

Chemical protection of *Calvia quatuordecimguttata* eggs against intraguild predation by the invasive ladybird *Harmonia axyridis*

Remy Lian Ware · Felipe Ramon-Portugal · Alexandra Magro ·
Christine Ducamp · Jean-Louis Hemptinne · Michael E. N. Majerus

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Abstract Intraspecific and interspecific predation of eggs is a well documented phenomenon amongst aphidophagous coccinellids. The invasive species *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is known to be a top intraguild predator and reported to attack the eggs of many coccinellid species both in a laboratory setting and in the wild. A previous laboratory study highlighted that while many species' eggs were highly palatable to *H. axyridis*, the eggs of *Calvia quatuordecimguttata* (Linnaeus) (Coleoptera: Coccinellidae) appeared to be extremely well protected from attack. Here we present the results of behavioural experiments testing the hypothesis that substances on the egg surface are responsible for this protection, and report preliminary results of GC-MS analysis of these compounds. When the coatings of *C. quatuordecimguttata* eggs were removed using hexane, they became significantly more susceptible to predation by neonate *H. axyridis* larvae. However, their overall palatability was not affected, in that complete consumption was never or rarely observed. This suggests that the surface compounds are a

R. L. Ware (✉) · M. E. N. Majerus
Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK
e-mail: r.ware@gen.cam.ac.uk

M. E. N. Majerus
e-mail: m.majerus@gen.cam.ac.uk

F. Ramon-Portugal · A. Magro · C. Ducamp · J.-L. Hemptinne
Laboratoire d'Agroécologie, UMR CNRS 5174 «Evolution et Diversité Biologique»,
Ecole Nationale de Formation Agronomique, BP 22687, 31326 Castanet-Tolosan, France

F. Ramon-Portugal
e-mail: felipe.ramonportugal@educagri.fr

A. Magro
e-mail: alexandra.magro@educagri.fr

C. Ducamp
e-mail: christine.ducamp@educagri.fr

J.-L. Hemptinne
e-mail: jean-louis.hemptinne@educagri.fr

true indicator of unpalatability in this species. The effect of hexane-washing on already palatable conspecific eggs was also analysed but had no significant effect on the susceptibility of eggs to cannibalism. We conclude that the eggs of at least one European species are effectively protected by surface deterrents from intraguild predation by *H. axyridis*. This effect might be due to both the diversity and abundance of hydrocarbons present within the egg coating, the presence of alkenes and/or the presence of patches of a red substance on the eggs' surface, which is thought to belong to the acid group. In conjunction with data on the susceptibility of other immature stages of *C. quatuordecimguttata*, this finding may indicate a decreased risk of the species falling victim to invasive *H. axyridis*, despite their coincident habitat ranges.

Keywords *Calvia quatuordecimguttata* · Chemical defence · Chemoecology · Coccinellidae · Cream-spot ladybird · Harlequin ladybird · *Harmonia axyridis* · Intraguild predation

Introduction

The egg stage represents a particularly vulnerable period for many vertebrates and invertebrates. Insect eggs are immobile and, in the absence of any parental protection, constitute an easy meal for a range of oophagous predators (Blum and Hilker 2002), in addition to being a target for various parasitoids and pathogens. Amongst aphidophagous coccinellids, the commonest cause of mortality at the egg stage is predation by conspecific or heterospecific larvae and adults (Banks 1956; Mills 1982; Majerus 1994; Dixon 2000; Cottrell 2004). Cannibalism of eggs by hatching larvae is known to dramatically increase survival of the latter (Pienkowski 1965; Majerus and Majerus 1997; Snyder et al. 2000). Through the process of intraguild predation, defined by Polis et al. (1989) as the killing and eating of a potential competitor, the consumption of an egg of another guild member has obvious benefits in that it can both provide a significant nutritional advantage and remove a future source of competition (Hemptinne et al. 2000a; Lucas 2005). Although non-conspecific eggs generally provide a poorer quality meal than conspecific eggs, intraguild predation is preferable to death from starvation and commonly occurs when local aphid populations decline (Agarwala et al. 1998; Obrycki et al. 1998; Agarwala and Yasuda 2001).

