and dyes, paint from the TexPen® (or Mark-Tex Tech-Pen as it was then known) was found to be the most durable when used for marking insects (Wineriter and Walkeor, 1984), and this paint is well suited for the marking ladybirds in studies of relatively short duration such as this. We used blue paint, since this color is easily distinguished from both the ladybird elytra and from foliage. Ladybirds were anesthetized under CO_2, marked and placed at 10 °C until the paint had dried and they had recovered. For marking a single dot of paint was applied to one of their elytra (left or right, depending on year) with a diameter of approximately 2 mm in *M. picta* and 4 mm in *H. axyridis*, which is about twice as large as *M. picta*. Laboratory tests and observations showed that ladybirds marked thus were still able to fly and exhibited no evident effects on their behavior or mortality. No marked adults of either species died in the laboratory over a period corresponding to the length of the field experiments (*H. axyridis*, $n = 10$; *M. picta*, $n = 22$). Ladybirds were marked several days prior to release.

2.4. Release of ladybirds

For aphidophagous ladybirds we can define a patch as a continuous area of aphid-bearing habitat through which the larvae can move to find aphid prey. Under this definition adults move between patches for reproduction and their larvae are restricted to a single patch during their development. This is most consistent with current theory on the patch dynamics of ladybirds (Dixon, 1997, 2000). The aphid *E. rileyi* appeared to be relatively uniformly distributed over pine trees rather than being concentrated on individual branches or buds; furthermore, in landscape plantings many of the trees planted are sufficient distances from each other that they do not touch. Consequently the patches used in this study were comprised of single trees not touching any close neighbors. One release of 100 ladybirds of each species was carried out in 2006 and one in 2007 on a single isolated tree that was part of a group of three to four trees. In the two years, releases were carried out on different trees at different locations on the University of Kentucky campus in Lexington.

Release times were determined by the aphid phenology and particularly by the phenology of wild resident ladybirds on the trees. We carried out regular surveys to determine the best tree and time to release the ladybirds. The specialist *M. picta* by virtue of its tolerance of lower aphid densities (Gagné and Martin, 1968) is likely to begin reproduction earlier at lower aphid densities than *H. axyridis*. A release of ladybirds before the habitat is suitable for *H. axyridis* oviposition is likely to lead to rapid abandonment of the tree by this generalist. It was therefore necessary that ladybirds were released at a point when aphid density was suitable to sustain *H. axyridis* oviposition. We therefore released ladybirds at the point when *H. axyridis* eggs were first observed on the tree. In 2006, this was on the 6th May. In 2007, an initial increase in aphids was observed in April, although no ladybird eggs were discovered. Cold weather led to an aphid population decline in late April and early May, and we were unable to release ladybirds until populations had recovered and *H. axyridis* eggs had been observed, on the 19th May. Subsequent observations suggested that a small amount of *H. axyridis* breeding had occurred during the earlier warm period (see Section 3).

For release, ladybirds were placed in cylindrical plastic containers (volume approximately 500 cm^3) with a lid. A square panel of approximately 25 cm^2 was cut out from the side and paper taped securely over this exit hole. Two containers per tree were used, each holding about 25 male and 25 female ladybirds of both species (with an exact total of 50 males and 50 females). We ensured that ladybirds from as many different families as possible (about 10 per species) were mixed in containers. Females and males from the same family were placed in different container to reduce inbred matings occurring in the released ladybirds: this meant that it was not always possible to exactly equalize the numbers of ladybirds in the two containers at 25. Ladybirds were placed in the containers in a walk-in refrigerator, since at the cooler temperatures it was possible to ensure that none escaped. They were allowed to warm for several hours afterwards before release. An excess of pea aphids were also placed in the containers, to ensure that ladybirds were satiated at release.

