

Intraguild predation of the aphid pathogenic fungus *Pandora neoaphidis* by the invasive coccinellid *Harmonia axyridis*

H. E. ROY^{1,2}, J. BAVERSTOCK³, R. L. WARE⁴, S. J. CLARK⁵,
M. E. N. MAJERUS⁴, K. E. BAVERSTOCK³ and J. K. PELL³

¹NERC Centre for Ecology and Hydrology, Monks Wood, Huntingdon, Cambridgeshire, U.K., ²Department of Life Sciences, Anglia Ruskin University, Cambridge, U.K., ³Department of Plant and Invertebrate Ecology, Rothamsted Research, Harpenden, Hertfordshire, U.K., ⁴Department of Genetics, Cambridge University, Cambridge, U.K. and ⁵Department of Biomathematics and Bioinformatics, Rothamsted Research, Harpenden, Hertfordshire, U.K.

Abstract. 1. The ladybird *Harmonia axyridis* is an invasive alien species in many countries and is predicted to have a negative impact on native biodiversity. However, little is known on the status of this aphidophage as an intraguild predator of natural enemies of aphids such as insect-pathogenic fungi.

2. The study assessed the predation of the aphid-specific pathogenic fungus *Pandora neoaphidis* by adult and larval *H. axyridis* collected from the U.K. (an invasive population) and Japan (a native population) relative to that of the ladybird *Coccinella septempunctata* (native to the U.K.) and the non-U.K. *C. septempunctata* subspecies *brucki* that were either starved or unstarved.

3. Overall, predation of uninfected aphids was greater than infected aphids and, when given a choice, a preference for aphids was shown. However, the extent of this preference was dependent on the species and origin of the coccinellid. *Harmonia axyridis* (U.K.) consumed the greatest quantity of fungal cadavers and showed little preference for uninfected aphids over infected aphids. In contrast, *C. septempunctata* rarely consumed infected aphids. Life stage had no direct effects on predation but starved coccinellids consumed more uninfected aphids than infected aphids.

4. *Harmonia axyridis* (U.K.) is a stronger intraguild predator of *P. neoaphidis* cadavers than the native species *C. septempunctata* and, therefore, may have an impact on the occurrence and persistence of *P. neoaphidis*. The differences in intraguild predation by *H. axyridis* collected in the U.K. and those from Japan suggests that individuals that invaded the U.K. could have a different genetic profile to those in its native range.

Key words. Allee effect, *Coccinella septempunctata*, *Harmonia axyridis*, *Pandora neoaphidis*, predation.

Introduction

Biotic homogenisation is considered among the greatest threats to biodiversity, with widely recognised ecological and evolu-

tionary consequences (Olden & Poff, 2004; Olden *et al.*, 2006). The rapid increase in introduced exotic species worldwide and the potential of these species to become invasive (Kajita *et al.*, 2006) further contributes to the problems associated with biotic homogenisation. Many accidentally introduced species have negligible effects and many of the species introduced with agriculture and forestry are beneficial and desirable (Williamson, 1999). However, the impact of some invaders is unquestionably negative (Williamson, 1996). The harlequin ladybird, *Harmonia*

Correspondence: Dr Helen Roy, Coordinator of Zoological Data & Research, Biological Records Centre, NERC CEH Monks Wood, Abbots Ripton, Huntingdon PE28 2LS, Cambridgeshire, UK. E-mail: hele@ceh.ac.uk

axyridis Pallas (Coleoptera: Coccinellidae), is predicted to be one such species (Majerus *et al.*, 2006; Roy *et al.*, 2006).

Harmonia axyridis has been used in many countries (U.S.A., South America, mainland Europe and the Middle East) as a classical biological control agent of pest insects, including aphids and scale insects (Gordon, 1985; Ipert & Bertand, 2001; Roy *et al.*, 2006). Consequently it has become established in many countries outside its native range of central and eastern Asia (Kuznetsov, 1997). This voracious predatory ladybird is now the dominant species of ladybird in many parts of North America (Teddars & Schaefer, 1994; Smith *et al.*, 1996; Hesler *et al.*, 2001) and has spread to regions far beyond intentional release sites. Indeed *H. axyridis* has not been deliberately released in the U.K. but in 2004 the first individual was recorded and it is now considered as an established invasive species in England (Majerus *et al.*, 2006). The arrival of *H. axyridis* in countries where it has not been deliberately released, and its expansion in areas where it has, is cause for considerable concern both from ecological and anthropogenic perspectives (Majerus *et al.*, 2006).

Recent attempts have been made to quantitatively predict the risk of an exotic species becoming invasive (van Lenteren *et al.*, 2003). Particular focus has been on classical biological control agents (Hokkanen *et al.*, 2003) and a recent methodology for risk assessment has been proposed for the regulation and release of exotic natural enemies (van Lenteren *et al.*, 2003). This general framework uses biological attributes [establishment, dispersal, host range, direct effects (on non-target herbivores and intra-guild predation) and indirect effects (competition with native natural enemies)] to produce numerical risk index values. Using this assessment process *H. axyridis* is allocated a high-risk index (van Lenteren *et al.*, 2003). *Harmonia axyridis* is a polyphagous coccinellid and, as such, is predicted to be a major threat to native biodiversity, both indirectly through resource competition and directly as an intra-guild predator (Roy *et al.*, 2005). Although studies on this are sparse there is evidence to suggest that *H. axyridis* is adversely affecting other aphidophages (Sato *et al.*, 2005). Indeed *H. axyridis* is one of the top predators within aphidophagous and coccidophagous guilds (Brown & Miller, 1998; Colunga-Garcia & Gage, 1998). Studies on the effect of predation by *H. axyridis* on other aphidophages such as the larvae of Neuroptera and some syrphids, parasitoids, and fungal pathogens of aphids are lacking and urgently needed.