As an adaptation against predation, eggs of many coccinellid species are protected by unpalatable or toxic chemicals. Chemical protection of eggs is well reported from a diverse array of insect species (Blum and Hilker 2002), and in coccinellids usually involves de novo synthesis of species-specific alkaloids, which are present throughout the life cycle (Pasteels et al. 1973; King and Meinwald 1996). Although the production of this defence can be costly (Holloway et al. 1991, 1993), the benefits in terms of deterring a range of predators are significant (Marples 1993; Hemptinne et al. 2000a; Blum and Hilker 2002; Sato and Dixon 2004). The possession of intrinsic protective chemicals is thought only to make evolutionary sense if their existence is 'flagged' in some way by a signal on the egg surface: they are of little value if the eggs are killed before a predator learns their distasteful or toxic properties (Hemptinne et al. 2000b). An alternative explanation may come in the form of kin selection in species laying their eggs in clutches: if a naïve predator samples one unpalatable egg, that egg is killed, but further eggs in the same clutch may be avoided (Agarwala and Dixon 1993).

Various studies report the apparent toxicity or unpalatability of the eggs of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) to larvae of other species (Burgio et al. 2002;

Cottrell 2004; Sato and Dixon 2004). Other life stages of *H. axyridis* appear equally well defended (Ware and Majerus 2007). This species is native to Asia but invasive in North America, continental Europe and more recently, the UK. The ability of *H. axyridis* to successfully exploit native members of the aphidophagous guild, while avoiding exploitation itself, is thought to be an important factor in its widespread establishment and spread (Sato and Dixon 2004; R. L. Ware et al. unpublished data). Dramatic declines in native coccinellids have been witnessed in North America following the establishment of *H. axyridis*, and have been attributed to both competition and intraguild predation of their immature stages (Hironori and Katsuhiko 1997; Cottrell and Yeorgan 1998; Michaud 2002). Intraguild predation of eggs and other immature stages is now thought to pose a significant threat to many European coccinellids.

A previous study investigated the vulnerabilities and relative palatabilities of the eggs of a range of British and Japanese coccinellids to *H. axyridis* (Ware et al., in press). While most species' eggs were readily consumed by first instar larvae, eggs of one British species, *Calvia quatuordecimguttata* (Linnaeus) (Coleoptera: Coccinellidae), were almost always avoided. Other workers have reported the reluctance of other coccinellid species to consume *C. quatuordecimguttata* eggs: Dyson (1996) demonstrated violent rejection behaviour of *Harmonia quadripunctata* (Pontoppidan), *Adalia bipunctata* (Linnaeus) and *Propylea quatuordecimpunctata* (Linnaeus) larvae towards *C. quatuordecimguttata* eggs, and Vanhove (1998) presented similar findings for *Calvia decempunctata* (Linnaeus) and *Adalia decempunctata* (Linnaeus) larvae. However, this repellent effect was not witnessed with conspecific larvae and cannibalism occurs commonly (Dyson 1996; Vanhove 1998). It has been suggested that the agent responsible for deterring non-conspecific predators is on the surface of the egg, as larvae withdraw rapidly upon first contact (Majerus 1994; Dyson 1996; Vanhove 1998; Ware et al., in press). Dyson (1996) reports preliminary results showing the deterrent to be hexane-soluble, as eggs washed in hexane became palatable to all predators studied. Hemptinne et al. (2000b) provide evidence for similar surface deterrents on *A. bipunctata* and *Coccinella septempunctata* Linnaeus eggs. Extracts of eggs washed in hexane were shown to be composed mainly of alkanes, which act as signals to both conspecific and heterospecific larvae.

Here we present the results of preliminary behavioural experiments testing the hypothesis that substances on the egg surface are responsible for protection of *C. quatuordecimguttata* eggs against intraguild predation by *H. axyridis*. We also present the results of chemical analysis of the coating of *C. quatuordecimguttata* eggs, and make comparisons with the substances already identified from *A. bipunctata* and *C. septempunctata* eggs. Finally, we discuss the results in relation to the threat posed to *C. quatuordecimguttata* by the establishment of *H. axyridis* in Europe, and the role of egg surface chemistry in providing signals to conspecific and heterospecific predators.