Releases were carried out just before dark; this allowed ladybirds to disperse more widely on the tree from the containers at initially lower temperatures, which are less likely to stimulate flight. The two containers were taped securely to low branches on opposite sides of the tree with the paper covered exit hole facing uppermost, so that ladybirds could not drop out of the container. Care was taken to ensure that none of the sticky side of the tape was left exposed, so that ladybirds could become stuck to it. After the containers were secured, the paper covering the exit hole was carefully cut out using scissors and a pine shoot inserted into the hole for the ladybirds to climb on. Containers remained on the tree for one day; by this time the majority of ladybirds had already left the containers. When containers were removed any ladybirds remaining inside were transferred manually to shoots on the tree.

2.5. Data collection

Counts of aphids and ladybirds were made on the lower branches of the tree (i.e. those that could be easily reached by the observer). Counts were first made immediately prior to release of the marked ladybirds and thereafter at regular intervals. In 2006 counts were made every two days for 12 days after release with a final count being made after a further five days (17 days after release). In 2007 due to warmer weather and a consequent expected higher activity rate, counts were made daily for the first week and then every two days for a further six days (to 13 days after release). Counts ceased when no further marked individuals of either species were recorded.

At each count aphids were counted on the terminal 10 cm of 25 randomly selected shoots, where they tend to occur. All branches within reach were carefully examined and marked and unmarked ladybirds and immature stages were recorded. It was possible to distinguish overwintered, reproducing *H. axyridis* adults from recently emerged adults of the spring generation by the ground color of the elytra which is a darker orange in the older adults (cf. Majerus, 1994). The immature stages of *M. picta* are easily distinguished from those of *H. axyridis*. The eggs of *M. picta* are smaller, as are the pupae; young larvae are lighter in color and less spiny than those of *H. axyridis* and older larvae have white dorsolongitudinal stripes unlike the larvae of *H. axyridis*, which have yellow longitudinal markings.

Data on mean temperature and rainfall during the study periods was obtained online from the US National Climatic Data Center database (http://www.ncdc.noaa.gov/oa/ncdc.html).

2.6. Analysis of marked ladybird data

In this work there is no *a priori* reason for assuming that emigration from the patch is constant or increases at a constant rate, as ladybirds of a species may potentially all remain in the patch until it becomes unsuitable and leave the patch at approximately the same time. Preliminary tests indicated that linear regression of log-transformed data (which would be suitable if emigration were constant or increased at a constant rate) generally provided a poor fit to the data. In any case emigration rates would be unnaturally high, given the large number of ladybirds introduced to a single tree (see Section 4). Analyses thus aimed exclusively to test our primary hypothesis about the two species' relative persistence in the patch rather than to quantify emigration rates. As an alternative to using log-linear regression, we adopted a different approach using Somers *d*-test, a non-parametric method for detecting an association between ordered variables (Siegel and Castellan, 1988). Rather than comparing the differences between two groups, this provides a measure of the degree of association of a dependent variable (in this case frequencies of marked individuals of the two species) with an independent one (time). If there is no association then the members of the two groups being tested leave the patch after a similar period. When the dependent variable is species frequency it may be considered ordered in respect of species specialization. The initial numbers of ladybirds released are not included in the analysis. The numbers of ladybirds observed on each occasion are likely to be a proportion of the total number present on the branches examined (i.e. not every ladybird is detected) and the proportion observed may vary for the two species: thus release and observational values are not comparable. Tests were one-tailed as a clear *a priori* alternative hypothesis existed (a positive relationship between specialization and length of time on the tree).

3. Results

3.1. 2006

In 2006, observations were made between 6th May (Day 0), when the ladybirds were released and the 23rd May (Day 17). During this time temperatures were below average, with mean daily temperature ranging between 10.6 and 17.2 °C; there was also a considerable amount of rain, most of it during the middle of the study (Fig. 1A). There was no obvious temporal pattern in aphid abundance over the course of the study with mean aphid density ranging between 1.5 and 4.9 aphids per shoot (Fig. 1B). There was evidence of both *H. axyridis* and *M. picta* presence on the tree at the start of observations (Fig. 1C and D), with *H. axyridis* adults and eggs and two third instar *M. picta* larvae counted on Day 0, prior to release of the marked ladybirds.