Pandora neoaphidis (Remaudière and Hennebert) Humber is an aphid-specific fungal pathogen and is the most common entomopathogen of aphids in temperate regions. It is capable of causing epizootics in field populations of aphids and has, therefore, received considerable attention as a potential biological control agent (Pell *et al.*, 2001). *Pandora neoaphidis* and aphidophagous coccinellids utilise the same resource (aphids) and are regarded as being in the same guild. Adult *Coccinella septempunctata* L. ladybirds also prey upon *P. neoaphidis*-infected aphids, making them intraguild predators of the fungus (Roy *et al.*, 1998, 2003; Roy & Pell, 2000). However, *C. septempunctata* does not consume entire *P. neoaphidis*-sporulating cadavers and has no overall negative impact on the transmission of the fungus (Roy *et al.*, 1998, 2003; Roy & Pell, 2000). This paper assesses the intraguild predation of *P. neoaphidis* by *H. axyridis* (Japanese and British origin) relative to that of

C. septempunctata and its Japanese subspecies, *C. septempunctata* subspecies *brucki* Mulsant.

Materials and methods

Rearing aphids

Apterous adult pea aphids, *Acyrtosiphon pisum* Harris, were used in the experiment. These aphids were maintained on broad bean plants, *Vicia faba* L. (cultivar: The Sutton), until 2–5 h prior to the start of the experiment, at which time they were removed from the host plant and placed at -20°C . This killed the aphids whilst ensuring that their tissues remained intact. It was important to immobilise the uninfected aphids to ensure comparability with the infected aphids. These aphid cadavers will henceforth be referred to as *uninfected aphids*.

Rearing coccinellids

Laboratory reared adult and fourth-instar coccinellid larvae were used in this experiment. These cultures were established using adult coccinellids collected from four field locations: *H. axyridis* collected from the U.K. (Battersea Park, London) and Japan (Fuchu, Honshu), *C. septempunctata* collected in the U.K. (Cambridge/Norfolk) and *C. septempunctata* subspecies *brucki* collected in Japan (Fuchu, Honshu). The experiment was carried out using the F_1 generation of U.K.-collected coccinellids and the F_2 generation of coccinellids collected in Japan. Adult coccinellids were all female and had undergone eclosion within 8 weeks of the experiment, whilst larvae were of mixed sex but had all undergone third ecdysis within the same week as each other. The coccinellids were kept in batches of five individuals within 90 mm diameter single-vented Petri dishes and maintained at 22°C (LD 14:10 h). All individuals were fed an excess of *A. pisum* up to and including the day of the experiment, unless starved individuals were required, in which case no food was provided for 12 h (larvae) or 24 h (adults) prior to the start of the experiment.

Cultivating Pandora neoaphidis

Pandora neoaphidis isolate X4 (from Rothamsted Research collection, original host species = *A. pisum*) was used in this experiment. The culture was maintained by regular *in vivo* passage through *A. pisum*, as described by Wilding (1970). *Pandora neoaphidis*-infected aphid cadavers were dried and stored at 4°C and 20% relative humidity in darkness until required and for no longer than 3 months. These infected cadavers will henceforth be referred to as *infected aphids*.

Bioassay procedure

Forty-eight observational arenas consisting of a 90 mm diameter Petri dish containing a piece of filter paper (90 mm diameter) that had been moistened using tap water were prepared for each replicate of the experiment. Six potential prey items were arranged

in a hexagonal formation 40 mm apart in each arena. Arenas contained either six uninfected aphids, six infected aphids, or three of each prey type (arranged alternately). The infected aphids were added to the arenas 16 h prior to the start of the experiment and maintained at >95% relative humidity to allow rehydration and sporulation to occur. Uninfected aphids were not added to the arenas until immediately prior to the start of the experiment. Finally, a single adult/larval coccinellid that was either starved or unstarved was placed in the arena. This gave a total of 48 treatment combinations (three arena designs \times two life stages \times four coccinellid types \times two starvation levels). The arenas were arranged in a row in randomised order and maintained under ambient laboratory conditions. The number of prey items consumed entirely was assessed after 30, 60, 90, 120, 150, and 180 min. The experiment was replicated three times per day on 2 days.

Statistical analyses

The numbers of whole prey items consumed by each time point in the *uninfected aphids only* and *infected aphids only* treatments were analysed using repeated measures ANOVA to assess the effect of treatment, predator type, life-stage, starvation, and time on predation levels. To assess the relative preference for uninfected and infected prey items in the *mixed* treatment, the numbers of uninfected (c_u) and infected (c_i) aphids consumed were first adjusted to allow for zero counts (i.e. $l = c_u + 0.05$ and $r = c_i + 0.05$, respectively), and then log ratios ($\log(l/r)$) calculated. Hence, where no preference was shown between prey types $\log(l/r) = 0$; where uninfected aphids were the preferred prey item $\log(l/r) > 0$; and where infected aphids were the preferred prey item $\log(l/r) < 0$. The log ratios were analysed using a repeated measures ANOVA to assess the effect of predator type, life-stage, starvation, and time. Standard error of the difference (SE_D) was calculated.