Materials and methods

Coccinellid rearing

All larvae and eggs used in experiments were derived from field collected pairs or F1 material. *H. axyridis* stocks were derived from samples sent into the Harlequin Ladybird Survey from around the UK. Stocks of *C. quatuordecimguttata* were collected from either Cambridge or London, UK. Mating pairs of all species were housed in 9 cm Petri dishes lined with filter paper and kept in the laboratory at 22°C and 16 hL: 8 hD. Adults were

supplied daily with excess pea aphids, *Acyrtosiphon pisum* (Harris), and filter papers were replaced daily. A few clutches of *H. axyridis* eggs were retained to generate the larvae required for the behavioural experiments, while the remaining *H. axyridis* eggs and all *C. quatuordecimguttata* eggs were subjected to the treatments detailed below.

Extraction of egg coating

Eggs were detached from the filter paper on which they were laid using the tip of a closed glass Pasteur pipette, after wetting the filter paper with a drop of de-ionised water to loosen the ovipositional glue. Half of the eggs available per day for each species were placed in a 5 ml test tube and washed in hexane (treatment A), while the other half were left unwashed but otherwise treated similarly (treatment B). Treatment A eggs were washed in 3 ml of n-hexane for liquid chromatography (Merck, Hohenbrunn, Germany) for 2 min, without agitation. The supernatant was carefully removed using a Pasteur pipette and the remaining solvent evaporated under a gentle stream of nitrogen for 3 min. After extraction, the eggs were observed under a dissecting microscope and, whenever broken eggs were detected, the extract was discarded. The extract of *C. quatuordecimguttata* eggs was conserved at 4°C until GC-MS analysis. Intact washed eggs were used for the behavioural experiments.

Gas chromatography-mass spectrometry (GC-MS) analysis

The extracts obtained from washing 42 *C. quatuordecimguttata* eggs were pooled. The solvent was evaporated under a gentle stream of nitrogen and the dry residue dissolved in 8.4 µl of n-hexane (20 µl per 100 eggs). Elucidation of the structure of the compounds in the n-hexane extract was carried out by GC-MS (Finnigan Trace 2000 operated by electron impact at 70 eV) using a helium carrier at 1.2 ml/min, operated under the following temperatures: source: 200°C, interface between GC and MS modules: 250°C, splitless injector: 280°C. Samples of 1 µl were injected using an apolar capillary column (Restek RTX-5MS, 30 × 0.25 mm², 0.25 µm film thickness). The temperature programme of the oven was set to: 50°C for 1 min, then from 50 to 140°C at 20°C per min, then from 140 to 300°C at 3°C per min, and finally held at 300°C for 3 min. The mass spectra were scanned from 60 to 450 m/z. On-line data acquisition was carried out with Xcalibur software (Thermo Finnigan, MA, USA). Detection limits are automatically established by the Xcalibur software, which only considers peaks over 0.4% of the highest peak. Compounds were identified by their fragmentation patterns compared with the NIST library and by comparison with previously injected known compounds. The quantification of each compound was carried out using a calibration curve at different concentrations of a mixture of hydrocarbons from C12 to C60 (Sigma-Aldrich, Tanfkirchen, Germany). The non-adeane [CH₃(CH₂)₁₇CH₃; Sigma-Aldrich] was used as internal standard. In order to quantify each detected compound, 3 µl of extract were mixed with 3 µl of a non-adeane solution (at 0.0781 mg l⁻¹ in n-hexane).

Behavioural experiments

Two neonate sibling larvae of *H. axyridis* were carefully removed from their clutch before dispersal, using a fine bristled paintbrush, and placed individually in clean 4 cm Petri

dishes. One larva was provided with a single *C. quatuordecimguttata* egg that had been washed in hexane (treatment A) while the other was provided with a single unwashed *C. quatuordecimguttata* egg (treatment B). Eggs were manipulated using a blunt mounted entomological pin. No other food or water was provided thereafter. The larvae were observed at regular intervals and times of death recorded. No larvae underwent ecdysis during this time. Survival times were calculated from the time of experimental set-up. After 24 h the 'status' of the egg was observed carefully using a 15× hand lens and placed into one of three categories as in Ware et al. (in press): not eaten (N), when the egg was completely untouched; partially eaten (P), when the chorion was broken but a substantial amount of yolk remained; and completely eaten (C), when the whole egg had been consumed or yolk remained only at the poles. The experiment was replicated 20 times. The reaction of *H. axyridis* larvae to washed and unwashed non-sibling conspecific eggs was also monitored in this way, with a view to assessing the effect of the washing process on a normally palatable egg. Survival controls involved placing a neonate larva in a dish with no egg (or other food or water), and noting its time of death.