Two days after release, nine marked *H. axyridis* and 16 marked *M. picta* were observed (Fig. 1E). After this time no further marked *H. axyridis* were observed on the tree, although marked *M. picta* continued to be observed until Day 12, when five *M. picta* were still recorded. No *M. picta* were recorded by Day 17 (Fig. 1E). The relationship between species and time on the tree is highly significant (one-tailed Somers' *d*-test, $d = 0.24$, $z = 2.67$, $P < 0.005$).

Although the marked *H. axyridis* rapidly left the tree, unmarked *H. axyridis* adults continued to be found on the tree at low levels for the entire course of the study (Fig. 1C): the dark color of their elytra indicated that these were overwintered, reproductively active adults. Egg clutches of *H. axyridis* peaked on the tree on Day 2, probably as a consequence of the release of the marked *H. axyridis*. *H. axyridis* egg clutches continued to be found on the tree for the entire duration of the study, although after Day 2, they slowly declined in number (Fig. 1C). The clutches recorded early on hatched long before the end of the study: it therefore appears that unmarked adults continued to reproduce on the tree. Larvae of *H. axyridis* were also observed on and after Day 2; however, no *H. axyridis* pupae were recorded during the study.

Larvae and unmarked adults of *M. picta* were intermittently observed on the tree during the study (Fig. 1D). However, although marked adults were observed mating and marked females were observed distended with eggs, no *M. picta* eggs were recorded until the last day of observations.

3.2. 2007

In 2007, observations were made between 19th May (Day 0), when the ladybirds were released, and the 1st June (Day 13). Temperatures were higher than in 2006, with an increase in mean daily temperature from 13.3 °C on Day 0 to 24.4 °C on Day 5, after which the temperature remained about the same (Fig. 2A). In contrast to 2006, there was virtually no rain in 2007 (Fig. 2A). Overall aphid abundance declined over the course of the study from a mean of 5.8 aphids per shoot on Day 0 to 0.8 aphids per shoot on Day 13 (Fig. 2B). Adults, eggs and larvae of *H. axyridis* were recorded on the tree on Day 0 (Fig. 2C). Two of the larvae were fourth instar, and the subsequent discovery of a pupa on Day 1, suggested that a small amount of *H. axyridis* breeding had occurred on the tree during an earlier warm period (see Section 2). No observations of *M. picta* were made on the tree on Day 0 (Fig. 2D).

The day after release, five marked *H. axyridis* and 20 marked *M. picta* were observed (Fig. 2E). After Day 1 no further marked *H. axyridis* were observed on the tree; however, marked *M. picta* continued to be observed until Day 11, although in smaller numbers than in 2006. No *M. picta* were recorded by Day 13 (Fig. 2E). The relationship between species and time on the tree is again significant (one-tailed Somers' *d*-test, $d = 0.15$, $z = 1.99$, $P < 0.025$).

Unmarked *H. axyridis* adults continued to be found on the tree at low levels during the study (Fig. 2C). The majority had dark elytra indicating that these were overwintered, reproductively active adults; however, a light colored recently emerged adult was observed at the end of the study. Egg clutches of *H. axyridis* peaked on the tree on Day 2, and thereafter declined rapidly, as they hatched, to none on Day 5 (Fig. 2C). Larvae of *H. axyridis* were also observed during the entire course of the study; pupae were also intermittently recorded.

No larvae, pupae or unmarked adults of *M. picta* were observed on the tree during the study (Fig. 2D). However, *M. picta* eggs, presumably from the marked adults, were recorded over the first seven days; no further eggs were recorded after Day 9.

4. Discussion

In both 2006 and 2007, marked individuals of the specialist *M. picta* were found on release trees longer than *H. axyridis*. The data are consistent with the view that specialist aphidophagous ladybirds are more sedentary than generalist ones and remain in patches for longer. However, the release of a large number of ladybirds into a relatively small area is likely to affect the number of aphids on the tree through predation and thus to affect the suitability of the tree for ladybirds. Because of this, such an experiment can only provide a qualitative comparison and cannot provide a quantitative measure of natural ladybird dispersal from a patch.