Results

Single choice treatments

Predation was affected by prey type ($F_{1,155} = 95.72$, $P < 0.001$), predator type ($F_{3,155} = 3.44$, $P < 0.05$), life stage ($F_{1,155}$

$= 4.97$, $P < 0.05$), starvation level ($F_{1,155} = 8.64$, $P < 0.01$), and time ($F_{2,1,341.8} = 103.85$, $P < 0.001$). The effects of prey type and predator type both varied with time ($F_{2,1,341.8} = 27.77$, $P < 0.001$, and $F_{6,4,341.8} = 3.23$, $P < 0.01$, respectively). Interactions also occurred between prey type and predator type ($F_{3,155} = 9.87$, $P < 0.001$), and between prey type and starvation level ($F_{1,155} = 13.95$, $P < 0.001$). Both these interactions varied over time [$F_{6,4,341.8} = 3.88$, $P < 0.001$ (Fig. 1), and $F_{2,1,341.8} = 4.52$, $P = 0.01$ (Fig. 2), respectively]. There was also an interaction between life stage and prey type ($F_{1,155} = 6.03$, $P < 0.05$; Fig. 3a), and between life stage and predator type ($F_{3,155} = 2.90$, $P < 0.05$; Fig. 3b). Both these interactions were consistent over time. No other terms were statistically significant at the 5% level.

After 180 min, on average, 2.17 uninfected aphids had been consumed compared with 0.59 infected aphids ($n = 96$, $SE_D = 0.137$), starved coccinellids had consumed 1.60 prey items and unstarved coccinellids only 1.16 ($n = 96$, $SE_D = 0.137$), and the greatest predation was by *C. septempunctata* subspecies *brucki* with a mean of 1.73 prey items consumed followed by *H. axyridis* U.K. (1.67 prey items) and finally *C. septempunctata* and *H. axyridis* Japan, which both consumed 1.06 prey items on average ($n = 48$, $SE_D = 0.194$).

Differences between the overall numbers of uninfected and infected aphids consumed were relatively large for *C. septempunctata* (difference = 1.49, $SE_D = 0.244$) and *C. septempunctata* subspecies *brucki* (2.14, $SE_D = 0.244$), but much smaller for *H. axyridis* (U.K.) (0.46, $SE_D = 0.244$) and *H. axyridis* (Japan) (0.69, $SE_D = 0.244$). However, these relative differences changed with time. The greatest predation of aphids was by *C. septempunctata* subspecies *brucki*, which consumed more uninfected aphids after 30 min (mean = 1.17) than any other predator [0.79, 0.46, and 0.38 for *C. septempunctata*, *H. axyridis* (Japan), and *H. axyridis* (U.K.), respectively, $n = 24$, $SE_D = 0.274$] and continued to consume this prey item until the final observation, at which point it had consumed a mean of 3.21 uninfected prey, approximately 50% more than the next most voracious predator of uninfected prey, *C. septempunctata* (2.08, $n = 24$, $SE_D = 0.274$). *Harmonia axyridis* (Japan) was the least voracious of the ladybirds tested having consumed only 1.54 uninfected aphids on average ($n = 24$) by the final observation. In contrast the greatest predation of infected aphids

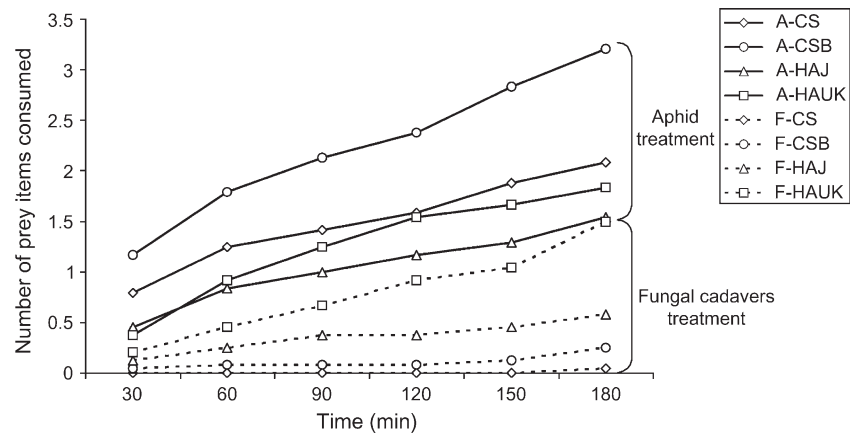


Fig. 1. Mean number ($n = 24$) of *Acyrtosiphon pisum* (A) or *Pandora neophidis*-sporulating fungal cadavers (F) consumed over a 180-min observation period in either the *aphid* or *fungal cadavers* treatments by *Coccinella septempunctata* (CS), *C. septempunctata* subspecies *brucki* (CSB), *Harmonia axyridis* (Japan) (HAJ) or *H. axyridis* (U.K.) (HAUK). SE_D (Standard Error of the Difference) for comparisons within each predator type/prey type combination = 0.136, SE_D for all other comparisons = 0.274.

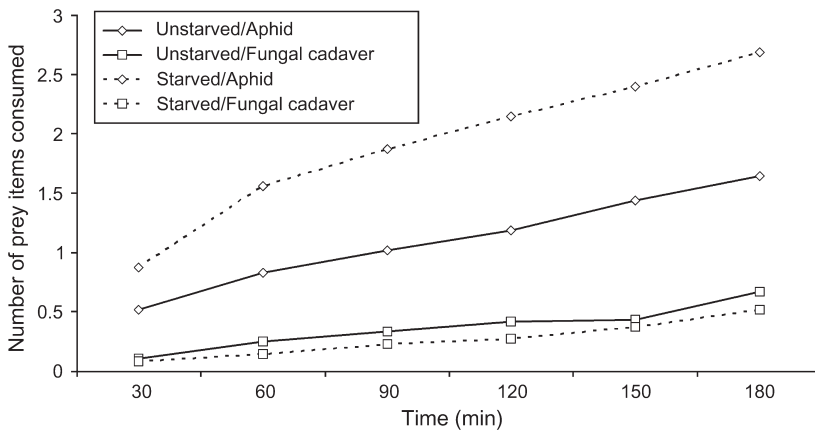


Fig. 2. Mean number ($n = 48$) of *Acyrtosiphon pisum* or *Pandora neoaphidis*-sporulating fungal cadavers consumed over a 180-min observation period in either the *aphid* or *fungal cadavers* treatments by coccinellids that had been provided with food until immediately prior to the start of the experiment (Unstarved) or had been starved prior to the start of the experiment. SE_D (Standard Error of the Difference) for comparisons within each starvation level–prey type combination = 0.096, SE_D for all other comparisons = 0.194.