When the chorion of an egg is broken by a sampling predator, the embryo within it is killed, whether it is subsequently eaten or dies through desiccation. From the perspective of the egg's individual fitness, therefore, there is essentially no difference between category 'C' and category 'P'. Eggs falling into category 'N' will be the only potential survivors. On the other hand, if an egg is palatable, it will presumably be completely consumed, rather than being sampled and then left behind. Thus, the proportion of eggs falling into category 'C' gives an indication of the palatability of the egg to the larva.

Statistical analysis

The effect of hexane washing on the number of eggs falling into each category was analysed for each species using a fully orthogonal approach. First, we tested for heterogeneity in the data using the Fisher-Freeman-Halton test on a 3×2 contingency table (2 *df*). If heterogeneity was found, Fisher's exact tests (1 *df*) were performed on the number of eggs falling into category 'C' versus 'P', and the numbers of eggs falling into 'C + P' versus 'N'. This analysis considers the palatability and the susceptibility of the eggs, respectively. Survival times were compared using the Kruskal-Wallis test and multiple pairwise Mann-Whitney *U*-tests adjusting for ties. Alpha values were adjusted according to the Bonferroni correction for multiple comparisons (referred to in text as α_B). The Fisher-Freeman-Halton test and Fisher's exact test were carried out in StatXact 7, and Minitab 14 was used to perform Kruskal-Wallis and Mann-Whitney *U*-tests.

Results

Gas chromatography-mass spectrometry (GC-MS) analysis

It is unfortunate that only a relatively small number of *C. quatuordecimguttata* eggs ($n = 42$) were available for GC-MS analysis, due to difficulty in manipulating the eggs without breaking them and logistical constraints of culturing this species in the time available. Normally the authors would aim to pool the extracts of three lots of 100 eggs each. We therefore report the following results as preliminary data only. Figure 1 shows the mass spectrum for the extract of 42 *C. quatuordecimguttata* eggs. The peaks are

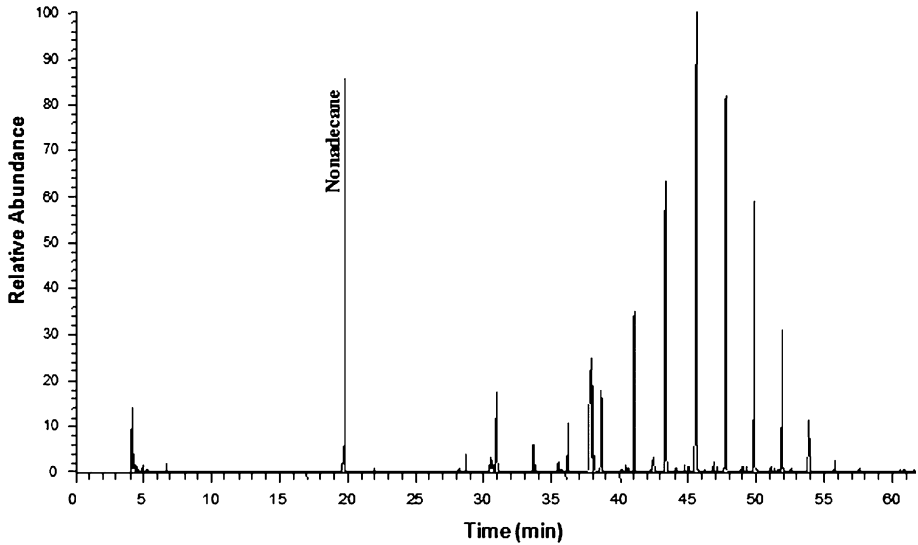


Fig. 1 Mass spectrum of the extract of *Calvia quatuordecimguttata* egg coatings. Non-adeane is the internal standard

identified and quantified in Table 1. Twenty-four peaks were obtained, of which 20 have been positively identified. A total quantity of 17.60 mg of surface hydrocarbons per 50 eggs was obtained, of which 90.28% were linear alkanes, 8.59% were alkenes, and the remaining 1.13% represented the four unidentified peaks. The alkane Hentriacontane was the major compound, and represented 20% of the total quantity (3.50 mg). These results, particularly in respect of the alkenes and double bond positioning, need to be confirmed by compound-specific chemical analysis.