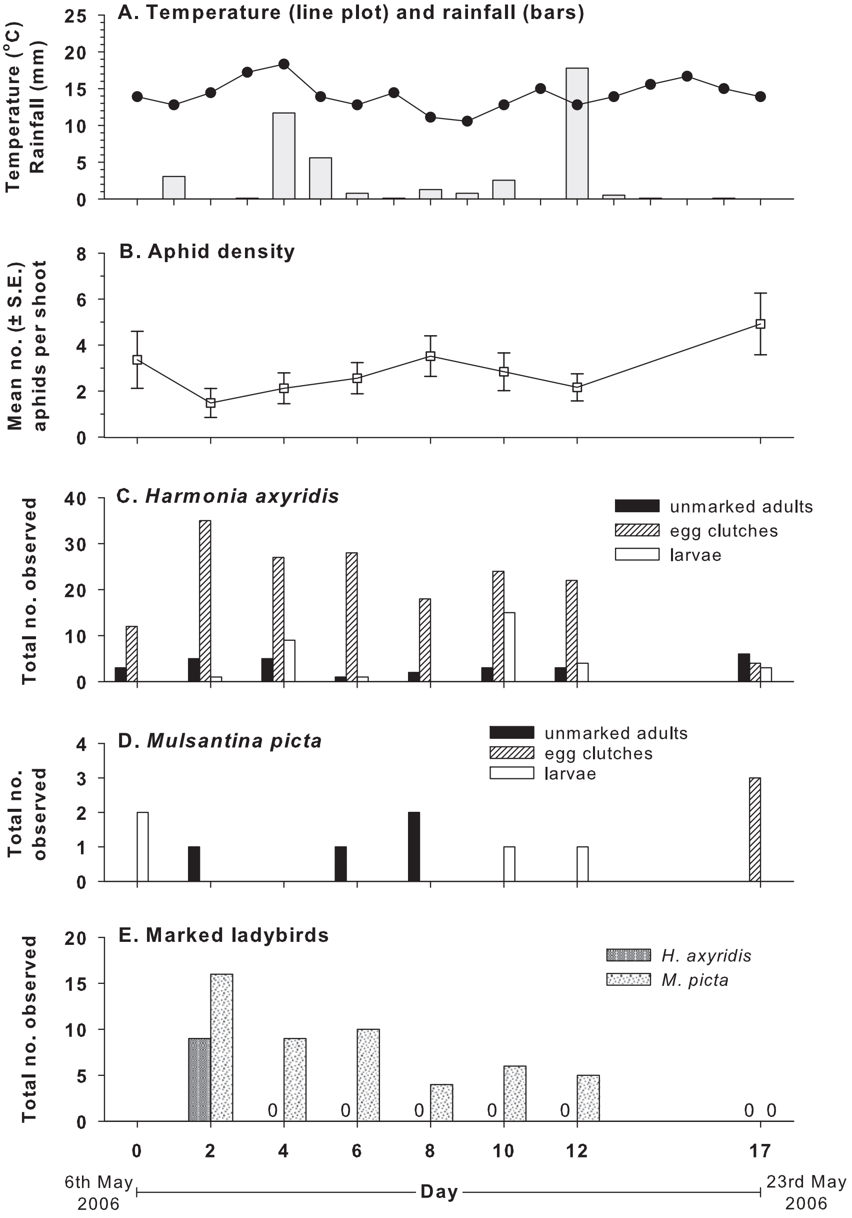

Fig. 1. Results for the 2006 release study. (A) Temperature (line plot) and rainfall (bars) over the study period. (B) Mean (±standard error) of the number of aphids (almost exclusively *E. rileyi*) on pine shoots ($n = 25$ for each sampling date). (C) Numbers of unmarked *H. axyridis* adults (all overwintered adults), egg clutches and larvae (no pupae were recorded). (D) Numbers of unmarked *M. picta* adults egg clutches and larvae (no pupae were recorded). (E) Numbers of marked *H. axyridis* and *M. picta* observed on each sampling date.