was by *H. axyridis* (U.K.), which consumed a mean of 1.5 of this prey item by the final observation compared with *H. axyridis* (Japan), *C. septempunctata* subspecies *brucki*, and *C. septempunctata*, which consumed on average 0.58, 0.25, and 0.04, respectively ($n = 24$, $SED = 0.274$). Although predation of infected aphids occurred within 30 min in treatments containing *C. septempunctata*

subspecies *brucki* and both the U.K. and Japanese strains of *H. axyridis*, *C. septempunctata* was not observed feeding on infected aphids in any replicate until the final observation when it still only consumed an average of 0.04 prey items ($n = 24$; Fig. 1).

Starved coccinellids consumed 63% more uninfected aphids (mean = 2.69) than did unstarved coccinellids (mean = 1.65)

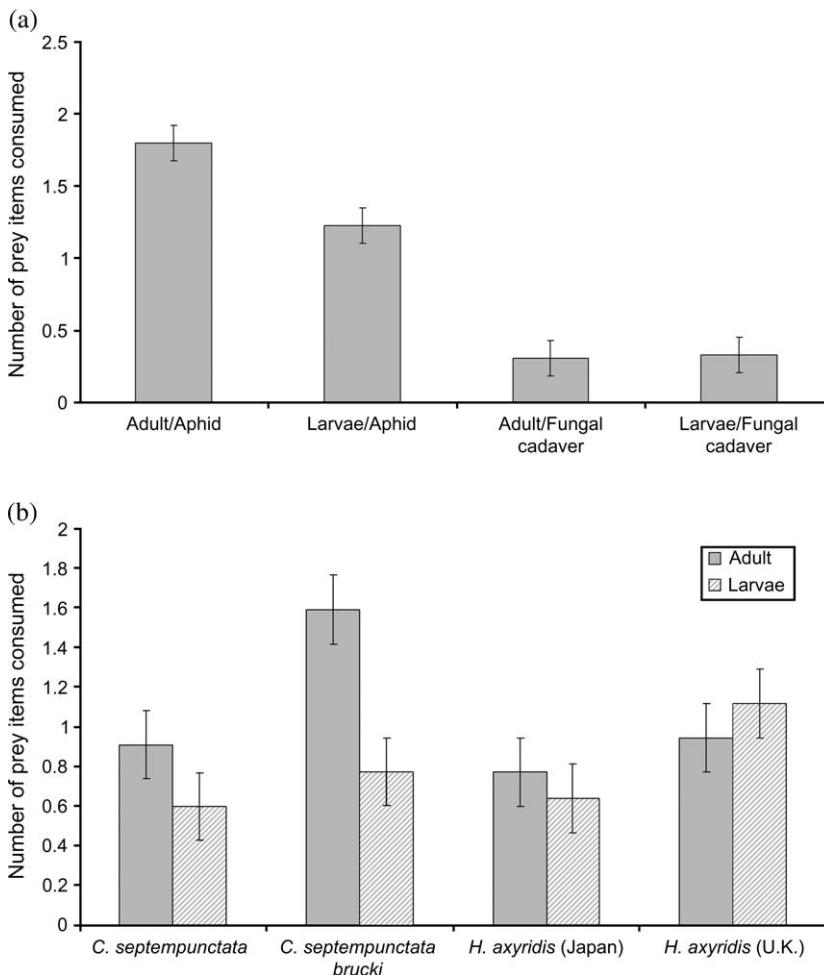


Fig. 3. (a) Mean number ($n = 288$) of *Acyrtosiphon pisum* or *Pandora neoaphidis*-sporulating fungal cadavers consumed in either the *aphid* or *fungal cadavers* treatments by adult or larval coccinellids. Means \pm SEM are shown; SE_D (Standard Error of the Difference) = 0.173. (b) Mean number ($n = 144$) of prey items consumed by adult or larval *H. axyridis* (U.K. and Japanese origin), *C. septempunctata*, and *C. septempunctata* subspecies *brucki*. Means \pm SEM are shown; $SE_D = 0.244$.

by the end of 180 min, whereas starved coccinellids (mean = 0.52) consumed 22% fewer infected aphids than did unstarved coccinellids (mean = 0.67, $n = 48$, $SE_D = 0.194$). However, the relative differences between prey types and starvation levels depended on time (Fig. 2).

On average, adults consumed 1.05 prey items and larvae 0.78 prey items ($n = 576$, $SE_D = 0.122$). However, adult coccinellids preyed more on uninfected aphids than did larvae (on average 1.80 and 1.23 aphids, respectively) whereas larval and adult coccinellids preyed similarly on infected aphids (on average 0.33 and 0.31 cadavers, respectively, $n = 288$, $SE_D = 0.173$; Fig. 3a). *Harmonia axyridis* (U.K.) larvae on average ate more prey items than adults of the same species; the reverse was true for the three other predator types, with adults of *C. septempunctata* subspecies *brucki* eating on average around twice the number of prey items compared to their larval counterparts (Fig. 3b).