During manipulation of *C. quatuordecimguttata* eggs, the authors observed the presence of patches of a red substance on their surface, visible under a 15 \times hand lens. This substance was found to be soluble in both water and hexane, which indicates that it could belong to the acid group with low relative molecular weight. Further analysis of its chemical nature is still needed.

Behavioural experiments

The effect of washing *C. quatuordecimguttata* eggs in hexane on its status after 24 h when left with a neonate *H. axyridis* larva is shown in Fig. 2. There is significant heterogeneity in the numbers of eggs falling into each category between treatments A and B ('C' versus 'P' versus 'N': Fisher statistic = 11.42, 2 *df*, $P = 0.002$). Further analysis indicates there is no difference in palatability between treatments ('C' versus 'P': Fisher statistic = 0.483, 1 *df*, $P \sim 1.00$) but that more washed eggs were killed than unwashed eggs ('C + P' versus 'N': Fisher statistic = 11.09, 1 *df*, $P = 0.002$).

The results of the hexane treatment of *H. axyridis* eggs on cannibalism are shown in Fig. 3. There was no significant heterogeneity within this data, showing that washing conspecific eggs in hexane did not affect how many were completely eaten, partially eaten, or not eaten, by larvae after 24 h. ('C' versus 'P' versus 'N': Fisher statistic = 2.94, 2 *df*, $P = 0.216$).

Table 1 Compounds identified from extracts of *C. quatuordecimguttata* egg coatings

Compounds	<i>C. quatuordecimguttata</i>		<i>A. bipunctata</i>		<i>C. septempunctata</i>	
	mg l ⁻¹	%	mg l ⁻¹	%	mg l ⁻¹	%
Heneicosane			0.50	11.90		
Docosane	0.01	0.04	0.10	2.38	0.04	3.64
6-Tricosene	0.03	0.19				
Unidentified	0.13	0.71				
Tricosane	0.20	1.15	0.30	7.14	0.13	11.82
7-Methytricosane			1.20	28.57		
9-Methytricosane			2.10	50.00		
Tetracosane	0.07	0.38			0.11	10.00
Unidentified	0.02	0.12				
6-Pentacosene	0.02	0.14				
Pentacosane	0.15	0.86			0.17	15.45
6-Hexacosene	1.24	7.06				
Hexacosane	0.29	1.65			0.05	4.55
6-Heptacosene	0.04	0.21				
Heptacosane	0.67	3.83			0.44	40.00
6-Octacosene	0.06	0.37				
Octacosane	1.50	8.53			0.04	3.64
Unidentified	0.02	0.11				
6-non-acosene	0.04	0.23				
Non-acosane	2.99	17.01			0.12	10.91
6-Triacontene	0.07	0.40				
Triacontane	3.01	17.08				
Unidentified	0.03	0.19				
Hentriacontane	3.50	19.87				
Dotriacontane	1.95	11.09				
Tritriacontane	1.16	6.60				
Tetratriacontane	0.38	2.19				
Total	17.60	100.00	4.20	100.00	1.10	100.00

Compounds from published data for *A. bipunctata* and *C. septempunctata* egg coatings (Hemptinne et al. 2000b) are given for comparison. Results are expressed in mg per litre for 50 eggs, and as relative % amounts