The inundative release method described here measures the patch residency of an individual group of ladybirds rather than giving an overall measure of a species' duration in the patch. In the case of *H. axyridis*, numbers of marked ladybirds declined very rapidly after release. A peak in *H. axyridis* egg clutches shortly after the release of marked individuals suggested that in both years some adults did oviposit onto the trees before dispersing. However, in both years reproductively mature unmarked adults occurred on the trees after the marked *H. axyridis* had dispersed, and in 2006 egg clutches continued to be found on the tree long after the eggs laid by the marked ladybirds would have hatched. In the latter case the patch clearly remained acceptable for oviposition for *H. axyridis* for longer than the marked adults remained there. Aphidophagous ladybirds appear to sample patches of aphids, passing through at low densities, but only accumulating in numbers in high quality patches (Evans, 2004). *H. axyridis* is extremely common in Lexington and the continued presence of reproductively mature adults is a likely consequence of individuals of this abundant ladybird continuing to sample the tree. The decision to oviposit is, in addition to being influenced by the quality of the patch, also influenced by the quality of recently visited patches (Fréchette et al., 2004). If few high quality patches were available in 2006, this might explain why *H. axyridis* oviposition continued on the tree, although not by the marked individuals.

A different pattern was observed in *M. picta*. Directly after release, as with *H. axyridis*, there was a sharp decline in numbers from the original 100, caused in large part by ladybirds spreading away from their point of release to other parts of the tree. However, unlike *H. axyridis*, marked *M. picta* continued to be detected for relatively long periods of time. The absence of detection of any *M. picta* eggs until the end of the study in 2006 led to concerns that some of the ladybirds might not have been fully active after