Mixed treatment

The mean numbers of whole uninfected and infected aphids consumed for each treatment combination are given in Table 1. Preferences for the two prey types differed amongst the times ($F_{2,5,202,1} = 7.79$, $P < 0.001$) and the predator type ($F_{3,75} = 6.50$, $P < 0.001$), and these predator differences changed with time ($F_{7,6,202,1} = 2.17$, $P < 0.05$) (Fig. 4). No other terms were statistically significant at the 5% level. Although no predator type showed a preference for infected aphids, the preference for uninfected aphids was considerably less for *H. axyridis* (U.K.) than for the remaining three predators at all observation times. After 180 min, the mean log ratio for *H. axyridis* (U.K.) was 0.29 (57% of consumed prey items were uninfected) compared to means of 0.75 (68%), 1.02 (74%), and 1.20 (77%) for *H. axyridis* (Japan), *C. septempunctata* subspecies *brucki*, and *C. septempunctata*, respectively ($n = 24$; $SE_D = 0.224$). After an initial preference for uninfected aphids, the mean log ratio for *H. axyridis* (U.K.) remained relatively small and constant over the observation period indicating no strong preference for one prey item over the other. This was in contrast to *C. septempunctata* and *C. septempunctata* subspecies *brucki*, which showed a consistent increase in their mean log ratio over the observation period, indicating an increasingly strong preference for uninfected aphids with time.

Discussion

Previous laboratory studies demonstrated that, in the absence of choice, *C. septempunctata* was an intra-guild predator of *P. neoaphidis* (Pell *et al.*, 1997; Roy *et al.*, 1998, 2003). The results from this study demonstrate that both fourth-instar larvae and adults of *H. axyridis* and *C. septempunctata* subspecies *brucki* also engage in intra-guild predation of *P. neoaphidis* in the no-choice experiments. From earlier work it was evident that *C. septempunctata* adults and larvae consumed significantly more uninfected aphids than infected aphids and rarely consumed entire cadavers (Pell *et al.*, 1997; Roy *et al.*, 1998, 2003). The

experiments described here also support these findings. Furthermore, both *C. septempunctata* subspecies *brucki* and *H. axyridis* (Japan) behaved similarly to *C. septempunctata* and consumed more uninfected aphids than infected aphids. Indeed *C. septempunctata* subspecies *brucki* was the most voracious predator in terms of consumption of uninfected aphids compared to the other ladybirds [*H. axyridis* (U.K. and Japan) and *C. septempunctata*] but was the least voracious when feeding on infected aphids. These results would suggest that in Japan, where *C. septempunctata* subspecies *brucki* and *H. axyridis* co-occur, *C. septempunctata* subspecies *brucki* may have a competitive advantage over *H. axyridis* in terms of aphid predation. However, *H. axyridis* (U.K.) consumed similar numbers of uninfected and infected aphids, suggesting they were equally palatable in the absence of choice. It is therefore possible that *H. axyridis* (U.K.) could have a greater negative impact on *P. neoaphidis* populations than *C. septempunctata*, *C. septempunctata* subspecies *brucki*, and even than *H. axyridis* (Japan). As in previous studies on *C. septempunctata*, all starved ladybirds evaluated here consumed more infected aphids than unstarved individuals (Pell *et al.*, 1997; Roy *et al.*, 1998, 2003). However, after a period of feeding in the choice experiment, the tendency to consume uninfected aphids as opposed to infected aphids increased over time for *C. septempunctata*, *C. septempunctata* subspecies *brucki*, and *H. axyridis* (Japan) but not for *H. axyridis* (U.K.), which failed to discriminate between different prey types over time and consumed a relatively high number of infected aphids. This is likely to increase its negative impact on *P. neoaphidis* further.

Roy *et al.* (1998) reported that feeding damage by *C. septempunctata* to moribund (infected but prior to sporulation) or sporulating aphid cadavers reduced subsequent conidia production but did not decrease transmission. It is likely that the impact of damage by other coccinellids, such as *C. septempunctata* subspecies *brucki* or *H. axyridis* (Japan), which only partially consume cadavers, or consume very few cadavers, would be similar, although this requires further investigation. The presence of foraging *C. septempunctata* ladybirds is also known to increase transmission of *P. neoaphidis* by increasing aphid movement and hence the probability of contact with the pathogen and also through direct pathogen vectoring. This is likely to be the case for *H. axyridis* and *C. septempunctata* subspecies *brucki* although this also requires further evaluation (Roy *et al.*, 2001). However, given that *H. axyridis* (U.K.) consumed entire cadavers and hence has the potential to reduce pathogen population density significantly more than the other coccinellids, enhanced transmission due to foraging activity may be impeded. As an initial study, these experiments were, of necessity, on a small spatial scale and used immobile prey items. Further work should be carried out on an increased scale and complexity, and focus on the impacts of *H. axyridis* (U.K.) on transmission.

The pronounced difference in prey consumption between *H. axyridis* originating from the U.K. and those from Japan suggests the influence of microevolution: change in the genetic composition of a population (Hartl & Clark, 1989). Differences in phenotypic traits, including feeding behaviour, between populations can be a consequence of Allee effects and exponential growth from a small founding population, rather than being due

Table 1. Mean number of *Acyrtosiphon pisum* (A) and *Pandora neoaphidis*-sporulating cadavers (F) consumed by *Coccinella septempunctata*, *C. septempunctata* subspecies *brucki*, *Harmonia axyridis* (Japan), or *H. axyridis* (U.K.) when given a choice of prey items over a 180-min observation period. The coccinellids were either adult or larvae and had been provided with food until immediately prior to the start of the experiment (UnStv.) or had been starved (Stv.).