There was a significant difference in survival between larvae subjected to the five different feeding regimes shown in Fig. 4 (Kruskal-Wallis test: $H = 53.07$, $df = 4$, $P < 0.001$). For larvae that had consumed an untreated or treated *C. quatuordecimguttata* egg before their death, survival was significantly increased relative to the control (Mann-Whitney U -tests: αB (10 tests) = 0.005: $W = 214.0$, $P < 0.001$ and $W = 219.0$, $P < 0.001$, respectively). However, there was no difference in survival between larvae that had received untreated or hexane-treated *C. quatuordecimguttata* eggs (Mann-Whitney U -test: αB (10 tests) = 0.005: $W = 309.5$, $P = 0.056$) (Fig. 3). Consumption of an untreated or treated conspecific egg significantly increased larval survival compared to the control (Mann-Whitney U -test: αB (10 tests) = 0.005: $W = 210.0$, $P < 0.001$ and $W = 231.0$, $P < 0.001$, respectively). There was no difference in survival between larvae that had consumed untreated conspecific eggs or hexane-treated conspecific eggs (Mann-Whitney

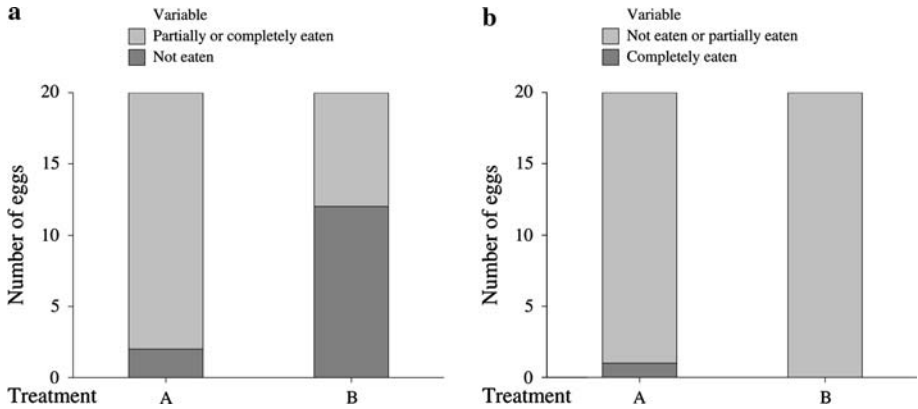


Fig. 2 (a) Susceptibility and (b) palatability of *Calvia quatuordecimguttata* eggs to intraguild predation by neonate *Harmonia axyridis* larvae after 24 h under treatments A (hexane washed) and B (unwashed). $n = 20$ for each treatment

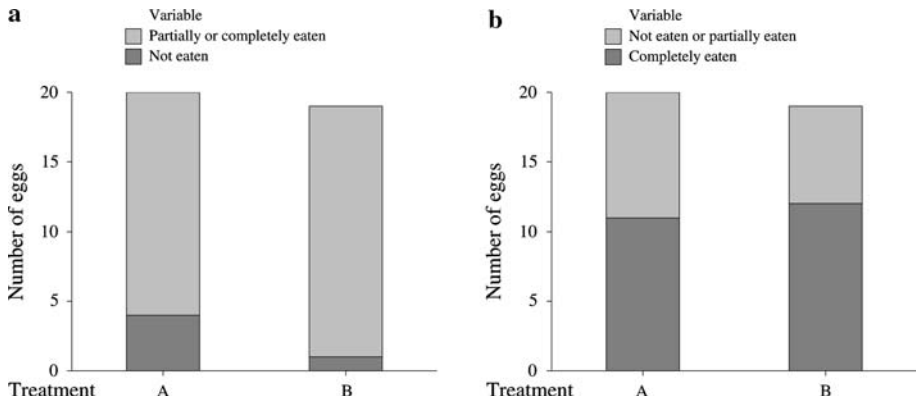


Fig. 3 (a) Susceptibility and (b) palatability of *Harmonia axyridis* eggs to cannibalism by neonate non-sibling larvae after 24 h under treatments A (hexane washed) and B (unwashed). $n = 20$ for each treatment

U -test: αB (ten tests) = 0.005: $W = 322.5$, $P = 0.596$). There was no statistically significant difference in survival between larvae that had consumed either treated or untreated conspecific eggs and those that had consumed untreated *C. quatuordecimguttata* eggs (Mann-Whitney U -test: αB (ten tests) = 0.005: $W = 225.0$, $P = 0.094$ and $W = 223.0$, $P = 0.051$, respectively). However, the consumption of treated *C. quatuordecimguttata* eggs resulted in a lower survival than the consumption of treated or untreated conspecific eggs (Mann-Whitney U -test: αB (ten tests) = 0.005: $W = 279.0$, $P = 0.002$ and $W = 256.5$, $P < 0.001$, respectively) (Fig. 4).