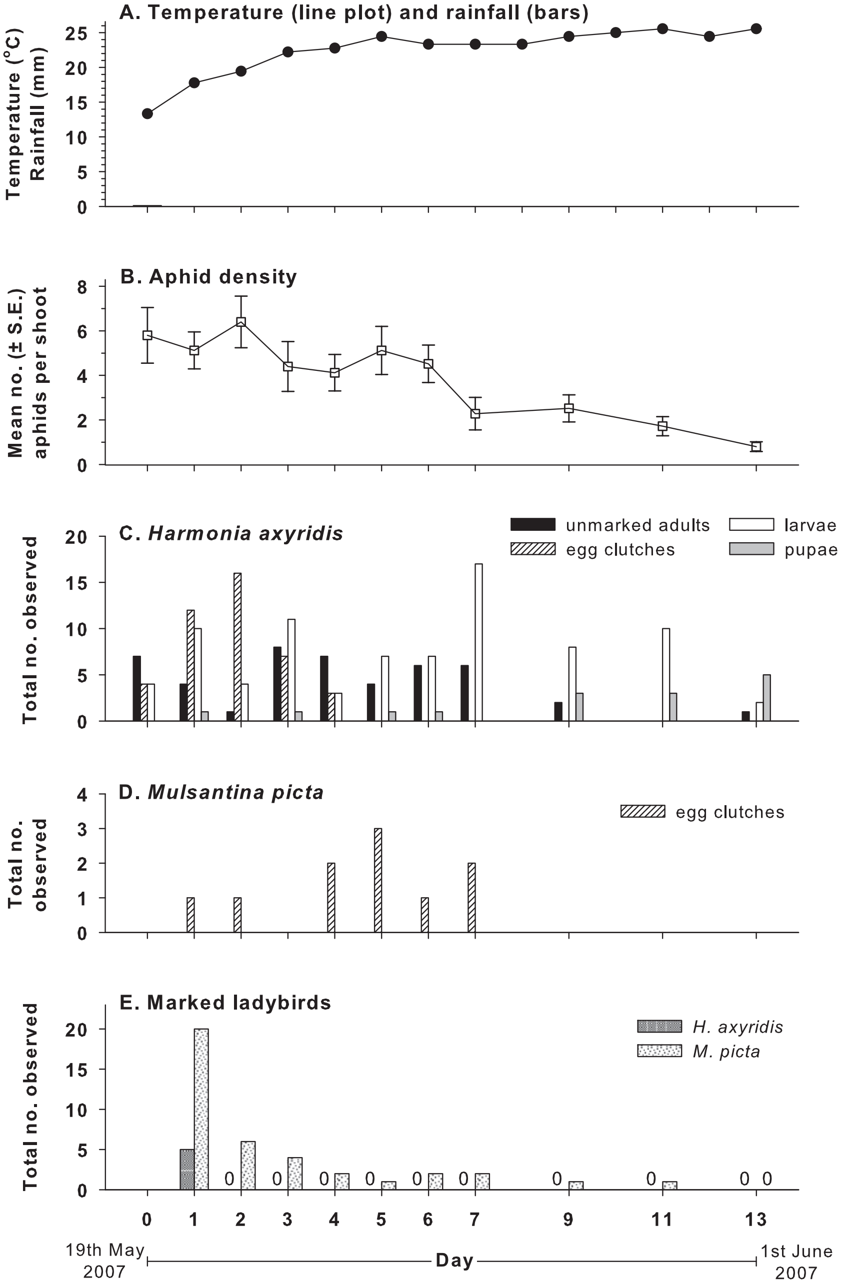

Fig. 2. Results for the 2007 release study. (A) Temperature (line plot) and rainfall (bars) over the study period. (B) Mean (±standard error) of the number of aphids (exclusively *E. rileyi*) on pine shoots ($n = 25$ for each sampling date). (C) Numbers of unmarked *H. axyridis* adults (all overwintered adults except on Day 13) and immature stages. (D) Numbers of *M. picta* eggs (no other life history stages were observed except the marked adults). (E) Numbers of marked *H. axyridis* and *M. picta* observed on each sampling date.

refrigeration in this year, and this led to the long residence times on the tree. However, similar results in 2007, along with the presence of eggs suggested this was not the case. It seems more likely that the absence of oviposition in 2006 may have been influenced by the wet weather (see Timms and Leather, 2007), which is more likely to pose more problems for foraging and oviposition for a small ladybird such as *M. picta* than the larger *H. axyridis*. In 2007, *M. picta* eggs, as well as marked adults, were detectable for longer than those of *H. axyridis*, as aphid densities declined.

A clear problem in a study such as this, involving only two species, is the question of whether the differences observed are truly a product of differing degree of specialization or result from some other factor. A possibility that cannot be entirely excluded, although is unlikely, is that *H. axyridis* were not sampled from the lower branches after the initial observations because they prefer to be higher in the tree than *M. picta* (see Berthiaume et al., 2007). It seems exceedingly improbable that even if this were the case, no *H. axyridis* would be observed at all after the initial observations in both study years. This is reinforced by the fact that *H. axyridis* also occurs regularly low-down on herbaceous plants (e.g. Sasaji, 1980; Majerus et al., 2006) and, in this study, unmarked *H. axyridis* and *H. axyridis* eggs were observed on the lower trees much of the time. The complete absence of observations clearly supports *H. axyridis* having really left the trees.

If the difference in patch residency is a real one, could other species-specific factors, unrelated to specialization, explain the difference? The very different weather in 2006 and 2007 make it unlikely that a differential species response to environmental conditions can explain the differences in patch residency. However, the two species still differ intrinsically in many other ways, most notably in body size, with *M. picta* being much smaller. It could be argued that *M. picta*, as a small ladybird, will naturally remain longer in patches, because its smaller size means it requires less food. This is undoubtedly true. However, the body size of aphidophagous ladybirds is strongly related to dietary breadth and specialization, due to its role in determining aphid capture efficiency and the amount of food required for reproduction (Sloggett, 2008a). In the case of *M. picta*, adults appear to be more tolerant of lower densities of *E. rileyi* (and similar American pine aphids) by virtue of their small size, which facilitates reproduction even at low aphid densities while also supporting high capture efficiencies of this small prey. Thus while body size is a more direct determinant of the difference in the relationship between the two ladybirds and their pine aphid prey the ultimate cause is their differing specialization. The same argument applies for other intrinsic differences between the species: specialization appears to interact with a diversity of biological characters in ladybirds including mobility and other aspects of foraging behavior (Gagné and Martin, 1968; Berthiaume et al., 2007), reproductive strategies (Timms and Leather, 2007; Sloggett and Lorenz, 2008) and even chemical defense (Sloggett, 2005).