Predator species	Time (min)											
	30		60		90		120		150		180	
	A	F	A	F	A	F	A	F	A	F	A	F
<i>Coccinella septempunctata</i>												
Adult												
UnStv.	0.33	0	1.17	0.33	1.33	0.33	1.67	0.33	1.83	0.33	1.83	0.33
Stv.	0.5	0	1	0	1.5	0	1.67	0	1.83	0	1.83	0
Larvae												
UnStv.	0.5	0	0.83	0	1	0	1.5	0	1.5	0	2	0
Stv.	0.17	0	0.67	0	1	0	1.33	0	1.5	0	2	0
<i>C. septempunctata</i> subspecies <i>brucki</i>												
Adult												
UnStv.	0.5	0	0.67	0	0.83	0	0.83	0	0.83	0	0.83	0
Stv.	0.33	0.17	1	0.17	1	0.33	1.83	0.5	1.83	0.67	2	0.83
Larvae												
UnStv.	0.33	0	0.5	0	0.83	0	0.83	0	1	0	1.3	0
Stv.	1.17	0	1.67	0	1.83	0	2	0	2.33	0	2.33	0
<i>Harmonia axyridis</i> (U.K.)												
Adult												
UnStv.	0.17	0	0.17	0	0.17	0.33	0.17	0.33	0.33	0.33	0.33	0.33
Stv.	0.33	0	0.33	0.17	0.67	0.17	0.83	0.17	0.83	0.33	1	0.33
Larvae												
UnStv.	0.17	0	0.5	0.17	0.67	0.17	1.17	0.5	1.33	0.5	1.33	0.67
Stv.	0.17	0	0.33	0.33	0.5	0.5	0.83	0.67	1	0.83	1.17	0.83
<i>H. axyridis</i> (Japan)												
Adult												
UnStv.	0.17	0	0.17	0	0.17	0	0.33	0	0.5	0	0.5	0
Stv.	0.67	0	0.83	0.17	1.33	0.17	1.83	0.33	1.83	0.33	2.17	0.33
Larvae												
UnStv.	0.17	0	0.17	0	0.17	0	0.33	0.17	0.33	0.17	0.83	0.33
Stv.	0.5	0	0.83	0	0.83	0.33	0.83	0.5	1.17	0.67	1.5	0.83

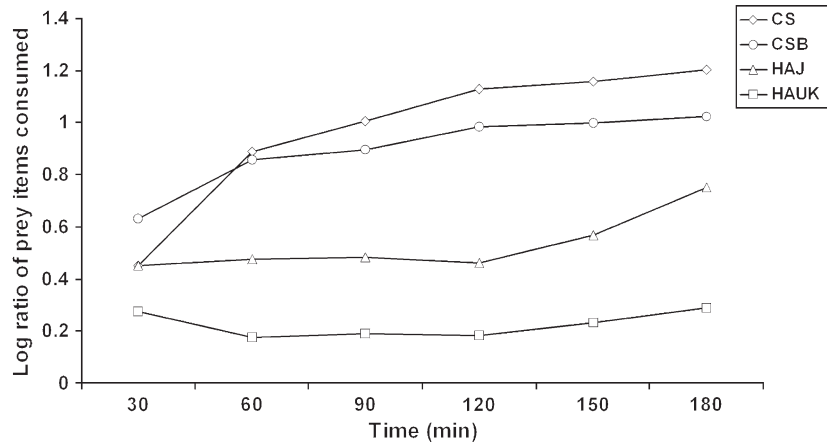
to adaptation (Roderick & Howarth, 1999; Hufbauer & Roderick, 2005). It is very likely that *H. axyridis* (U.K.) originated from founding individuals released as biological control agents in mainland Europe (Majerus *et al.*, 2006). It is therefore possible, but speculative, that *H. axyridis* (U.K.) individuals represent a highly polyphagous and voracious subpopulation that is not representative of the *H. axyridis* populations found in Japan. This requires further investigation and the authors plan to investigate genetic and ecological variability amongst *H. axyridis* subpopulations. However, our studies are the first to indicate fundamental differences in behaviour between *H. axyridis* (U.K.) and *H. axyridis* (Japan).

Alternatively, these differences could also be attributed to adaptation to mass production imposed selection criteria. Insects produced as classical biological control agents will have been subject to both intentional and unintentional selection criteria during commercial development. For example, it is common practice to rear *H. axyridis* on the eggs of the lepidopteran *Ephestia kuehniella* Zeller and also on liver-based artificial diet during mass production (Ferran *et al.*, 1997; Specty *et al.*, 2003). Indeed complete development of *H. axyridis* is possible on

E. kuehniella (Specty *et al.*, 2003). It is interesting to note that the liver-based artificial diet used in laboratory culture of *H. axyridis* is highly susceptible to fungal growth and, therefore, it is likely that *H. axyridis* (U.K.) would have been exposed to fungal contaminated food sources in previous generations. In contrast *H. axyridis* (Japan) were collected from the field in Japan, without any deliberate selection criteria, and have only been in laboratory culture for two generations.

The recognition that significant levels of genetic variability exist between populations of insects has influenced the regulation of biological control (Hufbauer & Roderick, 2005). For example, permission to release new arthropod agents of invasive weeds is now commonly given for a single population on which host specificity tests have been performed. This is a very different approach from that advocated by DeBach (1964), who suggested that individuals from many populations should be introduced, and has arisen from the recognition that individuals from separate locally adapted populations may result in unintended consequences (Hoffman *et al.*, 2002). Our studies further demonstrate that individuals of a particular species released for biological control may not be representative of the overall

Fig. 4. Means of the logged ratios of numbers of uninfected aphids consumed (plus offset of 0.05; l) relative to the number of infected aphids consumed (plus offset of 0.05; r) over a 180-min observation period by *Coccinella septempunctata* (CS), *C. septempunctata* subspecies *brucki* (CSB), *Harmonia axyridis* (Japan) (HAJ) or *H. axyridis* (U.K.) (HAUK). All values presented represent an average preference for uninfected aphids as $\log(l/r) > 0$ in each case. SE_D (Standard Error of the Difference) for comparisons within each predator = 0.1267, SE_D for all other comparisons = 0.2244.



population. The importance of assessing the host specificity of the subpopulation to be released should be carefully considered, particularly with a species such as *H. axyridis* which is attributed high-risk status as a biological control agent (van Lenteren *et al.*, 2003).