Discussion

Hemptinne et al. (2000b) argue that unpalatability or toxicity of coccinellid eggs should be advertised by a chemical signal on the egg's surface. They show that eggs of *A. bipunctata*

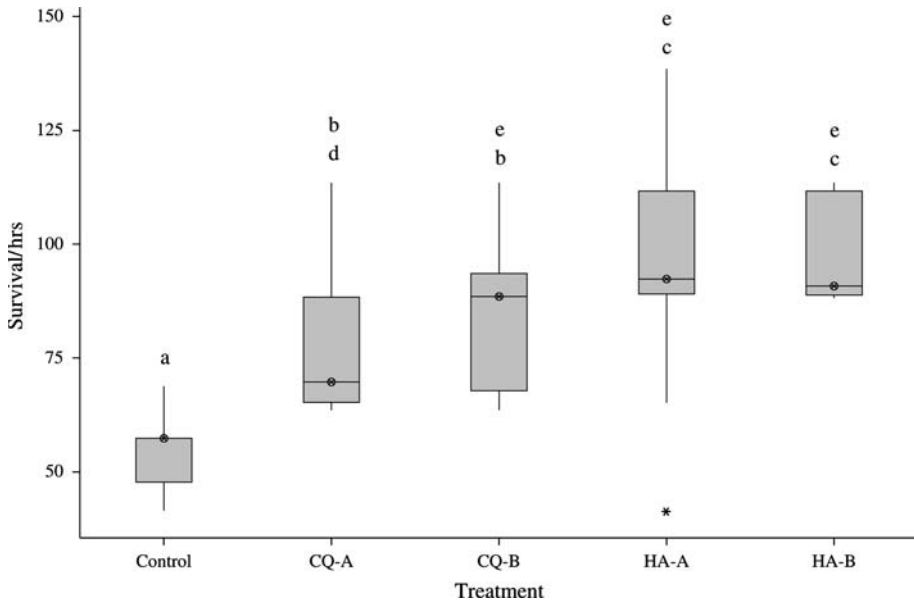


Fig. 4 Survival of neonate *Harmonia axyridis* larvae after having partially or completely consumed one egg of *Calvia quatuordecimguttata* (CQ) or of its own species (HA) under treatments A (hexane washed) and B (unwashed). No statistically significant difference was found between treatments plotted with the same letter. Medians (circles with stars), interquartile ranges (boxes), ranges (vertical lines) and outliers (stars) shown

are coated with hexane-soluble hydrocarbons that deter predation by both conspecific larvae and larvae of *C. septempunctata*, and that these chemicals are similar to those used in larval tracks (Doubbia et al. 1998; Magro et al. 2007) and mate recognition (Hemptinne et al. 1998). Meanwhile, *C. septempunctata* eggs are coated in surface deterrents that prove an effective defence against *A. bipunctata* larvae but not conspecific larvae (Hemptinne et al. 2000b). Other studies suggest that when eaten, the eggs of these two species impose a nutritional cost on larvae of the other species (Agarwala and Dixon 1992; Hemptinne et al. 2000a; Sato and Dixon 2004). This implies that surface chemical deterrents are honest signals of the cost of intraguild predation between these two species.

This study has confirmed that eggs of *C. quatuordecimguttata* are also protected by chemical deterrents on their surface. Preliminary chemical analysis indicates that the egg coating consists mainly of hydrocarbons, and highlights another, as yet unidentified, substance, thought to be a member of the acid group. Hydrocarbons are long-lasting and highly resistant molecules that are found commonly in nature and used by a range of species as cues for communication purposes. Hydrocarbons have been identified in the egg coatings of *A. bipunctata* and *C. septempunctata* by Hemptinne et al. (2000b). There are, however, some striking differences between the compounds found on the eggs of *C. quatuordecimguttata* and these two species (Table 1). First, the egg coating of *C. quatuordecimguttata* contains four times the amount of hydrocarbons found in *A. bipunctata*, and 16 times the amount isolated from *C. septempunctata* eggs. Second, a more diverse range of compounds were isolated from *C. quatuordecimguttata* compared to the other two species: the extract from *C. quatuordecimguttata* contained 24 different compounds, while those of *A. bipunctata* and *C. septempunctata* contained only five and

eight, respectively. If the chemical results are confirmed, the extract of *C. quatuordecimguttata* is unique in containing alkenes.