Thus it seems highly likely that the more specialized *M. picta* exploiting aphid patches for longer is an ultimate consequence of its specialization, although further studies are desirable to establish the generality of the relationship between specialization and patch residency. The developing larvae of *M. picta* also appear to exhibit foraging adaptations for greater efficiency, searching more actively for aphids (Gagné and Martin, 1968). It is probable that they are less affected by the aphid population decline than larvae of the generalist *H. axyridis*. The adults of *M. picta* are likely also to colonize pine aphid patches earlier than *H. axyridis*, when aphid densities are also low. Although this was not tested in the present study, it is worth noting that in 2006 third instar *M. picta* larvae were observed on the tree at the start of the study, although no *H. axyridis* larvae were found at this time. *M. picta* thus appears to start reproduction earlier than *H. axyridis* in patches of pine aphids and to continue to reproduce for longer.

The proximate cue for adult ladybirds to leave a patch of aphids is generally considered to be chemical cues from conspecific larvae in the patch, which inhibit oviposition (e.g. Růžička, 2001; Laubertie et al., 2006). The effect of cues from larvae increases with increasing larval density (e.g. Doumbia et al., 1998; Oliver et al., 2006) and this enables ladybirds to avoid high levels of intraspecific predation or competition for their larvae (Hemptinne et al., 1992; Dixon, 1997). Both *H. axyridis* and *M. picta* from northern populations have been found to be sensitive to such larval cues (Yasuda et al., 2000; Sloggett, unpublished data). Because *H. axyridis* is much commoner than *M. picta* and individuals lay many more eggs, even on pines, *H. axyridis* larvae reach critical densities for oviposition deterrence earlier in this species, leading to the longer residence of *M. picta* on the tree. Beyond this system, the "boom and bust" population dynamics of generalist species (e.g. Majerus and Majerus, 1996) may make population size or growth a natural regulating factor for the differences in patch residency of specialists and generalists. However, while of likely applicability to this study, this may not be the only factor responsible for differences between the two ecotypes. Ladybirds appear to integrate larval cues with aphid density to determine oviposition decisions (Oliver et al., 2006) and the differences in the acceptability of low aphid densities to specialists and generalists may thus play a direct role in regulating patch residency. It would nonetheless be of interest to compare the patch residence times of specialists and generalists in a habitat where the former outnumber the latter.

The idea that specialists remain active in patches and habitats for longer than generalists is integral to much of the current argument relating to specialization in aphidophagous ladybirds. An ability to reproduce for longer on lower densities of aphids means that fewer different habitats are used during the breeding season, which itself is specialization (Sloggett, 2008b). This study also serves to emphasize that the more sedentary behavior of specialists makes them highly suitable as biocontrol agents in habitats in which they occur. If they are released or somehow encouraged to naturally colonize a suitable area, they are likely to remain there and reproduce rather than dispersing elsewhere. Their exploitation of low aphid densities may make them particularly important early in the season in preventing aphid pests reaching high densities later on. The rearing method used here also illustrates that specialists are no more difficult to breed for inundative releases than generalists. In fact the majority of aphidophagous specialists can be bred in captivity on aphids such as the pea aphid (e.g. Majerus and Kearns, 1989), as described for *M. picta* here, and some may even perform moderately well on non-aphid prey (e.g. Niijima et al., 1986; Cook and Webb, 1995). Specialist species do tend to be concentrated in certain types of aphid-bearing habitats, such as arboreal ones, whereas they are largely absent in others, such as many field crops. Nonetheless for systems where they do occur, including conifer stands (e.g. Day et al., 2006; Berthiaume et al., 2007), crop trees (e.g. Cecilio and Ilharco, 1997) and rice paddies (Goidanich, 1943) specialist ladybirds appear to display many desirable characteristics to provide better control of aphid prey, with fewer undesirable side-effects.

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