This paper has explored the unidirectional intra-guild interactions between *H. axyridis*, *C. septempunctata*, and *C. septempunctata* subspecies *brucki* and the aphid pathogenic fungus *P. neoaphidis*. It is concluded that all these coccinellids prey on fungal infected cadavers but that *H. axyridis* (U.K.) is a stronger intra-guild predator in these interactions than the other coccinellids tested. Intra-guild predators can impact dramatically on the population dynamics of intra-guild prey (Polis *et al.*, 1989). Indeed intra-guild predation can be a major mortality factor limiting populations of such prey below their carrying capacities (Leving & Franks, 1982; Leving & Adams, 1984; Polis *et al.*, 1989; Burgio *et al.*, 2002). The efficacy of exotic predatory coccinellids as biological control agents of introduced plant pest species has been demonstrated in a number of field studies (Hodek & Honek, 1996; Smith *et al.*, 1996). The issues of non-target impacts of introduced (classical) biological control agents have also been widely addressed (Howarth, 1991; Samways, 2005). Two ladybird species, *H. axyridis* and *C. septempunctata*, have been widely documented as successful invaders (Teddners & Schaefer, 1994; Evans, 2000, 2004; Majerus *et al.*, 2006). It is hypothesised that a major factor contributing to the success of these two invasive species is their tendency to engage in intra-guild predation of other ladybirds and indeed other aphidophagous and coccidophagous predators (Kajita *et al.*, 2000, 2006).

Acknowledgements

J.B., K.E.B., and J.K.P. are supported by the Department for Environment, Food and Rural Affairs. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. H.E.R. is supported by the Environmental Science Research Centre, Anglia Ruskin University. We would also like to thank Ian Wright for technical support. This work was part-funded by Defra through the NBN Trust.

References

- Brown, M.W. & Miller, S.S. (1998) Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. *Entomological News*, **109**, 143–151.
- Burgio, G., Santi, F. & Maini, S. (2002) On intra-guild predation and cannibalism in *Harmonia axyridis* (Pallas) and *Adalia bipunctata* L. (Coleoptera: Coccinellidae). *Biological Control*, **24**, 110–116.
- Colunga-Garcia, M. & Gage, S.H. (1998) Arrival, establishment, and habitat use of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Michigan landscape. *Environmental Entomology*, **27**, 1574–1580.
- DeBach, P. (1964) *Biological Control of Insect Pests and Weeds*. Chapman & Hall, London.
- Evans, E.W. (2000) Morphology of invasion: body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. *European Journal of Entomology*, **9**, 469–474.
- Evans, E.W. (2004) Habitat displacement of North American ladybirds by an introduced species. *Ecology*, **85**, 637–647.
- Ferran, A., Gambier, J., Parent, S., Legendre, K., Tourniere, R. & Giuge, L. (1997) The effect of rearing the ladybird *Harmonia axyridis* on *Ephestia kuehniella* eggs on the response of its larvae to aphid tracks. *Journal of Insect Behaviour*, **10**, 129–144.
- Gordon, R.D. (1985) The Coccinellidae (Coleoptera) of America North of Mexico. *Journal of the New York Entomological Society*, **93**, 1–912.
- Hartl, D.L. & Clark, A.G. (1989) *Principles of Population Genetics*. Sinauer Associates, Sunderland, Massachusetts.
- Hesler, L.S., Keickhefer, R.W. & Beck, D.A. (2001) First record of *Harmonia axyridis* (Coleoptera: Coccinellidae) in South Dakota and notes on its activity there and in Minnesota. *Entomological News*, **112**, 264–270.
- Hodek, I. & Honek, A. (1996) *Ecology of Coccinellidae*. Kluwer Academic, Dordrecht, the Netherlands.
- Hoffman, J.H., Impson, F.A.C. & Volchansky, C.R. (2002) Biological control of cactus weeds: implications of hybridisation between control agent biotypes. *Journal of Applied Ecology*, **39**, 900–908.
- Hokkanen, H.M.T., Bigler, F., Burgio, G., van Lenteren, J.C. & Thomas, M.B. (2003) Ecological risk assessment framework for biological control. *Environmental Impacts of Microbial Insecticides* (ed. by H. M. T. Hokkanen and A. E. Hajek), pp. 1–14. Kluwer Academic, Dordrecht, the Netherlands.
- Howarth, F.G. (1991) Environmental impacts of classical biological control. *Annual Review of Entomology*, **36**, 485–509.