Dyson (1996) had already noted the presence of patches of a red substance on the surface of *C. quatuordecimguttata* eggs and speculated that they were a deterring agent. However, we suggest that their solubility in water would make them rather unreliable as a form of chemical defence. Further analysis is in progress in order to fully understand the nature and role of these patches. Nevertheless, the work presented here clearly shows that the removal of surface chemicals from *C. quatuordecimguttata* eggs using a hexane solvent significantly increases their susceptibility to attack by neonate *H. axyridis* larvae, and is therefore in agreement with the results previously found using larvae of other coccinellid species (Dyson 1996). Furthermore, the results show that while the removal of surface molecules increases the proportion of eggs attacked, they remain significantly unpalatable to *H. axyridis* larvae and are rarely completely consumed within 24 h. This suggests that here the surface signals are a reliable indicator of intrinsic toxic or distasteful chemicals, as theorised by Hemptinne et al. (2000b). We predict that coating *H. axyridis* eggs with the surface extract from *C. quatuordecimguttata* will make them unpalatable to *H. axyridis* larvae. Work to test this prediction is in progress.

When *H. axyridis* larvae did consume all or part of a *C. quatuordecimguttata* egg, no toxic effect was shown, in that it did not reduce survival below that of a larva given no food at all. We therefore suggest that the intrinsic chemicals are significantly distasteful to *H. axyridis* but not toxic, at least in the short-term. This should be confirmed by studies into the longer-term effects on development. While the consumption of a *C. quatuordecimguttata* egg can provide some short-term nutritional benefit to a neonate *H. axyridis* larva, it seems less than that afforded by a conspecific egg. This is particularly evident when considering larvae that had consumed treated *C. quatuordecimguttata* eggs, although this may simply be due to the larger sample size of individuals that sampled an egg when the egg had been washed. This result is in accordance with other work citing the increased value of consuming conspecific eggs over heterospecific eggs (Agarwala and Yasuda 2001; Cottrell 2004; Sato and Dixon 2004; Omkar et al. 2004).

It is thought that co-occurring species should be protected from intraguild predation by each other, either by means of chemical defence, or through behavioural adaptations that reduce the likelihood of encounters (Sato and Dixon 2004). For example, in Japan, *H. axyridis* and *C. septempunctata brucki* Mulsant are frequently found together in alfalfa fields (Takahashi 1989). *H. axyridis* is relatively resistant to intraguild predation from *C. septempunctata brucki* due the possession of defensive chemicals and robust physical defence structures (Ware and Majerus 2007). Moreover, as *C. septempunctata brucki* oviposits earlier in the year than *H. axyridis*, the eggs and younger larvae of *C. septempunctata brucki* are rarely encountered by *H. axyridis* larvae (Takahashi 1989; Sato and Dixon 2004). While sympatric species might be expected to possess co-evolved defence strategies against intraguild predation, such co-evolved strategies are unlikely between taxa whose ranges do not overlap. Ware et al. (in press) discuss the risk posed to many British species by the arrival of invasive *H. axyridis*. One exception appears to be *C. quatuordecimguttata*. This species is usually considered to be a deciduous tree specialist (Majerus 1991) and although the habitat range of *H. axyridis* is considerably more generalist (Roy et al. 2006), it is likely that the two species will interact in both Britain and Europe where their ranges overlap. However, this study has provided evidence that *C. quatuordecimguttata* eggs are effectively protected from predation by *H. axyridis*, and Ware and Majerus (2007) have also shown its larvae and pupae to be relatively well defended from attack. It

therefore seems that at least one European species is already well-equipped to deal with the encroachment of *H. axyridis*.

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