- Hufbauer, R.A. & Roderick, G.K. (2005) Microevolution in biological control: mechanisms, patterns and processes. *Biological Control*, **35**, 227–239.
- Iperti, G. & Bertand, E. (2001) Hibernation of *Harmonia axyridis* (Coleoptera: Coccinellidae) in South-Eastern France. *Acta Societatis Zoologicae Bohemicae*, **65**, 207–210.
- Kajita, Y., Takano, F., Yasuda, H. & Agarwala, B.K. (2000) Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. *Applied Entomology and Zoology*, **35**, 473–479.
- Kajita, Y., Takano, F., Yasuda, H. & Evans, E.W. (2006) Interactions between introduced and native predatory ladybirds (Coleoptera, Coccinellidae): factors influencing the success of species introductions. *Ecological Entomology*, **31**, 58–67.
- Kuznetsov, V.N. (1997) *Ladybeetles of Russian Far East*. Memoir Series of the Center for Systematic Entomology No 1. Saint Lucie, Florida.
- van Lenteren, J.C., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H.M.T., Kuske, S. *et al.* (2003) Environmental risk assessment of exotic natural enemies used in inundative biological control. *Bio-Control*, **48**, 3–38.
- Leving, S. & Adams, E. (1984) Intra- and inter-specific territoriality in *Nasutitermes* (Isoptera: Termitidae) in a Panamanian mangrove forest. *Journal of Animal Ecology*, **53**, 705–714.
- Leving, S. & Franks, N. (1982) Patterns of nest dispersion in a tropical ground ant community. *Ecology*, **63**, 338–394.
- Majerus, M.E.N., Strawson, V. & Roy, H.E. (2006) The potential impacts of the arrival of the Harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. *Ecological Entomology*, **31**, 207–215.
- Olden, J.D. & Poff, N.L. (2004) Ecological processes driving biotic homogenisation: testing a mechanistic model using fish faunas. *Ecology*, **85**, 1867–1875.
- Olden, J.D., Poff, N.L. & McKinney, M.L. (2006) Forecasting faunal and floral homogenisation associated with human population geography in North America. *Biological Conservation*, **127**, 261–271.
- Pell, J.K., Eilenberg, J., Hajek, A.E. & Steinkraus, D.C. (2001) Exploring the potential of entomophthorales in pest management. *Fungi as Biocontrol Agents: Progress, Problems and Potential* (ed. by T. M. Butt, C. W. Jackson and N. Magan), pp. 71–153. CABI, Wallingford.
- Pell, J.K., Pluke, R., Clark, S.J., Kenward, M.G. & Alderson, P.G. (1997) Interactions between two aphid natural enemies, the entomopathogenic fungus *Pandora neoaphidis* Remaudiere & Hennebert (Zygomycetes: Entomophthorales) and the predatory beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Journal of Invertebrate Pathology*, **69**, 261–268.
- Polis, G.A., Myers, C.A. & Holt, R.D. (1989) The ecology and evolution of intra-guild predation: potential competitors that eat each other. *Annual Review of Entomology*, **20**, 297–330.
- Roderick, G.K. & Howarth, F.G. (1999) Invasion genetics: natural colonisations, non-indigenous species and classical biological control. *Biological Invasions of Pests and Beneficial Organisms* (ed. by E. Yano, K. Matsuo, M. Shiyomi and D. Andow), pp. 98–108. National Institute of Agro Environmental Sciences, Tsukuba, Japan.
- Roy, H.E., Brown, P. & Majerus, M.E.N. (2006) *Harmonia axyridis*: a successful biocontrol agent or an invasive threat? *An Ecological and Societal Approach to Biological Control* (ed. by J. Eilenberg and H. Hokkanen), pp. 295–309. Kluwer Academic, Dordrecht, the Netherlands.
- Roy, H.E., Brown, P., Rowland, F. & Majerus, M.E.N. (2005) Ecology of *Harmonia axyridis*. *British Wildlife*, **16**, 403–407.
- Roy, H.E. & Pell, J.K. (2000) Interactions between entomopathogenic fungi and other natural enemies: implications for biological control. *Biocontrol Science and Technology*, **10**, 737–752.
- Roy, H.E., Pell, J.K. & Alderson, P.G. (2001) Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the aphid predator *Coccinella septempunctata*. *Biocontrol Science and Technology*, **11**, 101–112.
- Roy, H.E., Pell, J.K. & Alderson, P.G. (2003) Effect of spatial heterogeneity on the role of *Coccinella septempunctata* as an intra-guild predator of the aphid pathogen *Pandora neoaphidis*. *Journal of Invertebrate Pathology*, **82**, 85–95.
- Roy, H.E., Pell, J.K., Clark, S.J. & Alderson, P.G. (1998) Implications of predator foraging on aphid pathogen dynamics. *Journal of Invertebrate Pathology*, **71**, 236–247.
- Samways, M.J. (2005) *Insect Diversity Conservation*. Cambridge University Press, Cambridge.
- Sato, S., Yasuda, H. & Evans, E.W. (2005) Dropping behaviour of larvae of aphidophagous ladybirds and its effect on incidence of intraguild predation: interactions between the intraguild prey, *Adalia bipunctata* (L.) and *Coccinella septempunctata* (L.), and the intraguild predator, *Harmonia axyridis* Pallas. *Ecological Entomology*, **30**, 220–224.
- Smith, W.M., Arnold, D.C., Eikenbary, R.D., Rice, N.R., Shiferaw, A., Cheary, B.S. *et al.* (1996) Influence of ground cover on beneficial arthropods in pecan. *Biological Control*, **6**, 164–176.
- Specty, O., Febvay, G., Grenier, S., Delobel, B., Piotte, C., Pageaux, J.F. *et al.* (2003) Nutritional plasticity of the predatory ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae): comparison between natural and substitution prey. *Archives of Insect Biochemistry and Physiology*, **52**, 81–91.
- Tedders, W.L. & Schaefer, P.W. (1994) Release and establishment of *Harmonia axyridis* (Coleoptera: Coccinellidae) in the south-eastern United States. *Entomological News*, **105**, 228–243.
- Wilding, N. (1970) The effects of temperature on the infectivity and incubation periods of the fungi *Entomophthora aphidis* and *E. thaxteriana* for the pea aphid *Acyrtosiphon pisum*. *Proceedings of the 4th International Colloquium on Insect Pathology*, pp. 84–88. College Park, Maryland.
- Williamson, M. (1996) *Biological Invasions*. Chapman & Hall, London.
- Williamson, M. (1999) Invasions. *Ecography*, **22**, 5–12.

Accepted 9 August 2007

First published online 24 January 2008